AS PER PCI REGULATIONS
THIRD YEAR B. PHARM

SEMESTER-V

PHARMACOLOGY-II

Dr. MANJUNATHA P. MUDAGAL

Dr. UDAY RAJ SHARMA

NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

A Text Book of

PHARMACOLOGY - II

As Per PCI Regulations

Third Year B. Pharm.

Semester - V

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Preface

It is our immense pleasure to bring out the "**First Edition**" of this book which is dedicated to the students and faculty of B. Pharma. institutes of this country. This book is designed and edited in accordance to the syllabus requirement of "**Pharmacology-II**" of third year (5^{th} semester) B. Pharm course in pharmacy prescribed in "**Bachelor of Pharmacy (B. Pharm) course regulations 2014**" by Pharmacy council of India.

Sincere efforts have been made to present theoretical aspects in details along with flowcharts/ pictorial for easy understanding the mechanism of actions of drugs and its pharmacological aspects. Most aspects are described stating examples with an intention to scaffold theoretical concepts and easy attempted during Pharmacology Practical sessions to various global organizations.

The major objective of this book is to provide students, collective information about subject in simple and lucid language. We have kept in mind the difficulties which the students generally face.

The salient features of the book are:

1. It covers all the topics prescribed in "**Bachelor of Pharmacy (B. Pharm) course regulations 2014**" by Pharmacy council of India.
2. The language used is simple and lucid.
3. Questions: The book contain MCQs, short, long questions on each chapter.

We hope that this book shall be found useful by the students and quick lessons for teaching faculty.

We are thankful to the management of Acharya & BM Reddy College of Pharmacy, Bengaluru for their keen interest and timely encouragement that made it possible to bring out this first volume.

We are highly indebted to **Dr. Divakar Goli**, Campus director, Acharya Institutes, Bengaluru for his constant motivation and guidance.

Suggestions and comments are always welcome and they shall be gratefully acknowledged.

Manjunatha P Mudagal

Uday Raj Sharma

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Syllabus

Unit I **[10 Hrs.]**

1. Pharmacology of Drugs Acting on Cardio Vascular System

(a) Introduction to Hemodynamic and Electrophysiology of Heart.
(b) Drugs used in Congestive Heart Failure.
(c) Anti-hypertensive Drugs.
(d) Anti-anginal Drugs.
(e) Anti-arrhythmic Drugs.
(f) Anti-hyperlipidemic Drugs.

Unit II **[10 Hrs.]**

1. Pharmacology of Drugs Acting on Cardio Vascular System

(a) Drug used in the Therapy of Shock.
(b) Hematinics, Coagulants and Anticoagulants.
(c) Fibrinolytics and Anti-platelet Drugs.
(d) Plasma Volume Expanders.

2. Pharmacology of Drugs Acting on Urinary System

(a) Diuretics.
(b) Anti-diuretics.

Unit III **[10 Hrs.]**

3. Autocoids and Related Drugs

(a) Introduction to Autacoids and Classification.
(b) Histamine, 5-HT and their antagonists.
(c) Prostaglandins, Thromboxanes and Leukotrienes.
(d) Angiotensin, Bradykinin and Substance P.
(e) Non-steroidal Anti-inflammatory Agents.
(f) Anti-gout Drugs.
(g) Antirheumatic Drugs.

Unit IV **[08 Hrs.]**

5. Pharmacology of Drugs Acting on Endocrine System

(a) Basic Concepts in Endocrine Pharmacology.
(b) Anterior Pituitary Hormones-analogues and their Inhibitors.
(c) Thyroid Hormones-analogues and their Inhibitors.
(d) Hormones regulating Plasma Calcium LEVEL-Parathormone, Calcitonin and Vitamin-D.
(d) Insulin, Oral Hypoglycemic Agents and Glucagon.
(e) ACTH and Corticosteroids.

Unit V **[07 Hrs.]**

5. Pharmacology of Drugs Acting on Endocrine System

(a) Androgens and Anabolic Steroids.
(b) Estrogens, Progesterone and Oral Contraceptives.
(c) Drugs acting on the Uterus.

6. Bioassay

(a) Principles and Applications of Bioassay.
(b) Types of Bioassay
(c) Bioassay of Insulin, Oxytocin, Vasopressin, ACTH, d-Tubocurarine, Digitalis, Histamine and 5-HT.

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Contents

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Unit I

Chapter ... 1

Introduction to Hemodynamic and Electrophysiology of Heart

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *Pathophysiology of hypertension, atherosclerosis, arrhythmia.*

1.1 CARDIOVASCULAR HEMODYNAMICS

Haemodynamics is the term used to describe the interactions of the physiological parameters that govern the behaviour of the CVS.

1.1.1 Introduction

- The cardiovascular system is concerned with the circulation of the blood. Essentially it consists of heart, which works as pump, and the blood vessels, which carry the blood. The blood carries oxygen and nutrients, and circulates through various tissue of the body.
- The word **hemodynamics – means circulation of blood in the human body.**
- Cardiovascular hemodynamics comprises of blood circulation to the heart and in turn the blood circulation regulated by the heart.

1.1.2 Coronary Blood Flow

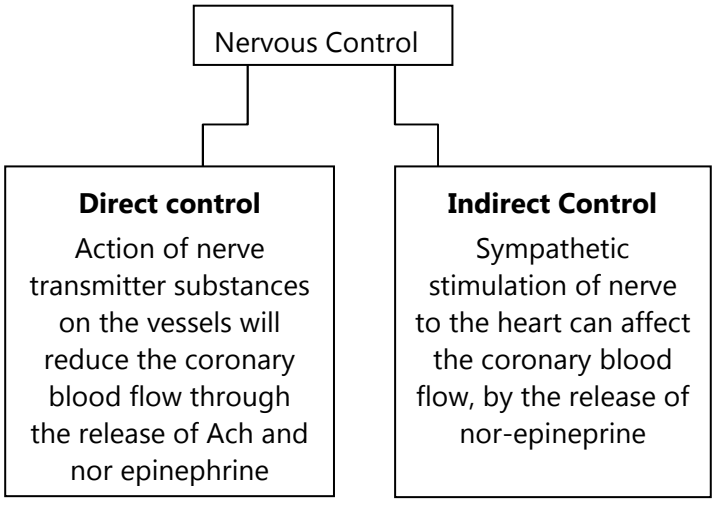
- Resting coronary blood flow in human average is approximately 225ml/minute, which is 0.7 to 0.8 ml per gram of the heart muscle.
- During the diastole, cardiac muscle relaxes completely and no longer obstructs the blood flow through left ventricular capillaries.
- This is phasic changes in coronary blood flow during cardiac muscle compression.
- During cardiac contraction – Intra myocardial pressure in the inner layer of the heart muscle is so much greater than the outer layer.
- It compresses the sub endocardial blood vessels far more than it compresses the outer vessel.

Control of Coronary Blood Flow:

- Oxygen demand is a major factor in local blood flow regulation.
- Determinants of oxygen consumption.
- Importance of increase in coronary blood flow in response to myocardial oxygen usage.
- Reactive hyperemia in coronary system.

Nervous Control:

- Stimulation of autonomic nerves to the heart can affect coronary blood flow in two ways.



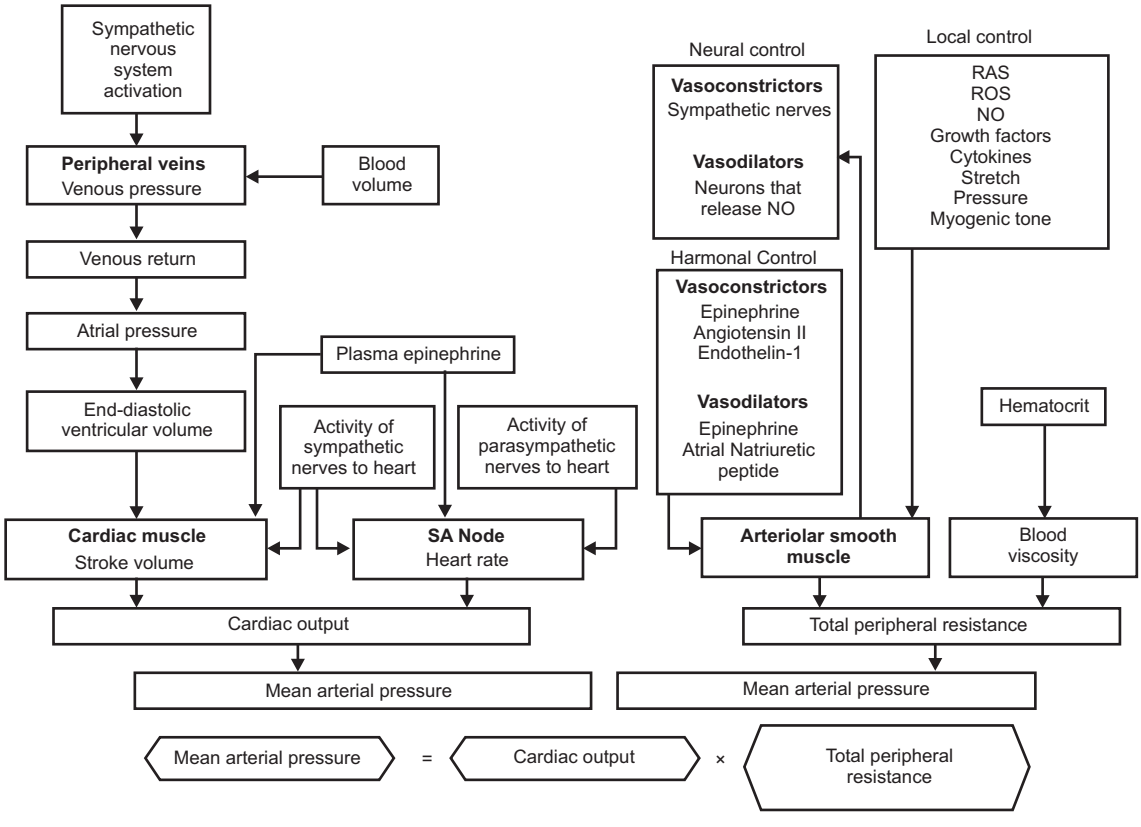


Fig. 1.1: Blood pressure regulation

Stroke Volume

- The amount of blood pumped by the left ventricle of the heart in one contraction. Normally only about 2/3rd of the blood in the ventricle is expelled with each beat.

Cardiac Output

- Flow of blood is usually measured in *l*/min.
- Total amount of blood flowing through the circulation = Cardiac output (CO)

  Cardiac Output = Stroke Volume × Heart Rate = 5 L/min.
- Influenced by blood pressure (force of blood against side wall) & resistance (Blood viscosity, Vessel length, Vessel Elasticity, Vasoconstriction/Vasodilation).

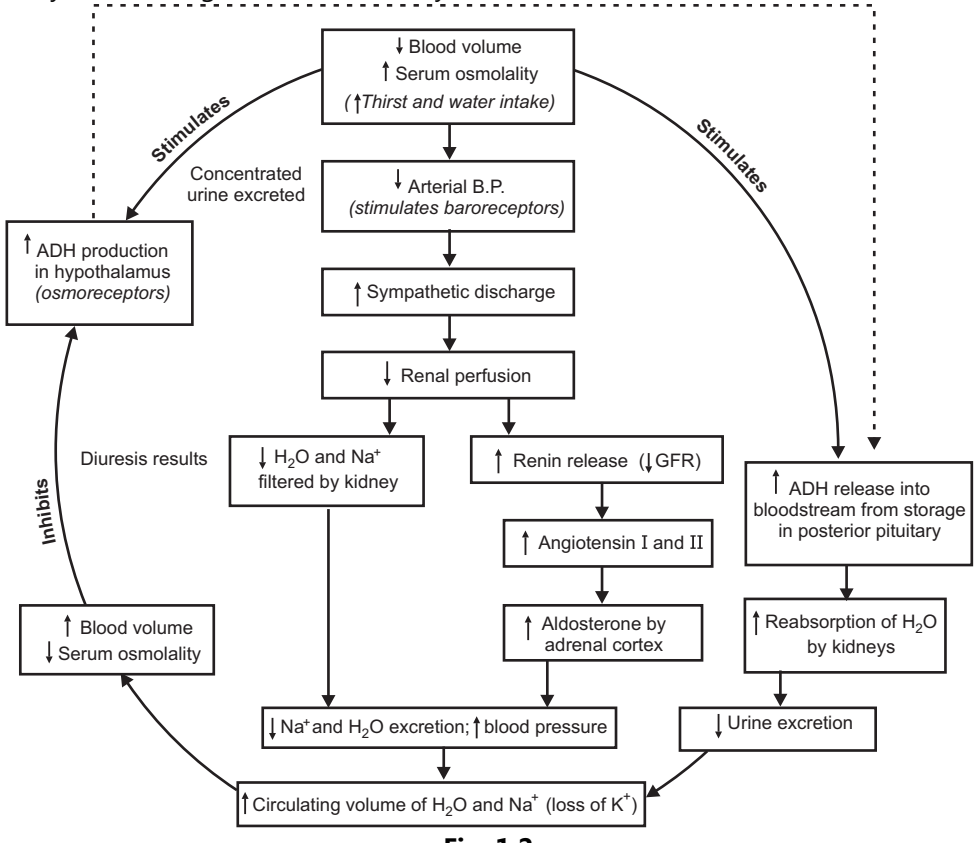


Fig. 1.2

Fluid regulation cycle, rennin angiotensin aldosterone (RAA) system and including anti-diuretic hormone (ADH).

Blood Pressure Regulation

- Blood pressure is determined by vascular resistance and cardiac output.
- Vascular resistance is regulated at the level of the arterioles, influenced by neural and hormonal inputs.
- Cardiac output is determined by heart rate and stroke volume, which is strongly influenced by blood volume.
- Blood volume in turn is regulated mainly by renal sodium excretion or resorption.

- Renin, a major regulator of blood pressure, is secreted by the kidneys in response to decreased blood pressure in afferent arterioles. In turn, renin cleaves angiotensinogen to angiotensin I; subsequent peripheral catabolism produces angiotensin II, which regulates blood pressure by increasing vascular smooth muscle cell tone and by increasing adrenal aldosterone secretion and, consequently, renal sodium resorption.

1.2 ELECTROPHYSIOLOGY OF HEART

1.2.1 Physiology of Cardiac Muscle

- Three Major types of cardiac muscle fiber:
  - Atrial muscle.
  - Ventricular muscle.
  - Specialized excitatory and conductive muscle fibers.
  - Atrial and ventricular muscle contract in a same way as skeletal muscle but the duration of contraction is longer.
  - The specialized excitatory and conductive fibers contract feebly because they contain few contractile fibrils. Exhibit automatic rhythmical electrical discharge in the form of action potentials.

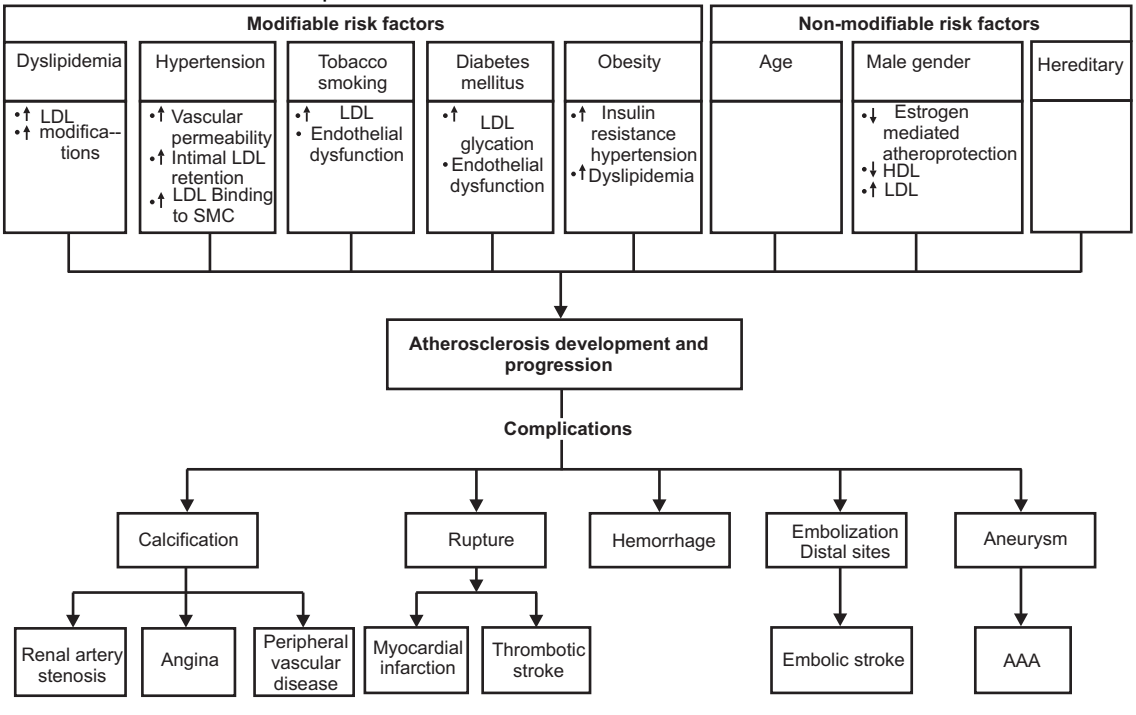


Fig. 1.3: Risk factors and complications of atherosclerosis

Atherosclerosis:

- Atherogenesis is driven by interplay of vessel wall injury and inflammation. The multiple risk factors for atherosclerosis all cause endothelial cell dysfunction and influence smooth muscle cell recruitment and stimulation.

- Atherosclerotic plaques develop and grow slowly over decades. Stable plaques can produce symptoms related to chronic ischemia by narrowing vessels, whereas unstable plaques can cause dramatic and potentially fatal ischemic complications related to acute plaque rupture, thrombosis, or embolization.
- Stable plaques tend to have a dense fibrous cap, minimal lipid accumulation, and little inflammation, whereas "vulnerable" unstable plaques have thin caps, large lipid cores, and relatively dense inflammatory infiltrates.

1.2.2 Action Potential in Cardiac Muscle

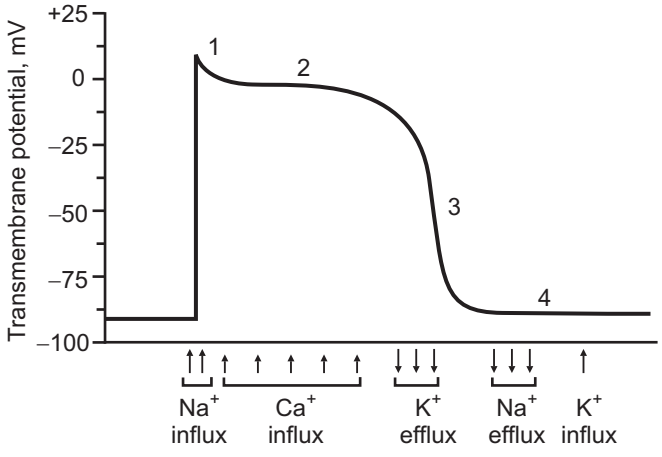


Fig. 1.4

Phase 0 (Rapid Depolarization):

External Stimulus to Excitable Tissue

↓

Opens the Voltage Gated Sodium Ion Channels

↓

Sodium Ions Enter the Cells down their Electrochemical Gradient

↓

Intracellular Movement of Sodium ion Depolarizes the Membrane

↓

Increases the Membrane Conductance to Sodium Ion displaces the Membrane Potential to +30mV

Phase 1 (Early Repolarization)

- Following phase 0, the membrane repolarizes rapidly and transiently to almost 0 mV because of the inactivation of sodium ion channel and simultaneous transient increases in outward potassium currents.

Phase 2 (Plateau)

- Membrane potential remains approximately 0 mV for a relatively prolonged duration.
- A balance between slow inward Ca^{2+} and outward K^{+} currents mediates the plateau phase of the action potential.

Phase 3 (Repolarization)

- Inactivation of Ca^{2+} channels and a simultaneous increase in outward K+ current through K^{+} channels produces a net outward movement of positive charge and repolarization of the membrane.

Phase 4 (Resting Membrane Potential)

- The membrane potential of ventricular myocytes remains at the resting membrane potential until the cell is stimulated again.
- The types of action potential in the heart can be separated into two categories:
  - **Fast-response action potentials**, which are found in the His-Purkinje system and atrial or ventricular cardio-myocytes.
  - **Slow- response action potentials**, which are found in the pacemaker cells in the SA and AV nodes.

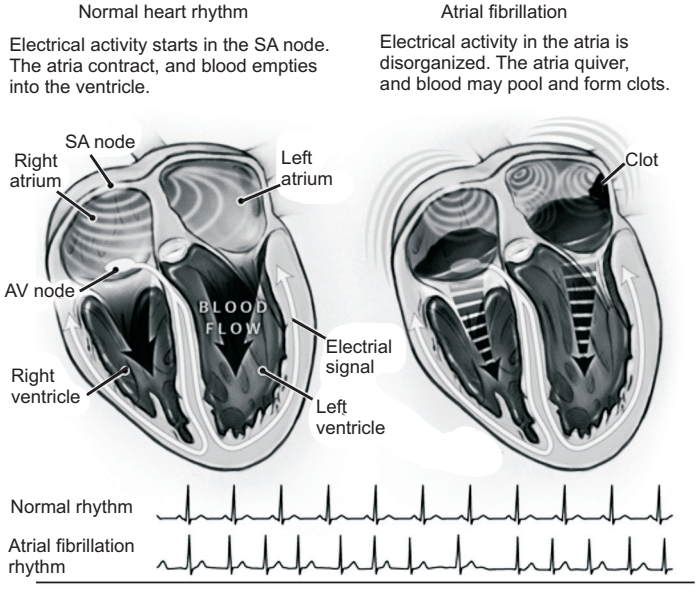


Fig. 1.5

Heart Failure

- CHF occurs when the heart is unable to provide adequate perfusion to meet the metabolic requirements of peripheral tissues; inadequate cardiac output usually is accompanied by increased congestion of the venous circulation.
- Left-sided heart failure is most commonly secondary to ischemic heart disease, systemic hypertension, mitral or aortic valve disease, or primary diseases of the myocardium; symptoms are mainly a consequence of pulmonary congestion and edema, although systemic hypoperfusion can cause renal and cerebral dysfunction.

- Right-sided heart failure is most often due to left heart failure and, less commonly, to primary pulmonary disorders; signs and symptoms are related chiefly to peripheral edema and visceral congestion.

Arrhythmias

- Arrhythmias can be caused by ischemic or structural changes in the conduction system or by myocyte electrical instability. In structurally normal hearts, arrhythmias more often are due to mutations in ion *channels* that cause aberrant repolarization or depolarization.
- Sudden cardiac death (SCD) most frequently is due to coronary artery disease leading to ischemia. Myocardial irritability typically results from non-lethal ischemia or from pre-existing fibrosis from previous myocardial injury. SCD less often is due to acute plaque rupture with thrombosis that induces a rapidly fatal arrhythmia.

■■■

Chapter ... 2

Drugs used in Congestive Heart Failure

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Classify drugs.*
- *Illustrate mechanism of action and ADR.*

2.1 DEFINITION

Congestive heart failure (CHF) is a clinical syndrome during which the center is unable to pump ample blood to fulfill the metabolic necessities of the body, or will do thus solely at an elevated filling pressure.

2.2 EPIDEMIOLOGY

- Heart failure may be a burgeoning downside worldwide, with over twenty million folks affected.
- The overall prevalence of HF within the adult population in developed countries is 2%.
- HF prevalence follow an exponential pattern, rising with age, and affects 6-10% of people over age 65.
- The incidence of HF is lower in women than in men.
- Although HF once was through to arise primarily within the setting of a depressed left chamber (LV) ejection fraction (EF), medical specialty studies have shown that around simple fraction of patients WHO develop HF have a standard or preserved EF (EF 40-50%).
- Accordingly, HF patients are now broadly categorized into one of two groups: (1) HF with a depressed EF (systolic failure) or (2) HF with a preserved EF (diastolic failure).

2.3 CLASSIFICATION OF CHF

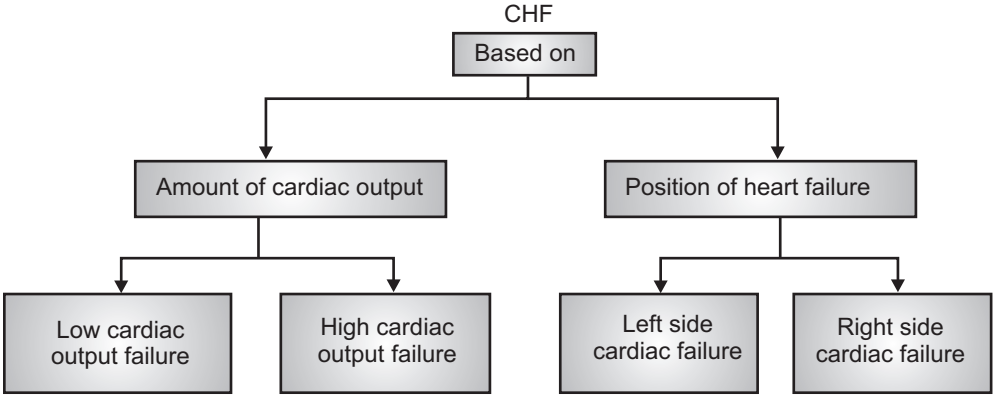


Fig. 2.1: Congestive heart failure

Based on amount of Cardiac Output:

Low cardiac Output Failure	High cardiac Output Failure
Most frequent.	Very rarely.
Metabolic demands of the body organs for oxygen are normal and within limits.	Metabolic demands of the body for oxygen is very high.
Myocardial fraction is prominent factor leading to the failure of systolic and diastolic function of the ventricles, ultimately results in low cardiac output failure.	Hyperthyroidism, anaemia, arteriovenous shunt causes high cardiac output failure.

Based on the position of Heart Failure:

Left side Cardiac Failure	Right side Cardiac Failure
Is the result of right side cardiac failure.	Is the result of left side cardiac failure
Inefficient pumping action of left ventricle is responsible for the accumulation of blood in the ventricles.	Inefficient pumping action of right ventricle is responsible for the accumulation of blood in right ventricle.
Left ventricle fails to accept/collect the blood from lungs due to back pressure.	Right ventricle fails to accept/collect the blood from peripheral organs.
Pulmonary congestion/oedema is the final result.	Peripheral generalized oedema is the final result.

2.4 PATHOPHYSIOLOGY OF CONGESTIVE CARDIAC FAILURE (C.C.F)

Cardiac membrane is lipoproteinous in nature. Normally Na^+ ion are concentrated extracellularly.

When Na^+ levels falls
↓
Sacroplasmic reticulum remains inactive
↓
Causes low level of Ca^{++}
↓
Leads to weak myocardial systole
↓
This causes accumulation of blood in ventricles
↓
Leads to ventricular distension
↓
Heart tries to expel this accumulated blood volume
↓
Gets exhausted and fails to work
↓
This failure due to accumulation of blood volume is defined as
↓
Congestive Heart Failure

Compensatory Mechanisms of CHF

To enhances the cardiac output, body compensates for the intrinsic cardiac effects in the following manner.

1. Increased sympathetic discharge.
2. To complete the remittent B.P., baroreceptors set within the arch of artery arterial blood vessel sinuses and walls of the center get excited and causes activation of beta-adrenergic receptors resulting in an increase in rate and force of contraction of heart.
3. An increase in blood vessel comes back (preload) is additionally seen because of the activation of alpha adrenergic receptors.
4. Increased rate associated force of contraction at the side of the enhanced preload ends up in an initial increase within the flow.
5. Vasoconstriction of the arteries due to alpha stimulation also causes an increase in after load, leading to fall in ejection fraction.

Activation of Renin Angiotensin Aldosterone (RAA)

- Fall within the flow decreases the urinary organ perfusion rate; as a result the RAA system gets activated.
- Angiotensin II may cause atrophic response in vascular smooth muscle (with vasoconstriction) and myocardial hypertrophy, attempting to restore wall stress to normal.

Cardiac Remodeling:

It is most vital mechanism by that body stipendiary for the intrinsic internal organ effects.

- It involves changes within the form of the center (from traditional to spherical) because of cardiac muscle hypertrophy.
- During cardiac remodeling, the connective tissue cells as well as the abnormal myocardial cells undergo proliferation and dilation instead of stretching under the influences of angiotensin-2.
- In the early stages, the remodeled heart maintains the cardiac performances.
- But later on, hypertrophy may exert certain adverse effects like ischaemic changes, decrease in the rate and force of contraction of heart. After certain period of time the antagonistic mechanisms get exhausted and worsen the cardiac performances. The stress on heart increases and a stage is reached where these mechanisms fails to maintain the adequate cardiac output.

Clinical manifestations/signs and symptoms

- Fluid retention
- Pulmonary congestion
- Dyspnoea and orthopnoea

CVS Manifestations

- Resting tachycardia
- Ventricular arrhythmias
- Enlargement of heart

Renal Manifestations

- Nocturia
- Oliguria

Other Manifestations

- Reduced cardiac output lead to poor perfusion of skeletal muscle resulting in fatigue.
- Reduced flow result in poor perfusion of muscle leading to fatigue.
- Reduced perfusion to brain results in altered mental states and confusion.
- Reduced perfusion might also cause the patient to seem pale with cold and perspiring hands.

Treatment

Non drug treatment/ non pharmacological approach:

- Physical exercise
- Salt intake
- Fluid intake
- Alcohol consumption
- Liquorice

2.5 DRUG THERAPY

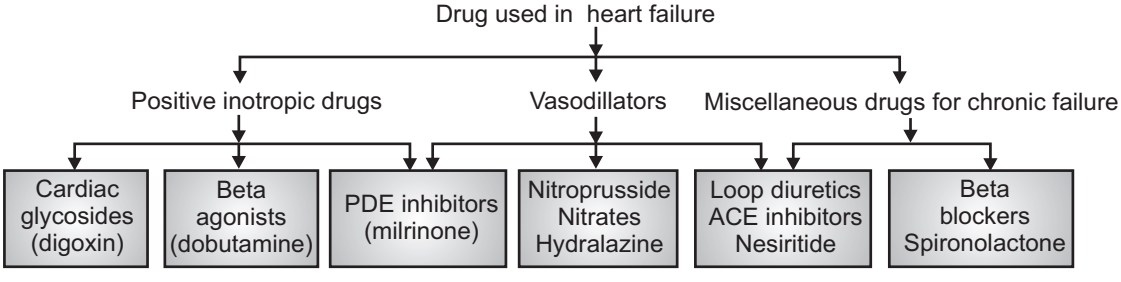


Fig. 2.2: Drugs used in heart failure

Inotropes:

- Increase force of contraction.
- All increase intracellular cardiac Ca^{++} concentration.

E.g. Digitalis (cardiac glycoside), Dobutamine (β-adrenergic receptor agonist), Milrinone.

Non-pharmacological Approaches:

CARDIAC GLYCOSIDES

Mechanism of action:

"Effect on cardio vascular function in CHF".

1. Digitalis derivatives when administrated to individuals suffering from CHF:

- Acts directly on the myocardium and increases conductivity, automaticity, rhythmicity and causes forceful contraction of heart. Because of powerful contractions, ventricular blood is forced from right side in to artery and from left ventricles to aorta.

  This causes complete emptying of heart.

- Digitalis derivatives block Na+ - K+ ATPase enzymes and improves level of Na+ and act as represented below:

Digitalis
↓
Blocks Na^+ - K^+ ATPase enzyme
↓
Increases Na^+ plasma level
↓
Activates sacroplasmic reticulum
↓
Releases Ca^{++}
↓
Combines with cardiac muscles
↓
Causes forceful contraction
↓
Leads to complete emptying of heart (ventricles)
↓
Thus relieves congestion
↓
Restore myocardial function

Thus digitalis derivatives, by their direct and indirect action, improve the force of contractility and thereby assure complete emptying of heart.

Thus digitalized heart can do work with less energy expenditure or more work with some energy expenditure.

Hence, digitalis is defined as "Cardiotonic".

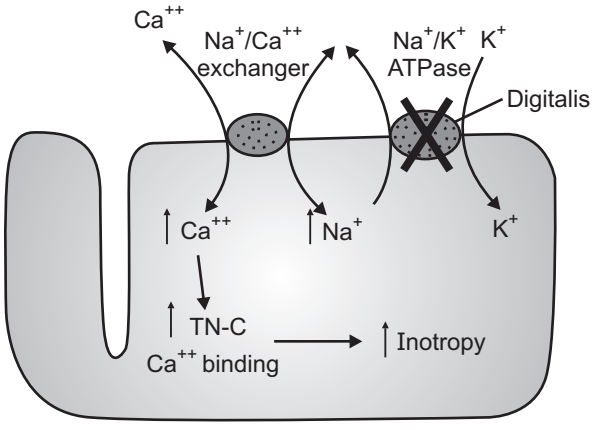


Fig. 2.3

2. Effect on automaticity, conductivity, contractility, blood pressure, heart rate:

- **Automaticity:** Digitalis increases the ability of purkinje cells and ventricular muscles to initiate impulses.
- **Conduction velocity:** The conduction velocity is slightly increased in the atria and ventricle by small doses of digitalis.
- **Blood pressure:** Digitalis increases mean arterial pressure in normal individuals only.
- **Heart rate:** Digitalis does not affect heart rate in normal individuals, but reduces it in CHF patients.

Extra Cardiac Actions:

- **On kidney:** Digitalis increases rate of excretion of Na+ and water by kidney and thus exerts diuresis.
- **On Gastro-intestinal Tract:** High doses of digitalis produces diarrhea, nausea, vomiting.

Side Effects:

- Anorexia, nausea, vomiting, diarrhoea.
- Headache, fatigue, insomnia.
- Yellow/green vision, blurred vision.
- Cardiac arrhythmia.

Therapeutic Uses:

- To treat heart failure.
- To treat atrial fibrillation.
- To treat atrial flutter.
- To treat paroxysmal atrial tachycardia.

Contraindication:

Digitalis is strictly contraindicated in following clinical conditions:

- Myocardial infraction
- Ventricular tachycardia

- Partial heart block
- Previous digitalis therapy
- Calcium administration.

Digitalis interaction:

- **Digitalis, Calcium:** Calcium ions increase the force of contraction of heart. High plasma calcium levels stimulate the myocardium so much, that it leads to cardiac arrest during systole. Digitalis is also known to increase the force of contraction of heart. Thus digitalis and calcium act synergetically and may prove to be toxic. Hence during digitalis therapy Ca^{++} ion administration must be avoided.
- **Digitalis, Quinidine:** Quinidine, when administrated in individuals taking digitalis, increases serum digoxin levels. This may cause adverse effects of digitalis and other clinical complexities. Hence must be avoided.

Treatment of over digitalisation:

- Immediately stop the administration of digitalis.
- Stop if any diuretic administration is in continuation.
- Mild tachycardia can be treated with atropine.
- Mild toxicity can be treated by administration of potassium salts – 5 to 7.5 g of potassium chloride orally daily.
- Ventricular tachycardia can be treated with phenytoin (250 mg well diluted).

Rapidly Acting Inotropic Agents

In critically sick infants with CHF, in those with renal dysfunction (e.g., infants or in postoperative cardiac patients with heart failure) quickly acting catecholamines with a short duration of action is preferable to digoxin.

- dopamine,
- dobutamine
- epinephrine

β_1 Blockers:

β_1-Blockers-bisoprolol, carvedilol, metoprolol.

Mechanism of Action:

- Heart failure is accompanied by an increase activation of sympathetic nervous system.
- This brings about structural and functional modification in the myocardium.
- β Blockers inhibit the sympathetic outflow of nor epinephrine and counteract the changes produced.
- The ventricular remodeling in heart failure is also reversed by β Blockers.
- Increases beta receptor sensitivity.

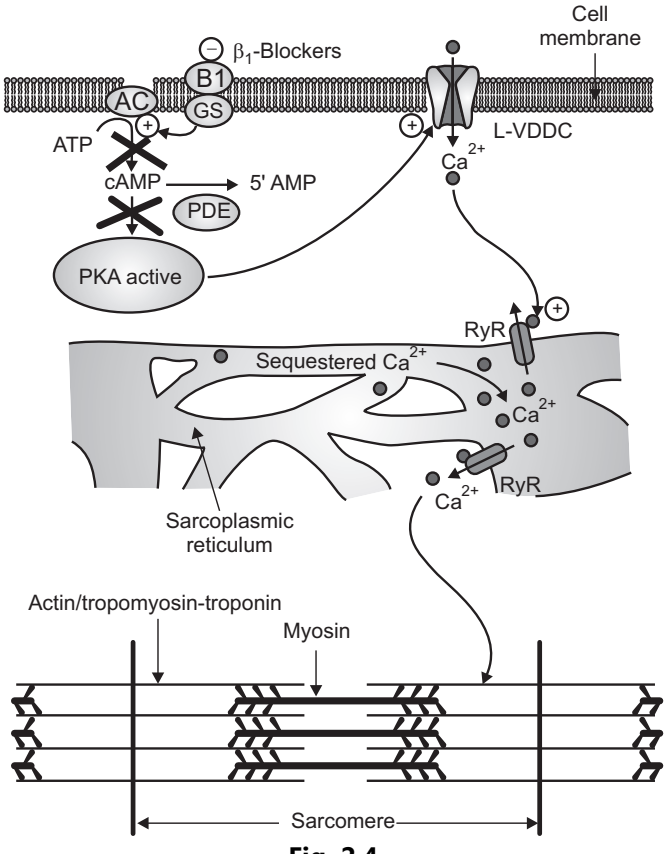


Fig. 2.4

Adverse drug reaction:

- Hypotension
- Bradycardia.

VASODILATORS

- Isosorbide dinitrate, isosorbide mononitrate, and hydralazine also used specially in patients who cannot tolerate ACE inhibitors.

Mechanism of Action:

- It directly relaxes the arterioles and arteries reducing the peripheral vascular resistances, preload and help to reduce after load.

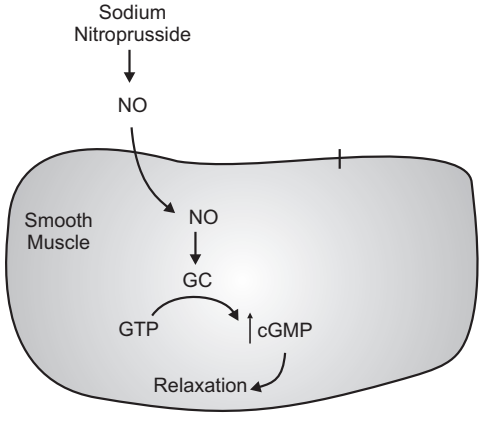


Fig. 2.5: Mechanism of Action of Vasodilators

Adverse drug reaction:

- Nausea
- Palpitation
- Tachycardia
- Salt and water retention on prolong therapy.

ACE INHIBITORS

- Captopril, Enalapril, Fosinopril, Lisinopril, Ramipril, Quinapril.

Mode of action:

- They inhibit the generation of angiotensin 2, a potent vasoconstrictor.
- They also inhibit the release of aldosterone and vasopressin, thereby inhibiting fluid and slat retention thus decreasing the preload.
- Elevate the levels of bradykinin.

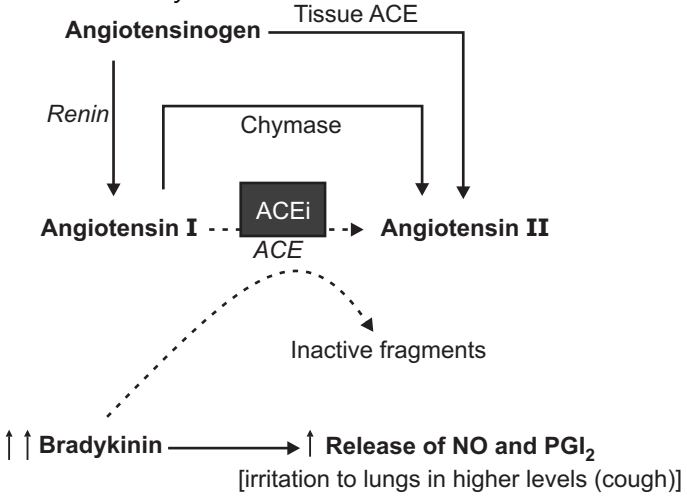


Fig. 2.6: Mechanism of Action of ACE incubators

Adverse drug reaction:

Postural hypotension, hyperkalemia, dry cough.

Angiotensin Receptor AT-1 blockers (ARB).

Losartan, Candesartan, Valsartan, Telmisartan.

Mode of Action:

- Angiotensin-2, a vasoconstrictor is concerned with ventricular remodeling and fluid retention.
- These drugs inhibit the binding of angiotensin 2 to its AT_1 receptor.
- Thus they preclude the above mentioned effects of angiotensin 2.
- These agents do not exert any action on bradykinin and thus do not produce cough.
- Has comparable effect to ACE I.
- Can be used in certain conditions when ACE I are contraindicated.

Adverse drug reactions:

- Orthostatic Hypotension.
- Hyperkalemia
- Headache
- Dizziness
- Impairment of renal functioning.

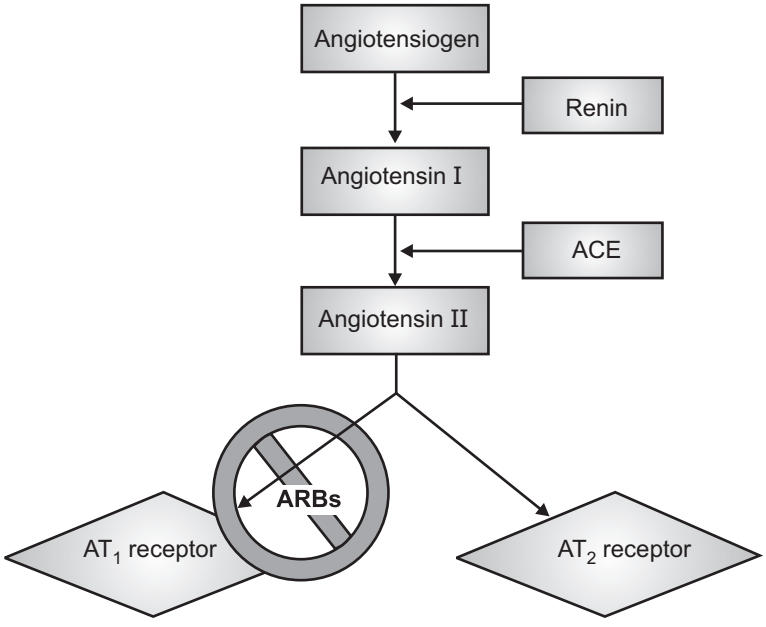


Fig. 2.7

DIURETICS

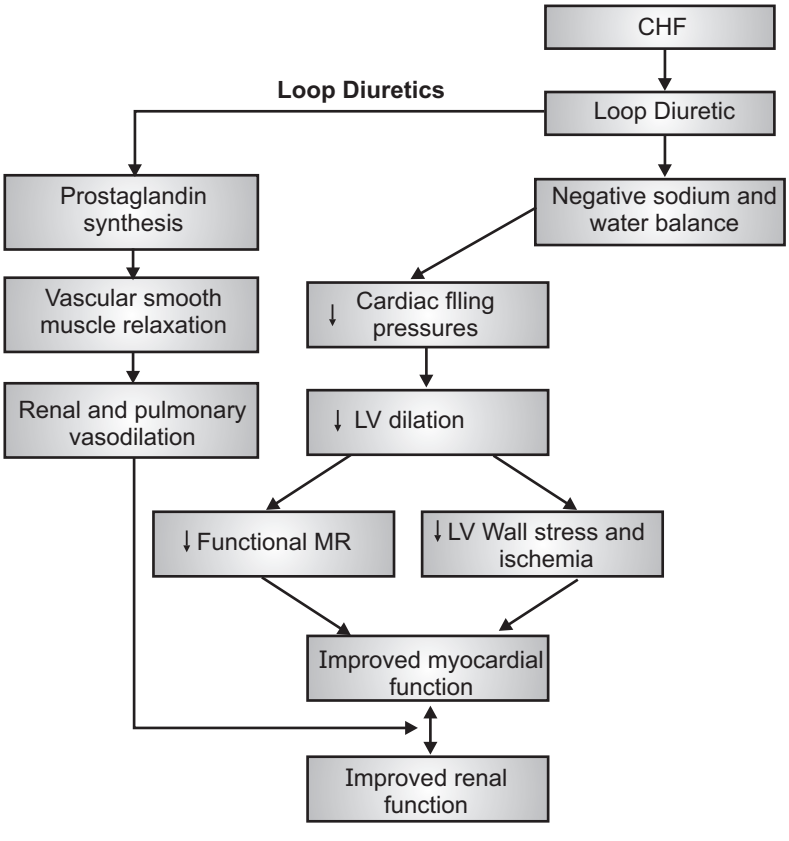


Fig. 2.8: Loop Diuretics

- Bumetanide, Furosemide, Metolazone, Torsemide.
- Diuretics remain the principal therapeutic agent to control pulmonary and systemic venous congestion.
- Diuretics reduce preload and improves congestive symptoms, but do not improve cardiac output or myocardial contractility.
- Loop diuretics commonly used.
- Aldosterone antagonists - used in conjunction with a loop diuretic.

QUESTIONS

1. Define cardiotonics. Explain the pharmacology of digitalis.
2. Explain the mechanism of action and toxicity of cardiac glycosides.

■■■

Chapter ... 3

Antihypertensive Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the classes of antihypertensive and their indications for use.*
- *Explain mechanism of action and ADR.*

3.1 DEFINITION

Hypertension is defined as bizarre raise in diastolic and/or systolic pressure.

3.2 CLASSIFICATION OF HYPERTENSION

Classification	Systolic (mmHg)	Diastolic (mmHg)
Normal	< 120	< 80
Pre-hypertension	120 - 139	80 - 89
Stage 1	140 - 159	90 - 99
Stage 2	> 160	> 100

Prevalence

Women with 55 years of age are more prone to hypertension than men.

Blood pressure levels increase with age and it (persistently elevated BP levels) is very common in the elderly.

ETIOLOGY

- In most patients, hypertension results from an unknown pathophysiologic etiology (*essential or primary hypertension*).

  This form of hypertension cannot be cured, but it can be controlled.

- A small percentage of patients have a specific cause of their hypertension (*secondary hypertension*).

There are many potential secondary causes that are either concurrent medical conditions or are endogenously induced.

If the cause can be identified, hypertension in these patients has the potential to be cured.

Essential Hypertension:

- More than 90% of individuals with hypertension have essential hypertension.
- Numerous mechanisms have been identified that may contribute to the pathogenesis of this form of hypertension, so identifying the exact underlying abnormality is not possible.
- Genetic factors may play an important role in the development of essential hypertension.

Secondary Hypertension:

- Fewer than 10% of patients have secondary hypertension where either a comorbid disease or drug is responsible for elevating BP.
- In most of these cases, renal dysfunction resulting from severe chronic kidney disease or renovascular disease is the most common secondary cause.
- Certain drugs, either directly or indirectly, can cause hypertension or exacerbate hypertension by increasing BP.

Diseases: Chronic kidney disease, Obstructive sleep apnea, Parathyroid disease, Pheochromocytoma, Thyroid disease.

Prescription drugs: Adrenal steroids, Amphetamines/anorexiants, Decongestants, Nonsteroidal anti-inflammatory drugs.

Food substances: Sodium, Ethanol, Licorice.

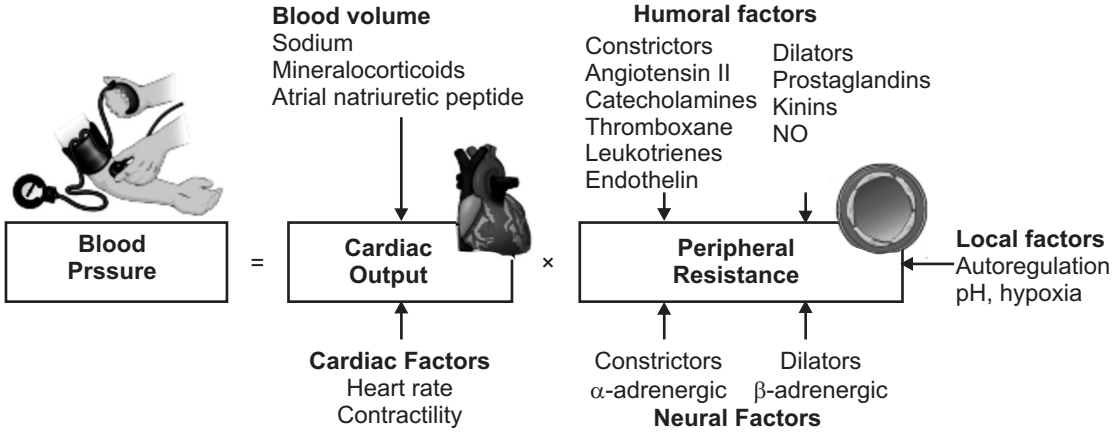


Fig. 3.1: Blood pressure regulation

3.3 ANTIHYPERTENSIVE DRUG CLASSES

Antihypertensive drugs are organized around a clinical indication – the need to treat a disease – rather than a receptor type. The drugs covered in this unit have a variety of mechanisms of action including diuresis, sympathoplegia, vasodilation, and antagonism of angiotensin, and many agents are available in most categories.

Note: Antihypertensive drugs have then never been more important. Along with diet and exercise, they remain one of the most effective methods to reduce high blood pressure.

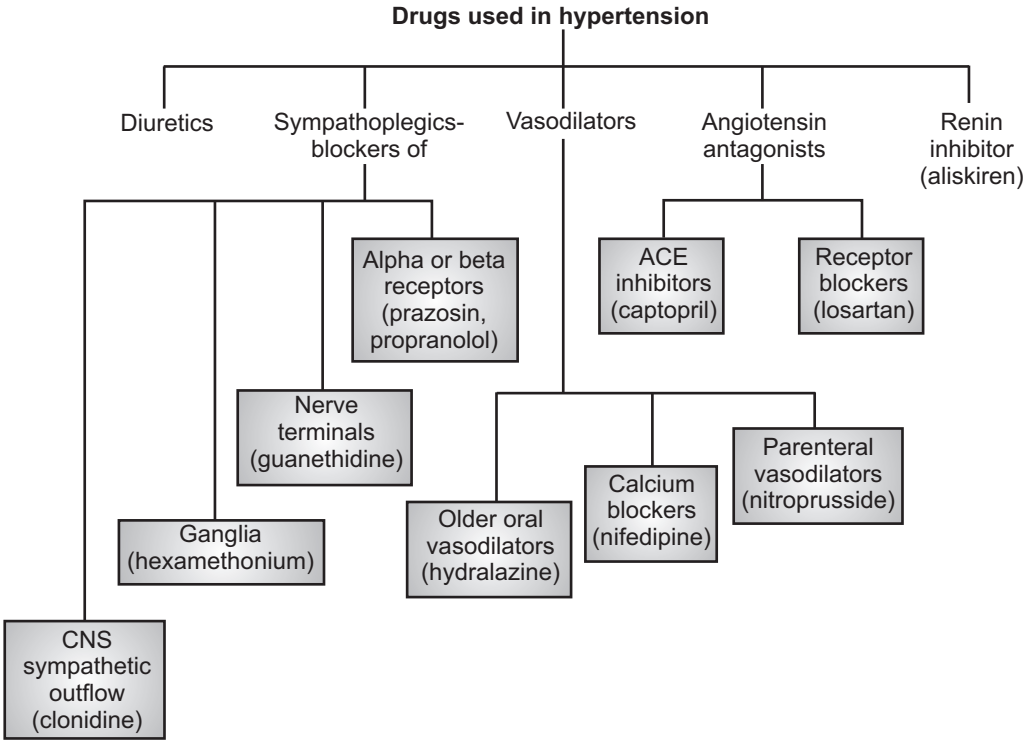


Fig. 3.2

Antihypertensive drug classes are as follows:

- ACE inhibitors
- Angiotensin II receptor antagonists
- Diuretics
- Calcium channel blockers
- β-blockers
- α-blockers
- α_2-blockers
- Renin inhibitors
- Vasodilators

For each drug class, we offer examples, their mechanism of actions (MOA), side effects and drug interaction profiles and any other relevant clinical features.

3.3.1. ACE Inhibitors (Angiotensin Converting Enzyme Inhibitors (ACEIs))

Example: Ramipril, Lisinopril, Perindopril.

MOA: Angiotensin converting enzyme inhibitors (ACEIs) are those drugs which are frequently used for the treatment of cardiovascular disorders including hypertension and heart failure. This group of drugs diminishes blood pressure and oxygen demand by dilating blood vessels. These drugs principally inhibit an angiotensin converting enzyme which is an integral part of RAAS (Renin-angiotensin-aldosterone system). The detailed mode of action is described in the following diagram.

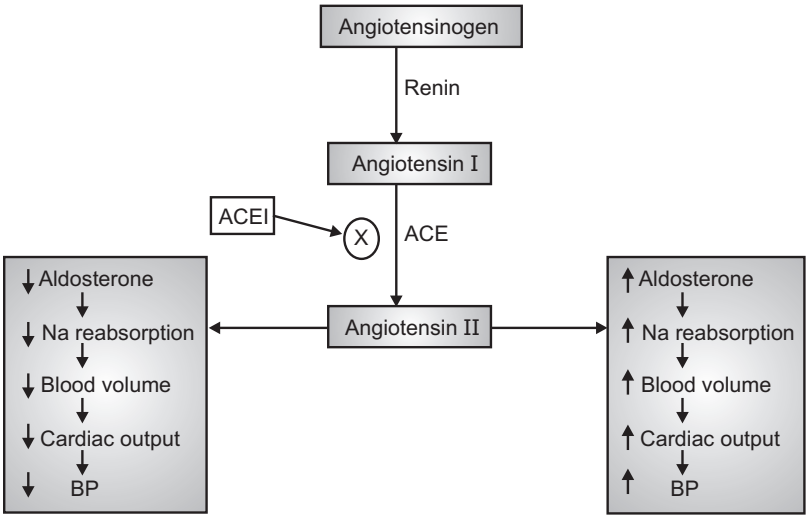


Fig. 3.3: MOA of ACEI

Side effects:

- Hypotension-first dose hypotension is prevalent with ACE inhibitors.
- Persistent dry cough-due to pulmonary kinin accumulation.
- Hyperkalemia-ACE inhibitors promote potassium retension.
- Other effects- fatigue, nausea, dizziness, headache.

3.3.2 Angiotensin II Receptor Antagonists

Angiotensin II receptor antagonist are also known as ARBs or angiotensin receptor blockers. They are sometime used in place of ACE inhibitors particularly where the persistent dry cough has become unbearable for the patient.

Examples: Candesartan, lrbesartan, Losartan, Telmisartan.

MoA:

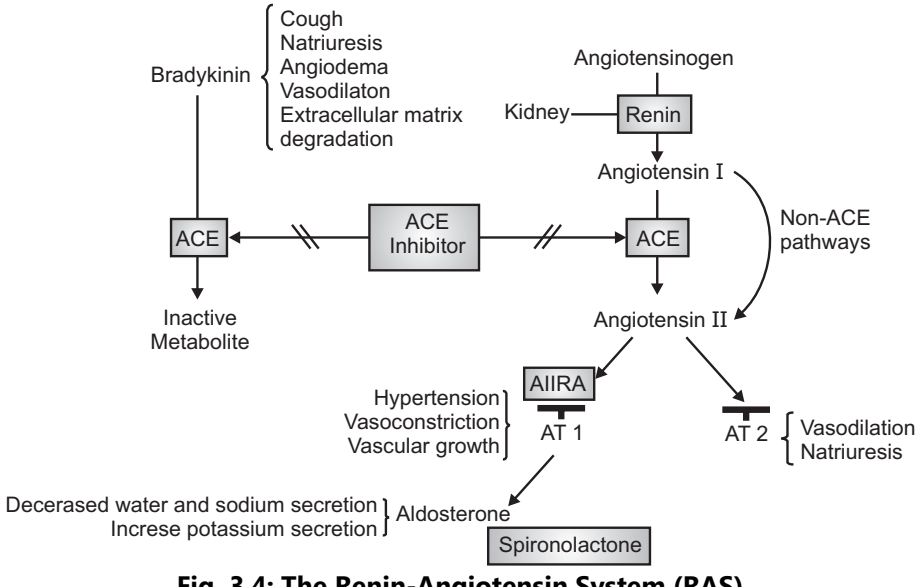


Fig. 3.4: The Renin-Angiotensin System (RAS)

Side effects: Hypotension, Hyperkalemia

Renal failure (as with ACE inhibitors):

- Cough-though less likely than with an ACE inhibitors.
- Due to risk of hyperkalemia, other potassium-elevating drugs should not be prescribed.
- This includes potassium supplements, potassium-sparing diuretics.
- As with ACE inhibitors, taking ARBs with NSAIDs increase risk of renal failure.

3.3.3 Diuretics

Diuretics are drugs that promote dieresis or water loss. There are many different diuretic classes, too many to review in detail here. However, we have examined many of the major diuretic classes elsewhere.

Diuretic drug classes:

- Loop diuretics-furosemide, bumetanide.
- Thiazide and thiazide - like diuretics - bendroflumethiazide, hydrochlorothiazide, indapamide, metolazone.
- Potassium-sparing diuretics- amiloride, spironolactone.
- The purpose of diuretics is to eliminate excess sodium and water from the body. Some diuretic classes also eliminate potassium, increasing the risk of hypokalemia.
- Other drugs though, such as a amiloride and spironolactone, retain potassium ions- increasing the risk of hyperkalemia. However, diuretic combinations are invariably used to balance and offset these risks.

Diuretics act at different points along the nephron:

For example:

- Loop diuretics act at the thick ascending limb.
- Thiazide diuretics act at the distal convoluted tubule.
- Potassium-sparing diuretics act at the cortical collecting duct.
- Osmotic diuretics, such as mannitol, act at the proximal tubule.
- Carbonic anhydrase inhibitors, such as acetazolamide, also act at the proximal tubule.

Side effects: In general terms, the broad side effects associated with diuretics include:

- **Loop diuretics:** Hypovolemia, hypokalemia, metabolic alkalosis, hyperuricemia.
- **Thiazides:** Associated with the side effects listed for loop diuretics, plus hypercalcemia and hyponatremia.
- **Hyperkalemia:** Amiloride, triamterene, spironolactone.

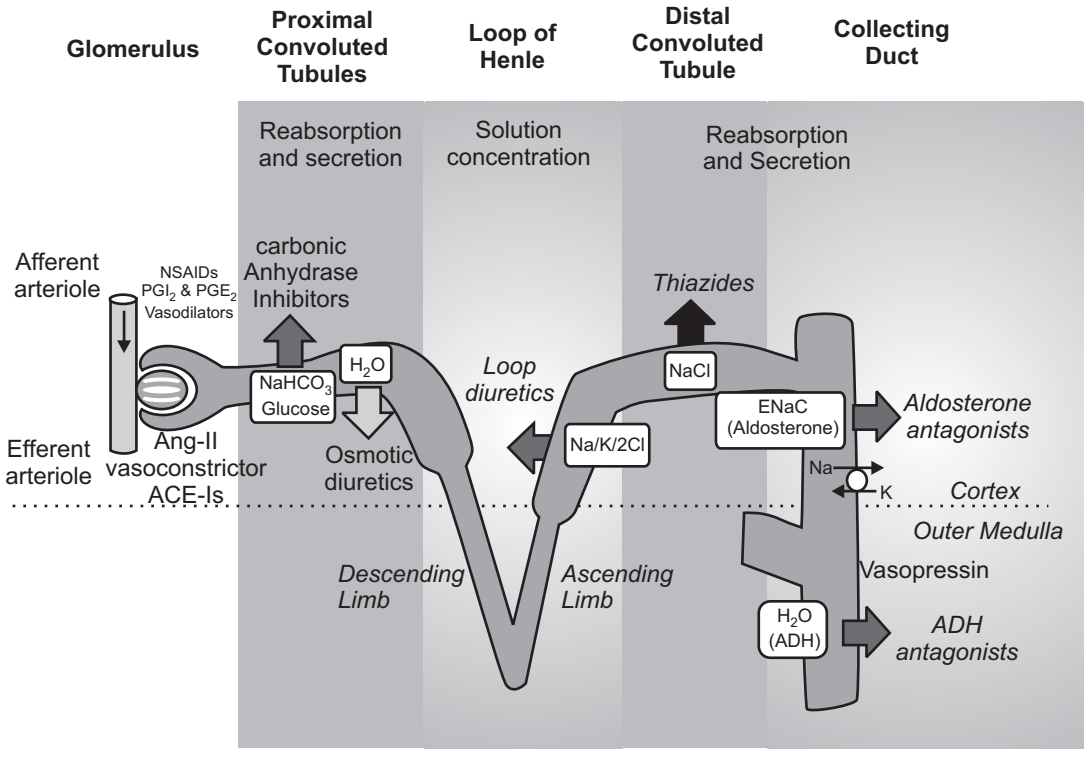


Fig. 3.5

3.3.4 Calcium Channel Blockers (CCB)

- Not all calcium channel blockers are used for their antihypertensive effects.

Examples:

- Amlodipine, Nifedipine, Diltiazem, Verapamil.
- Amlodipine and nifedipine may be used for hypertension whereas diltiazem and verapamil are predominantly used to control heart rate/ arrhythmias.

MoA: Calcium channel blockers reduce calcium entry into vascular and cardiac cells. This reduces intracellular calcium concentration which, in turn causes relaxation on vasodilation in atrial smooth muscle. Calcium channel blockers also reduce myocardial contractility in the heart.

Side effects: Because diltiazem and verapamil (the non-dihydropyridine CCBs) are used as class III anti-arrhythmic drugs, the side effects profile below focuses on amlodipine and nifedipine.

Flushing, Headache, Ankle swelling, palpitations, Light headedness.

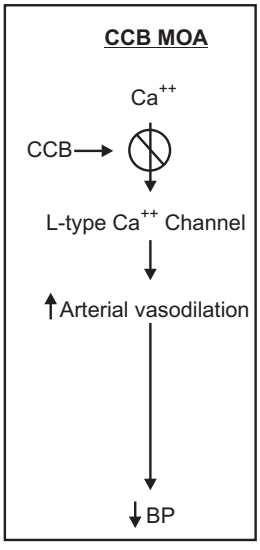


Fig. 3.6: MoA of CCB

3.3.5 β-blockers

β-blockers are not just used in the treatment of hypertension (though not generally given as initial therapy), they also used in the treatment of ischemic heart disease, chronic heart failure, atrial fibrillation and supraventricular tachycardia.

Examples: Metoprolol, Bisoprolol, Labetalol, Nebivolol.

MoA: β-blockers act through a variety of means for hypertension they act to reduce renin secretion from the kidney-an effect ordinarily mediated by β-1 receptors. Recall that β-1 receptors are located mainly in the heart, whereas β-2 receptors are mainly located in the smooth muscle of blood vessels and in the airways.

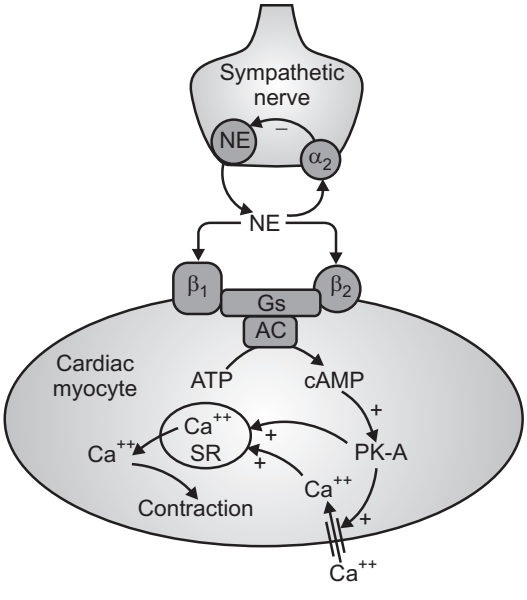


Fig. 3.7

Side effects: Fatigue, Cold extremites, Headache, Nausea.

3.3.6. α-blockers

α-blockers may be used to treat hypertension in resistant cases where other drugs –such as ACE inhibitors, calcium channel blockers and thiazide diuretics-have proven ineffective.

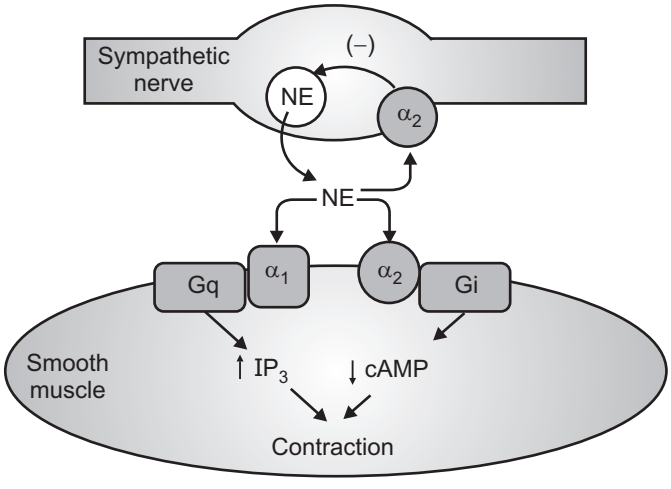


Fig. 3.8

Examples: Alfuzosin, Tamsulosin, Doxazosin.

MoA: α-1 receptors are predominantly found in smooth muscle, such as blood vessels or the urinary tract stimulation produces concentration and inhibition causes relaxation α blockers are highly selective for α-1 receptor, causing vasodilation and a reduction in blood pressure.

Side effects: Postural hypotension, Dizziness, Faintness.

These three effects are more pronounced after first dose.

3.3.7 α-2 Agonists

As with the α-2 agonists are rarely used they are typically only used when all other conventional options have been exhausted, when used they are usually taken alongside a diuretics.

Examples: Clonidine, Methyldopa, Moxonidine

MoA: More specifically, α-2 agonists are classified as centrally-acting α-2 agonists, these receptors are activated in the brain which, once activated, open peripheral blood vessels around the body, reducing blood pressure.

Side effects: Sedation, Dry nasal mucosa, Dry mouth, Rebound hypertension, Postural hypotension, Headache, Fatigue.

3.3.8 Renin Inhibitors

Renin is a protein and enzyme secreted by the kidneys.

It works by cleaving angiotensinogen (hepatic-produced) into angiotensin I. ACE then converts angiotensin-I into angiotensin-II and, in turn angiotensin-II causes increased secretion of aldosterone-increasing blood pressure. Renin inhibitors are then, an effective way to block the effects of angiotensin-II and reduce blood pressure.

Examples: Aliskiren

MoA:

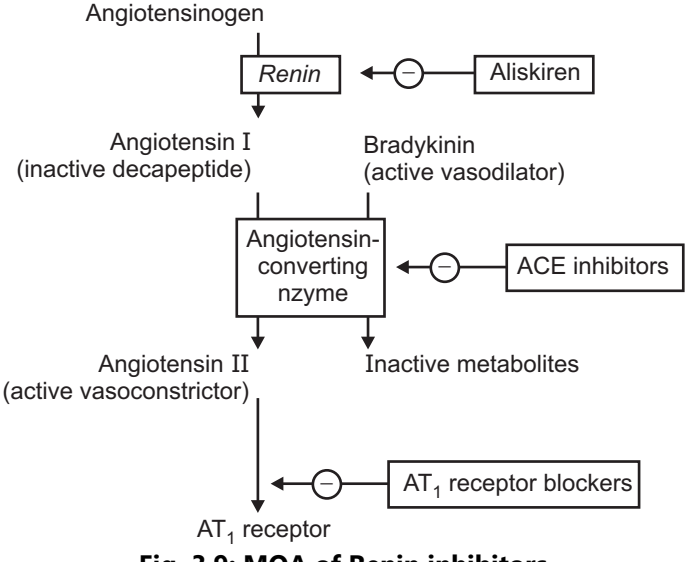


Fig. 3.9: MOA of Renin inhibitors

Side effects: Angioedema, Hyperkalemia, Hypotension, Diarrhea, Headache, Dizziness.

Aliskiren should not be taken alongside an angiotensin-receptor blocker or ACE inhibitor in diabetic patients due to an increased risk of stroke, hyperkalemia and kidney complications.

Due to the above risk and side effect profile, many clinicians regard aliskiren as more harmful than beneficial.

3.3.9 Vasodilator

Sodium Nitroprusside: Sodium nitroprusside has been used in cases of hypertensive emergency. It is administered via intravenous route and for this reason, has a rapid onset of effects.

Sodium nitroprusside deploys nitric oxide for its antihypertensive effect. Nitric oxide works to reduce total peripheral resistance and venous return. This reduces both preload and afterload.

Side effects associated with sodium nitroprusside include: Hypotension, Methemoglobinemia, Cyanide poisoning, Bradyarrhythmia, Palpitation, Tachyarrhythmia, Confusion, Dizziness, Renal azotemia.

Hydralazine: Hydralazine is used to treat hypertension, though it is more often used to treat high blood pressure, gestational hypertension. Like sodium nitropruside, it may also be used in hypertensive emergency.

VASODILATORS:

DIRECT

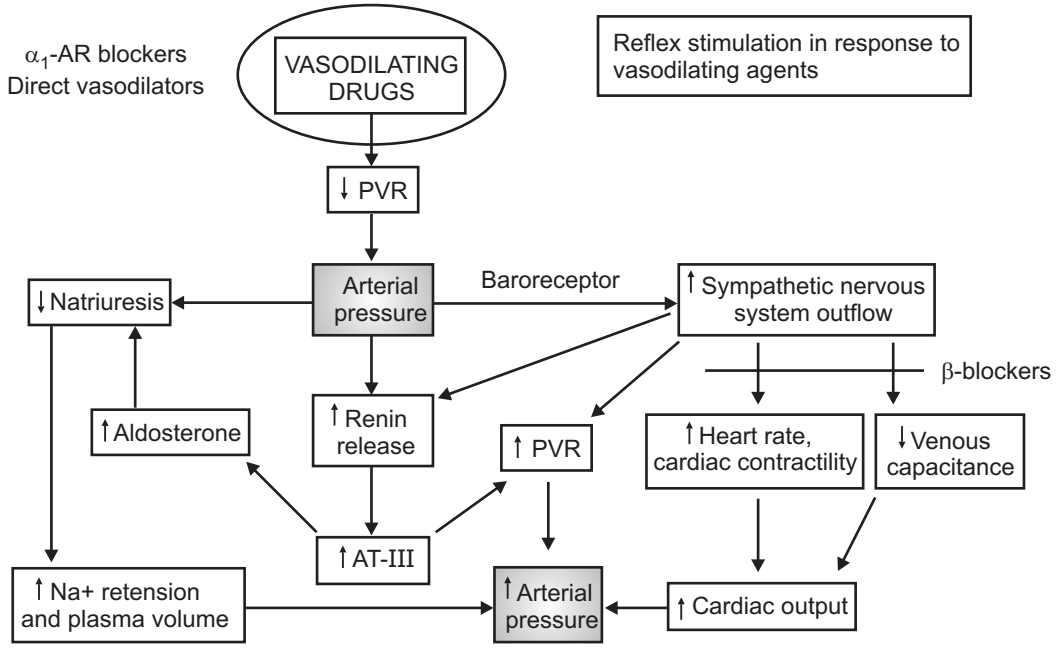


Fig. 3.10: MOA of Vasodilators

Side effects: Headache, Tachycardia, Palpitations, Hypotension, Aching/ swelling joints, Flushing.

It works as a direct acting smooth muscle relaxant, working as a vasodilator in resistance arterioles-decreasing total peripheral resistance and lowering blood pressure.

QUESTIONS

1. Classify antihypertensive drugs with examples. Explain the pharmacology of centrally acting antihypertensive and ACE inhibitors.
2. Classify anti-hypertensive with examples. Write the pharmacology of phenoxy-benzamine.
3. Define hypertension. Classify anti hypertensive drugs. Explain the pharmacology of Atenolol.
4. Discuss the pharmacology of Angiotensin – II receptor antagonist.
5. Discuss the mode of action, adverse effects and therapeutic applications of clonidine.
6. Highlight on Potassium channel activators as antihypertensive agents.

■■■

Chapter ... 4

Anti-Anginal Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Recall classification of Anti-Anginal drugs.*
- *State the mechanism of action and ADR.*

4.1 DEFINITION

Angina Pectoris is a symptom of myocardial ischemia and occurs due to an imbalance between oxygen demand and oxygen supply of myocardium.

- Characteristic abrupt, severe, pressing-like substernal chest pain radiating towards neck, jaw, back, and arms. Patients may also experience dyspnea or atypical symptoms such as indigestion, nausea, vomiting, or diaphoresis.
- Usually precipitated by exercise, excitement or a heavy meal.
- Transient episodes (15 seconds to 15 minutes) of myocardial ischemia (stable angina) do not results cellular death; as occurs in myocardial infarction (MI).

4.2 TYPES OF ANGINA

Typical Angina (Classical Angina)

- Pain is commonly induced by exercise, excitement or a heavy meal.
- Secondary to advanced atherosclerosis of coronary vessels.
- Associated with ST-segment depression on ECG.
- Usually lasts 1-15 minutes.

Variant Angina (Prinzmetal Angina)

- Pain is induced while at rest.
- Symptoms are caused by decreased blood flow to the heart muscle from the spasm of the coronary artery.
- Although individuals with this form of angina may have significant coronary atherosclerosis, the angina attacks are unrelated to physical activity, heart rate, or blood pressure.
- Associated with ST-segment elevation on ECG.
- Generally responds promptly to coronary vasodilators, such as nitroglycerin and calcium-channel blockers.
- But β-blockers are contraindicated.

Unstable angina (Acute Coronary Syndrome)

- May involve coronary spasm and may also have the component of atherosclerosis.
- The duration of manifestation is longer than the first two and has the manifestation of myocardial infarction (MI).
- Lies between stable angina and MI.
- The pathology is similar to that involved in MI: a platelet-fibrin thrombus associated with a ruptured atherosclerotic plaque but without complete occlusion of the blood vessel.

4.3 CLASSIFICATION OF ANTI-ANGINAL DRUGS

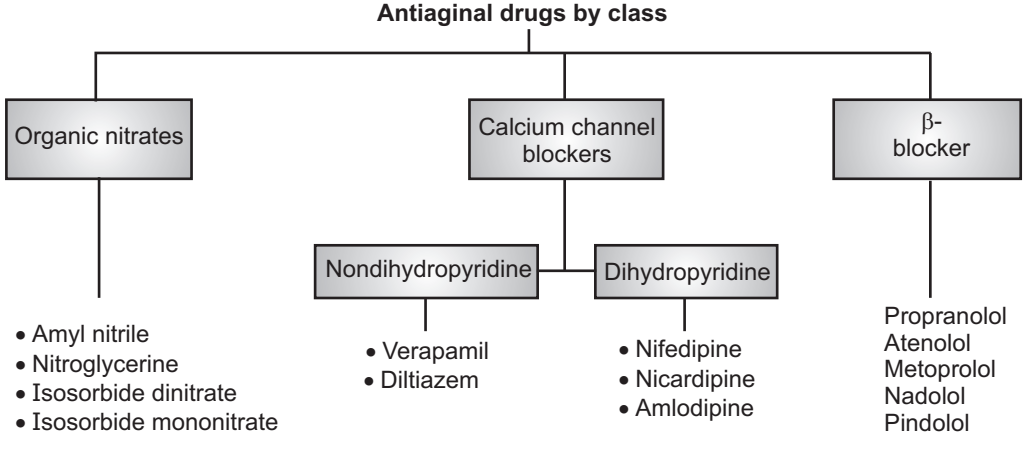


Fig. 4.1: Antianginal Drugs

4.3.1 Nitrates/Organic Nitrates

Nitroglycerine, Isosorbide dinitrate, Isosorbide mononitrate.

- Preload reduction: Peripheral pooling of blood → decreased venous return (preload reduction).
- After load reduction: Nitrates also produce some arteriolar dilation → slightly decrease total peripheral resistance or after load on the heart.
- Redistribution of coronary flow: In the arterial tree, nitrates preferentially relax bigger conducting coronary arteries than arterioles or resistance vessels.

MOA:

- The organic nitrate agents are prodrugs that are sources of NO. **NO activates the soluble isoforms of guanylyl cyclase,** thereby **increasing intracellular levels of cGMP.** In turn, **cGMP promotes the dephosphorylation** of the myosin light chain and the **reduction of cytosolic Ca++ and leads to the relaxation of smooth muscles** cells in a broad range of tissues.

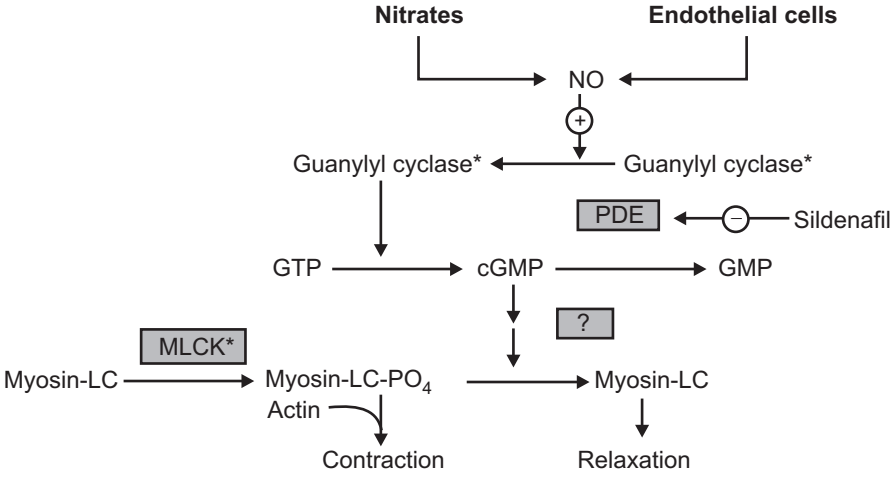


Fig. 4.2: MOA of Nitrates

Adverse effects:

- Headache is the most common adverse effect of nitrates. High doses of nitrates can also cause postural hypotension, facial flushing and tachycardia.
- Phosphodiesterase type 5 inhibitors such as **sildenafil** potentiate the action of the nitrates. To preclude the **dangerous hypotension** that may occur, this combination is contraindicated.

Tolerance:

- Tolerance to the action of nitrates develops rapidly as the blood vessels become desensitized to vasodilation. Tolerance can be overcome by providing a daily "**nitrate free interval**" to restore sensitivity to the drug.

Dependence:

- Sudden withdrawal after prolonged exposure has resulted in spasm of coronary and peripheral blood vessels. Withdrawal of nitrates should be gradual.

4.3.2 β-Blockers

Atenolol, Bisoprolol, Metoprolol, Proranolol

- Atenolol, metoprolol, propranolol, bisoprolol are used only for prophylactic therapy of angina; they are of no value in an acute attack.
- Effective in preventing exercise-induced angina.
- But are ineffective against the vasospastic form.
- Cardioselective β-blockers, such as metoprolol or atenolol, are preferred. Thus, Propranolol is not preferred.
- Agents with intrinsic sympathomimetic activity (for example, pindolol) are less effective and should be avoided in angina.

The dose should be gradually tapered off over 5 to 10 days to avoid rebound angina or hypertension.

MOA: Suppress the activation of the heart by blocking B1 receptors.

I. Decrease the heart rate, resulting in:
   1. decreased myocardial oxygen demand.
   2. Increased oxygen delivery to the heart.

II. Decrease myocardial contractility, helping to conserve energy/ decrease demand.

III. Reduce the work of the heart by decreasing COP and causing a slight decrease in BP
   - ↓ HR,
   - ↓ contractility,
   - ↓ systolic wall tension,
   - ↑ perfusion time

Reasons for Using Nitrates and β-Blockers in Combination in Angina

- β-Blockers prevent reflex tachycardia and contractility produced by nitrate-induced hypotension.
- Nitrates prevent any coronary vasospasm produced by β- Blockers.
- Nitrates prevent increases in left ventricular filling pressure or preload resulting from the negative inotropic effects produced by β-Blockers.

4.3.3 Calcium Channel Blockers (CCBs)

Amlodipine, Diltiazem, Felodipine, Nicardipine, Nifedipine, Verapamil.

MOA

- Calcium is essential for muscular contraction.
- The CCBs protect the tissue by inhibiting the entrance of Ca^{+2} into cardiac and smooth muscle cells of the coronary and systemic arterial beds.

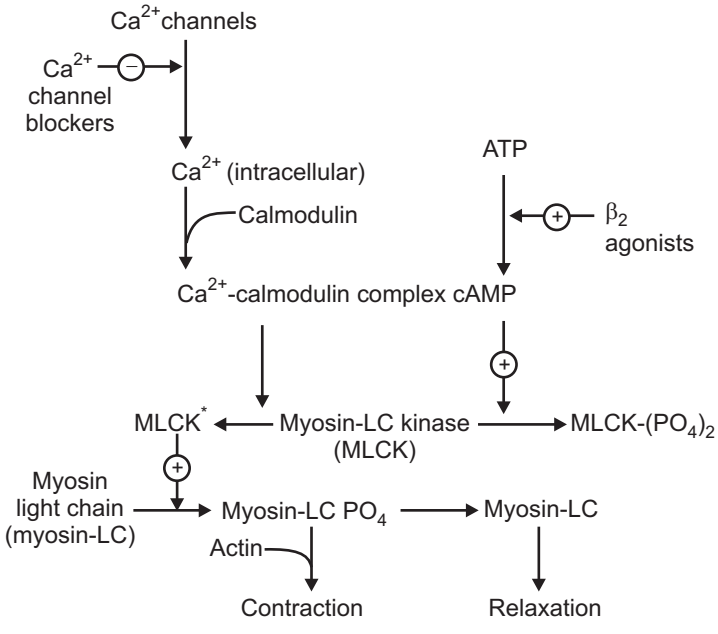


Fig. 4.3: MOA of Ca^{+} Channel Blockers

- All CCBs are therefore arteriodilators that cause a decrease in vascular resistance. Cause peripheral arterial vasodilation.
- Reduce myocardial contractility (-ve inotropic action).
- Result: decreased myocardial oxygen demand.

Pharmacological actions:

Smooth muscle: The CCBs causes relaxation by decreasing intracellular availability of Ca^{++}. The dihydropyridines (DHPs) have the most marked smooth muscle relaxant and vasodilator action; verapamil is somewhat weaker followed by diltiazem.

Heart: Calcium influx is increased in ischemia because of the membrane depolarization that hypoxia produces. The calcium channel blocker protect the tissue by inhibiting the entrance of calcium into cardiac and smooth muscle cells of the coronary and systemic arterial beds and decreases smooth muscle tone and vascular resistance, afterload.

Calcium Channel Blockers:

Phenyl alkylamine: Verapamil:

- It dilates arterioles and decreases total peripheral resistance.
- It slow atrioventricular (AV) conduction directly and decreases heart rate, contractility, blood pressure and oxygen demand.
- It also has some α adrenergic blocking activity.
- Verapamil has greater negative inotropic effects than amlodipine, but it is a weaker vasodilators.
- Verapamil should not given with β blocker, digoxin, cardiac depressants like quinidine and disopyramide

Benzothiazepines: Diltiazem:

- Diltiazem also slows AV conduction, decreases the rate of firing of the sinus node pacemaker, and is also a coronary artery vasodilator.
- Diltiazem can relieve coronary artery spasm and is particularly useful in patients with variant angina.
- It is somewhat less potent vasodilators than nifedipine and verapamil, and has modest direct negative inotropic action, but direct depression of SA node A-V conduction is equivalent to verapamil.

Dihydropyridine (DHP) Calcium channel blockers: Nifedipine:

- Nifedipine is the prototype DHP with a rapid onset and short duration of action. It causes arteriolar dilation and decreases total peripheral resistance.
- Nifedipine is usually administered as an extended-release oral formulation.
- It causes direct depressant action on heart in higher dose.

ADR: Frequent side effects are palpitation, flushing, ankle edema, hypotension, headache, drowsiness and nausea. Nifedipine has paradoxically increased the frequency of angina in some patients.

Other dihydropyridine (DHP) calcium channel blockers:

- Amlodipine, an oral dihydropyridine, functions mainly as an arteriolar vasodilators.
- Nitrendipine is a calcium channel blocker with additional action of vasodilation action. Vasodilation action is due to release NO from the endothelium and inhibits cAmp phosphodiesterase.
- Lacidipine, is a highly vasoselective newer DHP.
- Nimodipine is short-acting DHP which penetrates blood-brain barrier very efficiently due to high lipid solubility.
- DHP with long duration of action: Lercanidipine, Benidipine.

Uses:

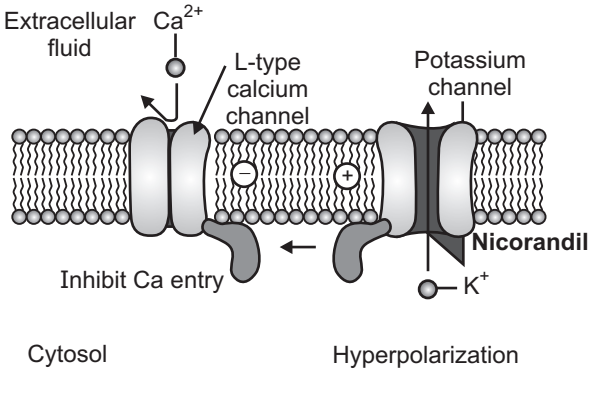
- Calcium channel blockers can be safely given to patients with obstructive lung disease and peripheral vascular disease in whom β blocker are contraindicated.
- CCB are used for the treatment of:
  - Angina pectoris
  - Hypertension
  - Cardiac arrhythmias
  - Hypertrophic cardiomyopathy

ADR: Lightheadedness. Low blood pressure. Slower heart rate.

Potassium Channel Openers:

Nicorandil:

- Antianginal action of nicorandil is mediated through ATP sensitive K^+ channels (KATP) thereby hyperpolarizing vascular smooth muscles.



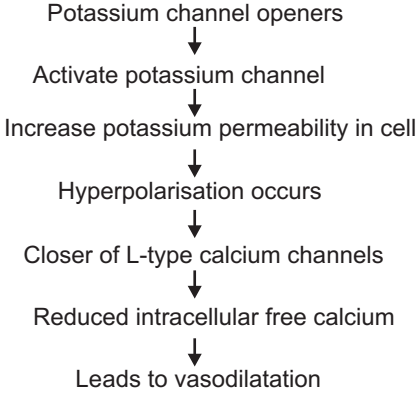


Fig. 4.4: MOA of Nicorandil

- Nicorandil is well absorbed orally, nearly completely metabolized in liver and excreted in urine. Administered I.V. during angioplasty for acute MI, it is believed to improve outcome.

ADR: Flushing, palpitation, weakness, headache, dizziness, nausea and vomiting.

4.4 OTHER ANTIANGINAL DRUGS

Drug	Pharmacological Action
Dipyridamol	• Dipyridamol inhibit platelet aggregation. • It is a powerful coronary dilator.
Trimetazidine	• This antianginal drugs act by nonhaemodynamic mechanisms. • The MOA of trimetazidine is uncertain, but it may improve cellular tolerance to ischemia by inhibiting mitochondrial long chain 3-ketoacyl-CoAhiolase.
Ranolazine	• This novel antianginal drug primarily act by inhibiting late Na+ current in the myocardium.
Ivabradine	• This 'pura' heart rate lowering antianginal drugs has been introduced recently as an alternative to β blocker. • It blocks cardiac pacemaker (sino-atrial) cell 'f' channels.
Oxyphedrine	• Improve myocardial metabolism.

Questions

1. Classify anti-anginal agents and write pharmacology of nitroglycerine.
2. What is angina pectoris? Classify anti-anginal agents. Describe the pharmacology of nitrates.
3. Explain the pharmacology of organic nitrates.

■■■

Chapter ... 5

Anti-Arrhythmic Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Enlist the anti-arrhythmic drugs.*
- *Describe mechanism of action and ADR.*

5.1 DEFINITION

Arrhythmia is defined as the variation of heart from normal rhythm.

Disturbances in the rhythm, heart rate, impulse generation or conduction of electrical impulses is responsible for membrane depolarization. These changes can escort to alterations in overall cardiac function that can be life-threatening.

5.2 MECHANISMS OF ARRHYTHMIAS

- Disturbances in generation of impulse may be due to:
  - Abnormal automaticity.
  - Delayed after depolarization.
- Disturbances in conduction of impulse:
  - By causing repeated activation (re-entry), impulse may re-circulate in heart.
  - Blocks conduction.

5.3 CLASSIFICATION OF ANTI-ARRHYTHMIC DRUGS

(Vaughan-Williams-Singh 1969)

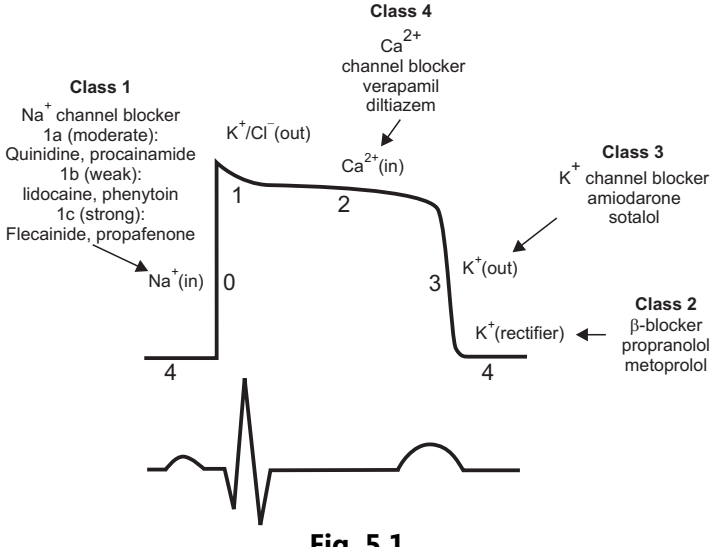


Fig. 5.1

Class I: Block Na+ channels	Ia (quinidine, Disopyramide, Lidocaine, Mexiletine, procainamide, Propafenone) Ib (lignocaine, phenytoin, mexiletine) Ic (flecainide)
Class II: ß-adrenoceptor antagonists	Atenolol, Esmolol, Metoprolol, Propranalol.
Class III: K^+ channel blocker-prolong action potential and prolong refractory period.	Amiodarone, Dofetilide, Sotalol, Dronedarone, Ibutilide.
Class IV: Ca^{2+} channel antagonists	Verapamil, Diltiazem.
Other anti-arrhythmic drugs	Adenosine.

Classification based on Clinical use:

- Drugs used for supraventricular arrhythmia`s.
  - Adenosine, verapamil, diltiazem.
- Drugs used for ventricular arrhythmias.
  - Lignocaine, mexelitine, bretylium.
- Drugs used for both supraventricular and ventricular arrhythmias.
  - Amiodarone, β-blockers, disopyramide, procainamide.

Na+ Channel Blocker:

- Bind to and block Na+ channels (and K+ also).
- Act on initial rapid depolarisation (slowing effect).
- Local Anaesthetic (higher concentration): block nerve conduction.
- Do not alter resting membrane potential (Membrane Stabilisers).
- At times, post repolarization refractoriness.
- Bind preferentially to the open channel state.
- **Use Dependence:** The more the channel is in use, the more drug is bound.

CLASS IA:

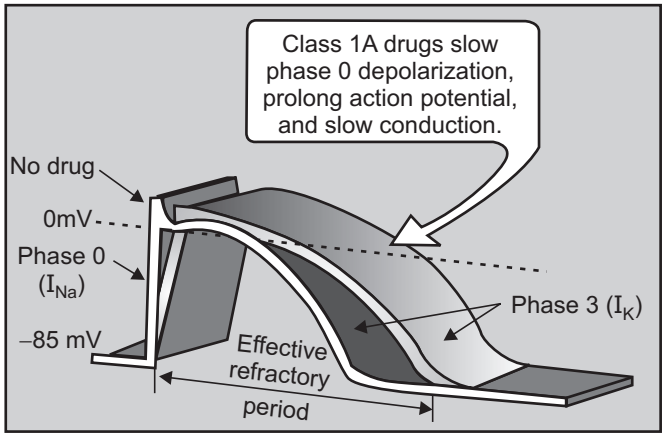


Fig. 5.2

Quinidine:

- D-isomer of quinine obtained from cinchona bark.
- Historically first antiarrhythmic drug used.

MOA:

- Blocks sodium channels.
- Decreases automaticity, conduction velocity and prolong S repolarization.
- Decreases phase 0 depolarization, increases action potential duration (APD) and effective refractory period (ERP).

Other actions:

- Decreases BP (α-block), skeletal muscle relaxation.

Clinical Pharmacokinetics

- Well absorbed.
- 80% bound to plasma proteins (albumin).
- Extensive hepatic oxidative metabolism.
- 3-hydroxyquinidine, is nearly as potent as quinidine in blocking cardiac Na^+ Channels and prolonging cardiac action potentials.

Uses:

- To maintain sinus rhythm in patients with atrial flutter or atrial fibrillation.
- To prevent recurrence of ventricular tachycardia or VF.

Adverse Effect:

Non-cardiac

- Diarrhea, thrombocytopenia.
- Cinchonism and skin rashes.

Cardiac:

- Marked QT-interval prolongation and torsades de pointes (2-8%).
- Hypotension.
- Tachycardia.

Drug Interactions:

- Metabolized by CYP450.
- Increases digoxin levels.
- Cardiac depression with beta blockers.
- Inhibits CYP2D6.

Disopyramide:

MOA:

Disopyramide produces a negative ionotropic effects that is greater than weak effect exerted by quinidine and procainamide, and unlike the latter drugs, disopyramide causes peripheral vasoconstriction.

Adverse Effects:

- Precipitation of glaucoma.
- Constipation, dry mouth.
- Urinary retention.
- Myocardial depression.

Drug Interactions:

Both metabolism of disopyramide and the accumulation of its metabolite are increased, in the presence of phenytoin, thus escalating the probability of anti-cholinergic properties.

Use:

- Ventricular tachycardia.
- Atrial flutter (AF) and Atrial fibrillation (AFI).

Procainamide:

- Procaine derivative, quinidine like action.

MOA:

- Procainamide binds to open and inactivated Na+ channels and prevents sodium influx, slowing the rapid upstroke during phase 0.

Adverse Effect:

- Hypotension.
- Hypersensitivity reaction.

Drug interactions:

- Cimitidine inhibits the metabolism of procainamide.

Uses:

- Premature atrial contractions.
- Paroxysmal atrial tachycardia.

CLASS IB

- They shorten Phase 3 repolarization.
- ↓ the duration of the cardiac action potential.
- Prolong phase 4.

IBL:

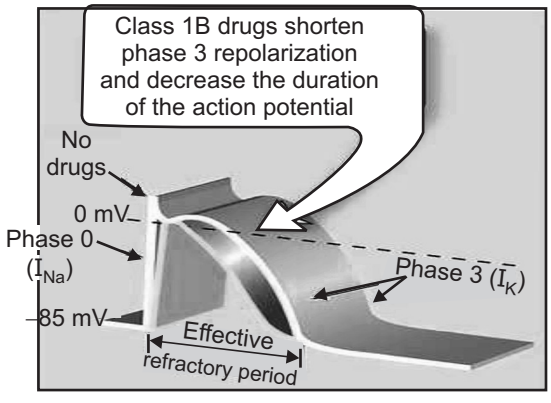


Fig. 5.3

Lidocaine

MOA

- It shorten phase 3 repolarization and decreases the duration of action potential.

Pharmacokinetics:

- High first pass metabolism.
- Metabolism dependent on hepatic blood flow.
- T½ = 8 min – distributive, 2 hrs – elimination.
- Propranolol decreases half life of lignocaine.
- Dose = 50-100 mg bolus followed by 20-40 mg every 10-20 min i.v.

Adverse Effects:

- Drowsiness.
- Slurred speech.
- Confusion and convulsions.

Drug Interaction:

- Propranolol increases its toxicity.
- The myocardial depressant effect of lidocaine is enhanced by phenytoin administration.

Uses:

- VA.
- Digitalis toxicity.

Phenytoin:

Phenytoin was originally introduced for the control of convulsive disorders but now also been shown to be effective in the treatment of cardiac arrythmias.

Adverse Effects:

- Respiratory arrest.
- Hypotension.

Drug Interaction:

- In the presence of chloramphenicol, disulfiram, and isoniazid, the plasma concentration of phenytoin is increased, since the later drugs inhibit the hepatic metabolism of phenytoin.

Uses:

- Anaesthesia.
- Open heart surgery.
- Digitalized induced and ventricular arrythmia in children.

Mexiletine

MOA

- By the oral route, it acts as local anesthetic as well as an active antiarrythmic; chemically and pharmacologically similar to lidocaine.
- It reduces automaticity in Purkinje Fibres (PF), both by decreasing phase IV slow and by increasing threshold voltage.
- It may convert one-way block to two-way block by reducing the rate of 0 phase depolarization in ischemic PF.

Adverse Effects:

- Tremor
- Hypotension
- Bradycardia

Drug Interactions:

- When mexiletine is administered with phenytoin or rifampin, since these drugs stimulate the hepatic metabolism of mexiletine, reducing its plasma concentration.

Uses:

- VA
- Congenital long QT syndrome.

CLASS IC

- **Markedly slow Phase 0** depolarization.
- Slow conduction in the myocardial tissue.
- **Minor effects on the duration of action potential and ERP**.
- Reduce automaticity by increasing threshold potential rather than decreasing slope of Phase 4 depolarization.

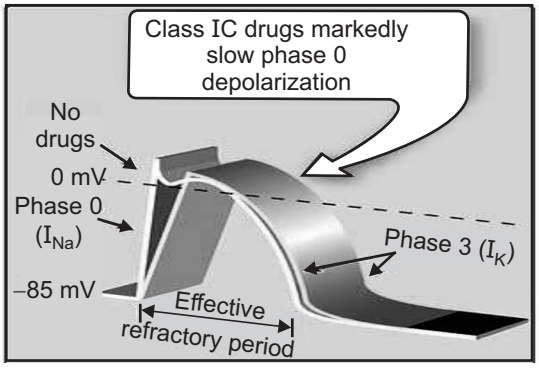


Fig. 5.4

FLECAINIDE

MOA

- Flecainide suppresses phase 0 upstroke in purkinje and myocardial fibers.
- This causes marked slowing of conduction in all cardiac tissues, with a minor effect on the duration of the action potential and refractoriness.
- Automaticity is reduced by an increase in the threshold potential rather than a decrease in the slope of phase 4 depolarization.
- Potent blocker of Na & K channels with slow unblocking kinetics.
- Maintain sinus rhythm in supraventricular arrhythmias.

Adverse Effect:

- Torsades de point, visual disturbances & headache.

Uses

- Ventricular arrhythmia.

Class II Drugs: β- Blockers

PROPRANOLOL, METOPROLOL, ESMOLOL, ACEBUTOLOL

β-receptor stimulation:

- Increase automaticity.
- Decrease AV conduction velocity.
- Increase refractory period.
- β-adrenergic blockers competitively block catecholamine induced stimulation of cardiac β-receptors.

β-blockers:

- Depress phase 4 depolarization of pacemaker cells,
- Slow sinus as well as AV nodal conduction: Decrease Heart Rate (HR), Increase Pulse Rate (PR).
- Increase ERP, prolong Action Potential (AP) Duration by Decrease AV conduction
- Reduce myocardial oxygen demand.

Propranolol

MOA

- Propanolol decreases the slope of phase 4 depolarization.
- Prolong the ERP of A-V node.

Adverse Effect:

- Hypoglycemia (infants)
- Asthma
- Bronchospasm

Uses:

- Atrial Fibrillation.
- Digitalis induced arrhythmias

Esomolol

MOA

- Esomolol is a short-acting β1-selective adrenoreceptor blocker.
- It doesn't possess membrane-stabilizing activity.
- Route of administration is through i.v.

Adverse Effects:

- Nausea
- Hypotension
- Headache

Uses:

- Supra ventricular tachyarrythmias.

Class III Drugs:

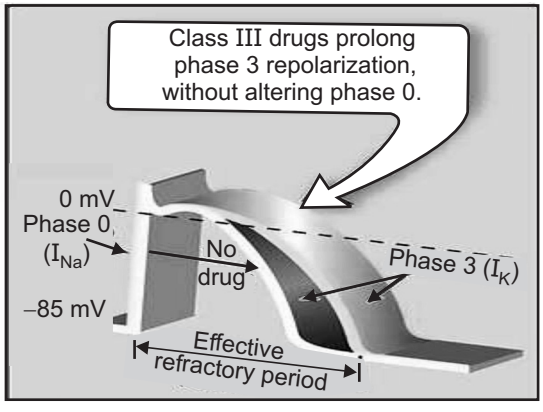


Fig. 5.5

Amiodarone:

- Iodine containing long acting drug.

MOA: (Multiple actions)

- Prolongs APD by blocking K^+ channels.
- Blocks inactivated sodium channels.
- β blocking action , Blocks Ca^{2+} channels.
- Decrease conduction, decrease ectopic automaticity.

Pharmacokinetics:

- Variable absorption 35-65%.
- Slow onset 2 days to several weeks.
- Duration of action: weeks to months.
- Many drug interactions.

Uses:

- Can be used for both supraventricular and ventricular tachycardia.

Adverse effects:

- Cardiac: hypotension, bradycardia, QT prolongation, heart block , cardiac failure.
- Pulmonary: pneumonitis leading to pulmonary fibrosis.
- Bluish discoloration of skin.
- GIT disturbances, hepatotoxicity.
- Causes hypothyroidism or hyperthyroidism by blocking peripheral conversion of T_4 to T_3.

Bretylium:

- Adrenergic neuron blocker used in ventricular arrhythmias.

Sotalol:

- Beta blocker.

Dofetilide:

- Selective K^+ channel blocker, less adverse events.
- Oral use in AF to convert or maintain sinus rhythm.

Ibutilide:

- K^+ channel blocker used as IV infusion in AF.

Class IV: Ca^{2+} channel Blockers

- Inhibit the inward movement of calcium.
- Decrease contractility, automicity and AV conduction.

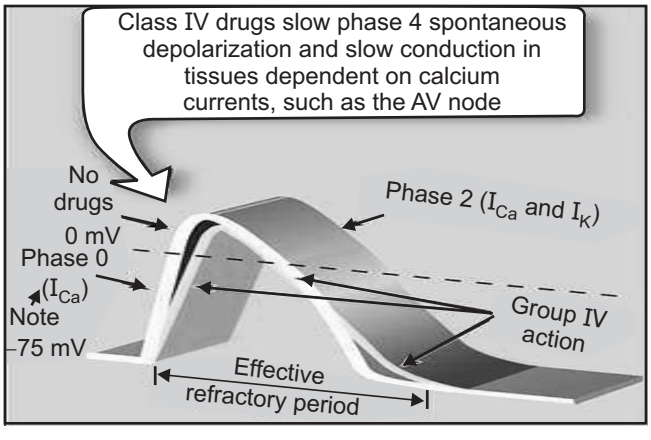


Fig. 5.6

Verapamil:

Uses:

- Terminate PSVT.
- Control ventricular rate in atrial flutter or fibrillation.

Drug interactions:

- Displaces digoxin from binding sites.
- Decrease renal clearance of digoxin.

Anti Arrhythmic Drugs:

Class	Mechanism	Action	Notes
I.	Na^+ channel blocker	Change the slope of phase 0.	Can abolish tachyarrhythmia caused by reentry circuit.
II.	β-blocker	Increase heart rate and conduction velocity.	Can indirectly alter K^+ and Ca^{2+} conductance.

contd. …

III.	K^+ channel blocker	1. Increase action potential duration (APD) or effective refractory period (ERP). 2. Delay repolarization.	Inhibit re-entry tachycardia
IV.	Ca^{++} channel blocker	Slowing the rate of rise in phase 4 of SA node.	Decrease conduction velocity in SA and AV node.

5.4 OTHER ANTI-ARRHYTHMIC

Adenosine:

- Purine nucleotide having short and rapid action.

MOA:

- It activates ACh sensitive K^+ channels and causes membrane hyperpolarization through interaction with A1 type of adenosine GPCRs on SA node coronaries.

Adverse events:

- Nausea, dyspnoea, flushing, headache.

QUESTIONS

1. Enlist anti-arrhythmic drugs with examples and mechanism of action of quinidine.
2. Discuss the action and uses of Propranolol.
3. Discuss about Lidocaine as an anti-arrhythmic agents.
4. Mention types of arrhythmias and classify anti-arrhythmic drugs.
5. Discuss the pharmacology of calcium channel blockers.

■■■

Chapter ... 6

Anti-Hyperlipidemic Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Enlist the drugs.*
- *Summarize mechanism of action and ADR.*

6.1 HYPERLIPIDEMIA

Hyperlipidemia is a broad term, it is also called as hyperlipoproteinemia, it is a common disorder in developed countries and major cause of coronary heart disease.

It is a result of abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoprotein.

Causes of Hyperlipidemia:

- Most of the time hyperlipidemia is caused due to lifestyle habits or treatable medical conditions.
- Obesity, not exercising, and smoking, diabetes, obstructive jaundice, and an under active thyroid gland inherit hyperlipidemia.

6.2 THE BIOCHEMISTRY OF PLASMA LIPIDS

Lipids: Lipids are the heterogenous mixtures of fatty acids and alcohol that are present in the body. The major lipids in the bloodstream are ***cholesterol*** and it's ***esters***, ***triglycerides*** and ***phospholipids.***

Cholesterol: It is C_{27}– steroid that serves as an important component of all cell membranes and important precursor molecule for the biosynthesis of bile acids, steroid hormones, and several fat-soluble vitamins.

Normal functions of ***cholesterol*** in the body:

- It is compulsory for new cells to form and for older cells to repair themselves after injury.
- It is also used by the adrenal glands to form hormones such as cortisol, by the testicles to form testosterone, and by the ovaries to produce estrogen and progesterone.

Normal functions of ***triglycerides and phospholipids*** in the body.

- ***Triglycerides*** supply energy for the body. Triglycerides are required to meet immediate energy needs in muscles or to store fat for future energy requirements.
- ***Phospholipids*** are compounds that are used to make cell membranes, generate second messengers, and store fatty acids for the use in generation of prostaglandins.

Lipoproteins:

- Blood and other body fluids are watery, so fats require a special transport system to travel around the body.
- Fats are travelling from one place to another mixing with protein particles, called lipoproteins.
- Four (or five) types of lipoproteins are there, each having particular distinct job.
- A **lipoprotein** contains both proteins and lipids, bound to another protein which is called ***apolipoproteins***, which allow fats to move through the water inside and outside cells. Provide structural support and stability, binds to receptors.

Classification of Lipoproteins:

Classification	Composition	Primary function
Chylomicrons	Triglyceride TGs 99%, 1% protein.	Transport dietary TGs to adipose tissue and muscle.
VLDL	Newly synthesized TGs. Lipid 90%, 10% protein.	Transport endogenous TGs to adipose tissue and muscle.
IDL	Intermediate between VLDL and LDL.	They are not usually detectable in the blood.
LDL	Lipid 80%, 20% protein.	Transport endogenous cholesterol from liver to tissues.
HDL	Lipid 60%, 40% protein.	Collect cholesterol from the body's tissues, and take it back to the liver.

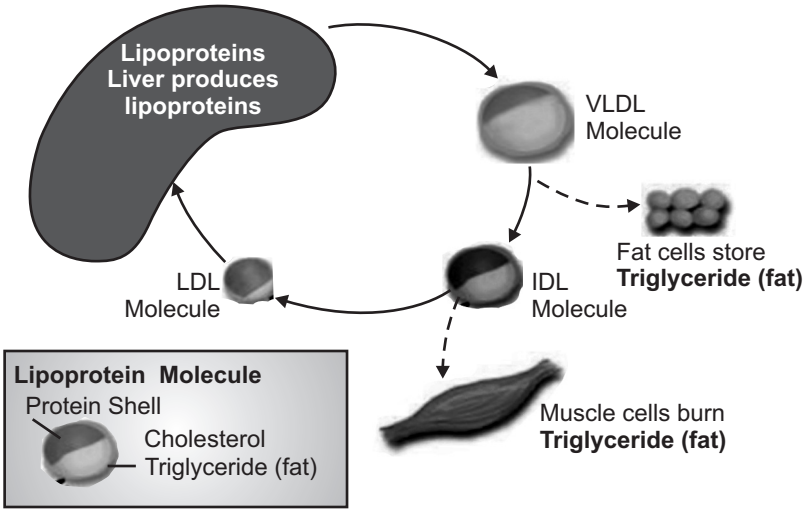


Fig. 6.1: Life cycle of Cholesterol-Carrying Lipoproteins

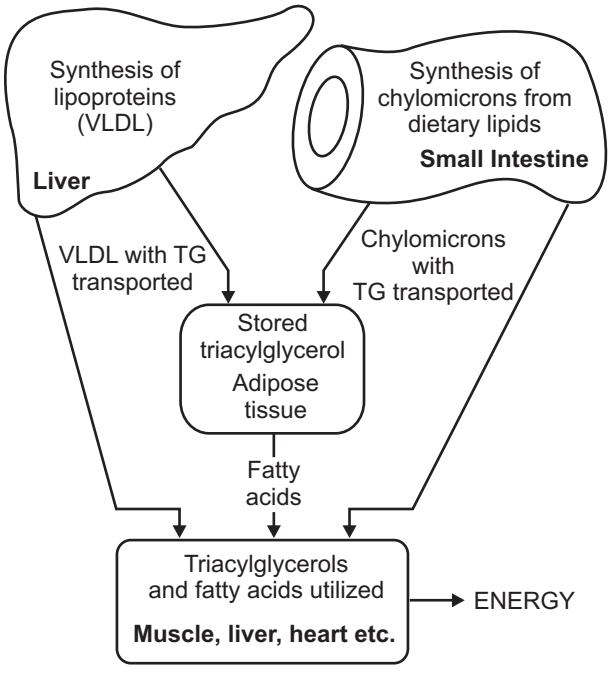


Fig. 6.2: Overview of Fat Metabolism

6.3 CLASSIFICATION OF ANTIHYPERLIPIDEMIC DRUGS

Sr. No.	Class	Example
1.	HMG CoA Reductase Inhibitors	Lovastatin, Simvastatin, Metastatin, Pravastatin, Fluvastatin, Atorvastatin, Pitavastatin, Rosuvastatin
2.	Fibrates	Clofibrate, Fenofibrate, Gemfibrozil, Ciprofibrate, benzafibrate, Fluvestatin.
3.	Bile acid sequestrants	Cholestyramine, Colestipol
4.	LDL oxidation inhibitor	Probucol
5.	Pyridine derivatives	Nicotinic acid, Nicotinamide
6.	Cholesterol absorption inhibitors	Ezetimibe
7.	Miscellaneous agents	β-Sitosterol, Dextrothyroxine

1. HMG- CoA Reductase inhibitors (STATINS):

- 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA).
- The enzyme that catalyzes the conversion of HMG-CoA to mevanolate.
- This reaction is the rate determining step in the synthetic pathway of cholesterol.

Statins:

- Lovastatin was isolated from Aspergillus terreus.

- There are two classes of statins:
  - **Natural Statins:** Lovastatin(mevacor), Pravastatin (pravachol), Simvastatin (Zocor).
  - **Synthetic Statins:** Atorvastatin (Lipitor), Fluvastatin (Lescol).
- Statins are competitive inhibitors of HMG-CoA reductase. They are bulky and "stuck" in the active site.
- Statins prevents the binding of enzyme with its substrate, HMG CoA.

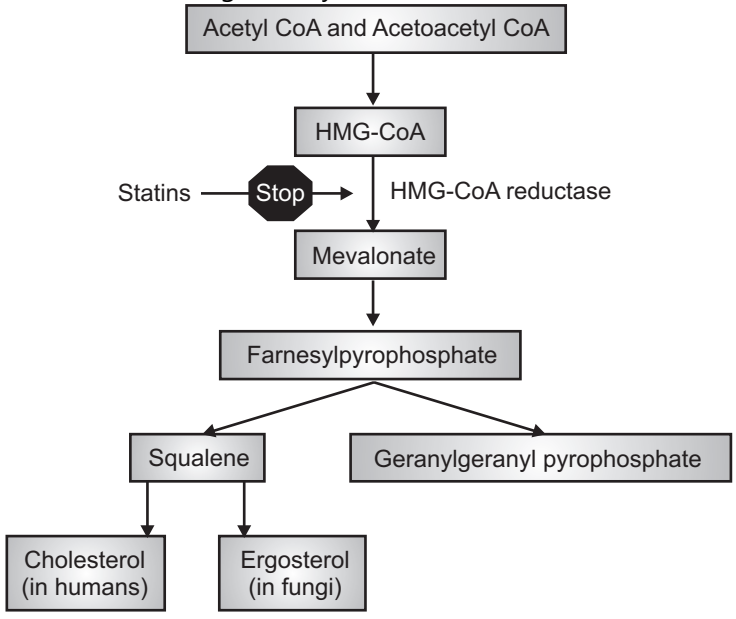


Fig. 6.3: MoA of Statins

Drug	ADR	Uses
Lovastatin	Increased creatinine phosphor-kinase, Flatulence, Nausea.	Antihyperlipoproteinemic agent.
Simvastatin	Headache, nausea, flatulence, heartburn, abdominal pain.	Antihyperlipdemic agent.
Pravastatin	GI disturbances, headache, insomnia, chest pain, rash.	Antihyperlipoproteinemic agent.
Atorvastatin	Headache, flatulence, diarrhea.	Primmary hyperlipidemia and secondary hyper cholesterolemia.
Rosuvastatin	Headache, dizziness, constipation, nausea, vomiting.	High LDL, total cholesterol, TGs.

2. Fibrates:

- Fibrates are antihyperlipidemic agents, these are used in the treatment of various forms of hyperlipidemia and hypercholesterolemia.
- These are 2-phenoxy-2-methyl propanoic acid derivatives.

- These drugs stimulate β-oxidation of fatty acids in mitochondria.
- These are specially using for decreasing plasma levels of fatty acid and triacylglycerol.

MOA:

- Fibrates are decreasing plasma TGs levels.
- This class of drugs acting through lowering of the blood triglyceride levels by ***decreasing*** the ***production of*** liver's VLDL (the triglyceride-carrying particle that circulates in the blood) by activation of lipoprotein lipase and enhances the removal of TGs from the blood. It is due to activation of PPAR-α.
- These drugs are acts by increasing ***blood HDL cholesterol*** and not effective in ***lowering LDL cholesterol***.

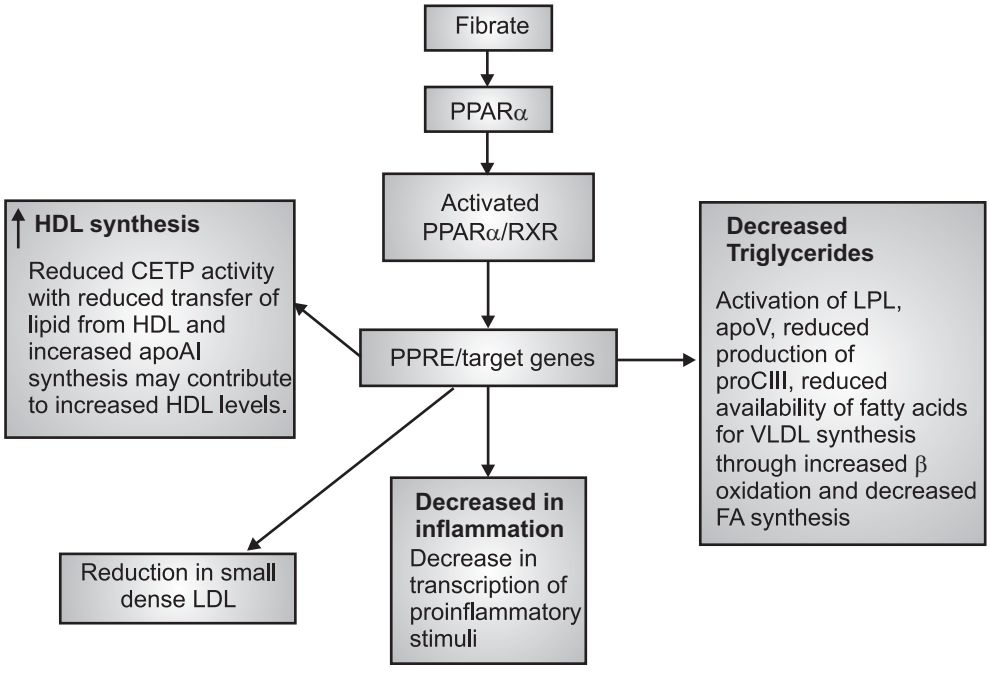


Fig. 6.4: MoA of Fibrates

Drug	ADR	Uses
Clofibrate	Cholecystitis, gall stone, eosinophilia, pneumonia.	Type III Hyperlipoproteinemias.
Gemfibrozil	Myositis syndrome, Cholelithiasis, GI disturbances, rash and headache.	Hyperlipidemia.
Fenofibrate	Headache, dizziness, asthaenia, fatigue, arrhythmia.	More potent hypercholesterolemic and triglycerides lowering agent.

3. Bile Acid Sequestrants:

- Cholestyramine (Questran)
- Colestipol hydrochloride (Colestid)
- Colesevelam (tablet form)

Also called bile acid–binding resins and ion-exchange resins.

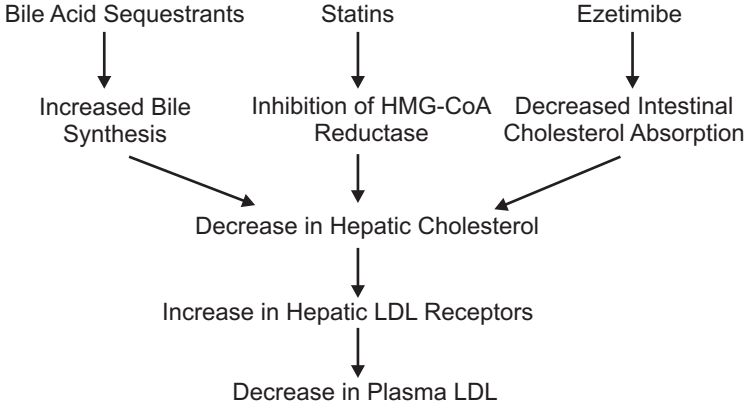


Fig. 6.5: MoA of Anti-hyper lipidemic drug

4. Cholesterol Absorption Inhibitor:

Ezetimibe (Zetia):

It is a drug that lowers plasma cholesterol levels. It acts by decreasing cholesterol absorption in the intestine.

MOA:

- Ezetimibe lowers the plasma cholesterol levels by inhibiting the absorption from intestine.
- This drug decreases the cholesterol delivery to the liver which in turn clears more cholesterol from the blood.
- It has selective action ((not interfere with TGs, lipidsoluble vitamins absorption)
- The levels of LDL-C in the serum are reduced by bile acid sequestrants.

Therapeutic Uses:

- As mono therapy or in combination with HMGRI for reduction of elevated total cholesterol.

5. Pyridine Derivatives:

- **Nicotinic Acid (Niacin):** Nicotinic acid is a water soluble vitamin of the B family; after converted into the amide, it is incorporated as NAD. For the effective action the dose should be 1.5 to 3.5 gm daily. It is available as sustained release dosage form.

Vitamin B_3:

- For Lipid-lowering action it requires higher doses than when used as a vitamin.
- Effective, inexpensive, also used in combination with other lipid-lowering drugs.

MOA:

- It Increases the activity of lipase, which breaks down the lipids.
- It decreases the metabolism of cholesterol and triglycerides.

Indications:

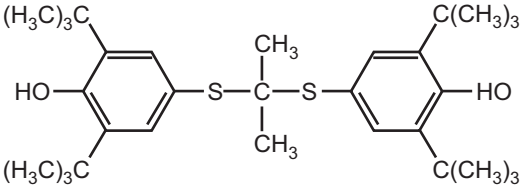
- It is effective in the lowering of triglycerides, total serum cholesterol, and LDL levels.
- It also increases the HDL levels.
- It is also effective in the treatment of types IIa, IIb, III, IV, and V hyperlipidemias

Adverse Effect:

- Flushing (due to histamine release)
- Pruritus
- GI distress
- Liver dysfunction and jaundice. Serious liver damage is the most important risk.

6. LDL Oxidation Inhibitor:

Probucol:



- Probucol has two tertiary butyl phenol groups linked by the help of dithiopropylidene bridge and gives a high lipophilic character along with high antioxidant properties.
- In humans, it reduces both liver and serum cholesterol levels and it does not alter plasma triglycerides.
- It reduces LDL levels.
- It decreases the extent of HDL levels by a unique mechanism that is still not clearly delineated.
- The decreased HDL level may be caused by the ability of probucol to inhibit the synthesis of apoprotein A-1, a major protein component of HDL.
- It is effective in reducing the levels of LDL and it is also used in hyperlipoproteinemias characterized by increased LDL levels.

ADR:

- GI disorders and prolongation of GI intervals.

Use:

- It is used as antihyperlipoproteinemic agent.

7. Miscellaneous Agent:

β-Sitosterol:

- Sitosterol is a plant sterol, whose structure is identical with that of cholesterol, except for the substituted ethyl group on C-24 of its side chain.

- Its hypolipidemic effect is not clearly understood, it is claimed that the drug inhibits absorption of dietary cholesterol from the gastrointestinal tract.
- It is absorbed poorly from the mucosal lining and it is competing with cholesterol for absorption site in the intestine.

ADR:

- Diarrhoea, constipation, GI disturbances.

Use:

- Anti cholesteremic agent and treatment of prostatic edema.

Questions

1. Classify hypolipidemic agents with examples, write the pharmacology of Atorvastatin.
2. Write the classification of hypolipidemics. Explain the complications of dyslipidemia.
3. Write the classification of hypolipidemics with examples. Describe the mechanism of Niacin.

■■■

Unit II

Chapter ... 7

Drugs used in the Therapy of Shock

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Discuss the general concepts associated with shock states, including physiologic response to shock, and shock progression.*
- *Classify drugs and illustrate mechanism of action and ADR*

7.1 INTRODUCTION

It is abnormal physiological state resulting from widespread and serious reduction of tissue perfusion that if prolonged will lead to generalized impairment of cellular function.

- A life-threatening clinical syndrome of cardiovascular collapse characterized by:
  - An acute reduction of effective circulating blood volume (hypotension).
  - An inadequate perfusion of cells and tissues (hypoperfusion).
- If uncompensated, these mechanisms may lead to impaired cellular metabolism and death.
- The clinical manifestations of shock are the result of stimulation of the sympathetic and neuroendocrine stress responses, inadequate oxygen delivery, end-organ dysfunction.

7.2 TYPES OF SHOCK

- Hypovolemic shock
- Cardiogenic shock
- Distributive shock
  - Septic shock
  - Neurogenic shock
  - Anaphylatic shock

Management of Shock:

- **Ionotrope:** An agent that changes myocardial contractility.
- **Vasopressor:** An agent that increases blood pressure.
- **Chronotrope:** An agent that changes heart rate.
- **Dromotrope:** An agent that increases cardiac conduction velocity.

Norepinephrine:

- Most widely used vasopressor.
- Potent α_1 agonist causing vasoconstriction in tissue beds.
- Resultant increase in SVR causes rise in blood pressure.
- Standard dose: 4 mg in 50 ml (0.08 mg/ml).

Epinephrine:

- Nature's vasopressor.
- Most commonly used during resuscitation cardiac arrest and anaphylaxis.

  α_1: Increases SVR.

  β_1: Increases HR and myocardial contractility.

  β_2: Bronchial smooth muscle relaxation.

Standard dose: 10 mg in 50 ml (0.2mg/ml).

Dopamine:

- Vasopressor agent.
- Use in cardiogenic and septic shock.
- Receptor stimulation depend on dose given.

Dobutamine:

- A synthetic cathecholamine.
- An inodilator.
- β_1 stimulation: Increase HR and increase cardiac contractility.
- β_2 mediated vasodilatation.
- Reduction in MAP is common with dobutamine.
- NE usually needed to offset vasodilatation.

Vassopressin:

- Peptide hormone released from posterior pituitary.
- Causes increase permeability of DCT and CT, increases water retention.(V2 receptor).
- V_1 receptor present in the smooth muscle of a arteriolar wall and stimulation causes smooth muscle contraction and vasoconstriction.

7.2.1 Hypovolemic Shock

Improper tissue perfusion as a result of severe loss of blood or other fluid from the body or inadequate fluid intake, any of which decrease intravascular volume.

Causes of Hypovolemic Shock;

- Haemorragic (acute blood loss)
- Burns
- Excessive vomiting and diarrhea

Pathophysiology of Hypovolemic shock

Hemorrhage from small venules and veins (50%)
↓
Decreased filling of right heart
↓
Decreased filling of pulmonary vasculature
↓
Decreased filling of left atrium and ventricle
↓
Left ventricular stroke volume decreases (Frank Starling)
↓
Drop in arterial blood pressure and tachycardia
↓
Poor perfusion to pulmonary arteries
↓
Cardiac depression and pump failure

Classsification of Hypovolemic Shock:

- **Hemorrhagic:** Trauma, gastrointestinal bleeding.
- **Non-Hemorrhagic:** external fluid loss, diarrhoea, vomiting, polyurea, fluid redistribution, burns, anaphylaxis.

Signs and Symptoms:

- Anxiety, restlessness, altered mental state.
- Hypotension.
- A rapid, weak, thready pulse.
- Cool, clammy skin.
- Rapid and shallow respirations.
- Hypothermia.
- Thirst and dry mouth.
- Distracted look in the eyes.

Compensatory Mechanisms:

1. Adrenergic discharge.
2. Hyperventilation.
3. Vasoactive hormones Angiotensin, Vasopressin, Epinephrine.
4. Collapse.
5. Re-absorption of fluid from interstitial tissue.
6. Resorption of fluid from intracellular to extracellular space.
7. Renal conservation of body water and electrolyte.

Clinical Monitoring:

- Blood pressure
- Respiration
- Urine output
- Central venous pressure

- ECG
- Swan-Ganz catheter
  - cardiac output.
  - mixed venous oxygen level.
  - vascular pressure.
- Pulmonary artery wedge pressure.

Diagnosis:

- In management of trauma patients, understanding the patterns of injury of the patient in shock will help direct the evaluation and management.
- Blood loss sufficient to cause shock is generally of a large volume (e.g. external, intrathoracic, intra-abdominal, retroperitoneal, and long bone fractures).
- Diagnostic and therapeutic tube thoracotomy may be indicated in unstable patients based on clinical findings and clinical suspicion.
- Chest radiographs, pelvic radiography, diagnostic ultrasound or diagnostic peritoneal lavage.

MANAGEMENT:

Objectives:

(a) Increase Cardiac Output
(b) Increase Tissue Perfusion

The plan of action should be based on:

(a) Primary problem
(b) Adequate fluid replacement
(c) Improving myocardial contractility
(d) Correcting acid-base disturbances

- Resuscitation
- Immediate control of bleeding: Rest, Pressure Packing.

Operative Methods:

- Extracellular fluid replacement:
  - Infusion of fluid is the fundamental treatment.
  - Crystalloids, for initial resuscitation for most forms of hypovolemic shock.
  - After the initial resuscitation, with up to several liters of crystalloid fluid, use of colloids.

Drugs:

1. Sedatives
2. Chronotropic agents
3. Inotropic

7.2.2 Distributive Shock

- As in hypovolemic shock, there is an insufficient intravascular volume of blood.
- This form of "relative" hypovolemia is the result of dilation of blood vessels which diminishes systemic vascular resistance.
- Examples of this form of shock:
  1. Septic shock
  2. Anaphylactic shock
  3. Neurogenic shock

1. **Septic Shock:** A type of distributive shock resulting from sepsis.
   - **Sepsis:** An abnormal body wide inflammatory response to an infection that can result in death.

Pathophysiology of Septic Shock:

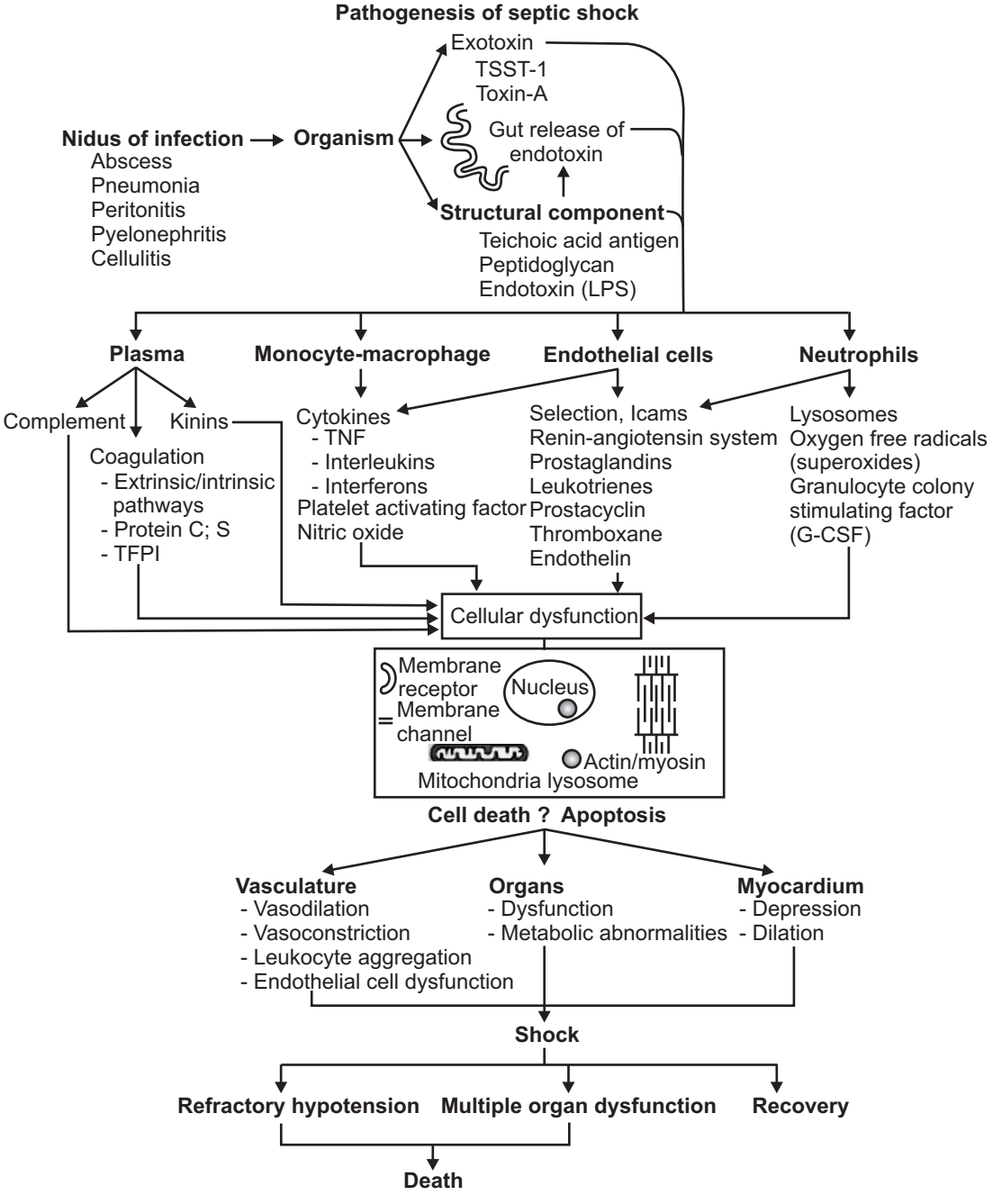


Fig. 7.1: Pathogenesis of Septic Shock

Clinical Signs:

- Hyperthermia
- Tachycardia
- Wide pulse pressure
- Low blood pressure (SBP < 90)
- Mental status changes

Treatment of Septic Shock:

- Fluid replacement.
- Supplemental oxygen.
- **Antibiotics:** Survival correlates with how quickly the correct drug given cover gram positive and gram negative bacteria:
  - Ceftriaxone 1 gram IV BD or
  - Imipenem 1 gram IV TDS.

Add additional coverage for:

- **Pseudomonas:** Gentamicin or Cefepime.

2. Anaphylactic Shock:

It develops following exposure to:

- Allergen and cross links IgE on mast cells causing mediator release (release of Histamine, Eicosanoids-LTs, PGs).

Clinical Presentation:

- Urticaria and angioedema.
- Bronchospasm.
- Hypertension and CV collapse.

Treatment:

Epinephrine is 1st line drug:

- **Standard Dose:** Inj. 0.5 ml (1:1000) IM.
- Repeat every 5-10 min if not improve.
- Inj. 0.5 ml (1: 10000),(1:100000) IV.

Antihistaminic:

- Diphenhydramine (H_1) administered IV.
- Ranitidine (H_2) administered IV.
- **β_2 agonist:** Salbutamol.
- **Corticosteroid:** Hydrocortisone 200 mg IV followed by oral prednisolone for 3 days.

3. Neurogenic Shock:

Develops secondary to a sudden loss of ANS functions following spinal cord injury resulting in vasomotor tone and impaired cellular metabolism.

Features:

- Hypotension
- Bradycardia
- Poikilothermia

Management:

- Airway support.
- Fluid replacement.
- Dopamine (>10 mcg/kg/min).
- Ephedrine (12.5 - 25 mg IV every 3-4 hr).
- Atropine for bradycardia. (0.5 mg IV every 3 to 5 mins – 3 mg).
- Treatment of the underlying cause.

7.2.3 Cardiogenic Shock

A state of inadequate cardiac output despite of adequate intravascular volume , resulting in hypoxia.

- Cool, mottled skin
- Tachypnea
- Hypotension
- Altered mental status
- Narrowed pulse pressure

Causes of Cardiogenic Shock:

- Acute myocardial infarction
- Myocarditis
- Myocardial contusion
- Aortic or mitral stenosis
- Acute aortic insufficiency

Pathophysiology of Cardiogenic Shock:

- Often after ischemia, loss of LV function
- CO reduction = lactic acidosis, hypoxia
- Stroke volume is reduced
- Tachycardia develops as compensation
- Ischemia and infarction worsens

Treatment of Cardiogenic Shock:

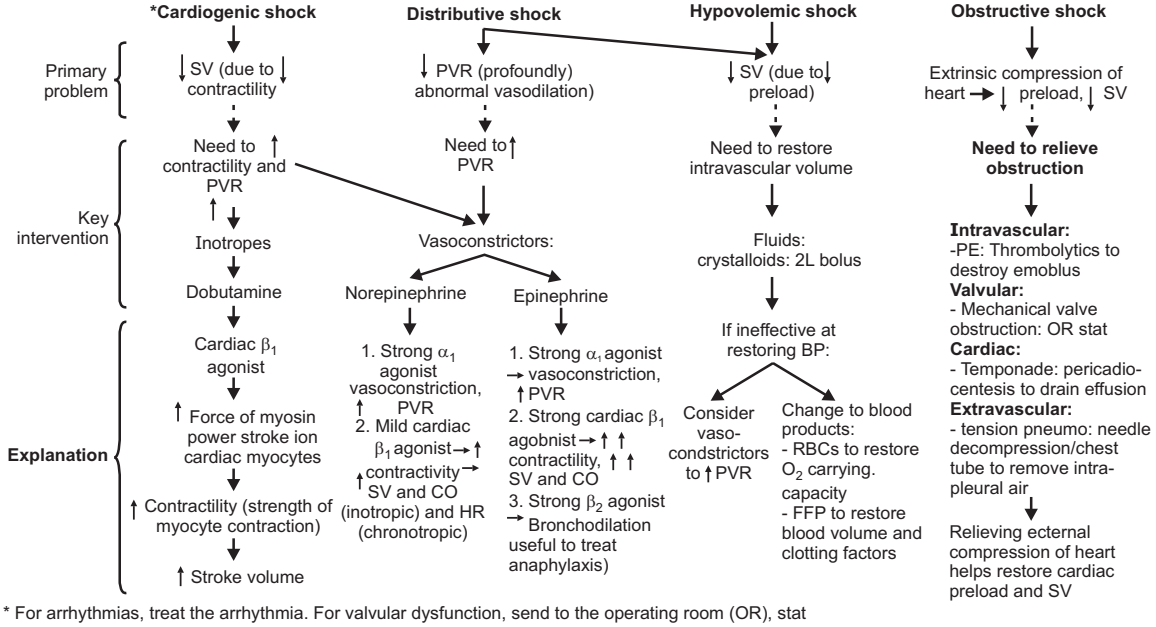
- Aspirin, beta blocker, morphine, heparin
- If no pulmonary edema, IV fluid
- If pulmonary edema
- Dopamine – will ↑ HR and thus cardiac work
- Dobutamine – May drop blood pressure
- Combination therapy may be more effective
- Thrombolytics(streptokinase, rt-PA)

7.3 TREATMENT OF SHOCK

Treatment of shock:

Always remember: BP = PVR × (SV × HR) - Identify which is the problem, and then fix it

CO



* For arrhythmias, treat the arrhythmia. For valvular dysfunction, send to the operating room (OR), stat

Fig. 7.2: Treatment of shock

QUESTIONS

1. Write a note on drugs used in the therapy of shock.

■■■

Chapter ... 8

Hematinics, Coagulants and Anticoagulants

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *The mechanism of drug action and its relevance in the treatment.*

8.1 HEMATINICS

- Agent that tends to stimulate blood cell formation or to increase the **hemoglobin** in the blood.
- **Or used for the prevention and treatment of *anemia*.**

Haemoglobin:

- Formed in red bone marrow.
- It is a conjugated protein, consisting of an iron containing pigment combined with ***histone*** (protein) is known as ***Globin***.
- The iron containing protein is a ***porphyrin*** consisting of 4 pyrrole rings.
- This porphyrin is designated as ***Heam.***
- ***Folic acid and vitamin B_{12}*** are capable of increasing the rate of **Heam** synthesis in the red cells.

Anemia:

- A condition in which the blood is deficient in the RBC (**erythrocytes**), in hemoglobin.
- Or deficiency in quality or in the quantity of blood.
- Erythrocytes are mainly responsible for the delivering oxygen to the tissues, less RBC means less oxygen to tissues.

4 types

- **Microcytic anemia:** Deficiency of iron (Fe).
- **Macrocytic anemia:** Deficiency of folic acid and B_{12}.
- **Hemolytic anemia:** Abnormal breakdown of RBCs.
- **Aplastic anemia:** Body stops producing new blood cells.

Hematinics:

- IRON.
- FOLIC ACID (pteroylglutamic acid).
- VITAMIN B_{12} (cyanocobalamin).

IRON:

- The human body contains about 3.5 gm of iron of which about 2/3 is contained in the blood.
- 5 - 10% of ingested iron is absorbed.
- Once ingested the acid in the stomach:
  1. Aids in ionization of iron
  2. Splits chelated food iron from chelator
  3. Maintains iron in soluble form
  4. Allows iron to remain in the absorbable form Fe^{3+}.

Mechanism of Iron Absorption:

- Iron absorption occurs all over the intestine.
- In the stomach, which contains HCL and reducing agent, convert the ferric to ferrous.
- Two separate iron transporters in the intestinal mucosal cells function to effect iron absorption.
- At the luminal membrane the divalent metal transporter 1 (DMT) carries ferrous iron into the mucosal cell.
- The ferroportin are bound with ferrous iron and pass through mucosal cell directly into the blood steam.

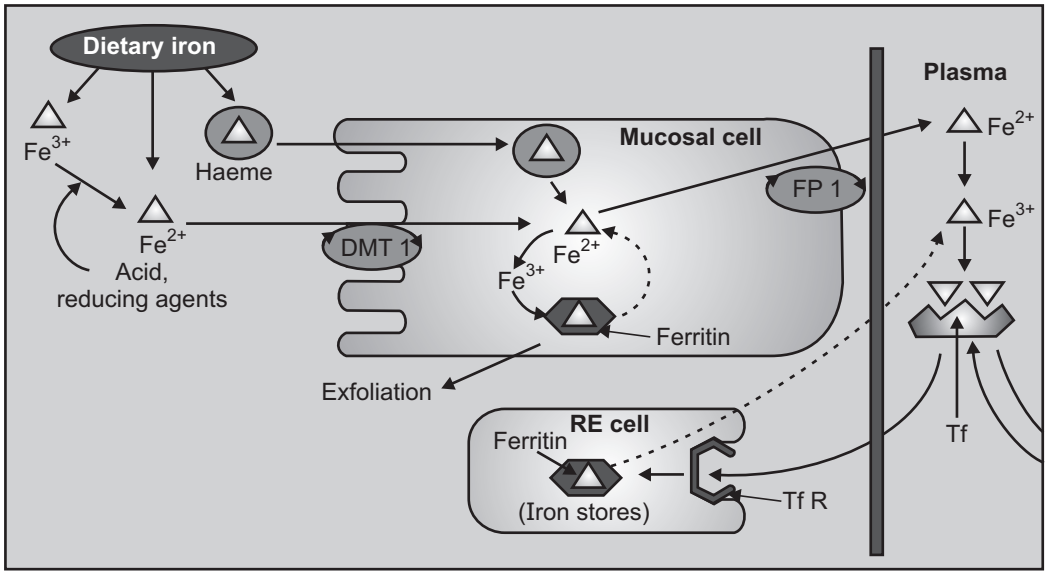


Fig. 8.1: MoA of Iron Absorption

Transport, Utilization, Storage and Excretion:

- As such, on entering plasma it is immediately converted to the ferric form and complexed with a glycoprotein transferring (T_f).

- Iron is transported into erythropoietic and other cells through attachment of transferring receptor (T_f Rs).
- The complex is engulfed by receptor mediated endocytosis.
- Iron dissociates from the complex at the acidic pH of the intracellular vesicles.
- The released iron is utilized for haemoglobin synthesis or other purposes.
- T_f and T_f R are returned to the cell surface to carry fresh loads.

Storage:

- Reticulo endothelial cells
- Spleen
- Bone marrow
- Hepatocytes and myocytes.

Therapeutic uses of Iron:

- Iron Deficient Anemia
- Pregnancy
- Premature Babies
- Blood loss
- Hookworn infestation
- Malabsorption Syndrome
- GI Bleeding due to:
    - Ulcers, Aspirin, Excess consumption of coffee.

Iron Preparations:

Oral Iron:

- Ferrous Sulfate (Feosol) – 300 mg tid.
- Side Effects are extremely mild:
    - Nausea, upper abdominal pain, constipation or diarrhea.
    - Cheapest form of Iron and one of the most widely used.

Parenteral

- Iron Dextran (Imferon) – IM or IV
- Indicated for patients who cannot tolerate or absorb oral iron or where oral iron is insufficient to treat the condition ie. Malabsorption syndrome, prolonged salicylate therapy, dialysis patients.

FOLIC ACID

- Source in food – yeast, egg yolk, liver and leafy vegetables.
- Folic Acid (F.A.) is absorbed in the small intestines.
- F.A. is converted to tetrahydrofolate by dihydrofolate reductase.
- Folic Acid deficiency (F.A. Deficiency) is also called Will's Disease.
- Deficiency may produce megaloblastic anemia; neural tube defect in fetus.

Therapeutic Uses of Folic Acid

1. **Megaloblastic Anemia due to inadequate dietary intake of folic acid:**
   - Can be due to chronic alcoholism, pregnancy, infancy, impaired utilization: uremia, cancer or hepatic disease.
2. **To alleviate anemia that is associated with dihydrofolate reductase inhibitors:**
   - i.e. Methotrexate (Cancer chemotherapy), Pyrimethamine (Antimalarial)
   - Administration of citrovorum factor (methylated folic acid) alleviates the anemia.
3. **Ingestion of drugs that interfere with intestinal absorption and storage of folic acid:**
   - **Mechanism:** Inhibition of the conjugases that break off folic acid from its food chelators.
   - **Example:** Phenytoin, Progestin/estrogens (oral contraceptives)
4. **Malabsorption:** Sprue, Celiac disease, partial gastrectomy.
5. **Rheumatoid arthritis:** Increased folic acid demand or utilization.

Dose:

- Synthetic folic acid daily 10-30mg orally is given.

Toxicity:

- Non toxic to man.

VITAMIN B_{12}:

Source:

- In food, especially in liver and kidneys. GI Microorganism synthesis, Vitamin Supplements (Cyanocobalamin).
- Necessary for normal DNA synthesis.

Absorption of B_{12}:

1. **Intrinsic Factor (low dose):** A protein made by stomach parietal cells that binds to B_{12} and delivers it from the ileum via a calcium mediated event.
2. **Mass Action (High dose):** 1000 mg/day, absorbed via passive diffusion.

Distribution of B_{12}:

Vitamin B_{12} is distributed to various cells bound to a plasma glycoprotein, Transcobalamin II.

Storage of B_{12}:

- Excess vitamin B12 (upto 300-500 microgram) is stored in liver.

Therapeutic Uses of B_{12}

- Daily Requirements - 0.6 - 1.0mh/day; $T_{1/2}$ ~ 1 year.
- Pernicious Anemia.

- Impaired GI absorption of B_{12}.
- Gastrectomy.
- Corrosive Injury of GI mucosa.
- Fish tape worm: worm siphons off B_{12}.
- Placebo abuse with B_{12}, especially in elderly patients.
- Malabsorption syndrome.

8.2 COAGULANTS

Haemostasis (arrest of blood loss) and blood coagulation involve complex interactions between the injured vessels wall, platelets and coagulation factors.

Coagulants:

Vitamin K : K_1 (from fat-soluble): phytonadione (phylloquinone)

: K_3 (synthetic)

- Fat soluble (Menadione, Acetomenaphthone)
- Water soluble (Menadione sod. Bisulfite, Menadione sod. Diphosphate.
- Miscellaneous: Fibrinogen (human), Antihaemophilic factors, Desmopressin, Adrenochrome monosemicarbazone, Rutin, Ethamsylate.

Vitamin K:

- Vit. K is a fat-soluble dietary principle required for the synthesis of clotting factors.
- Daily requirements: Vit. K_2 produced by colonic bacteria and 3-10 µg/day external source may be sufficient. The total requirement of Vit. K for an adult has been estimated to be 50-100 µg/day.

Mechanism of Action:

- Vit. K acts as a cofactor at a late stage in the synthesis by liver of coagulation proteins – prothrombin, factors VII, IX and X.

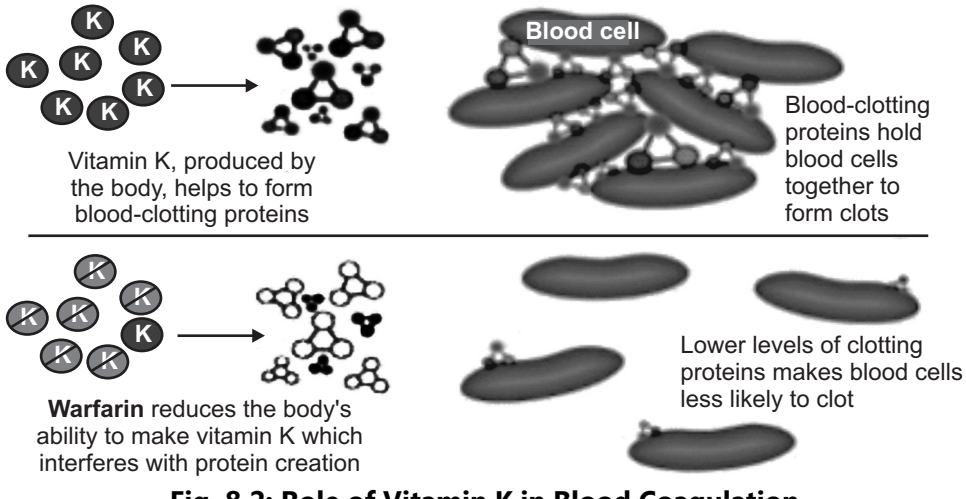


Fig. 8.2: Role of Vitamin K in Blood Coagulation

Uses:

- The only use of Vitamin K is in prophylaxis and treatment of bleeding due to deficiency of clotting factors.

Plasma Fractions:

- Deficiencies in plasma coagulation factors can cause bleeding.
- Factor VIII deficiency (classic hemophilia or hemophilia A) and factor IX deficiency (Christmas disease, or hemophilia B) account for most of the heritable coagulation defects. Concentrated plasma fractions and recombinant protein preparations are available for the treatment of these deficiencies.

Desmopressin Acetate:

- Desmopressin (DDAVP) stimulates the release of von willebrand factor (V_{wf}) from the Weibel-palade bodies of endothelial cells, thereby increasing the levels of V_{wf} (as well as coagulant factor VIII) 3 to 5 fold.
- It is also used to promote the release of V_{wf} in patients with coagulation disorders such as von willebrand disease, mild hemophilia A and thrombocytopenia.

Cryoprecipitate:

- It is a plasmaprotein fraction obtainable from whole blood. It is used to treat deficiencies or qualitative abnormalities of fibrinogen.
- It may also be used for patients with factor VIII deficiency and Willbrand disease.

8.3 ANTICOAGULANT

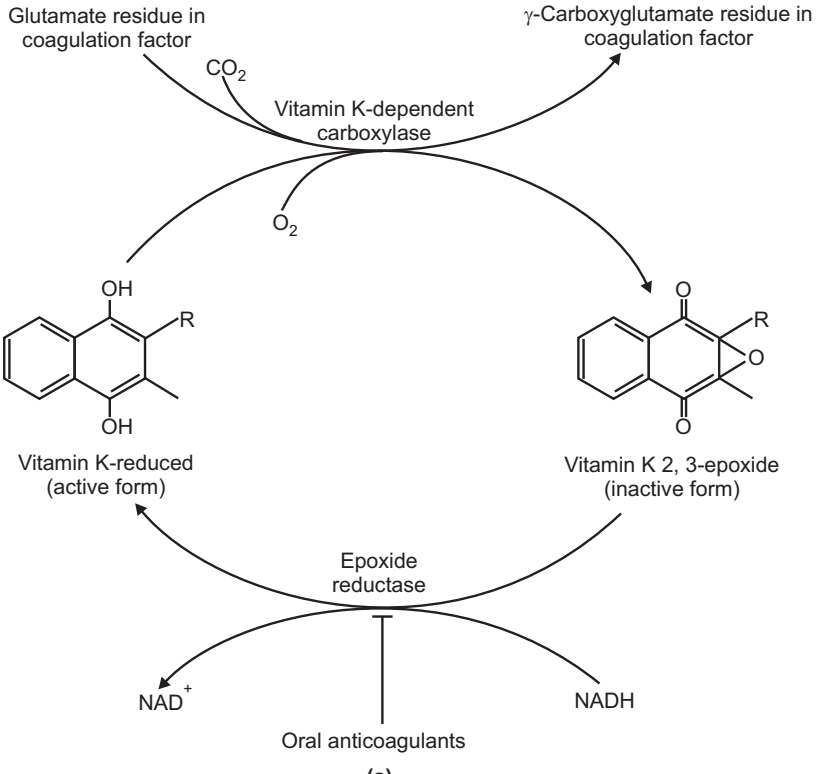
Drugs that **prevent blood coagulation** and stop the occurrence or expansion of a thrombus.

Classification:

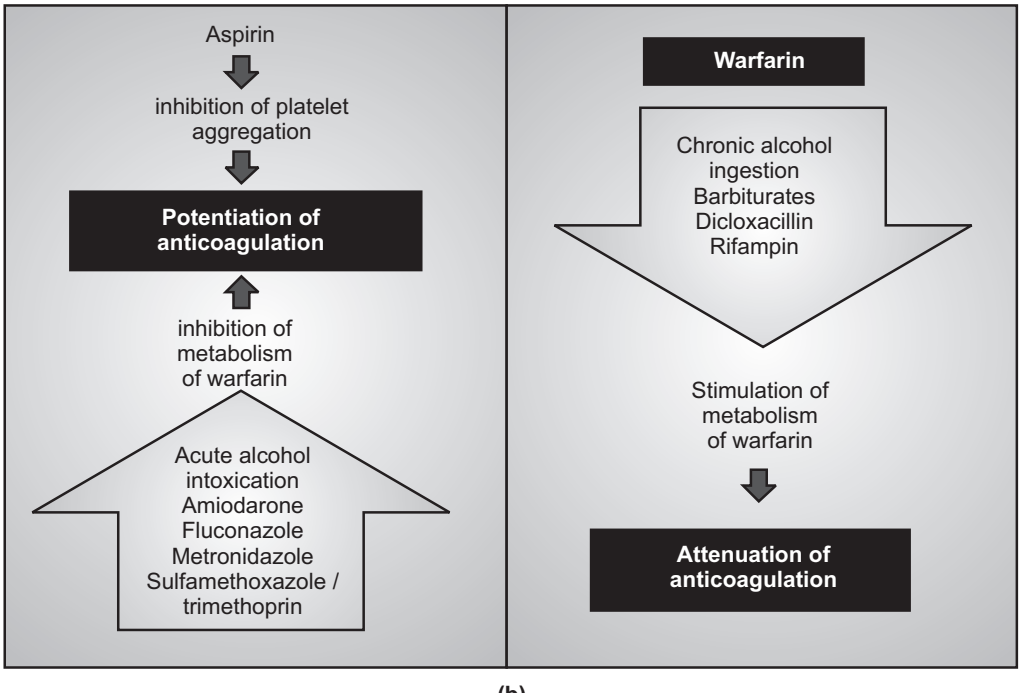
1. Vitamin K antagonist - Warfarin.
2. Heparin and related drugs:
   (a) Heparin.
   (b) LMWH (Enoxaparin, Dalteparin, Tinzaparin).
   (c) Synthetic heparin derivatives (Fondaparinux – longer acting).
3. Direct thrombin inhibitors:
   (a) Parenteral → Hirudin, Lepirudin, Argatroban, Bivalirudin.
   (b) Oral → Dabigatran.
4. Active factor Xa inhibitor → Rivaroxaban, Apixaban.

Warfarin:

- Competitively inhibits vitamin K epoxide reductase and inhibits the post-translational carboxylation of glutamate residues on vitamin K dependent coagulation **factors II (prothrombin), VII, IX, and X.**


Glutamate residue in coagulation factor
γ-Carboxyglutamate residue in coagulation factor
CO_2
Vitamin K-dependent carboxylase
O_2
OH
R
OH
O
R
O
O
Vitamin K-reduced (active form)
Vitamin K 2, 3-epoxide (inactive form)
Epoxide reductase
NAD^+
NADH
Oral anticoagulants

(a)


Aspirin
inhibition of platelet aggregation
Potentiation of anticoagulation
inhibition of metabolism of warfarin
Acute alcohol intoxication
Amiodarone
Fluconazole
Metronidazole
Sulfamethoxazole / trimethoprin
Warfarin
Chronic alcohol ingestion
Barbiturates
Dicloxacillin
Rifampin
Stimulation of metabolism of warfarin
Attenuation of anticoagulation

(b)

Fig. 8.3

Heparin:

- Antithrombin III-Irreversibly inactivates thrombin and factor Xa.
- Heparin potentiates anti-thrombin III activity.

Advantages of LMWH:

1. Can be administered s.c.
2. Effects are consistent and dosing less frequent (Long $t_{1/2}$ and elimin. By 1st order kinetics).
3. Dose is given in mg (not in units) can be easily calculated on body weight basis.
4. Chance of haemorrhage is less.
5. Risk of osteoporosis is decreased.

	Heparin	**Warfarin**
Route of administration	I.v., S.c.	Oral
Onset of action	Immediate	Delayed
Mechanism	Activ. Of AT-III	Decrease activ. Of c.f. 2,7,9,10
Antagonist	Protamine sulphate	Vitamin K
Use	To initiate therapy	For maintenance

Uses of anti-coagulants:

1. Myocardial infarction
2. Unstable angina
3. Rheumatic heart disease
4. Cerebrovascular disease
5. Haemodialysis
6. Defibrination syndrome (DIC).

QUESTIONS

1. Write a note on haematinics agents.
2. Write a note on oral anticoagulants.
3. Discuss the factors in blood coagulation.
4. Explain drugs used for different types of Anaemia.
5. Discuss the pharmacology of warfarin.
6. Classify anti-coagulants with examples. Write the mechanism of action of heparin.

■■■

Chapter ... 9

Fibrinolytics and Anti-Platelets Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *The mechanism of drug action and its relevance in the treatment.*

9.1 FIBRINOLYTIC SYSTEM

- The process of dissolution of clot is called fibrinolysis.

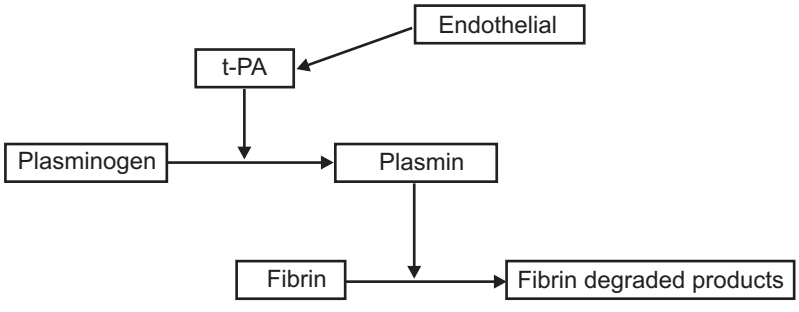


Fig. 9.1

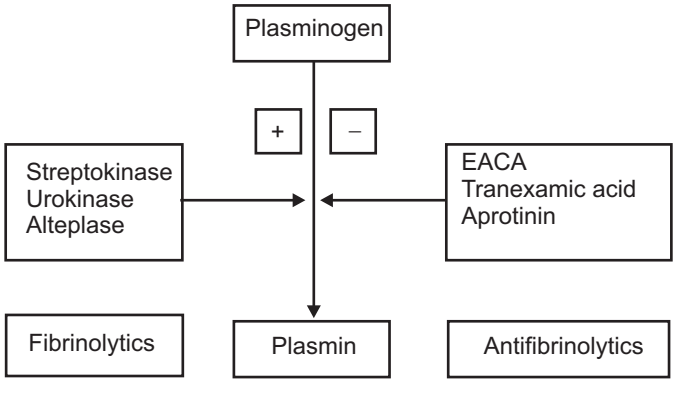


Fig. 9.2

FIBRINOLYTICS

- Used to lyse the thrombi / clot to re-channelize the occluded blood vessel (mainly coronary artery).
- Work by activating the Fibrinolytic system:
  - **STREPTOKINASE**
  - **UROKINASE**

- **RETEPLASE** (analogue of alteplase)
- **ALTEPLASE (tissue - Plasminogen Activator [t-PA])**
- **TENECTEPLASE**

Streptokinase:

- Obtained from - hemolytic streptococci.
- Binds with circulating plasminogen to form plasmin.
- Complex that activates plasminogen to plasmin.
- t½ = 30 – 80 min.
- Antigenic, Pyrogenic.
- Destroyed by circulating antistreptococcal antibodies.
- Hypotension and Arrhythmia can occur.

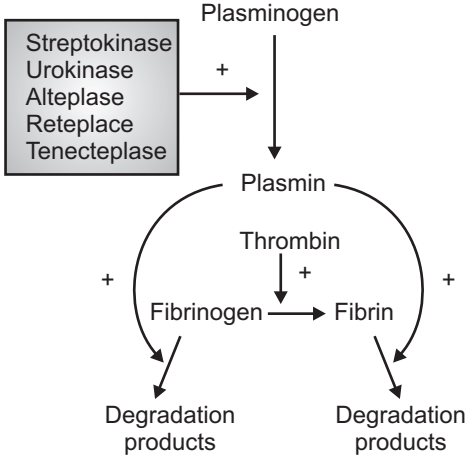


Fig. 9.3: MoA of Fibrinolytics

Uses:

- Acute myocardial infarction, – 7.5 to 15 lac IU; I.V over 1 hr period.
- Deep vein thrombosis , Pulmonary embolism.

Adverse Effects:

- Bleeding, hypotension, allergic reactions, fever, arrhythmias.

Contraindications:

- Recent trauma, surgery, abortion, stroke, severe.
- hypertension, peptic ulcer, bleeding disorders.

Urokinase:

- Enzyme isolated from human urine, now prepared from cultured human kidney cells.
- Direct plasminogen activator.
- t ½ of 10 to 15 min.
- Non-antigenic, Non-allergenic.
- Fever can occur but hypotension rare.
- Indicated in patients in whom streptokinase has been for an earlier episode.

Alteplase:

- Recombinant tissue Plasminogen Activator (rt-PA)
- Selectively activates plasminogen bound to fibrin

- Non-antigenic, not destroyed by antibodies
- Rapid acting, more potent
- Superior in dissolving old clots
- Short half life 4-8 min
- Nausea, mild hypotension, fever may occur
- Expensive.

Newer Recombinant Tissue Plasminogen Activators:

- **Reteplase:**
  - Modified rt-PA.
  - Longer half life 15 -20 min, but less specific for fibrin bound plasminogen.
- **Tenecteplase:**
  - Genetically engineered mutant form of alteplase.
  - Higher fibrin selectivity and longer half life – 2 hrs.
  - Single bolus dose 0.5 mg/kg sufficient.
  - Very expensive.

Uses of Fibrinolytics:

- Acute myocardial infarction.
- Deep vein thrombosis.
- Pulmonary embolism.
- Peripheral arterial occlusion.
- Ischemic Stroke.

9.2 ANTIPLATELET DRUGS

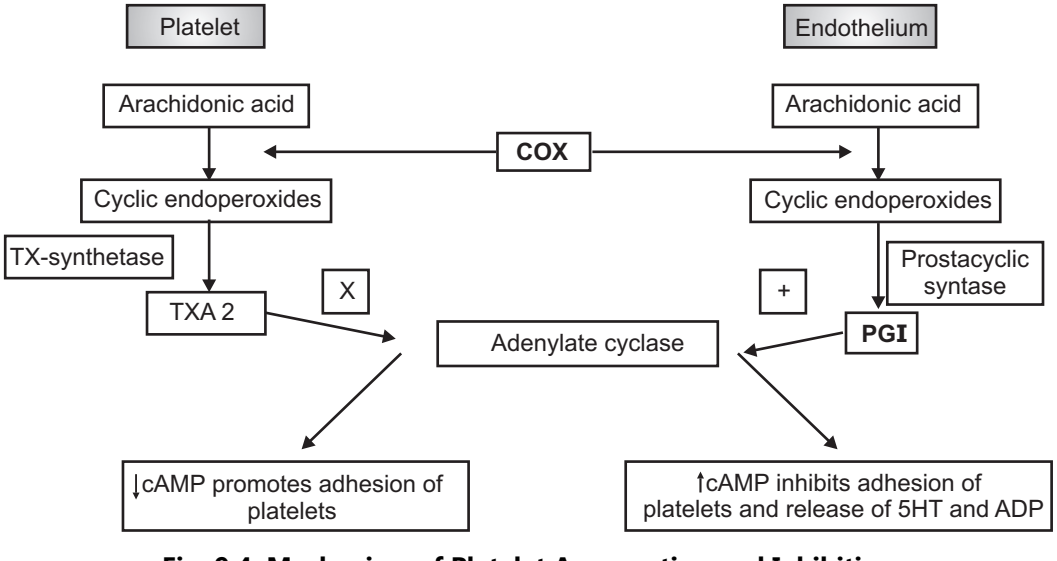


Fig. 9.4: Mechanism of Platelet Aggregation and Inhibition

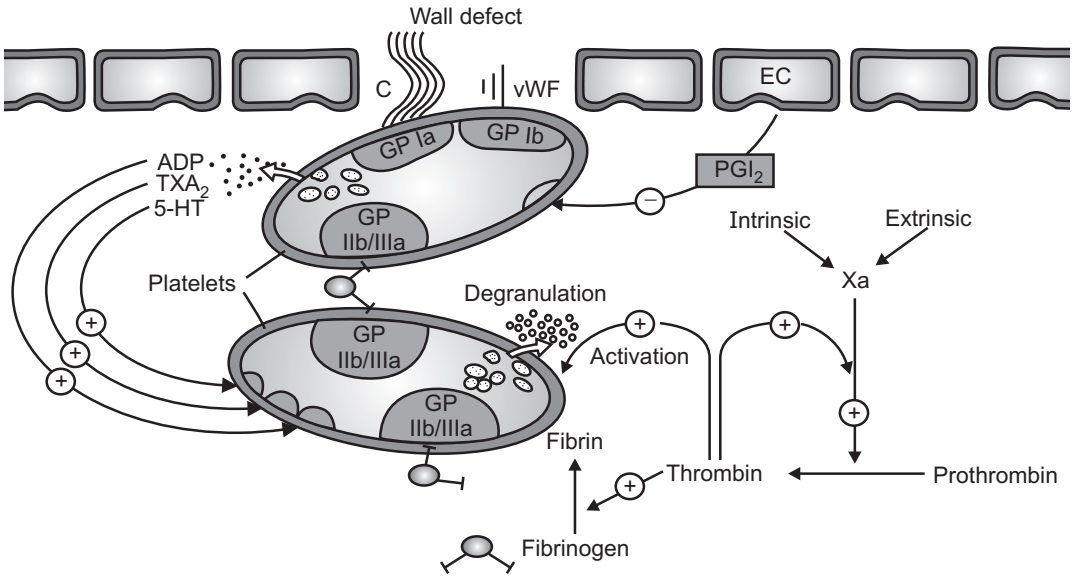
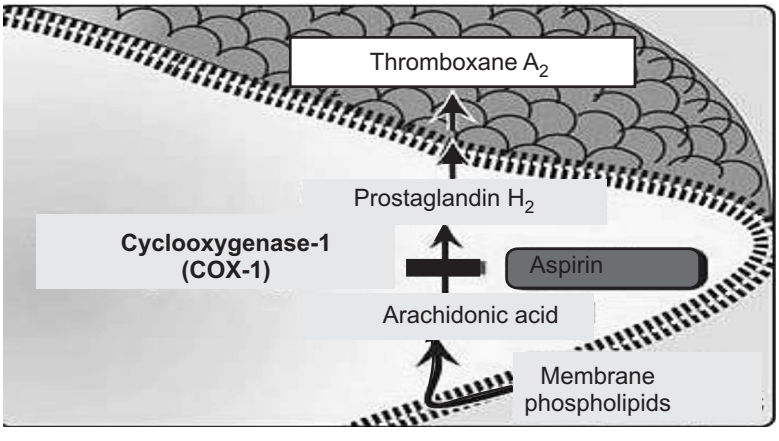


Fig. 9.5

Antiplatelet Drugs (Classification):

- TXA2 synthesis inhibitor:
  - Low dose aspirin
- Phosphodiesterase inhibitor:
  - Dipyridamole, cilostazole
- Thienopyridine derivatives (ADP antagonists):
  - Ticlodipine, clopidogrel
- Gp-IIb/IIIa receptor antagonists
  - Abciximab, eptifibatide, tirofiban
- Others
  - PGI2, daltroban, dazoxiben, clofibrate

Aspirin:



(a)

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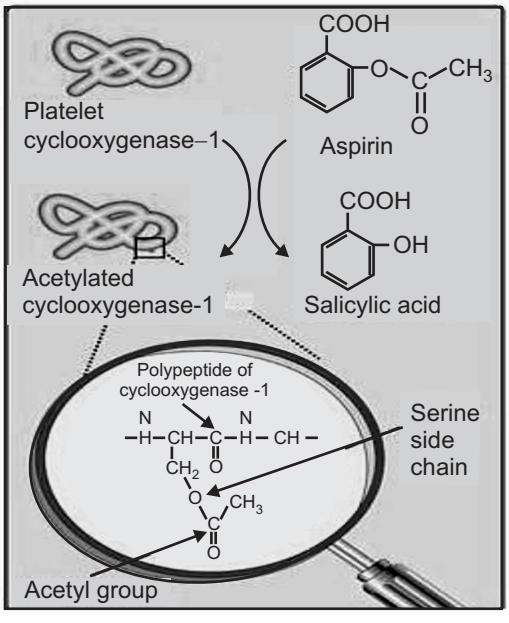

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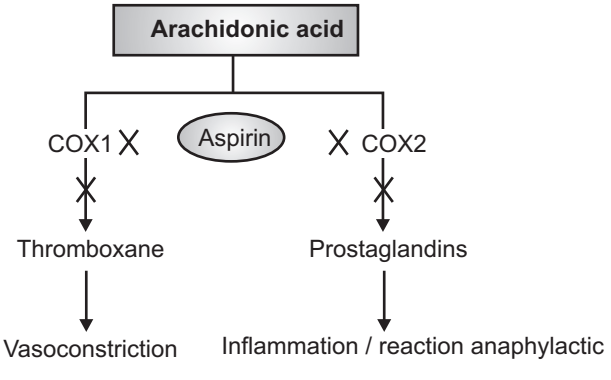


(c)

Fig. 9.6: MoA of Aspirin

Dipyridamole:

- Coronary vasodilator and relatively weak antiplatelet drug.

Mechanism of Action:

- Potentiates effect of endogenous prostacycline.
- In high concentration inhibits Phosphodiesterase, so increase cAMP.
  - Dose = 100 mg BD/TDS.
  - Used with aspirin to prevent ischemic stroke in patients of TIA.

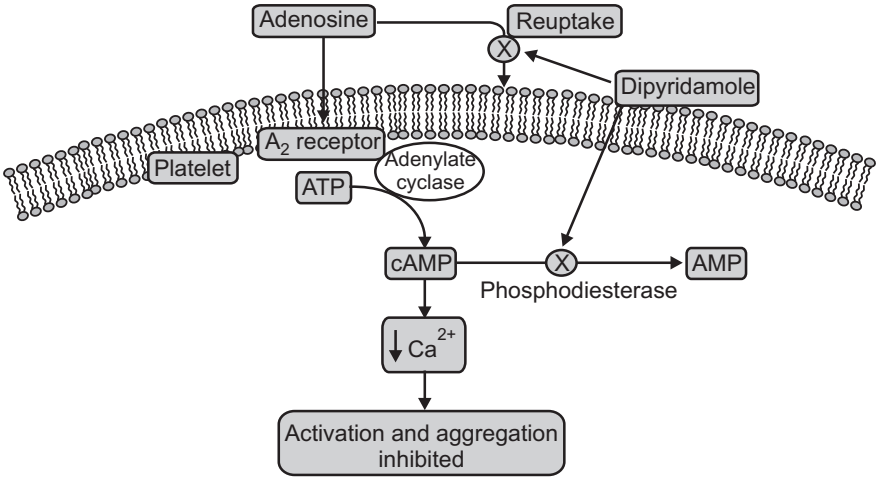


Fig. 9.7: Dipyridamole mechanism of action

Ticlodipine and Clopidogrel:

- ADP antagonists, inhibit binding of ADP to its receptors irreversibly.
- Also Inhibit fibrinogen induced platelet aggregation without modifying GPIIb/IIIa.
- Synergistic action with aspirin.
- Both are prodrugs have long duration of antiplatelet effect.
- Clopidogrel a congener of ticlodipine is safer and better tolerated.

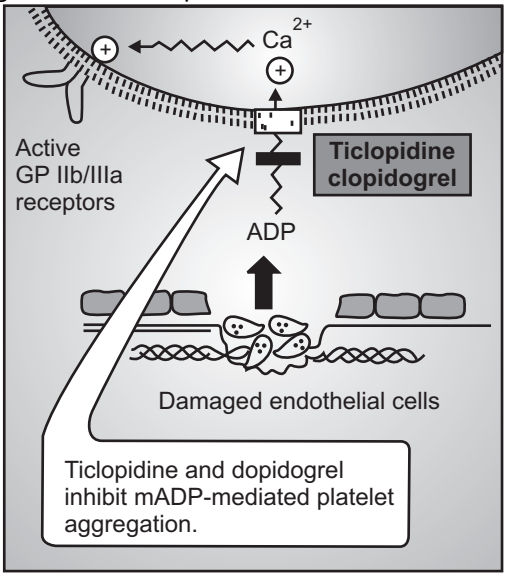


Fig. 9.8: MoA of Clopidogrel and Ticlopidine

Ticlodipine:

- **Adverse effects:**
  - Diarrrhoea, vomiting, abdominal pain
  - Headache, tinnitus, skin rash
  - Bleeding, neutropenia, thrombocytopenia
- Dose = 250 mg BD.

Clopidogrel:

- Adverse effects:
  - Bleeding most IMP
  - Less bone marrow toxicity
  - Diarrhoea, epigastric pain, rashes
- Dose = 75 mg OD.

Abciximab:

- Fab fragment of Chimeric monoclonal antibody against GP-IIb/IIIa.
- Used to prevent platelet aggregation in patients having PCI, administered along with aspirin & heparin or LMW heparin.
- Most common A/E is bleeding.
- May cause thrombocytopenia, hypotension, bradycardia.
- Non antigenic.

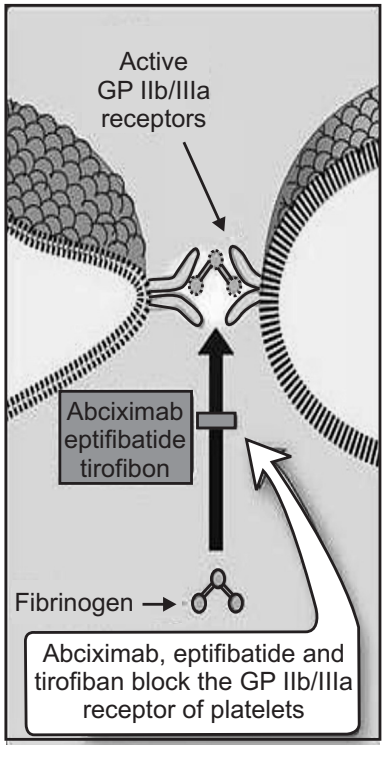


Fig. 9.9

Uses of Antiplatelet Drugs:

- Prosthetic heart valves & A-V shunts
- Peripheral vascular disease

- Coronary artery diseases
  - Myocardial infarction
  - Unstable angina
  - Primary & secondary prevention of MI
- Coronary angioplasty, stents, bypass implants
- Cerebrovascular transient ischemic attacks
- Venous thrombo-embolism.

QUESTIONS

1. Explain the mechanism of action of streptokinase and urokinase.
2. Adverse effects and therapeutic use of thrombolytic agents.
3. Add a note on aspirin as anti-platelet agent.

■■■

Chapter ... 10

Plasma Volume Expanders

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Evaluate the use of blood volume expanders.*

10.1 INTRODUCTION

Blood:

Is a fluid connective tissue that circulates continuously around the body, allowing constant communication between tissues distant from each other.

Plasma:

Plasma is a clear, straw colored, watery fluid in which several different types of blood cells are suspended.

- Plasma expanders are agents that have relatively high molecular weight and boost the plasma volume by increasing the osmotic pressure.
- They are used to treat patients who have suffered hemorrhage or shock.
- Volume expanders are the intravenous fluid solutions that are used to increase or retain the volume of fluid in the circulating blood.
- Generally volume expanders are used to replace fluids that are lost due to illness, trauma or surgery.
- These are used to correct hypovolemia due to loss of plasma or blood.

10.2 TYPES OF VOLUME EXPANDERS

- There are two main types of volume expanders:
  1. **Crystalloids:** Crystalloids are aqueous solutions of mineral salts or other water-soluble molecules. E.g. normal saline, dextrose, Ringer's solution etc.
  2. **Colloids:** Colloids are larger insoluble molecules, such as dextran, human albumin, gelatin, blood. Blood itself is a colloid.
- The larger molecules of colloids are retained more easily in the intravascular space & increase osmotic pressure. So, more effective resuscitation of plasma volume occurs by colloids than produced by that of crystalloids.
- Duration of action of colloid relatively longer than crystalloid.

Colloid:

- Increase plasma volume.
- Less peripheral edema.
- Smaller volume for resuscitation.
- Intravascular half life 3-6 hrs.

Crystalloid:

- Inexpensive.
- Use for maintenance of fluid and initial resuscitation.
- Intravascular half life 20-30 minutes.

Ideal properties of PVEs (Plasma Volume Expanders):

- Iso-oncotic with plasma.
- Distributed to intravascular compartment only.
- Pharmacodynamically inert.
- Non-pyrogenic, non-allergenic & non-antigenic.
- No interference with blood grouping or cross-matching.
- Stable, easily sterilizable and cheap.

Generally used Plasma Expanders:

- Human albumin.
- Dextran.
- Degraded gelatin polymer (Polygeline).
- Hydroxyethyl starch (Hetastarch/HES).
- Polyvinyl pyrrolidone –PVP.

Mechanism of Action:

- Generally works on the principle of osmosis.
- Increases plasma osmotic pressure, drawing water into plasma from interstitial fluid.
- Since the lost blood is replaced with a suitable fluid, now the diluted blood flows more easily, even in small vessels.
- As a result of chemical changes, more oxygen is released to the tissues.

Uses of Plasma Expanders:

- Used in conditions where blood or plasma has been lost or has moved to extravascular compartments e.g., in burns, hypovolaemic shock, endotoxin shock, severe trauma and extensive tissue damage.
- Can also be used as a temporary measure in cases of whole blood loss till the same can be arranged.
- **Note:** They do not have oxygen carrying capacity.

1. Human Albumin:

- It is obtained from pooled human plasma.
- It can be used without regard to patient's blood group and doesn't interfere with coagulation.
- It is free of risk of transmission of hepatitis because the preparation is heat treated.
- Crystalloid solution must be infused concurrently for optimum benefit.
- It has been used in acute hypoproteinaemia, acute liver failure and dialysis.
- It is comparatively expensive.
- Available products:
  - Albudac, Albupan 50, 100 ml inj.,
  - Albumed 5%, 20% infusion (100 ml)

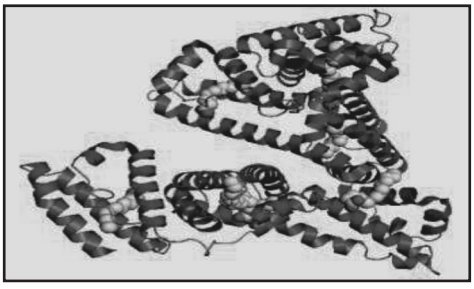


Fig. 10.1: Structure of Albumin

2. Dextran:

- It is highly branched polysaccharide molecule obtained from sugar beat.
- It is produced by using the bacterial enzyme dextran sucrase from the bacterium *Leuconostoc mesenteroides* which grows in a sucrose medium.
- Most commonly used plasma expanders and is available in two forms.
  1. Dextran 70
  2. Dextran 40

(a) Dextran 70:

1. It is most commonly used preparation.
2. It expands plasma volume for nearly 24 hrs.
3. Excreted slowly by glomerular filtration as well as oxidized in body over weeks.
4. Some amount is deposited in retuculoendothelial cells.

Dextran 70 has nearly all the properties of an ideal plasma except:

- It may interfere with blood grouping and cross matching.
- It can interfere with coagulation and platelet function and thus prolong bleeding time.
- Some polysaccharide reacting antibodies, if present, may cross react with dextran and trigger anaphylactic reaction like Urticaria, itching, bronchospasm, fall in BP.

(b) Dextran 40:

- It is 10% solution in Dextrose or Saline.
- It acts more rapidly than dextrose - 70.
- It reduces blood viscosity .
- It is excreted through renal tubules and occasionally may produce acute renal failure.
- The total dose should not exceed 20 ml/kg in 24 hr.
- Dextrans can be stored for 10 years and are cheap so are the most commonly used plasma expanders.

Caution: Dextran doesn't provide necessary electrolytes and can cause hyponatremia or other electrolyte disturbances.

3. Degraded Gelatin Polymer (Polygeline);

- It is synthetic polymer (polypeptide) of MW-30,000.
- It doesn't interfere with blood grouping and cross matching and is non-antigenic.

- Expands plasma volume for 12 hrs.
- It is more expensive than dextran and can also be used for priming of heart-lung and dialysis machines.

Brands:

Haemaccel; Seraccel 500 ml vaccine.

4. Hydroxyethyl starch(Hetastarch)

- It is a complex mixture of ethoxylated amylopectin of various molecular sizes; average MW 4.5 lacs.
- It maintains blood volume longer.
- It doesn't cause acute renal failure or coagulation disturbances.
- It improves hemodynamic status for 24 hrs.

Adverse effects:

- Vomiting, mild fever, itching, chills, flu like symptoms, swelling of salivary glands, Urticaria, bronchospasm etc.

Brand:

- Expan 6% inj. (100 , 500 ml vac).
- It has also been used to improve harvesting of granulocytes because it accelerates erythrocyte sedimentation.

Adverse effects:

- Anaphylactic reactions, mild fever, chilling, periorbital edema, Urticaria, itching.

5. Polyvinylpyrrolidine(PVP)

- It is a synthetic polymer of average MW 40,000 used as a 3.5% solution.
- PVP was used as blood plasma expander for trauma victims after the 1950s.
- It interferes with blood grouping and cross matching and is histmine releaser.
- It binds to penicillin and Insulin.
- It is excreted by kidney and small amounts by liver into bile.
- A fraction is stored in RE cells for prolonged periods.
- It is less commonly used plasma expander.

Uses of PVP:

- PVP is also used in personal care products such as shampoos and toothpaste, hair sprays and gels.
- It is used as binder in many pharmaceutical tablets.
- PVP added to Iodine forms a complex called Povidone- Iodine that posses disinfectant properties. And known under the trade name of **Betadine and Pyodine.**

Some Crystalloids:

1. Normal Saline (Isotonic):

- It is the crystalloid fluid containing 0.9% NaCl.
- The pH of isotonic saline is considerably lower than the plasma pH.
- NS is frequently used in patients who cannot take fluids orally and have developed dehydration or hypovolemia.

2. Lactated Ringer's solution:

- It was introduced in 1880 by Sydney ringer, a British physician.

- The solution was designed to promote the contraction of frog hearts and was contained with calcium and potassium in a NaCl diluents .
- It is contraindicated as diluents for blood transfusions.

3. Dextrose solutions:

- Generally 5% dextrose solutions are used which provides 170 kcal/lit.
- It is IV sugar solution which provides some energy to the body parts.
- Osmolarity is lower than serum.
- Useful when kidney function is impaired.

Contraindications to Plasma Expanders:

- Allergy
- Heart failure
- Severe anaemia
- Thrombocytopenia
- Pulmonary edema
- Renal insufficiency.

Some commercially used Plasma volume expanders:

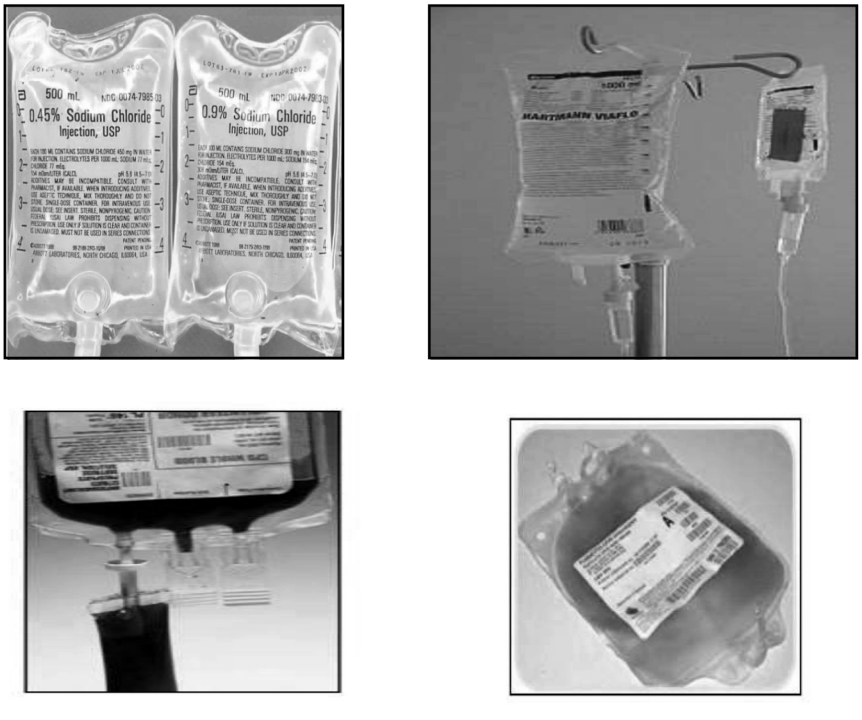


Fig. 10.2

QUESTION

1. Write a note on plasma volume expanders.

■■■

Chapter ... 11

Pharmacology of Drugs Acting on Urinary System

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the primary therapeutic uses of diuretics and the broad types of adverse effects.*
- *List the four major classes of diuretics; describe mechanism / site of action in nephron, clinical uses, side effects, and any key considerations.*

11.1 DIURETICS

Diuretics are the materials that promote the excretion of urine.

- Promotes the excretion of the (Na^+), (Cl^-) or (HCO_3^-) and water.
- Net result being:
  - Increase the urine flow.
  - Change urine pH.
  - Change the ionic composition of the urine and blood.
- Diuretics are very effective in the treatment of edema, CHF, pregnancy and nutritional nephrotic syndrome, hypertension, cirrhosis of liver and also lower the intracellular and CSF pressure.

Normal Physiology of Urine Formation

- **Kidney:** 1.3 million nephron each.
- **Glomerular Filtration:**
  - Receive 25% of cardiac output
  - Filtration rate: 100-120 ml/minute
  - 180 L of glomerular filtrate/day.
- **Tubular Reaborption:**
  - Reabsorption of 99% of glomerular filtrate
  - 1.5 L/day of urine.

- **Tubular Secretion:**

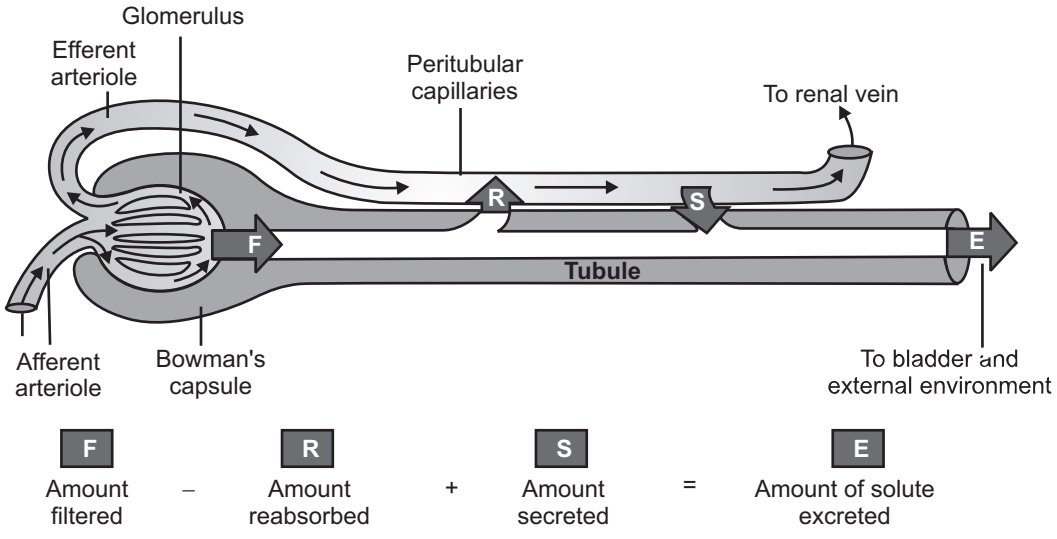
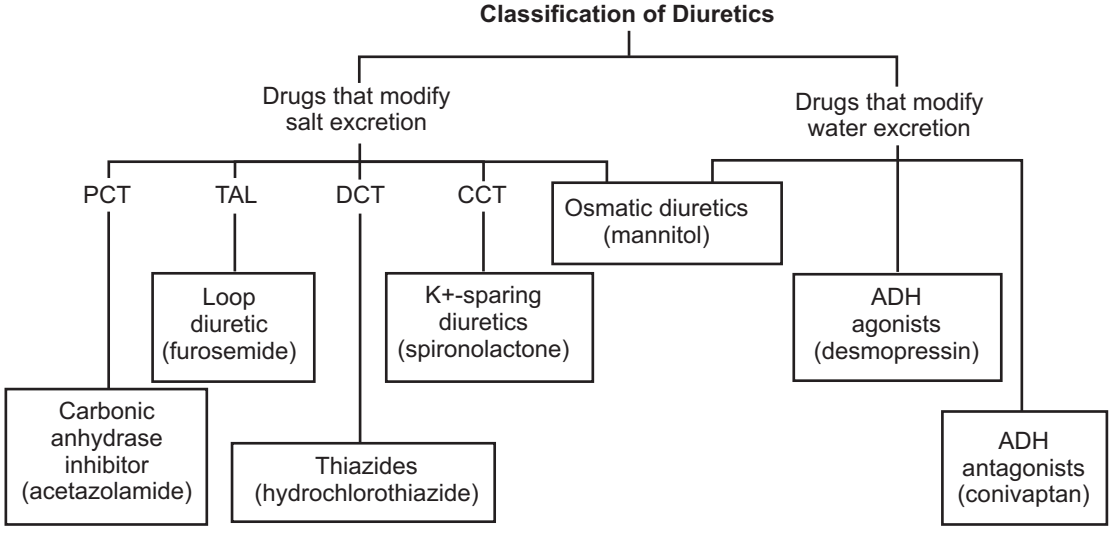


Fig. 11.1

11.2 CLASSIFICATION OF DIURETICS



Proximal Convoluted Tubule (PCT), Thick Ascending Limb of the loop of Henle (TAL), Distal Convoluted Tubule (DCT) and Cortical Collecting Tubule (CCT).

Fig. 11.2

11.3 SITE AND MECHANISMS OF ACTIONS OF DIURETICS

Diuretics	Site of Action	Mechanism
Osmotic Diuretic	1. Proximal tubules 2. Loop of Henle 3. Collecting duct	Inhibition of water and Na^+ reabsorption.
Carbonic Anhydrase Inhibitor (CA-I)	Proximal tubules	Inhibition of bicarbonate reabsorption.
Loop Diuretic	Loop of Henle (*thick ascending limb*)	Inhibition of Na^+, K^+, Cl cotransport.
Thiazide	Early distal tubule	Inhibition of Na^+, Cl cotransport
K^+ sparing diuretics	Late distal tubule Collecting duct	Inhibition of Na^+ reabsorption and K^+ secretion.

1. Carbonic Anhydrase Inhibitor:

Acetazolamide

Mechanism of Actions:

- **Kidney:** Self Limited Diuresis 2-3 days.
  - Carbonic anhydrase catalyzes: $CO_2 + H_2O \rightarrow H_2CO_3$
  - H^+ ion produced by the breakdown of H_2CO_3, exchanged for Na^+ and is also combined with HCO^{3-} in the lumen of the PCT.
  - Inhibition of Bicarbonate (HCO^{3-}) reabsorption.
  - Reduces Na^+ - H^+ -exchange $NaHCO_3$ is excreted along with H_2O.

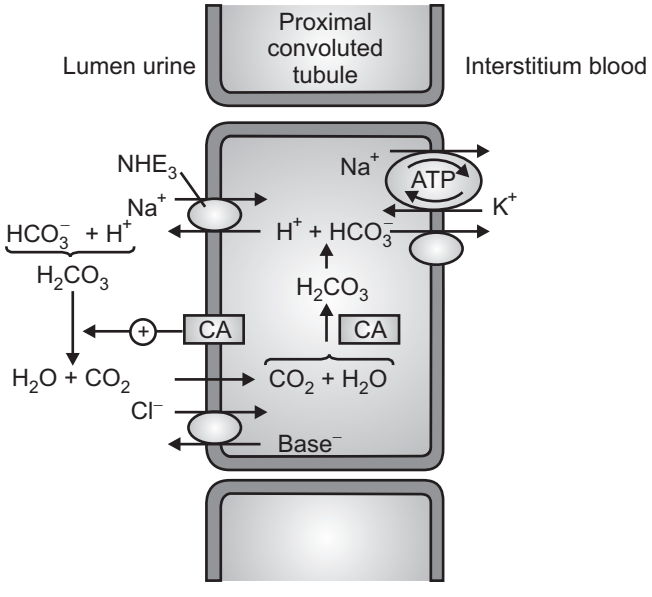


Fig. 11.3: MoA of Carbonic Anhydrase inhibitor

Adverse Effects and Contraindications:

- Metabolic acidosis.
- Renal stones (Phosphate and Calcium stone).
- Renal potassium wasting; ($NaHCO^{3-}$) enhances K^+ secretion.
- Diuresis is self limiting within 2-3 days.
- AE: Drowsiness, paresthesia, disorientation, renal stone (in case of urine alkalinization).

Contraindication:

- Liver cirrhosis (CA-I inhibits conversion of NH_3 to NH_4) $\rightarrow$ NH_3 increased $\rightarrow$ encephalopathy.

Indications of CA-I:

- Glaucoma (Eye: not self limiting effect):
  - Orally acetazolamide
  - Topically dorzolamide, brinzolamide
- Prevent mountain sickness (high altitude) sickness inhibit sec of bicarbonate by the choroid plexus; Acidosis of the CSF results in hyperventilation.
- **Urinary alkalinization:** Preventing uric acid and cystine stones.
- Used for their diuretic effect only if edema is accompanied by significant metabolic alkalosis.

2. Thiazides:

- Hydrochlorothiazide (prototype), Chlorothiazide, Bendroflumethiazide, Chlorthalidone, Metolazone, Indapamide.
- All are sulfonamide derivatives, $t^{1/2}$ – 6-12 hrs.

Mechanism of Actions:

- Thiazides are secreted by proximal tubules but works in DCT.
- Inhibit Na^+Cl symporter from the lumen to tubular cells $\rightarrow$ increase Na^+, Cl^- excretion (and water)
- Some thiazides have weak CA-I effect.

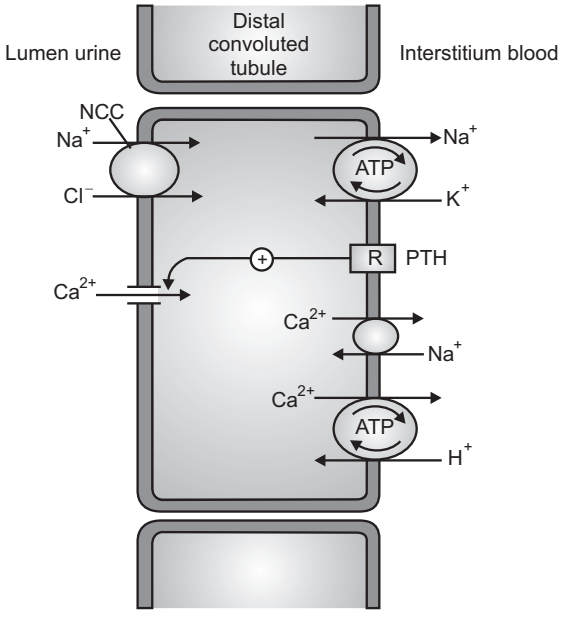


Fig. 11.4

Effects on Electrolytes:

- Increases Na^+ and Cl^- excretion.
- Hypokalemic metabolic alkalosis; K^+ excretion also increase associated with increased Na^+ in distal tubules.
- Inhibits uric acid secretion → hyperuricemia and gout.
- Decreases Ca^{2+} excretion.
- Tends to increase plasma Ca^{++}.
- Retards osteoporotic process.
- Increases Mg^{2+} excretion.

Adverse Effects:

- Hypo K^+ → Increased risk of digitalis toxicity.
- Hypo Na^+, Hypo Mg^{++}.
- Hyperuricemia → caution in gout arthritis.
- Hyperglycemia and hypercholesterolemia → not favorable for DM and dyslipidemia (although not contraindicated).
- Indapamide has less effects on lipid and uric acid.
- Hypercalcemia (long-term).
- Sexual dysfunction.

Interactions:

- Increases the risk of arrhythmia when combined.
- With digitalis, quinidine and other antiarrhythmias.
- Reduces efficacy of anticoagulant and uricosuric.
- Reduces the efficacy oral antidiabetics.
- NSAID reduce the efficacy of thiazide.

Indications of Thiazides:

- Hypertension (single drug or in combination).
- Chronic, mild-heart failure.
- Edema (loop diuretic is preferable).
- Nephrogenic Diabetes insipidus.
- Prevention of Ca^{++} excretion in osteoporosis and Calcium nephrolithiasis.

3. Loop Diuretics:

- Furosemide, torasemide, bumetanide: Are sulfonamide derivatives.
- Ethacrynic acid is a phenoxyacetic acid derivative.
- Site of action: thick ascending limb of Henle.

Mechanism:

- Loop diuretics should be excreted into the lumen.
- Inhibits Na^+, K^+, $2Cl^-$ symporter → significantly increases the excretion of Na^+, K^+, Cl^-.

- Osmotic gradient for water reabsorption is also decreased → increasing water excretion.
- Ca^{2+} and Mg^{2+} are excreted as well.

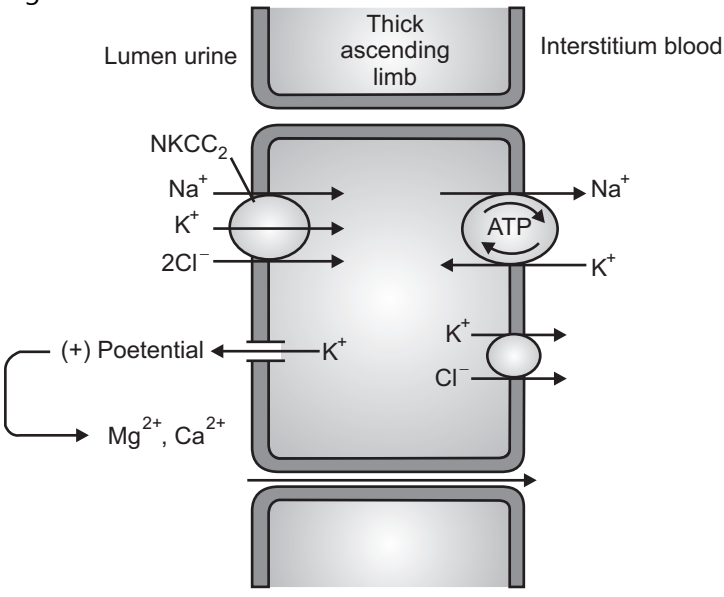


Fig. 11.5: Mechanism of action of Loop diuretics

Adverse effects:

- Hypovolemic metabolic alkalosis.
- Ototoxicity.
- Typical sulfonamide allergy.

Interactions:

- Concomitant use with aminoglycoside or cisplatin increases the risk of nephro-toxicity and ototoxicity.
- PGs are important in maintaining GF; NSAID reduces the effects of diuretics.
- Probenecid reduces the effects of diuretics by inhibiting its secretion into the lumen.

Indications:

- Congestive heart failure (1st line drug).
- Acute pulmonary edema.
- Edema due to renal failure, nephrotic syndrome, ascites.
- Hypercalcemia (that induced by malignancy).
- Severe hypertension.
- Force diuresis during drug/chemical intoxication (drug that excreted through the kidney in active form).

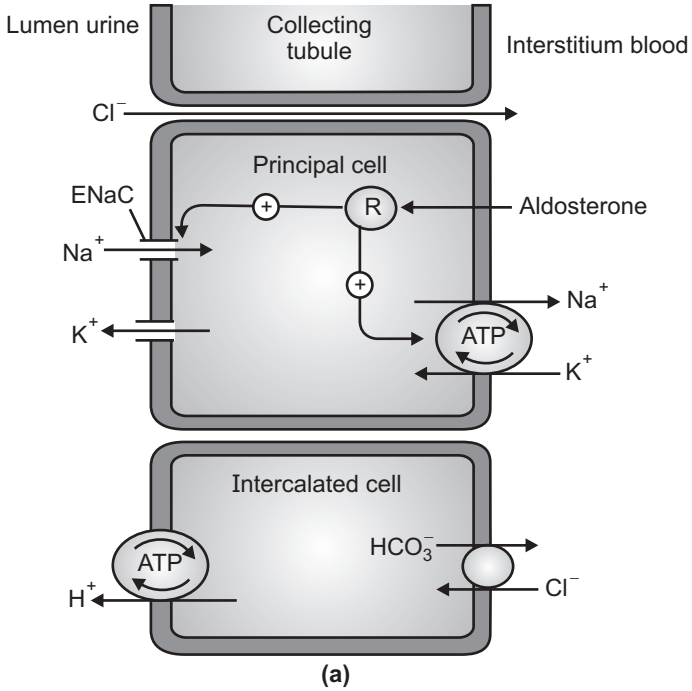
4. Potassium Sparing Diuretics:

(i) Na^+ channel inhibitor (Amiloride, triamterene):

- Inhibit Na^+ reabsorption → Na^+ excretion.
- Reduced K^+ secretion → K^+ retention.

(ii) Aldosterone antagonist (Spironolactone, Eplerenone) Steroid Derivatives:

- Aldosterone induces the expression of Na/K ATPase and Na+ channel.
- Spironolactone and eplerenone blocks aldosterone receptor → reduces Na^+ reabsorption and K^+ secretion.



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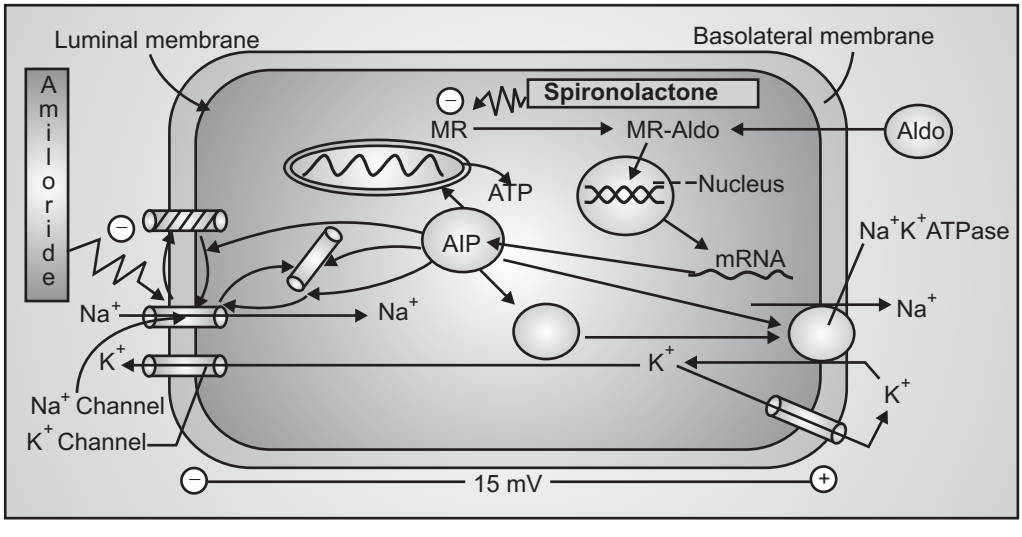


(b)

Fig. 11.6: MoA of Potassium Sparing Diuretics

- Potassium sparing diuretic has a weak diuretic action.

- Usually used in combination with other diuretic:
  - Potentiation of diuretic and antihypertensive effects.
  - Prevents hypokalemia.
- Spironolactone is metabolized to its active metabolite, canrenone.
- Long term use of spironolactone can prevent myocardial hypertrophy and myocardial fibrosis.

Adverse Effects:

- Hyperkalemia
- Antiandrogenic effect
  - gynecomastia,
  - decrease of libido, impotence,
  - menstrual disturbance.
- Megaloblastic anemia: Triamterene (folate antagonist).

Indications:

- Antihypertension.
- In combination with other antihypertensives.
- To increase the effect and to prevent hypokalemia.
- Aldosteronism (that occur in cirrhosis).

Contraindications/Precautions:

- Conditions that prone to hyperkalemia.
- Renal failure.
- Should never be combined with ACE-inhibition, ARB.
- NSAID, K^+ supplementation.

5. Osmotic Diuretics (OD):

- Mannitol (prototype).
- Others rarely used: urea, glycerin, isosorbide.
- Properties of osmotic diuretics:
  - Freely filtrated by glomerulus.
  - Negligible tubular reabsorption.
  - Chemically inert.
  - Usually non-metabolized.

Mechanism of Action:

- OD is filtrated and increases osmotic pressure in tubular lumen.
- Hence, increases excretion of water and electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}, HCO^{3-}, phosphate) ↑volume of urine and rate of urine flow through the tubule.

- Mannitol can also reduce brain volume and intracranial pressure by osmotically extracting water from the tissue into the blood.
- A similar effect occurs in the eye.

Adverse Effects:

- Removal of water from the I.C compartments → Initial increase of plasma volume → potentially dangerous in heart failure and pulmonary edema → Hypo → Na^+ → headache, nausea, vomiting.
- Water excretion → Hypovolemia → Hypernatremia.
- Hypersensitivity reaction.
- Vein thrombosis, pain if extravasation (urea).
- Hyperglycemia, glycosuria (glycerin).

Pharmacokinetics:

- **Mannitol and urea:** Intravenous.
- **Glycerin and isosorbide:** Can be administered orally.

Metabolism:

- Glycerin 80% metabolized.
- Mannitol 20%.
- **Urea, isosorbide:** Not metabolized.
- **Excretion:** Renal.

Indications:

- Glaucoma (rare) ↓ IOP (Intra-Ocular Perssure).
- Brain edema, ↓brain volume and pressure.
- Mannitol and urea are given before and after brain surgery.
- Disequilibrium syndrome after hemodialysis.
- Solute overload in sever hemolysis and rhabdomyolysis.
- Prophylaxis of ATN (acute tubular necrosis) due to contrast media, surgery, and trauma.
- NaCl 0.45% can also be used.

11.4 ANTIDIURETICS

An antidiuretic is a substance that helps to control fluid balance in a living body by reducing urination, opposing diuresis.

Antidiuretics: Drug List

- Antidiuretic Hormone (ADH, Vasopressin)
  - Desmopressin, Lypressin, Terlipressin
- Thiazide Diuretics:
  - Amiloride
- Miscellaneous:
  - Indomethacin,
  - Chlorpropamide,
  - Arbamazepine

Antidiuretic Hormone (ADH)

ADH is a hormone (protein) secreted by posterior pituitary (neurohypophysis). Rate of ADH Release controlled by:

- Osmoreceptors present in hypothalamus.
- Volume receptors present in left atrium, ventricles and pulmonary veins.
- ADH and Desmopressin are ADH Agonists.
- Secretion of ADH increase in response to:
  - Plasma osmolarity
  - Hypovolemia, hypotension (bleeding, dehydration)
- Demeclocycline and conivaptan are ADH antagonists
- Lithium has ADH antagonist effect but never used for this purpose.

ADH Receptors:

V1 Receptors:

- At all sites except for sites of V2 (i.e. Collecting Duct cells).
- Further classified as V1a and V1b.

V1 (a) Vascular smooth muscles (including that of vasa recta in renal medulla), uterine, visceral smooth muscles, interstitial cells in renal medulla, cortical CD cells, adipose tissue, brain, platelets, liver, etc.

V1 (b) Anterior pituitary, certain areas in brain and in pancreas.

V2 Receptors: More sensitive:

- **Collecting Duct Principal cells in Kidney:** Regulates their water permeability.
- **Also present in AscLH cells:** Activates Na^+K^+2Cl cotransporter.
- **Endothelium:** Vasodilator.

ADH: Action on Various Organs

- **Kidneys:**
  Acts on CD principal cells---- renders them water permeable --- water absorbed---- concentrated urine (equilibrating with hyperosmolar medulla) passed.
- **Blood Vessels:**
  Constricts through V1 receptors: raises blood pressure.
  Dilates through V2 receptors: endothelium dependent NO production.
- **GIT:**
  Increased peristalsis: evacuation and expulsion of gases.
- **Uterus:**
  Contracted by acting on oxytocin receptors.
- **Central Nervous System:**
  Endogenous AVP may be involved in regulation of temperature, learning of tasks.

Others:

Induces platelet aggregation, hepatic glycogenolysis. Release of factor VIII and von Willebrand's factor from vascular endothelium : V2 mediated.

Mechanism of Action:

- Works in ascending limb of Henle's loop and collecting ducts.
- Two kind of receptors:
  - V1: Vascular smooth muscle – vasoconstriction.
  - V2: Kidney – increase water permeability of tubular epithelium – water reabsorption.
- ADH facilitates water reabsorption from the collecting tubule by: activation of V2 receptors (coupled to GS, stimulate AC) increase cAMP.
- Cause insertion of additional aquaporin AQP2 water channels into the luminal membrane in this part of the tubule.

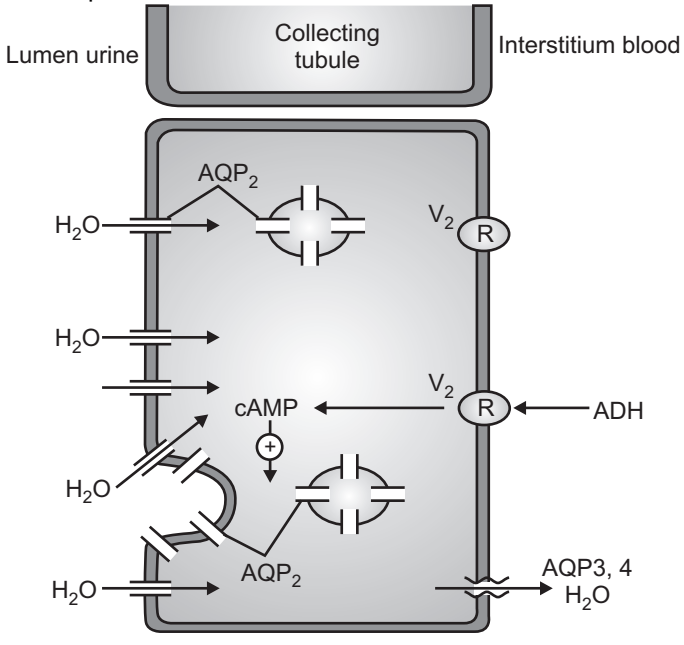


Fig. 11.7

Clinical uses of ADH Agonists:

- ADH and desmopressin reduce urine volume and concentrate it, are useful in Pitutary Diabetes Insipidus (DI).
- Gastrointestinal bleeding due to portal hypertension.

ADH Antagonists:

- Conivaptan is an ADH inhibitor at V1a and V2 receptors.
- Demeclocycline and Li inhibit the action of ADH at some point distal to the generation of cAMP and presumably the insertion of water channels into the membrane.

Clinical uses of ADH Antagonists:

- Oppose the actions of ADH and other naturally occurring peptides (certain tumors; small cell carcinoma of the lung) that act on the same V2 receptors, leads to significant water retention and dangerous hyponatremia.
- Syndrome of inappropriate ADH secretion.
- (SIADH) can be treated with demeclocycline and conivaptan.

AVP (Arginine vasopressin) Interactions:

- **Lithium, demelocycline:** Partially antagonise AVP action (limiting cAMP formation).
- Used in patients with inappropriate ADH secretion.
- **NSAIDs (Indomethacin):** Augments AVP (increased renal PG synthesis).
- Carbamazepine, chlorpropamide: potentiates AVP action on kidney.

AVP: Uses

Based on V2 Actions:

- Diabetes Insipidus (Neurogenic).
- Bedwetting in children and nocturia in adults.
- Renal Concentration Test.
- Hemophilia, von Willebrand's Disease.

Based on V1 Actions:

- Bleeding Esophageal Varices.
- Before abdominal radiography.

Vasopressin: Adverse Effects

- Selective drugs produce lesser side effects.
- Transient headache and flushing: frequent.
- **Local Application:** Nasal irritation, congestion, rhinitis, ulceration, epistaxis.
- **Systemic Side effects:** belching, nausea, vomiting, abdominal cramps, pallor, urge to defecate, backache. In females (uterine contraction) Fluid retention, hyponatremia.

Thiazide: Hydrochlorthiazide

- Paradoxical Effect.
- **Furosemide:** effective but less desirable: short and brisk action.
- Effective in both neurogenic as well as nephrogenic DI.

Mechanism of Action:

1. Similar to Salt Restriction:

- State of sustained electrolyte depletion.
- Glomerular filtrate completely reabsorbed iso-osmotically in PT.
- Urine passing has low solutes – presented to cortical DT – salt reabsorption decreases – less dilute urine presented to CD – same is passed out.

2. Reduces glomerular filtration rate – reduced fluid load on tubules.

Other Antidiuretics:

Indomethacin:

- Decreases renal prostaglandin synthesis, reduced polyuria in nephrogenic DI.
- Combined with thiazide +/- amiloride.

Chlorpropamide:

- Long acting sulfonylurea oral hypoglycaemics.
- Effective in neurogenic DI: sensitizes kidney to ADH.

Carbamazepine:

- Anticonvulsant.

QUESTIONS

1. Classify diuretics. Discuss the mechanism of actions and uses of thiazide diuretics.
2. Discuss the pharmacology of frusemide.
3. Write down the mode of action, adverse effect and therapeutic uses of loop diuretics.
4. Write a note on osmotic diuretics.
5. Classify diuretics with examples. Explain the mechanism of action, adverse effects and uses of Acetazolamide.
6. MOA, ADR and uses of vasopressin.

■■■

Unit III

Chapter ... 12

Autocoids and Related Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the drugs that can be used in various form of allergic disorders.*

12.1 INTRODUCTION

The word autacoids come from the Greek "autos" (self) and "acos" (relief; i.e., drug). **Autacoids** or "autocoids" are biological factors (molecules) which act like local hormones, have a brief duration, and act near their site of synthesis. Vasodilator **autacoids** are released during periods of exercise.

The effects of autacoids are primarily local, though large quantities can be produced and moved into circulation. Autacoids may thus have systemic effects by being transported via the circulation. These regulating molecules are also metabolized locally. In sum, these compounds typically are produced locally, act locally and are metabolized locally. Autacoids can have a variety of different biological actions, including modulating the activities of smooth muscles, glands, nerves, platelets and other tissues.

Some autacoids are chiefly characterized by the effect they have on specific tissues, such as smooth muscle. With respect to vascular smooth muscle, there exist both vasoconstrictor and vasodilator autacoids. Vasodilator autacoids are released during periods of exercise. Their main effect is seen in the skin, where they facilitate heat loss.

These are local hormones; they therefore have a paracrine effect. Some notable autacoids are: eicosanoids, angiotensin, neurotensin, NO (nitric oxide), kinins, histamine, serotonin, endothelins and palmitoylethanolamide.

Recently, research on autacoids has given rise to the nascent field of "Autacoid Medicine" particularly since new lipid autacoids have been found to be of utility in the treatment of chronic disorders, where inflammation plays a role. In 2015, a new definition of autacoids was proposed, which helps to more specifically describe Autacoid Medicine.

Definition:

These are heterogeneous substances produced by a wide variety of cells having widely different structures and intense biological activity but generally act locally at the site of its synthesis and release.

"Autacoids are locally produced modulating factors, influencing locally the function of cells and/or tissues, which are produced on demand and which subsequently are metabolized in the same cells and/or tissues".

Classification of Autacoids:

1. **Amine Autacoids (Decarboxylated autacoids amines):** Hiatamine, 5-hydroxy tryptamine (Serotonin).
2. **Peptide autacoids (Polypeptide):** Bradykinin, Kallidin, Vasoactive intestinal pepetides.
3. **Lipid derived autacoids (Eicosonoids):** Prostaglandins, Thromboxane, Leucotrines, Platelets activating factors (PAF).

12.2 CLASSIFICATION OF AUTACOIDS

Different autacoids are:

- Histamine
- 5 Hydroxytryptamine (5HT)
- Bradykinin and Kallidin
- Cytokines
- Autacoids derived from membrane phospholipid
  - Eicosanoids – arachidonic acid
  - (PG, PGI, TXA_2, LT)
  - Modified phospholipids – PAF

12.3 HISTAMINE

12.3.1 Synthesis

Histamine is a basic amine formed from histidine by *histidine decarboxylase*.

Storage: It is found in most tissues but is present in high concentrations in the lungs and the skin, and in particularly high concentrations in the gastrointestinal tract. At the cellular level, it is found largely in mast cells but non-mast cell histamine occurs in 'histaminocytes' in the stomach and in *histaminergic neurons* in the brain.

In mast cells and basophils, histamine is complexed in intracellular granules with an acidic protein and a high-molecular-weight heparin termed *macroheparin*. Stored in granules of mast cells, basophils and secreted when complement interact with cell IgE .

Others – GIT, lungs, skin, heart, liver, neural tissue, reproductive mucosa, rapidly growing tissues and body fluids.

Produces effects by acting on H_1, H_2, H_3 receptors.

12.3.2 Histamine Release

Histamine is released from mast cells by exocytosis during inflammatory or allergic reactions. Stimuli include C3a and C5a that interact with specific surface receptors, and the

combination of antigen with cell-fixed IgE antibodies. In common with many secretory processes, histamine release is initiated by a rise in cytosolic Ca^{2+}. Various basic drugs, such as **morphine** and **tubocurarine**, release histamine through a non-receptor action. Agents that increase cAMP formation (e.g. β-adrenoceptor agonists;) inhibit histamine secretion. Replenishment of secreted histamine by mast cells or basophils is a slow process, which may take days or weeks, whereas turnover of histamine in the gastric histaminocyte is very rapid. Histamine is metabolised by *histaminase* and/or by the methylating enzyme *imidazole* N-*methyltransferase*.

12.3.3 Histamine-Synthesis and Metabolism

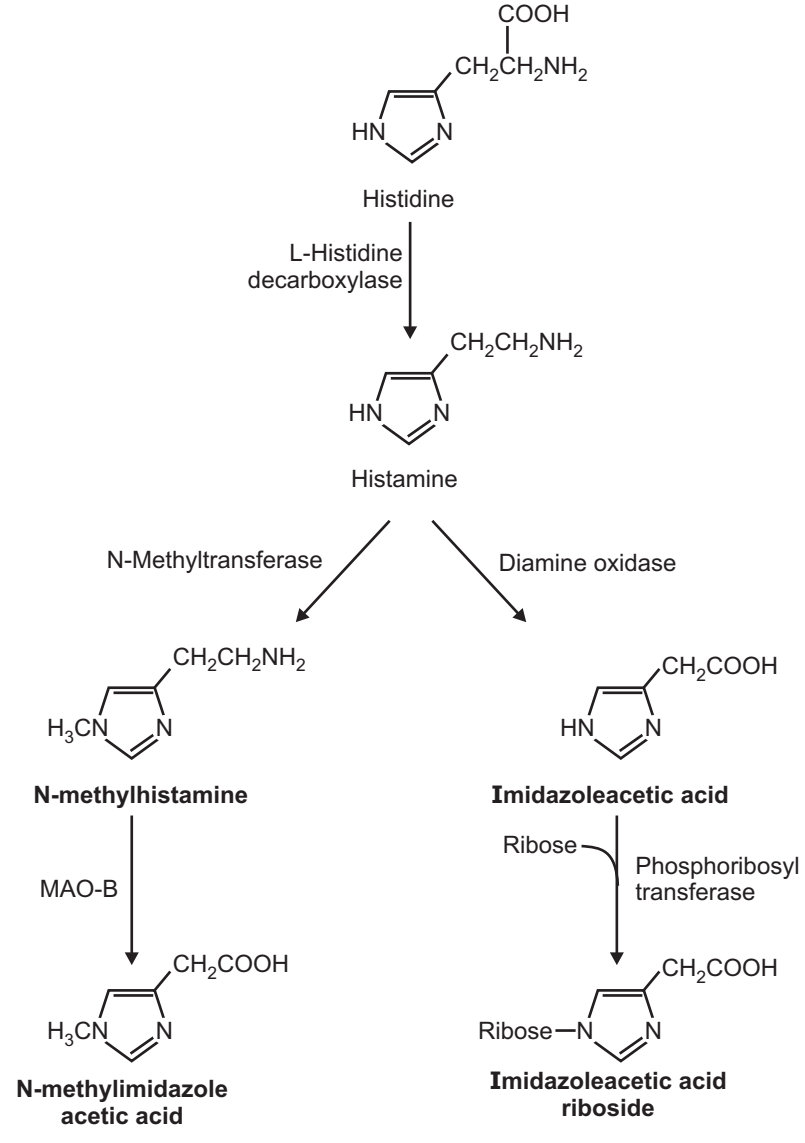


Fig. 12.1: Synthesis and Metabolism of histamines

MOA:

- Histamine act on H_1 (Gq, protein coupled) receptor cause activation of IP3 and DAG and protein kinase and release NO.
- Histamine acts on H_2 (GS protein coupled) receptor and cause activation of cAMP and release of Ca^{2+}. Activation of H/K^+ ATPase pumps and increases HCL secretion.
- Histamine acts on H_3 (Gi protein coupled) receptor and inactivate cAMP and decreased influx of calcium and opening of K^+ channels.

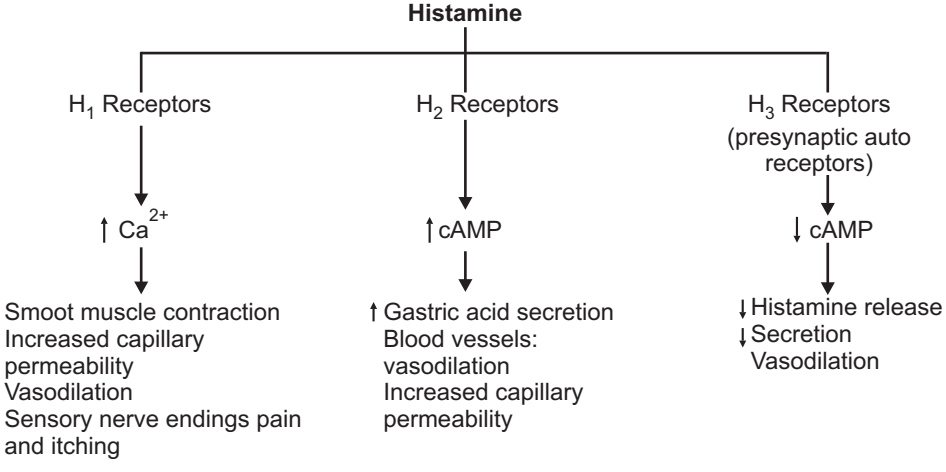


Table 12.1: Selected Actions of Histamine in Humans:

H_1 - Located in post synaptic membrane: • Smooth muscles - Contraction • Blood vessels - Vasodilation • Sensory nerve endings - Pain, itching. **H_2** - Located in post synaptic membrane: • Gastric glands - Acid secretion • Blood vessels - Vasodilation • Heart - Increased FOC and HR. **H_3** • Brain presynaptic - Decreases histamine , NE, Ach release. • Lung, Spleen, Gastric mucosa- Decreases histamine release. **H_4** • Eosinophils. • Neutrophil. • CD4 T cells.	**H_1, H_2** - Located in post synaptic membrane. **H_3** - Presynaptic. **H_1** - Predominant in endotracheal and smooth muscle. **H_2** - Facial veins, carotid a, pulm. a, heart gastric mucosa, heart, smooth muscle and some immune cells. **H_3** - Several areas in CNS. **Triple response** - Wheal, flare and redness.

Pharmacological Action:

1. **CVS:** H_1 mediated response

   Blood vessels:

   - Contraction of major blood vessels like artery and veins.
   - Dilation of minor blood vessels like capillaries, venules and cranial blood vessels.
   - Net effects – vasodilatation.

   Blood pressure:

   - Moderate dose - Hypotension.
   - High dose - Prolonged hypotension.

   Heart:

   - Increase forced and frequency of ventricular contraction.
   - Increased coronary blood flow.
   - Large dose - Ventricular arrhythmias.

2. **Allergic Reaction:** H_1 mediated response.

   Itching occurs if histamine is injected into the skin or applied to a blister base, because it stimulates sensory nerve endings by an H_1-dependent mechanism. Injected intradermally, histamine causes the 'triple response':

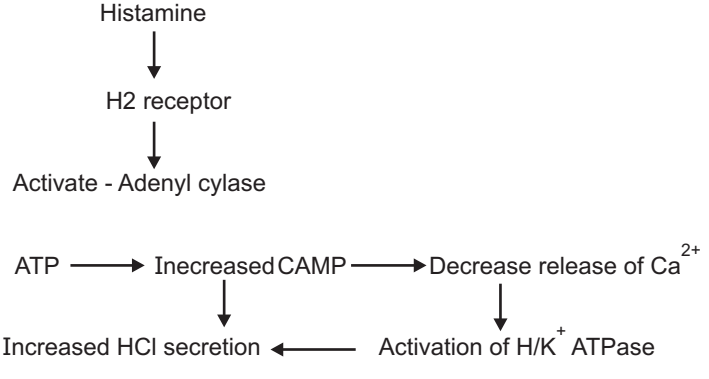
   - **Reddening /Flush** (local vasodilatation),
   - **Flare** (bright flare, irregular outline extended upto 1-5 mm beyond flush, it is develop from an 'axon' reflex in sensory nerves releasing a peptide mediator) and
   - **Weal** (direct action on blood vessels, developments of localized edema due to escape of fluid from localized capillaries)

3. **Smooth muscle:** H_1 mediated response.

   - Moderate dose - Contraction of smooth muscles of GIT urethral.
   - High dose - Abdominal cramps, colic and increase intestinal contraction.

4. **Exocrine glands:** H_2 mediated response.

   Powerful stimulation of gastric acid and pepsin secretion. Histamine acts on H_2 (Gs protein coupled) receptor and cause activation of cAMP and release of Ca^{2+}. Activation of H/K^+ ATPase pumps and increases HCl secretion.



5. **CNS:** H_3 mediated response.

   Histamine do not cross BBB its synthesized locally from histidine. Central physiological role is not clear, intracerabral and intravetebral injection may cause hypothermia and vomiting.

6. **Autonomic ganglion and adrenal medulla:**

   Histamine at high concentration stimulate both and cause release of Adrenaline.

Metabolism:

- **Major pathways:** Histamine is metabolised by histaminase and/or by the methylating enzyme imidazole N-methyltransferase, and converts to N-methly histamine, which is act by MAO, after oxidation excreted in urine as methyl imidazole acetic acid.
- **Deamination:** Small intestine, liver, kidney and monocytes. Methylation – small intestine, liver, skin, kidney, thymus and leukocytes. **N-methylimidazole acetic acid** – principal urinary metabolite

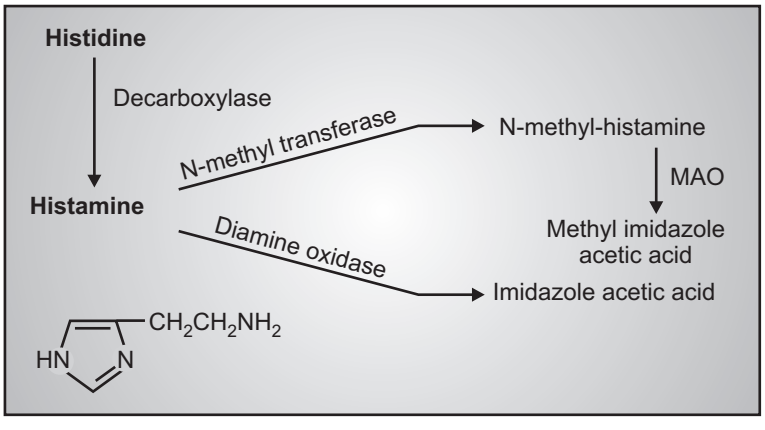


Fig. 12.2: Metabolism of Histamine

Uses:

1. Role in allergic responses – Ag + IgE (bound to mast cells and basophils).
2. Preformed mediators.
3. Most important mechanism of release/controlled by H2 esp. in skin and blood.
4. Release of other autacoids.
5. Release by drugs (morphine, urase, amines), peptides, venoms and other agents.
6. Release by urticarias.
7. Gastric secretagogue.
8. Neurotransmitter → increased wakefulness, thermoregulation.

ADR:

- Hypotension, Flushing, Headache, Visuals distribution, Allergic reaction like-Flush, flare and weal, Anaphylaxis shock.

12.4 ANTI HISTAMINE

Definition:

These are the drugs antagonized various actions of histamine that liberate in the body or exogenously administered.

These drugs are use mainly for symptomatic relief of allergic disorders and peptic ulcer.

These acts in 3 Ways:

- Physiological Antagonist: Adrenaline
- By inhibiting the release of histamine from the sensitive mast cells following:
  - Antigen Antibody reaction - Disodium chromoglycote, Nedocromil Sodium, Calcium channels blockers, Loratidine, Citrizine
- Receptor antagonists - Which prevents histamine to reach at the site of actions.

Competitively inhibits the action of Histamine at Histamine Receptor:

- These are the competitive Antagonists at all the H receptors.
- H_1 antagonists: Triprolidine, Chlorpheniramine.
- H_2 antagonists: Ranitidine, Famotidine.
- H_3 antagonists: Iodophenprofit, Chlobenpropit.
- H_4 antagonists: Thioperamide.

Classifications of H1 Antagonists:

1. **Potent and Sedative:** Diphenhydramine, Promethazine, Dimenhydramine.
2. **Potent and non-Sedative:** Tripalanamine, Chlorcyclizine, Chlorpheniramine.
3. **Less Potent and Less sedative:** Pheniramine, Phenindone, Mepyramine.
4. **Non sedative:**
   - **First generation:** Loratidine, Cetrizine, hydroxyzine.
   - **Second generation:** Astimazole, Acrivastine, Terfenamide, Desloratidine, Fexofenatidine.
   - **First generation ones are short/ intermediate acting; more sedating; more anti muscarinic effect.**
   - **Second generation have longer duration of action, poor permeability to BBB, so less sedating.**

   Histamine H2 receptor blocker: Cimetidine, Ranitidine, Famotidine.

MOA:

Antihistamine blocks histamine receptor and antagonize the action produce by histamine.

Pharmacological Action:

H1 Receptor Blocking Action:

CNS:

It produced CNS depression that may leads to sedation, hypnosis, drowsiness and sleep.

These drugs also may be used in motion sickness due to various degree of CNS depression.

Smooth Muscles:

It antagonizes contraction of bronchial smooth muscles produced by histamine and relax bronchial smooth muscles.

The relaxation of smooth muscles of intestine, uterus and gall bladder is lesser extent compare to bronchial smooth muscles.

These all action mediated through H1 receptor blocking action.

CVS:

It produces membrane stabilizing action which leads to anti-arrhythmic effects.

It also produced relaxation of vascular smooth muscles.

Anti-Parkinsonism effects:

It produced anti cholinergic effects which may leads to decreased acetyl choline and caused relief from Parkinsonism.

Local anesthetics action:

Due to its membrane stabilizing action it produces local anaesthesia.

General action: It blocks histamine receptor and cause relief from all allergic reaction including triple response.

H2 Receptor Blocking Action:

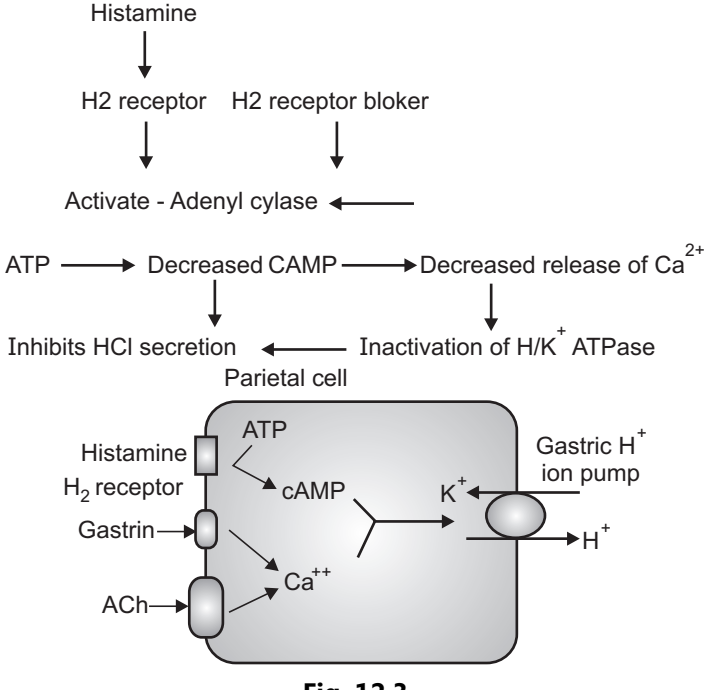


Fig. 12.3

ADME: Well absorbed by oral and parenteral routes of administration, 50-60% binds to plasma proteins, metabolized in liver by hydroxylation and followed by glucuronide conjugation and excreted in urine.

Therapeutic uses: H_1 Blocker:

- Prevents or treats symptoms of allergic rhinitis and urticaria.
- First generation ones cause sedation and are better for itching so used in atopic dermatitis.
- Second generation ones are non sedative, preferred for hay fever.
- Most of them show anti muscarinic effect, though as a side effect.
- So used in motion sickness(cyclizine, meclizine).
- Used as anti emetics(hydroxyzine, promethazine).
- Suppressing Parkinsonism Symptoms(Diphenhydramine, promethazine).

ADR: H_1 Blocker:

- Sedation and anti cholinergic actions like dry mouth, urinary retention, constipation.
- Drug allergy with topical agents.
- Tolerance after prolonged use.
- Teratogenic effects by hydroxyzine, cyclizine.
- Toxic doses leads to excitement, hallucinations, convulsions and coma.
- Drowsiness, Euphoria, diplopia, tinnitus, weakness.
- High Dose- Anti cholinergic effects- Gastric distress, Dryness of mouth and throat, Blurred vision, Lightness of chest, Hypotension.

Therapeutic uses: H_2 Blocker:

- In peptic ulcer.
- Dudenal ulcer.
- Zollinger Ellison syndrome.
- Gastro-esophageal reflux.
- Heart burn.

ADR: H_2 Blocker:

- Headache, fatigue, myalgia, constipation.
- Mental status change occurs with cimetidine.
- Endocrinial effects with Cimetidine.
- Male impotence.
- Skin rashes, headache, dizziness, gynaecomastia, Impotence, Mental confusion, Hepatotoxicity.

Histamine Release Inhibitors

- Examples are Cromolyn Sodium and Nedocromyl Sodium.
- They prevent degranulation of mast cells hence inhibit histamine release.
- Used as pulmonary inhalants in the treatment of bronchial asthma.
- Nasal and ophthalmic formulations are used to reduce symptoms of allergic rhinitis and conjunctivitis.

12.5 5-HYDROXY TRPTAMINE (5-HT) / SEROTONIN

- In humans, present in GI enterochromaffin cells (90%), platelets and brain.
- Synthesized from tryptophan (in diet) in two steps.
- Platelets do not synthesize but take up from blood (active uptake process in platelets and nerve terminals).

12.5.1 Distributions

Widely distributed amine (animals + plants). In humans, present in Small intestine-(90%, found in enterochromaffin cells) platelets, mast cells, lungs, bone marrow, pineal gland and CNS

Sources: Tunicates, mollusks, anthropods, colenterates, fruits, nuts, wasps and scorpions, Cell storage in granules similar to catecholamines.

12.5.2 Synthesis and metabolism of 5-HT

- Competition at the level of brain and neuronal uptake
- Rate limiting enzyme not saturated usually
- No end-product negative feedback
- 5-OHTr decarboxylase same as DOPA decarboxylase
- 5-OHIAA actively extruded from CNS (probenecid-sensitive) and excreted in urine.

Tryptophan

Tryptophan hydroxylase

↓

5- hydroxy tryptophan

L-amino acid decarboxylase

↓

5 hydroxytryptamine (Serotonin)

MAO

↓

5-hydroxyindole acetaldehyde

Aldehyde dehydrogenase

↓

5-hydroxyindole acetic acid

Receptors:

$5HT_1$ - Subtypes:

$5HT_{1A}$:

- Located in CNS.
- Inhibitory pre-synaptic receptor.
- Behavioural effects, Sleep, anxiety, thermoregulation, Decreased CAMP.

$5HT_{1B}$:

- Located in CNS, Vascular smooth muscle.
- Pulmonary contraction, Behavioural effects, Inhibitory pre-synaptic receptor decreased CAMP.
- Act by decreasing IP_3 – DAG system.

$5HT_{1D}$:

- Most parts of the brain, cranial blood vessels.
- Cerebral vasoconstriction.
- Behavioral effects, decreased CAMP.
- Act by decreasing IP_3 – DAG system.

$5HT_2$:

- Located mainly CNS, Smooth Muscles and Platelets.

Subtypes:

$5HT_{2A}$:

- CNS, PNS, Smooth Muscles and Platelets.
- Excitements, Platelets aggregation.
- Behavioral effects, GIT and bronchial smooth muscle contraction.
- Act by Increasing IP_3 – DAG system.

$5HT_{2B}$:

- Located in gastric fundus.
- Contraction.
- Act by Increasing IP_3 – DAG system.

$5HT_{2C}$:

- Located in CNS, Choroid plexus and hippocampus.
- CSF secretion.
- Act by Increasing IP_3 – DAG system.

$5HT_3$:

- Located in PNS and CNS.
- Neuronal excitation, emesis, anxiety and behavioral effects.
- Act by increasing IP_3 – DAG system.

$5HT_4$:

- Located in PNS and CNS.
- Neuronal motility and excitation.
- Act by increasing IP_3 – DAG system.

$5HT_5$:

- Located in CNS, hippocampus
- Decrease CAMP
- Act by Decreasing IP_3 – DAG system

$5HT_6$:

- CNS, Straitum
- Increased cAMP
- Act by Increasing IP_3 – DAG system

$5HT_7$:

- CNS, Hippocampus
- GIT, Blood vessels
- Increased cAMP
- Act by Increasing IP_3 – DAG system

Endogenous Function:

- Central neurotransmitter
- Precursor of melatonin
- GI tract: uncertain; motility?
- In carcinoid tumors: large amounts released leading to diarrhea, bronchoconstriction and edema
- Platelets: 5-HT_2 receptors → aggregation and vasoconstriction

Pharmacological actions of 5HT:

- Serotonin mediated actions through large number of receptors which posses diverse characteristics.
- Among these many subtypes receptors lack any specific physiological roles, So common actions are:

CNS:

- It is very important neurotransmitters in CNS - in brain, brain stem, hypothalamus, raphae nuclei, limbic system, pituitary glands.
- 5HT cause regulation of mood, behavior, sleeps, depression, pain, sexual activity, thermoregulations, Pain perception and Sleep/Wakefulness.
- Various behaviors normal/abnormal: depression, schizophrenia, obsessive compulsive behavior, etc.
- Neuroendocrine regulation - controls hypothalamic cells involved in release of several anterior pituitary hormones.
- Hypothalamic controls releases pituitary hormones.
- 5HT in pineal gland is precursor for synthesis of melatonin, a melanocyte stimulating hormones, which controls/ influences sleeps.

CVS:

- 5HT acts on $5HT_2$ receptor dilates blood vessels of skin, heart, smooth muscles and skeletal muscles.
- It also produces bradycardia, decreased COP - Overall result is decreased BP/Hypotension, though there is minor pheripheral vasoconstriction.

Platelets Aggregation:

- It cause released of more 5HT by acting on $5HT_{2A}$ receptor and also cause release of platelets, result forms clot and decreased blood shade out of damage organ.

GIT:

- Small intestine very sensitive to serotonin → intense rhythmic contractions due to direct and indirect (ganglia in wall) effects.
- Increased GI peristalsis partly by acting on $5HT_2$ and partly by acting on $5HT_3$ and $5HT_4$.
- Increased gastric acid secretion which result in GIT irritation and nausea, vomiting.

Respiratory system:

- Broncho constriction if asthmatic; stimulation of aortic and carotid chemo receptors → ↑ RR and minute vol.

Miscellaneous:

- Also stimulates vomiting (5-HT_3 receptors on vagal afferents and centrally).
- 5HT cause contraction of Bronchial smooth muscles.
- It produces anorexia.
- It increased pain perception and itching.

ADME: Well absorbed and rapidly degraded

5 hydroxytryptamine (Serotonin)

MAO

↓

5-hydroxyindole acetaldehyde

Aldehyde dehydrogenase

↓

5-hydroxyindole acetic acid

It is excreted through urine.

Therapeutic use:

- Useful in various inflammatory responses.

ADR: GIT irritation, vertigo, insomnia, hypotension, edema, nausea and vomiting.

Drugs acting on 5HT Receptors:

Serotonin Agonists:

- **Sumatriptan:** 5-HT_{1D} agonist; contraindicated in patients with angina.
- **Fluoxetine:** Selective serotonin uptake inhibitors for depression and other indications.
- **Buspirone:** 5-HT_{1A} agonist for anxiety.
- **Cisapride:** 5-HT_4 agonist to ↑ GI motility and decrease G-E reflux (Removed from US market due to fatal arrhythmias).
- **LSD:** $5HT_{1A}$ – hallucinogen.
- **Ergot alkaloids:** 5-HT_1 and 2 and other receptors.

Serotonin Antagonists:

- **Methysergide and Cyproheptadine:** $5HT_2$ antagonists. In carcinoid, migraine.
- **Ketanserin:** $5HT_2$ and Alpha antagonist – used as antihypertensive.
- **Ondansetron:** $5HT_3$ antagonist for chemotherapy induced nausea and vomiting
- **Clozapine:** $5HT_{2A/2C}$ antagonist: for schizophrenia.

5HT Receptor Agonists

Buspirone:

- Newer anti-anxiety drugs, Non Benzodiazapine group.
- Partial agonist of $5HT_1$ receptor in CNS.

Sumatriptine:

- Selective agonist at $5HT_{1B}$ and $5HT_{1D}$.
- Cerebral vasoconstrictor agents regularly use in migraine attack.

Cisapride and Renzapride:

- $5HT_4$ receptor agonists, Increased GI motility use to treat gastro esophageal reflux.

5HT Receptor Antagonists:

Ketanserin:

- $5HT_{2A}$ receptor antagonists also block $5HT_{2C}$ Receptor.
- Effective antihypertensive agents with mild effects-Dizziness, Lethargy, nausea and drymouth.

Ritanserin:

- More selective $5HT_{2A}$ antagonists than Ketanserin.
- Significant α_1 blocking action. It inhibits thromboxane formation and platelets aggregation and increased bleeding time.

Ondansetron, Granisetron, Dolasetron:

- $5HT_3$ antagonists
- Use in chemotherapy induced emesis.

Risperidone:

- Potent $5HT_{2A}$ and D_2 receptor antagonists. Use as antipsychotic agents

Methiserzide:

- Ergot group of alkaloids
- Potent $5HT_{2A\text{-}2C}$ antagonists.
- Prophylaxis of Migraine and post gastrectomy.

Cyproheptidine:

- Potent $5HT_2$ receptor antagonists.
- Also having antihistaminic and anticholinergic action.
- Possesses significant CNS depression action.
- Reduced allergic reactions.
- Stimulate appetite probably by acting on hypothalamus, increased weight gain from first week of therapy and decreased once its stops.
- It decreases Aldosterone production.
- It also controls secretion of ACTH secretion by hypothalamus.

Therapeutic uses:

- Relief from pruritis, urticaria, dermatitis, itching, skin disease, rashes, patches.
- Treatment of Gastrectomy.

ADR:

- Dryness of mouth, Ataxia, Mental confusion, Headache, Visual hallucination.

12.5.3 Migraine

Clinical Presentations:

- Often accompanied by brief aura (visual scotomas, hemianopia).
- Severe, throbbing, usually unilateral headache (few hours to a few days in duration).

Migraine Pathophysiology:

- Vasomotor mechanism -- inferred from:
  - increased temporal artery pulsation magnitude.
  - pain relief (by ergotamine) occurs with decreased artery pulsations.
- Migraine attack associated with (based on histological studies):
  - sterile neurogenic perivascular edema.
  - inflammation (clinically effective antimigraine medication reduce perivascular inflammation).

Migraine: Drug Treatment:

Ergotamine: best results when drug administered prior to the attack (prodromal phase) –less effective as attack progresses.

- Combined with caffeine: better absorption
- Potentially severe long-lasting Vasoconstriction.

Dihydroergotamine (IV administration mainly): may be appropriate for intractable migraine.

Nonsteroidal Antiinflammatory Drugs (NSAIDs):

Sumatriptan: Alternative to ergotamine for acute migraine treatment; not recommended for patients with coronary vascular disease risk.

- Formulations: subcutaneous injection, oral, nasal spray.
- Selective serotonin-receptor agonist (short duration of action).
- Probably more effective than ergotamine for management of acute migraine attacks (relief: 10 to 15 minutes following nasal spray).

Migraine: Prophylaxis:

Methysergide

- Effective in about 60% of patients.
- NOT effective in treating an active migraine attack or even preventing an impending attack.
- Methysergide toxicity: retroperitoneal fibroplasia, subendocardial fibrosis. Recommend 3-4 week drug holiday every six months.

Propranolol:

- Most common for continuous prophylaxis
- Best established drug for migraine attack prevention.

Amitriptyline (TCA):

- Most frequently used among the tricyclic antidepressants.

Valproic acid (Antiepileptic)

- Effective in decreasing migraine frequency.

Nonsteroidal antiinflammatory drugs (NSAIDs)

- Used for attack prevention and aborting acute attack.

12.5.4 Platelet-activating factor (PAF)

- **Platelet-activating factor**, also known as **PAF**, it is a potent phospholipid activator and mediator of many leukocyte functions, platelet aggregation and degranulation, inflammation, and anaphylaxis. It is also involved in changes to vascular permeability, the oxidative burst, chemotaxis of leukocytes, as well as augmentation of arachidonic acid metabolism in phagocytes.

- PAF is produced by a variety of cells, but especially those involved in host defense, such as platelets, endothelial cells, neutrophils, monocytes and macrophages. PAF is continuously produced by these cells but in low quantities and production is controlled by the activity of PAF acetyl-hydrolases. It is produced in larger quantities by inflammatory cells in response to specific stimuli.
- Platelets are involved primarily in coagulation and thrombotic phenomena but also play a part in inflammation. They have low-affinity receptors for IgE, and are believed to contribute to the first phase of asthma. In addition to generating thromboxane (TX) A_2 and PAF, they can generate free radicals and proinflammatory cationic proteins. Platelet-derived growth factor contributes to the repair processes that follow inflammatory responses or damage to blood vessels.

Synthesis:

Membrane acyl-PAF

↓

Phospholipase A_2

↓

Lyso PAF

↓

Acetyl CoA

↓

PAF

PAF is biosynthesized from acyl-PAF in a two-step process. The action of PLA_2 on acyl-PAF produces lyso-PAF, which is then acetylated to give PAF. PAF, in turn, can be deacetylated to the inactive lyso-PAF.

Sources of platelet-activating factor:

Platelets stimulated with thrombin and most inflammatory cells can release PAF under the right circumstances.

Pharmacological action:

- PAF receptor is a G-protein coupled receptor. Activate IP_3-DAG system

↓

Increased Ca^{2++} release

- By acting on specific receptors, PAF is capable of producing many of the signs and symptoms of inflammation.
- Chemotactic to neutrophil and esinophils.
- Activate leucocytes.
- Activate platelets aggregation.
- It produces vasodilatation (and thus erythema), increased vascular permeability and weal formation.

- Higher doses produce hyperalgesia. It is a potent chemotaxin for neutrophils and monocytes, and recruits eosinophils into the bronchial mucosa in the late phase of asthma.
- It can activate PLA_2 and initiates eicosanoid synthesis.
- On platelets, PAF triggers arachidonate turnover and TXA_2 generation, producing shape change and the release of the granule contents. This is important in haemostasis and thrombosis.
- PAF has spasmogenic effects on both bronchial and ileal smooth muscle.
- The anti-inflammatory actions of the glucocorticoids may be caused, at least in part, by inhibition of PAF synthesis.
- Competitive antagonists of PAF and/or specific inhibitors of lyso-PAF acetyltransferase could well be useful anti-inflammatory drugs and/or antiasthmatic agents.
- The PAF antagonist **lexipafant** is in clinical trial in the treatment of acute pancreatitis.

12.5.5 Leukotriene

- Any of a group of biologically active compounds, originally isolated from leucocytes.
- Leuko - because they are made by white cells, and trienes because they contain conjugated a triene system of double bonds.
- Synthesized from arachidonic acid by lipoxygenase catalysed pathways.
- Mainly found in lung, platelets, mast cells and white blood cells.
- Leukotrienes together with prostaglandins and other related compounds are derived from 20 carbon fatty acids that contains double carbon. Hence this group is called Eicosanoids.
- It produced along with histamine, unlike histamine they are more potent and have longer duration and called as Slow Reacting Substances (SRS).
- Main enzyme in this group is 5-lipoxygenase.
- On cell activation, this enzyme translocates to the nuclear membrane, where it associates with a crucial accessory protein, affectionately termed FLAP (five-lipoxygenase activating protein.
- The 5-lipoxygenase incorporates a hydroperoxy group at C5 in arachidonic to form 5-hydroperoxytetraenoic acid (5-HPETE) leading to production of unstable acid leukotriene(LTA4) which is enzymatically converted to LTB4.
- LTB4, utilising a separate the glutathione, to with conjugation involving pathway cysteinyl-containing leukotrienes LTC 4, LTD4, LTE4 and LTF4 (sulfidopeptide leukotrienes by mainly)which are produced eosinophils, mast cells, basophils macrophages.

- LTB4 is produced mainly by neutrophils.
- Lipoxins and other active products, some of which have anti inflammatory properties, are also produced from arachidonate.

Receptors and Actions:

- Receptors are termed BLT if the ligand is LTB4, CysLT for the cysteinyl leukotrienes.
- Receptors couple with Gq protein and function through the IP3/DAG transducer mechanism.
- BLT receptors are chemotactic and primarily expressed in leucocytes and spleen. BLT1 receptor has high, while BLT2 receptor has lower affinity for LTB4.

Leukotrienes B4 Receptors (BLT):

Includes:

1. BLTR1
2. BLTR2

- G Protein coupled receptors associated with Gq, activated upon binding of cells.
- Gq stimulates the membrane bound phospholipase C which then cleaves PIP2 into two second messengers IP3 and DAG.
- DAG remains bound to the membrane and IP3 is released.
- IP3 binds to IP3 receptors within cells particularly Calcium channels in endoplasmic reticulum and cause increase in release of Ca.

Cysteinyl Leukotrienes:

They include

1. LTC4
2. LTD4
3. LTE4

- Signaling pathway is similar to LTB receptors.
- Mainly expressed in bronchial and intestinal muscle and has higher affinity for LTD4 than for LTC4.
- The primary location of cysLT2 receptor is leucocytes and spleen, and it shows no preference for LTD4 over LTC4.
- Cysteinyl leukotrienes may mediate the cardiovascular changes of acute anaphylaxis.
- Agents that inhibit 5-lipoxygenase are therefore obvious candidates for anti-asthmatic and anti-inflammatory agents.
- All **Leukotrienes** acts through IP_3 DAG system.

Pharmacological Action:

The respiratory system:

- Cysteinyl leukotrienes are potent spasmogens contraction of human , causing dose-related muscle *in vitro* bronchiolar
- LTE4 is less potent than LTC4 and LTD4, mucus secretion in lasting. All cause an increase but its effect is much longer.

The cardiovascular system:

- Small amounts of LTC4 or LTD4 given pressure, and rapid, short lived fall in blood intravenously cause a constriction of small coronary resistance vessels.

The role in inflammation:

- LTB4 is a potent chemotactic agent for neutrophils and macrophages.
- Regulates membrane adhesion molecule expression on neutrophils, and increases the production of toxic oxygen products and the release of granule enzymes.

On macrophages and lymphocytes:

- It release and stimulates proliferation and cytokine control and regulation.

Membrane Phospholipids:

- Control and regulation is dependent on factors like availability and amount of integral fatty acids.
- Alpha linoleic acid-present in the plasma membrane.
- Fatty acids are broken down to arachidonic by lipoxygenase for the leukotriene synthesis.

Metabolism:

- LTB**4** is metabolized by a unique membrane-bound cytochrome P450 enzyme in neutrophils and then further oxidised to 20-carboxy-LTB**4**
- LTC**4** and LTD**4** are metabolized to LTE**4**, which is excreted in the urine

Therapeutic Uses:

- Abortion
- Induction of labour
- Post partum haemorrhage
- Cervical ripening
- Peptic ulcer
- Glaucoma
- To avoid platelet damage

ADR:

- Nausea
- Vomiting
- Diarrhoea
- Uterine cramps

- Forceful uterine contractions
- Flushing
- Shivering
- Fever
- Fall in BP
- Tachycardia
- Chest pain.

12.6 BRADYKININ

Bradykinin and lysyl bradykinin (*kallidin*) are active peptides formed by proteolytic cleavage of circulating proteins termed *kininogens* through a protease cascade pathway.

12.6.1 Source and Formation of Bradykinin

- An outline of the formation of bradykinin from high-molecular-weight kininogen in plasma by the serine protease *kallikrein*.
- Kininogen is a plasma α-globulin that exists in both high and low molecular weight forms. Kallikrein is derived from the inactive precursor *prekallikrein* by the action of *Hageman factor* (factor XII).
- Hageman factor is activated by contact with negatively charged surfaces such as collagen, basement membrane, bacterial lipopolysaccharides, urate crystals and so on.
- Hageman factor, prekallikrein and the kininogens leak out of the vessels during inflammation because of increased vascular permeability, and exposure to negatively charged surfaces promotes the interaction of Hageman factor with prekallikrein.
- The activated enzyme then 'clips' bradykinin from its kininogen precursor.
- Kallikrein can also activate the complement system and can convert plasminogen to plasmin.
- In addition to plasma kallikrein, there are other kinin-generating isoenzymes found in pancreas, salivary glands, colon and skin. These *tissue kallikreins* act on both high- and low-molecular-weight kininogens and generate mainly kallidin, a peptide with actions similar to those of bradykinin.

12.6.2 Metabolism and Inactivation of Bradykinin

- Specific enzymes that inactivate bradykinin and related kinins are called *kininases*.
- One of these, *kininase II*, is a peptidyl dipeptidase that inactivates kinins by removing the two C-terminal **amino acids**.
- This enzyme, which is bound to the luminal surface of endothelial cells, is identical to *angiotensin-converting enzyme* which cleaves the two C-terminal residues from the inactive peptide angiotensin I, converting it to the active vasoconstrictor peptide angiotensin II.

- Thus kininase II inactivates a vasodilator and activates a vasoconstrictor.
- Potentiation of bradykinin actions by ACE inhibitors may contribute to some side effects of these drugs (e.g. cough).
- Kinins are also metabolised by various less specific peptidases, including a serum carboxypeptidase that removes the C-terminal arginine, generating *des-Arg*^{9}-*bradykinin*, a specific agonist at one of the two main classes of bradykinin receptor.

12.6.3 Bradykinin Receptors

- There are two bradykinin receptors, designated B_1 and B_2.
- Both are G-protein-coupled receptors and mediate very similar effects.
- B_1 receptors are normally expressed at very low levels but are strongly induced in inflamed or damaged tissues by cytokines such as IL-1.
- B_1 receptors respond to des-Arg^9-bradykinin but not to bradykinin itself. A number of selective peptide antagonists are known.
- It is likely that B_1 receptors play a significant role in inflammation and hyperalgesia, and there is recent interest in developing antagonists for use in cough and neurological disorders.
- B_2 receptors are constitutively present in many normal cells and are activated by bradykinin and kallidin, but not by des-Arg^9-bradykinin.
- Peptide and non-peptide antagonists have been developed, the best known being icatibant. None are yet available for clinical use.

12.6.4 Actions and Role of Bradykinin in Inflammation

- Bradykinin causes vasodilatation and increased vascular permeability.
- Its vasodilator action is partly a result of generation of PGI_2 and release of NO.
- It is a potent pain-producing agent, and its action is potentiated by the prostaglandins. It stimulates pain nerve endings.
- Bradykinin also has spasmogenic actions on intestinal, uterine and bronchial smooth muscle (in some species).
- The contraction is slow and sustained in comparison with that produced by histamine (hence *brady*, which means 'slow').
- Although bradykinin reproduces many inflammatory signs and symptoms, its role in inflammation and allergy has not been clearly defined, partly because its effects are often part of a complex cascade of events triggered by other mediators.
- However, excessive bradykinin production contributes to the diarrhoea of gastrointestinal disorders, and in allergic rhinitis it stimulates nasopharyngeal secretion.
- Bradykinin also contributes to the clinical picture in pancreatitis.

- Physiologically, the release of bradykinin by tissue kallikrein may regulate blood flow to certain exocrine glands, and influence secretions.
- It also stimulates ion transport and fluid secretion by some epithelia, including intestine, airways and gall bladder.

12.7 EICOSANOIDS

- Unlike histamine, *eicosanoids* are not preformed in cells but are generated from phospholipid precursors on demand.
- They are implicated in the control of many physiological processes, and are among the most important mediators and modulators of the inflammatory reaction.
- Interest in eicosanoids arose in the 1930s after reports that semen contained a lipid substance that contracted uterine smooth muscle.
- The substance was believed to originate in the prostate, and was saddled with the misnomer *prostaglandin*.
- Later, it became clear that prostaglandin was not a single substance but a whole family of compounds that could be generated from 20-carbon unsaturated fatty acids by virtually all cells.

Structure and biosynthesis

- In mammals, the main eicosanoid precursor is *arachidonic acid* (5,8,11,14-eicosatetraenoic acid), a 20-carbon unsaturated fatty acid containing four double bonds (hence *eicosa*, referring to the 20 carbon atoms, and *tetraenoic*, referring to the four double bonds).
- In most cell types, arachidonic acid is esterified in the phospholipid pool, and the concentration of the free acid is low.
- The principal eicosanoids are the *prostaglandins*, the *thromboxanes* and the *leukotrienes*, although other derivatives of arachidonate, for example the *lipoxins*, are also produced.
- (The term *prostanoid* will be used here to encompass both prostaglandins and thromboxanes.)
- In most instances, the initial and rate-limiting step in eicosanoid synthesis is the liberation of arachidonate, either in a one-step process or a two-step process from phospholipids by the enzyme *phospholipase* A_2 (PLA_2).
- Several species exist, but the most important is probably the highly regulated *cytosolic* PLA_2. This enzyme generates not only arachidonic acid (and thus eicosanoids) but also *lysoglyceryl-phosphorylcholine* (*lyso-PAF*), the precursor of *platelet activating factor*, another inflammatory mediator.
- Cytosolic PLA_2 is activated (and hence arachidonic acid liberated) by phosphorylation.
- This occurs in response to signal transduction events triggered by many stimuli, such as thrombin action on platelets, C5a on neutrophils, bradykinin on fibroblasts, and antigen-antibody reactions on mast cells.

Metabolism:

- General cell damage also triggers the activation process. The free arachidonic acid is metabolised by several pathways, including the following.
- *Fatty acid cyclo-oxygenase (COX).* Two main isoform forms, COX-1 and COX-2, transform arachidonic acid to prostaglandins and thromboxanes.
- *Lipoxygenases:* Several subtypes synthesise leukotrienes, lipoxins or other compounds.

12.8 PROSTANOIDS

- The term *prostanoids* encompasses the prostaglandins and the thromboxanes.
- Cyclo-oxygenases (COXs) oxidise arachidonate, producing the unstable intermediates prostaglandin (PG) G_2 and PGH_2.
- There are two main COX isoforms: COX-1, a constitutive enzyme, and COX-2, which is often induced by inflammatory stimuli.
- Cyclo-oxygenase-1 is present in most cells as a constitutive enzyme that produces prostanoids that act as homeostatic regulators (e.g. modulating vascular responses), whereas COX-2 is not normally present but it is strongly induced by inflammatory stimuli and therefore believed to be more relevant to inflammation therapy (see next chapter for a full discussion of this point).
- Both enzymes catalyse the incorporation of two molecules of oxygen into every arachidonate molecule, forming the highly unstable endoperoxides PGG_2 and PGH_2.
- These are rapidly transformed by *isomerase* or *synthase* enzymes to PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and TXA_2, which are the principal bioactive end products of this reaction.
- The mix of eicosanoids thus produced varies between cell types depending on the particular endoperoxide isomerases or synthases present. In platelets, for example, TXA_2 predominates, whereas in vascular endothelium PGI_2 is the main product.
- Macrophages, neutrophils and mast cells synthesise a mixture of products. If *eicosatrienoic acid* (three double bonds) rather than arachidonic acid is the substrate, the resulting prostanoids have only a single double bond, for example PGE_1, while *eicosapentaenoic acid*, which contains five double bonds, yields PGE_3.
- The latter substrate is significant because it is present in abundance in some fish oils and may, if present in sufficient amounts in the diet, come to represent a significant fraction of cellular fatty acids.
- When this occurs, the production of the proinflammatory PGE_2 is diminished and, more significantly, the generation of TXA_2 as well.
- This may underlie the beneficial anti-inflammatory and cardiovascular actions that are ascribed to diets rich in this type of marine product.

Catabolism of the prostanoids

- This is a multistep process. After carrier-mediated uptake, most prostaglandins are rapidly inactivated by 'prostaglandin-specific' enzymes, and the inactive products are further degraded by general fatty acid-oxidising enzymes.
- The prostaglandin-specific enzymes are present in high concentration in the lung, and 95% of infused PGE_2, PGE_1 or $PGF_{2\alpha}$ is inactivated on first passage. The half-life of most prostaglandins in the circulation is less than 1 minute.
- Prostaglandin I_2 and TXA_2 are slightly different. Both are inherently unstable and decay rapidly (5 minutes and 30 seconds, respectively) in biological fluids into inactive 6-keto-$PGF_{1\alpha}$ and TXB_2.

Prostanoid receptors

- There are five main classes of prostanoid receptors, all of which are typical G-protein-coupled receptors.
- They are termed *DP*, *FP*, *IP*, *EP* and *TP receptors*, respectively, depending on whether their ligands are PGD_2, $PGF_{2\alpha}$, PGI_2, PGE_2 or TXA_2.
- Some have further subtypes; for example, the EP receptors are subdivided into three subgroups.
- Act by IP_3/DAG system.

Pharmacological Actions of the prostanoids

The prostanoids affect most tissues and exert a variety of effects.

- *PGD_2* causes vasodilatation, inhibition of platelet aggregation, relaxation of gastrointestinal and uterine muscle, and modification of release of hypothalamic/pituitary hormones. It has a bronchoconstrictor effect through an action on TP receptors.
- *$PGF_2\alpha$* causes myometrial contraction in humans, luteolysis in some species (e.g. cattle) and bronchoconstriction in other species (cats and dogs).
- *PGI_2* causes vasodilatation, inhibition of platelet aggregation, renin release and natriuresis through effects on tubular reabsorption of Na^+.
- *TXA_2* causes vasoconstriction, platelet aggregation and bronchoconstriction (more marked in guinea pig than in humans).
- PGE_2 is prominent in inflammatory responses and is a mediator of fever. Main effects are:
    - EP_1 receptors: contraction of bronchial and gastrointestinal tract (GIT) smooth muscle
    - EP_2 receptors: relaxation of bronchial, vascular and GIT smooth muscle
    - EP_3 receptors: inhibition of gastric acid secretion, increased gastric mucus secretion, contraction of pregnant uterus and of GIT smooth muscle, inhibition of lipolysis and of autonomic neurotransmitter release.

- $PGF_{2\alpha}$ acts on FP receptors, found in uterine (and other) smooth muscle, and corpus luteum, producing contraction of the uterus and luteolysis (in some species).
- PGD_2 is derived particularly from mast cells and acts on DP receptors, causing vasodilatation and inhibition of platelet aggregation.
- In their own right, PGE_2, PGI_2 and PGD_2 are powerful vasodilators and synergise with other inflammatory vasodilators such as histamine and bradykinin.
- It is this combined dilator action on precapillary arterioles that contributes to the redness and increased blood flow in areas of acute inflammation.
- Prostanoids do not directly increase the permeability of the postcapillary venules, but potentiate this effect of histamine and bradykinin.
- Similarly, they do not themselves produce pain, but potentiate the effect of bradykinin by sensitising afferent C fibres to the effects of other noxious stimuli.
- The anti-inflammatory effects of the NSAIDs stem largely from their ability to block these actions of the prostaglandins.
- Prostaglandins of the E series are also pyrogenic (i.e. they induce fever). High concentrations are found in cerebrospinal fluid during infection, and there is evidence that the increase in temperature (attributed to cytokines) is actually finally mediated by the release of PGE_2. NSAIDs exert antipyretic actions by inhibiting PGE_2 synthesis in the hypothalamus.
- However, some prostaglandins have *anti-inflammatory* effects under some circumstances. For example, PGE_2 decreases lysosomal enzyme release and the generation of toxic oxygen metabolites from neutrophils, as well as the release of histamine from mast cells. Several prostanoids are available for clinical use.

The role of the prostanoids in inflammation

Mediators derived from phospholipids:

- The main phospholipid-derived mediators are the eicosanoids (prostanoids and leukotrienes) and platelet-activating factor (PAF).
- The eicosanoids are synthesised from arachidonic acid released directly from phospholipids by phospholipase A_2, or by a two-step process involving phospholipase C and diacylglycerol lipase.
- Arachidonate is metabolised by cyclo-oxygenase (COX)-1 or COX-2 to prostanoids, or by 5-lipoxygenase to leukotrienes.
- PGI_2 (prostacyclin), predominantly from vascular endothelium, acts on IP receptors, producing vasodilatation and inhibition of platelet aggregation.
- Thromboxane (TX) A_2, predominantly from platelets, acts on TP receptors, causing platelet aggregation and vasoconstriction.

PAF is derived from phospholipid precursors by phospholipase A_2, giving rise to lyso-PAF, which is then acetylated to give PAF.

The inflammatory response is inevitably accompanied by the release of prostanoids. PGE_2 predominates, although PGI_2 is also important. In areas of acute inflammation, PGE_2 and PGI_2 are generated by the local tissues and blood vessels, while mast cells release mainly PGD_2. In chronic inflammation, cells of the monocyte/macrophage series also release PGE_2 and TXA_2. Together, the prostanoids exert a sort of yin-yang effect in inflammation, stimulating some responses and decreasing others. The most striking effects are as follow.

Therapeutic uses of Prostanoids:

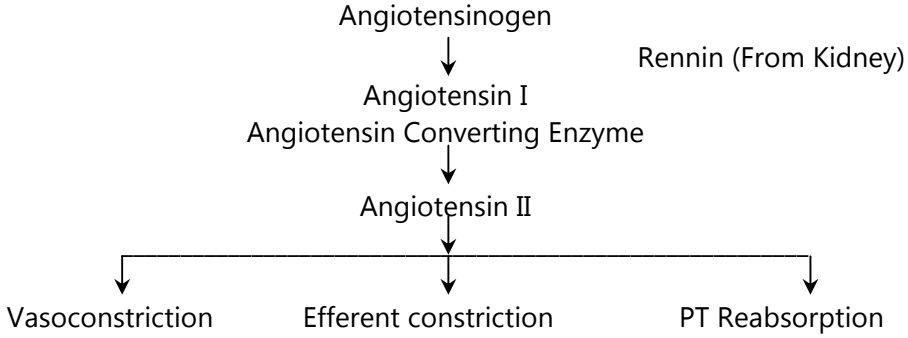
- **Gynaecological and Obstetric:**
  - termination of pregnancy: **gemeprost** or **misoprostol** (a metabolically stable prostaglandin (PG) E analogue)
  - induction of labour: **dinoprostone** or **misoprostol**
  - postpartum haemorrhage: **carboprost**.
- **Gastrointestina:**
  - to prevent ulcers associated with non-steroidal anti-inflammatory drug use: **misoprostol**
- **Cardiovascular:**
  - to maintain the patency of the ductus arteriosus until surgical correction of the defect in babies with certain congenital heart malformations: **alprostadil** (PGE_1)
  - to inhibit platelet aggregation (e.g. during haemodialysis): **epoprostenol** (PGI_2), especially if heparin is contraindicated
  - primary pulmonary hypertension: **epoprostenol**
- **Ophthalmic**
  - open-angle glaucoma: latanoprost eye drops.

12.9 ANGIOTENSIN

- The **renin–angiotensin system** (**RAS**), or **renin–angiotensin–aldosterone system** (**RAAS**), is a hormone system that regulates blood pressure and fluid and electrolyte balance, as well as systemic vascular resistance.
- When renal blood flow is reduced, juxtaglomerular cells in the kidneys convert the precursor prorenin (already present in the blood) into rennin and secrete it directly into circulation.
- Plasma renin then carries out the conversion of angiotensinogen, released by the liver, to angiotensin I. Angiotensin I is subsequently converted to angiotensin II by the angiotensin-converting enzyme (ACE) found on the surface of vascular endothelial cells, predominantly those of the lungs.
- Angiotensin II is a potent vasoconstrictive peptide that causes blood vessels to narrow, resulting in increased blood pressure. Angiotensin II also stimulates the secretion of the hormone aldosterone from the adrenal cortex.

- Aldosterone causes the renal tubules to increase the reabsorption of sodium and water into the blood, while at the same time causing the excretion of potassium (to maintain electrolyte balance). This increases the volume of extracellular fluid in the body, which also increases blood pressure.
- If the RAS is abnormally active, blood pressure will be too high. There are many drugs that interrupt different steps in this system to lower blood pressure.
- These drugs are one of the primary ways to control high blood pressure, heart failure, kidney failure, and harmful effects of diabetes.
- The system can be activated when there is a loss of blood volume or a drop in blood pressure (such as in hemorrhage or dehydration). This loss of pressure is interpreted by baro receptors in the carotid sinus.
- It can also be activated by a decrease in the filtrate sodium chloride (NaCl) concentration or a decreased filtrate flow rate that will stimulate the macula densa to signal the juxtaglomerular cells to release renin.
  1. If the perfusion of the juxtaglomerular apparatus in the kidney's macula densa decreases, then the juxtaglomerular cells (granular cells, modified pericytes in the glomerular capillary) release the enzyme rennin.
  2. Renin cleaves a decapeptide from angiotensinogen, a globular protein. The decapeptide is known as angiotensin I.
  3. Angiotensin I is then converted to an octapeptide, *angiotensin II* by angiotensin-converting enzyme (ACE), which is thought to be found mainly in endothelial cells of the capillaries throughout the body, within the lungs and the epithelial cells of the kidneys. One study in 1992 found ACE in all blood vessel endothelial cells.
  4. Angiotensin II is the major bioactive product of the renin–angiotensin system, binding to receptors on intraglomerular mesangial cells, causing these cells to contract along with the blood vessels surrounding them and causing the release of aldosterone from the zona glomerulosa in the adrenal cortex. Angiotensin II acts as an endocrine, autocrine/paracrine, and intracrine hormone.

Renin-Angiotensin System



Increased Aldosterone release, CD and Reabsorption

RAS system in details

Hypotension/ Hypovolumic

↓

Renal Infusion — Activation of aortic/carotid baro receptors

Lower afferent arteriole pressure — NaCl delivery to mucosa densa sympathetic tone

Renin Release
From juxta glomerular cells of nephrone

Angiotensinogen (release from liver)

Angiotensinogen-I
↓ ACE
Angiotensinogen-II
Vasoconstriction
Increased Aldosterone release
Increased Na absorption
Endothelial dysfunction
Promthrombotic effects (Increased PAI-I)
↓
Increased Blood Volume
Increased BP

Cardiovascular Effects:

It is believed that angiotensin I may have some minor activity, but angiotensin II is the major bio-active product. Angiotensin II has a variety of effects on the body:

- Throughout the body, angiotensin II is a potent vasoconstrictor of arterioles.
- In the kidneys, angiotensin II constricts glomerular arterioles, having a greater effect on efferent arterioles than afferent.
- As with most other capillary beds in the body, the constriction of afferent arterioles increases the arteriolar resistance, raising systemic arterial blood pressure and decreasing the blood flow.
- However, the kidneys must continue to filter enough blood despite this drop in blood flow, necessitating mechanisms to keep glomerular blood pressure up. To do this, angiotensin II constricts efferent arterioles, which forces blood to build up in the glomerulus, increasing glomerular pressure. The glomerular filtration rate (GFR) is thus maintained, and blood filtration can continue despite lowered overall kidney blood flow.

- Because the filtration fraction has increased, there is less plasma fluid in the downstream peri tubular capillaries. This in turn leads to a decreased hydrostatic pressure and increased oncotic pressure (due to unfiltered plasma proteins) in the peri tubular capillaries.
- The effect of decreased hydrostatic pressure and increased oncotic pressure in the peri tubular capillaries will facilitate increased reabsorption of tubular fluid.
- Angiotensin II decreases medullary blood flow through the vasa recta. This decreases the washout of NaCl and urea in the kidney medullary space.
- Thus, higher concentrations of NaCl and urea in the medulla facilitate increased absorption of tubular fluid. Furthermore, increased reabsorption of fluid into the medulla will increase passive reabsorption of sodium along the thick ascending limb of the Loop of Henle.
- Angiotensin II stimulates Na^+/H^+ exchangers located on the apical membranes (faces the tubular lumen) of cells in the proximal tubule and thick ascending limb of the loop of Henle in addition to Na^+ channels in the collecting ducts. This will ultimately lead to increased sodium reabsorption.
- Angiotensin II stimulates the hypertrophy of renal tubule cells, leading to further sodium reabsorption.
- In the adrenal cortex, angiotensin II acts to cause the release of aldosterone. Aldosterone acts on the tubules (e.g., the distal convoluted tubules and the cortical collecting ducts) in the kidneys, causing them to reabsorb more sodium and water from the urine.
- This increases blood volume and, therefore, increases blood pressure. In exchange for the reabsorbing of sodium to blood, potassium is secreted into the tubules, becomes part of urine and is excreted.
- Angiotensin II causes the release of anti-diuretic hormone (ADH), also called vasopressin – ADH is made in the hypothalamus and released from the posterior pituitary gland. As its name suggests, it also exhibits vaso-constrictive properties, but its main course of action is to stimulate reabsorption of water in the kidneys.
- ADH also acts on the central nervous system to increase an individual's appetite for salt, and to stimulate the sensation of thirst.
- These effects directly act together to increase blood pressure and are opposed by atrial natriuretic peptide (ANP).

Local Renin–angiotensin Systems:

- Locally expressed renin–angiotensin systems have been found in a number of tissues, including the kidneys, adrenal glands, the heart, vasculature and nervous system, and have a variety of functions, including local cardiovascular regulation, in association or independently of the systemic renin–angiotensin system, as well as non-cardiovascular functions.

- Outside the kidneys, renin is predominantly picked up from the circulation but may be secreted locally in some tissues; its precursor pro renin is highly expressed in tissues and more than half of circulating prorenin is of extra renal origin, but its physiological role besides serving as precursor to renin is still unclear.
- Outside the liver, angiotensinogen is picked up from the circulation or expressed locally in some tissues; with renin they form angiotensin I, and locally expressed angiotensin-converting enzyme, chymase or other enzymes can transform it into angiotensin II. This process can be intracellular or interstitial.
- In the adrenal glands, it is likely involved in the paracrine regulation of aldosterone secretion; in the heart and vasculature, it may be involved in remodeling or vascular tone; and in the brain, where it is largely independent of the circulatory RAS, it may be involved in local blood pressure regulation. In addition, both the central and peripheral nervous systems can use angiotensin for sympathetic neurotransmission.
- Other places of expression include the reproductive system, the skin and digestive organs. Medications aimed at the systemic system may affect the expression of those local systems, beneficially or adversely.

Fetal Renin–angiotensin System:

- In the fetus, the renin–angiotensin system is predominantly a sodium-losing system, as angiotensin II has little or no effect on aldosterone levels. Renin levels are high in the fetus, while angiotensin II levels are significantly lower; this is due to the limited pulmonary blood flow, preventing ACE (found predominantly in the pulmonary circulation) from having its maximum effect.

Clinical Significance:

- ACE inhibitors–inhibitors of angiotensin-converting enzyme are often used to reduce the formation of the more potent angiotensin II. Captopril is an example of an ACE inhibitor. ACE cleaves a number of other peptides, and in this capacity is an important regulator of the kinin–kallikrein system, as such blocking ACE can lead to side effects.
- Angiotensin II receptor antagonists, also known as angiotensin receptor blockers, can be used to prevent angiotensin II from acting on its receptors.
- Direct renin inhibitors can also be used for hypertension. The drugs that inhibit renin are aliskiren and the investigational remikiren.
- Vaccines against angiotensin II, for example CYT006-AngQb, have been investigated.

QUESTIONS

1. Write a note on autocoids and classification.
2. Classify antihistamine with examples.
3. Write the mechanism of action and therapeutic uses of Prostaglandins, Thromboxanes and Leukotrienes.
4. Discuss the pharmacology of Angiotensin, Bradykinin and Substance P.

■■■

Chapter ... 13

Non-steroidal Anti-Inflammatory Agents

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the drugs that can be used in inflammation, pain and fever.*

13.1 INTRODUCTION

- These are also called non-narcotic analgesics.
- Non-steroidal anti-inflammatory drugs (NSAIDs) are the groups of drugs that reduce pain, decrease fever, and, in higher doses, decrease inflammation, they are less potent than narcotic analgesic.
- They reduce dull aching pain but not useful in visceral pain.
- Useful in the treatment of postoperative, dental, menstrual pain, Muscle pain, Joint pain, headaches and migraine.
- They posses non-narcotic analgesic, antipyretics and anti inflammatory action.

Non-narcotic analgesic: Reduced pain without CNS depression.

Antipyretics: Which reduce elevated body temperature.

Anti inflammatory: Useful to produced symptomatic relief from pain.

13.2 CLASSIFICATION

(A) Nonselective COX Inhibitors (Traditional NSAIDs)

1. **Salicylates:** Aspirin, Methyl Salicylate, Sodium salicylate.
2. **Propionic acid derivatives:** Ibuprofen, Naproxen, Ketoprofen, Flurbiprofen.
3. **Anthranilic acid derivative:** Mephenamic acid, Enfenamic acid, Flufenamic acid.
4. **Aryl-acetic acid derivatives:** Diclofenac, Aceclofenac.
5. **Oxicam derivatives:** Piroxicam, Tenoxicam.
6. **Pyrrolo-pyrrole derivative:** Ketorolac.
7. **Indole derivative:** Indomethacin, Sulindac.
8. **Pyrazolone derivative:** Phenylbutazone, Oxyphenbutazon.

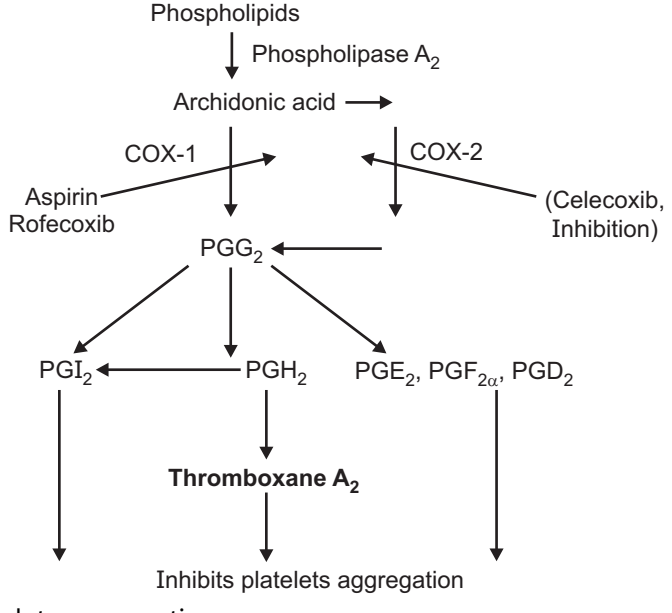
(B) Preferential COX-2 Inhibitors: Nimesulide, Meloxicam, Nabumeton.

(C) Selective COX-2 Inhibitors: Celecoxib, Etoricoxib, Parecoxib.

(D) Analgesic-antipyretics with Poor Anti inflammatory Action:

1. **Para aminophenol derivatives:** Paracetamol, Phenacitn.
2. **Pyrazolone derivative:** Metamizol, Propiphenazone.
3. **Benzoxazocine derivative:** Nefopam.

13.3 MECHANISM OF ACTION



- Inhibits Platelets aggregation
- Inhibits pain sensation - Analgesic action.
- Reset hypothalamus at lower temperature - Anti pyretic action.
- Reduced capillary permeability, Decreased tissue edema - Anti inflammatory action.

COX inhibitors cause inhibition of COX enzyme and inhibits conversion of Arachidonic acid into **PGG_2 later PGG_2 to PGH_2, PGI_2, PGE_2, $PGF_{2\alpha}$, PGD_2 and Thromboxane A_2.**

So result: Inhibits pain sensation - Analgesic action, Reset hypothalamus at lower temperature - Anti pyretic action, reduced capillary permeability, decreased tissue edema - Anti inflammatory action and inhibits pain sensation - Analgesic action.

13.4 PHARMACOLOGICAL ACTIONS

Analgesic effect:

The analgesic effect by:

- Peripheral inhibition of prostaglandin production.
- May also be due to the inhibition of pain stimuli at a sub cortical site.
- It also inhibits pain sensitizing mechanism induced by TNF_α and Bradykinin.
- Prevent the potentiating action of prostaglandins on endogenous mediators of peripheral nerve stimulation.
- Aspirin is a weaker analgesic than morphine type drugs.

- Effectively relieves inflammation, tissue injury, connective tissue and integumental pain, but is relatively ineffective in severe visceral and ischaemic pain.
- No sedation, subjective effects, tolerance or physical dependence.

Antipyretic effect:

- Inhibition of production of prostaglandins induced by interleukin-1 (IL-1) and interleukin-6 (IL-6) in the hypothalamus.
- "Resetting" of the thermoregulatory system, leading to vasodilatation and increased heat loss.

Anti-inflammatory effect:

- Due to the inhibition of the enzymes that produce prostaglandin (cyclooxygenase, or COX), which converts arachidonic acid to prostaglandins, and to TxA_2 and prostacyclin.
- Inhibits accumulation of fluids.
- Reduced capillary permeability.
- Reduced exudation of fluid.
- Reduced tissue swelling.
- Result anti-inflammatory effect.

Metabolic and Endocrine effects:

- Significant only at anti-inflammatory doses (High dose).
- Cellular metabolism is increased, especially in skeletal muscles, due to uncoupling of oxidative phosphorylation.
- Increased utilization of glucose blood sugar may decrease and liver glycogen is depleted.
- Chronic use of large doses cause negative N_2 balance by increased conversion of protein to carbohydrate.
- Plasma free fatty acid and cholesterol levels are reduced.
- It stimulate adrenal medulla and increase adrenaline secretion which intern produced hypoglycaemia.

Respiratory System:

- Effects are dose dependent.
- It increased consumption of oxygen by skeletal muscles and increased production of carbon dioxide.
- It stimulates medullar respiratory centers so result increased rate of respiration.
- At anti-inflammatory doses, respiration is stimulated by peripheral (increased CO_2 production) and central (increased sensitivity of respiratory centre to CO_2) actions.
- Hyperventilation is prominent in salicylate poisoning.
- Further rise in salicylate level causes respiratory depression; death is due to respiratory failure.

Acid-base and Electrolyte Balance:

- Anti-inflammatory doses produce significant changes in the acid-base and electrolyte composition of body fluids.
- Initially, respiratory stimulation predominates.
- Still higher doses cause respiratory depression with CO_2 retention, leading to respiratory acidosis.

CVS:

- Aspirin has no direct effect in therapeutic doses.
- Larger doses increase cardiac output to meet increased peripheral O_2 demand and causes direct vasodilatation.
- Toxic doses depress, vasomotor centre: BP may fall.
- Because of increased cardiac work as well as Na^+ and water retention, CHF may be precipitated.
- Decreased prothrobin levels and inhibits platelets aggregation.

Blood:

- Aspirin, irreversibly inhibits TxA_2 synthesis by platelets, bleeding time is prolonged to twice.
- Long-term intake of large dose decreases synthesis of clotting factors in liver and predisposes to bleeding; can be prevented by prophylactic Vit. K therapy.

GIT:

- Aspirin and released salicylic acid irritate gastric mucosa, cause epigastric distress, nausea and vomiting.
- It also stimulates CTZ.
- Prolonged use may leads to ulcer formation as it inhibits PGE_2, $PGF_{2\alpha}$ synthesis, which intern inhibits gastric mucosa production.

Kidney:

- It inhibits PGE_2, $PGF_{2\alpha}$ result decreased glomerular filtration and decreased urine outputs.
- It also increases Na^+ and Water retention, which leads to increased BP and edema formation.

Local Action:

- It produced fungistatics, keratolytics and antiseptic action.
- It caused irritation of GIT which leads to nausea and vomiting.

Pharmacokinetics:

- Absorbed from the stomach and small intestines.
- Poor water solubility is the limiting factor in absorption: micro fining the drug particles and inclusion of an alkali enhances absorption.
- 50-60% binds to plasma protein.

- Rapidly deacetylated in the gut wall, liver, plasma and other tissues to release salicylic acid which is the major circulating and active form.
- Slowly enters brain but freely crosses placenta.
- The metabolites are excreted by glomerular filtration as well as tubular secretion.

Therapeutic Uses:

- Analgesic.
- Antipyretic.
- Acute rheumatic fever.
- Rheumatoid arthritis.
- As antiplatelets agents.
- Local application for Fungistatics, Antiseptics and Keratolytics.

Adverse Effects:

- GIT - Nausea, vomiting, Diarrhoea, Ulceration, perforation and hemorrhage.
- Intolerance - Skin rashes, urticaria, pruritis, bronchial asthma, anaphylactic shock.
- Bone marrow depression - Agranulocytosis, Thrombocytopenia, Aplastic anaemia.

Prequations to be taken while using Aspirin:

- It should not be taken in empty stomach.
- Patients suffering from ulceration.
- During delivery as it increases bleeding time.
- Allergic or sensitive to salicylates.

Salicylism:

- Headache, Tinnitus, Giddiness, Difficulty in hearing, Mental confusion, vomiting, diarrhea, respiratory alkalosis.

Acute poisoning:

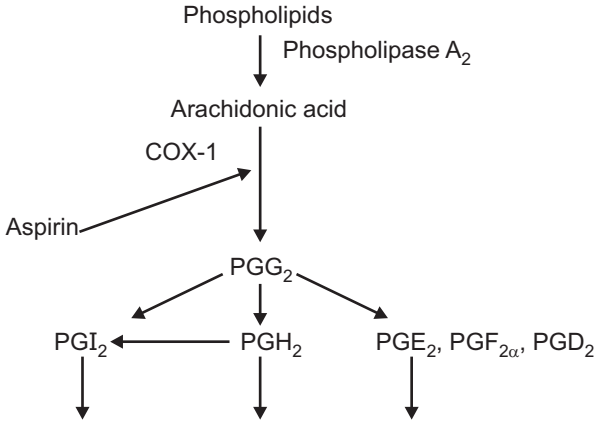
- Hyperglycemia, acidosis, dehydration, GIT irritation, hemorrhage, restlessness, excitement, delirium, tremors, euphoria, convulsion, hallucination.

Treatment:

- Gastric leavage.
- Correction of dehydration, acid base balance.
- Administration of alkalies and fluids (0.9% saline with 2% $NaHCO_3$) prevents metabolic acidosis and increased excretion of salicylates.

13.5 PARACETAMOL

- Most commonly use as antipyretics and analgesics effects.
- Analgesic - one of the most commonly used non-narcotic antipyretic agents.
- Weak anti-inflammatory activity.
- Effects of does not share the gastric or platelet side the other NSAIDs.



- Inhibits Prostaglandins synthesis.
- Inhibition of production of prostaglandins induced by interleukin-1 (IL-1) and interleukin-6 (IL-6) in the hypothalamus.
- Reset hypothalamus at lower temperature - Anti pyretic action.

Pharmacokinetics:

- Well absorbed when given orally, with min. 30-60 in reaching concentrations plasma peak.
- The plasma half-life of therapeutic doses is 2-4 h.
- Paracetamol metabolized to N-Acetyl P-benzoquinone imine and inactivated in the liver, being conjugated to give glucuronide.
- Excreted in urine.

Therapeutic Uses:

- Analgesics
- Anti-pyretic

Adverse Effects:

- With therapeutic doses, side effects are few and uncommon.
- Allergic skin reactions.
- Regular intake of large doses over a long period cause kidney damage.
- Toxic doses cause potentially fatal hepatotoxicity.

13.6 SELECTIVE COX-2 INHIBITORS

Celecoxib and Etoricoxib

- Symptomatic relief in the treatment of osteoarthritis and rheumatoid arthritis.
- Extensively metabolized in the liver.
- High plasma protein binding.
- Dizziness, skin. Common adverse effects are headache, rashes and peripheral oedema caused by fluid retention.

Parecoxib:

- Prodrug of valdecoxib.
- Short-term treatment of postoperative pain.
- Plasma protein binding is high.
- Drug should also be given with caution to patients with impaired renal function, and renal failure has been reported.
- Postoperative anaemia may also occur.

QUESTIONS

1. Give the mechanism and uses of Aspirin.
2. Pracetamol poisoning.
3. Write the classification, mechanism of action and therapeutic applications of NSAIDS.
4. Classify NSAIDS with example. Write the pharmacology of salicylates.

■■■

Chapter ... 14

Anti-gout Drugs

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the drugs that can be used in gout disease.*

14.1 INTRODUCTION

- Gout is a common and complex form of arthritis that can affect anyone. It's characterized by sudden, severe attacks of pain, **swelling**, redness and tenderness in the joints, often the joint at the base of the big toe.
- It is metabolic disorders characterized by hyperuricemia. Purine metabolized to uric acid by xanthine oxidase. In gout patients either increased in uric acid production or unable to excrete uric acid normally.
- So excess of uric acid in blood, this uric acid combine with sodium and forms sodium ureate, which accumulates in typical sites like kidney, cartilage, joints and ears.
- Uric acid is the final product of the metabolism of endogenous and exogenous purine in man. An excess of uric acid, measured in the plasma as sodium urate, constitutes **hyperuricaemia**.
- This excess may be caused by an overproduction or under excretion of urate. It is influenced by genetic and environmental factors and may be classified as primary (mainly idiopathic) or secondary.
- An increase in urate production may be caused by excessive dietary purine intake, certain cancers or their treatment, or, more rarely, enzyme defects of purine metabolism.
- Reduced urate excretion may be caused by renal disease, hypertension, or the intake of certain drugs such as thiazide diuretics. Other factors contributing to hyperuricaemia include hyperlipidaemia, obesity, alcohol consumption, and lead exposure.
- Gout is a form of inflammatory arthritis that develops in some people who have high levels of uric acid in the blood.
- The acid can form needle-like crystals in a joint and cause sudden, severe episodes of pain, tenderness, redness, warmth and swelling.
- Gout is caused initially by an excess of uric acid in the blood, or **hyperuricemia**.
- Uric acid is produced in the body during the breakdown of purines - chemical compounds that are found in high amounts in certain foods such as meat, poultry, and seafood.

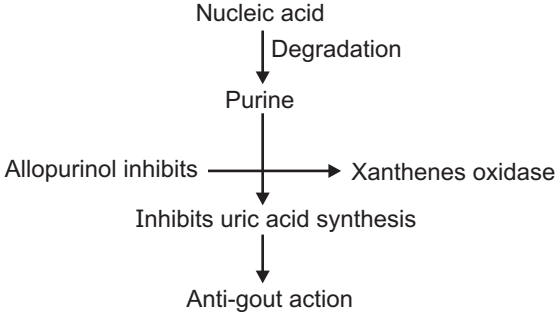
14.2 CLASSIFICATIONS OF DRUGS USE IN THE TREATMENT OF GOUT

Drugs which acts in Acute Gout:

- Drugs which inhibits neutrophils migration in joint: Colchicines.
- Drugs which inhibits inflammation and pain: NSAIDS, Prednisolone.

Drugs used in Chronic Gout:

- Drugs which inhibits uric acid synthesis: Allopurinol, Febuxostat.
- Drugs which increase uric acid excretion: Probencid, Sulphinpyrazone, Benzhromarone.



Therapeutic Uses:

- Anti gout drugs in chronic and acute gout.
- Secondary hyperuricemia.

ADR:

- Hypersensitivity reaction, Skin rashes, Arthralgia, pain, Fever, hepatitis, GIT distress, Nausea, Peripheral neuritis, Cataract formation.

Probencid:

- Increased uric acid excretion by inhibiting its active reabsorption from renal tubules. Result increased uric acid excretion. Uricosuric effect.

Therapeutic Uses:

- Chronic gout.
- Hyperuricemia.

ADR:

- Git distress, Allergic dermatitis, dyspepsia, nephritic syndrome.

Sulfinpyrazone:

- Increased uric acid excretion by inhibiting its active reabsorption from renal tubules. Result increased uric acid excretion. Uricosuric effect.

Therapeutic Uses:

- Chronic gout
- Hyperuricemia

ADR:

- GIT disturbance, Nausea, Vomiting, hyperuricemia.

Prednisolone:

MOA:

- Inhibits gene transcription of COX_2, cytokines, interleukins, as a result produce relief from inflammation.
- Increase release and synthesis of annexin-1, which is potent anti-inflammatory to cells hence produce anti-inflammatory actions.
- Symptomatic pain relief.
- Inhibition of chemotactic migration of leucocytes.

ADR:

- Prolonged use leads to toxic effect.

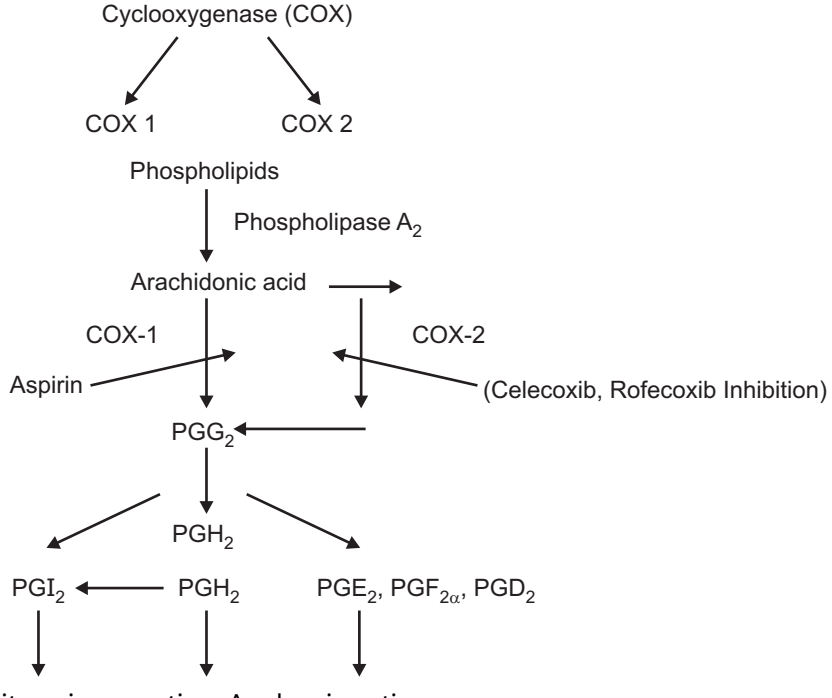
Therapeutic Uses:

- Acute gout.
- Hyperuricemia.

NSAIDS:

MOA:

- NSAIDs reduce inflammation and pain but it does not have any effect on disease progression.



- Inhibits pain sensation-Analgesic action.
- Reduced capillary permeability, decreased tissue edema-Anti inflammatory action.
- These release Prostaglandins which are responsible for inflammation, pain etc.

- COX inhibitors cause inhibition of COX enzyme and inhibits conversion of arachidonic acid into **PGG_2 later PGG_2 to PGH_2, PGI_2, PGE_2, $PGF_{2\alpha}$ and PGD_2.** So inhibits pain sensation-Analgesic action, decreased tissue edema-Anti inflammatory action.
- Symptomatic pain relief
- Inhibition of chemotactic migration of leucocytes.
- Inhibits crystal urate formation.

Therapeutic Uses:

- Acute gout
- Hyperuricemia

Adverse Effects:

- Nausea, Vomiting, Constipation, Diarrhea, Dizziness, Edema, Kidney failure, Ulcers.

Colchicines:

- Drugs which inhibits neutrophils migration in joint. Inhibition of chemotactic migration of leucocytes.
- Relief from gout.

Therapeutic Uses:

- Acute gout.
- Hyperuricemia.

ADR:

- GIT disturbance, Nausea, Vomiting, hyperuricemia.

QUESTIONS

- Discuss the pharmacology of anti-gout drugs.
- MoA of drugs used as anti-gout drug.

■■■

Chapter ... 15

Rheumatoid Arthritis

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the drugs that can be used in rheumatoid arthritis.*

15.1 INTRODUCTION

- Rheumatoid arthritis is an autoimmune disease in which the normal immune response is directed against an individual's own tissue, including the joints, tendons, and bones, resulting in inflammation and destruction of these tissues.
- Attacks normal joint tissues, causing inflammation of joint lining (synovial joint).
- It is a long-term autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints.
- Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body.
- The prevalence of rheumatoid arthritis in most Caucasian populations approaches 1% among adults 18 and over and increases with age, approaching 2% and 5% in men and women, respectively, by age 65.
- The incidence also increases with age, peaking between the 4th and 6th decades. The annual incidence for all adults has been estimated at 67 per 100,000.
- Both prevalence and incidence are 2-3 times greater in women than in men. Genetic factors have an important role in the susceptibility to rheumatoid arthritis.

Sign and Symptoms:

- Fatigue, Joint pain, Joint Swelling, Joint Redness, Joint warmth, Stiffness of joints in morning, Fever, Weight loss.

Drugs Used To Treat Rheumatoid Arthritis:

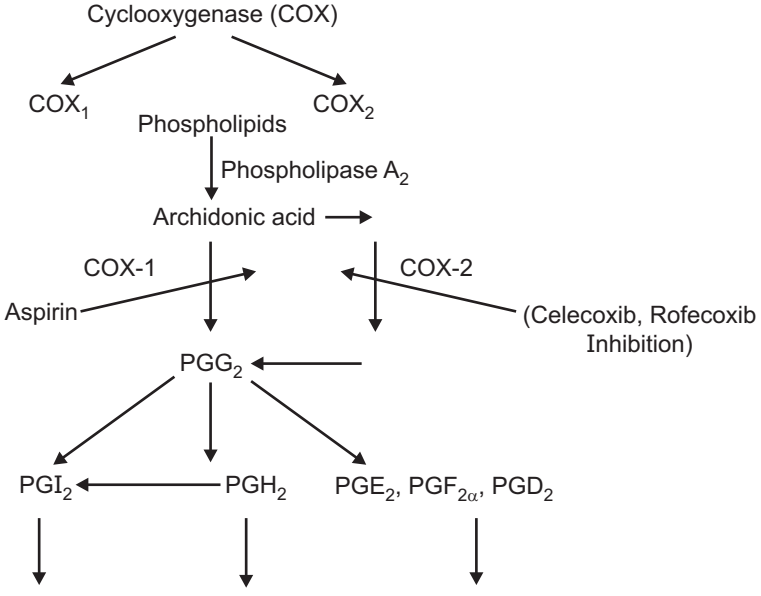
- Non steroidal anti-inflammatory drugs (NSAIDs).
- Disease modifying anti-rheumatic drugs (DMARDs).
- Biological Response Modifiers(Biologics).
- Glucocorticosteroids.

1. Non Steroidal Anti Inflammatory Drugs (NSAIDs):

- They are mainly of two types:
  - Non selective Cyclooxygenase Inhibitors: Aspirin, Ibuprofen.
  - Selective Cyclooxygenase 2 Inhibitors: Nimesulide, Meloxicam.

MOA:

- NSAIDs reduce inflammation and pain but it does not have any effect on disease progression.



- Inhibits pain sensation-Analgesic action.
- Reduced capillary permeability, Decreased tissue edema-Anti inflammatory action.
- These release Prostaglandins which are responsible for inflammation, pain etc.
- COX inhibitors cause inhibition of COX enzyme and inhibits conversion of arachidonic acid into **PGG_2 later PGG_2 to PGH_2, PGI_2 , PGE_2, $PGF_{2\alpha}$ and PGD_2.** So result**:** Inhibits pain sensation-Analgesic action, Decreased tissue edema-Anti inflammatory action.

Adverse Effects:

- Nausea, Vomiting, Constipation, Diarrhea, Dizziness, Edema, Kidney failure, Ulcers.

2. Disease Modifying Anti Rheumatic Drugs (DMARDs)

- The effects of these drugs take few weeks to several months to become evident, so they are also called Slow Acting Anti Rheumatic (SAARDs).
- Drugs (SAARDs): Methotrexate, Sulphasalazine, Chloroquine, Gold compounds etc.

Methotrexate:

MOA:

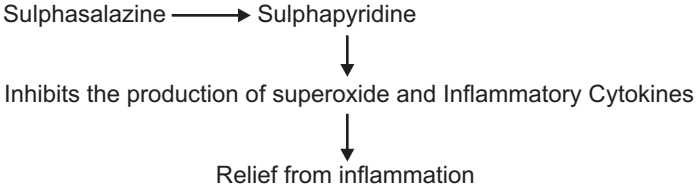
- It is a folate antagonist.
- It inhibits chemotaxis of neutrophils.
- Hence, production of pro-inflammatory cytokines are reduced and cell mediated immunity is suppressed.

ADR:

- Nausea, Vomiting, Mucosal ulcers.
- It is used in a very low dose and is contraindicated in pregnancy, liver disease and peptic ulcers.

Sulphasalazine:

MOA:



ADR:

- Nausea, Vomiting, Headache, Diarrhea, Skin rashes.

3. Biologics:

- These are preparations made from organisms or their products.
- They are administered parentally.
- They are used to treat rheumatoid arthritis which does not respond to DMARDs.

15.2 DRUGS UNDER BIOLOGICS

Etanercept

MOA:

- TNF alpha antagonist.
- TNF alpha is a potent inflammatory cytokine.
- TNF alpha antagonist reduce or inhibit the production of pro inflammatory cytokines hence produce relief from inflammation.

Anakinra

MOA:

- Interleukins 1(IL-1) antagonist.
- IL-1 is also a pro inflammatory cytokine.
- IL-1 antagonist reduces or inhibits the pro-inflammatory cytokine hence produce relief from pain.

Abatacept

MOA:

- T-cell Modulating Agent.
- It prevents and cures infection.

Rituximab

MOA:

- B-Lymphocyte Depletor.
- Destroy the B cells with rheumatoid arthritis.

ADR:

- Prolonged use leads to tuberculosis, urinary tract infection.

15.3 GLUCOCORTICOIDS

- The drugs are Prednisolone, Triamcinolone.

MOA:

- Inhibits gene transcription of COX2, cytokines, interleukins as a result produce relief from inflammation.
- Increase release and synthesis of annexin-1, which is potent anti-inflammatory to cells hence produce anti-inflammatory actions.

ADR:

- Prolonged use leads to toxic effect.

QUESTION

1. Write a note on mechanism of action behind anti-rheumatic drugs.

■■■

Unit IV

Chapter ... 16

Basic Concepts in Endocrine Pharmacology

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the endocrine system, different hormones and functions.*

16.1 INTRODUCTION

- Endocrinology deals with hormone secretion, hormone action, their feedback control mechanism and treatment of endocrine disorder.

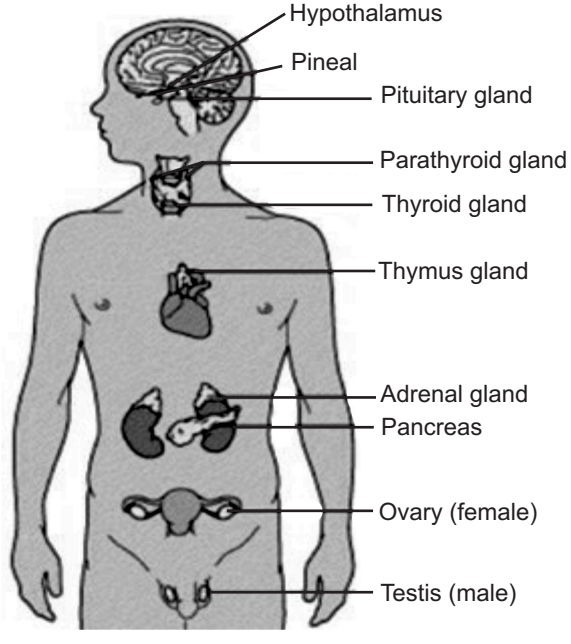


Fig. 16.1: Endocrine System

16.2 HORMONES

- Hormones are the chemical substance/chemical messengers synthesized by endogenous glands and secreted directly in to the blood circulation, but only target cells are equipped to respond.

16.2.1 Classification of Hormone

- Chemically hormone can be divided in to seven major classes.
  1. Amino acid derivatives – Dopamine, Catecholamine, Thyroid hormone.
  2. Neuropeptides – Gonadotropin releasing hormone, Somatostatin.
  3. Simple proteins – Corticotropin (ACTH).
  4. Large proteins – Insulin, Luteinising hormone (LH), Parathyroid hormone (PTH).
  5. Glycoprotein – Thyroid stimulating hormone (TSH).
  6. Steroids – Cortisol, Estrogen, Progesterons, Glucocortocoids.
  7. Vitamins – Vitamine D.
- The hormones also can be divided into two general classes based on their solubility in water.
- The water soluble - amine (epinephrine) and peptide/protein hormones.
- The lipid soluble hormones - thyroid hormone, steroid hormones and Vitamin D_3.

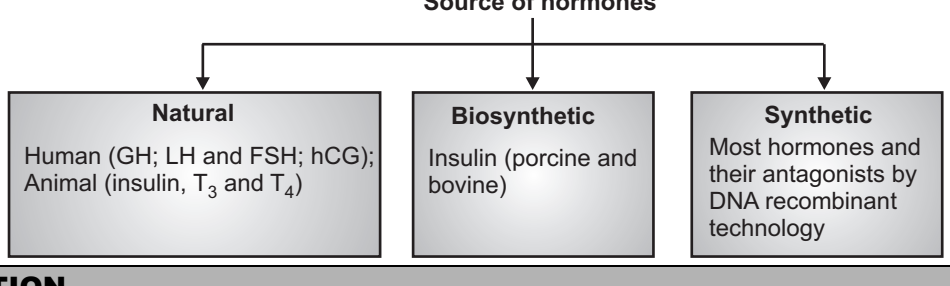
Functions of Hormones:

- Growth and differentiation.
- Maintenance of Homeostasis.
- Regulation and integration of reproductive function.

16.2.2 Hormone Receptors

- Hormone receptors are divided into 3 classes.

1. **Membrane receptor:**
   - Mainly neuropeptide hormone and catecholamine binds with membrane receptor.
   - There are several types of membrane receptors:
     - G-protein coupled receptor – NE, E, DA,FSH, LH, PTH, ACTH (through adenylase-cAMP system), Oxytocin, vasopressin (through phospholipase-C-inositol system.
     - Tyrosine-kinase receptor – Insulin, growth hormone, growth factor.
     - Cytokine receptor – Prolactin and growth factor.
     - Serine kinase receptor – transforming growth factor-β, bone morphogenic prorein.
2. **Cytosolic receptors:** For steroidal hormones (glucocorticoids, mineralocorticoids, estrogens, progestines and androgens.
3. **Nuclear receptors:** For Throid hormones T_3 and T_4.



QUESTION

1. Define endocrine gland and functions of hormones.

■■■

Chapter ... 17

Anterior Pituitary Hormones - Analogues and their Inhibitors

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the pituitary hormones and their functions.*

17.1 THE PITUITARY GLAND

- Pituitary Gland is divided into 2 areas.

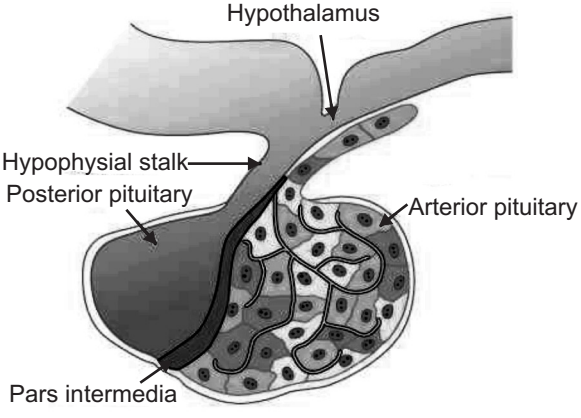


Fig. 17.1: Pituitary gland

The Anterior Pituitary:

- Under the regulation of the hypothalamus, anterior pituitary makes and releases hormone.

Example:

- Growth Hormone (**GH**).
- Thyroid-stimulating Hormone (**TSH**).
- Adrenocorticotropin (**ACTH**).
- Follicle-stimulating Hormone (**FSH**).
- Leutinizing Hormone (**LH**).
- Prolactin.

The Posterior Pituitary:

- Stores and secretes hormones that are made in the hypothalamus.
- Oxytocin and anti-diuretic hormone (**ADH**).

17.2 ANTERIOR PITUITARY HORMONES

17.2.1 Growth Hormone

- GHRH (Growth Hormone Releasing Hormone) released from hypothalamus is regulating the secretion of Growth hormone (GH).
- GH secretion is high in newborn, till 4 yr of age.
- Growth of brain and eye independent of growth hormone.
- Insulin-like growth factor 1 (IGF-1) released from the liver inhibits GH secretion by stimulating somatostatin secretion from the hypothalamus.
- **Sermorelin – a synthetic analogue of GHRH.** Which is used as a diagnostic agent for testing childhood short stature.

Pharmacological Action:

- Promotes retention of nitrogen and other tissue constituents.
- Induces lipolysis especially in adipose tissue.
- Increases hepatic glucose output.
- Glycogenolysis in liver.
- Is protein anabolic hormone.

Growth Hormone Deficiency:

- It is occurs as a result of damage to the pituitary or hypothalamus by a tumor, infection, surgery, or radiation therapy.
- In childhood: short stature and adiposity, hypoglycemia.
- **Adults:** Generalized obesity, reduced muscle mass.
- Lack of GH can cause dwarfism.

Growth Hormone Excess:

- It leading to benign pituitary tumor.
- In adults causes acromegaly.
- If this occurred before the long bone epiphyses close, it leads to the rare condition, gigantism.

Treatment of Excess GH Disorders:

- Synthetic Somatostatin (Octreotide).
- DA agonists (Bromocriptine).
- Surgical removal / Radiotherapy of the tumor.
- GH Antagonists (Pegvisomant).

ADR:

- Hypothyroidism, Pancreatitis, Gynecomastia.

Somatostatin:

- It is a growth hormone release-inhibiting hormone **(GHRIH).**
- It is inhibiting the secretion of GH. Also inhibiting the secretion of TSH, insulin and gastrin.
- Because of short half life and lack of specificity use of somatostatin is very limited.
- Octreotide, Lanreotide, Seglitide are somatostatin analogues.

17.2.2 Thyroid-stimulating Hormone (TSH) / Thyrotrophin

- TSH stimulates secretion of thyroxine (T4) and triiodothyronine (T3).
- Synthesis and release of TSH by pituitary is controlled by hypothalamus.
- Inappropriate TSH secretion results in hypo or hyperthyroidism.

17.2.3 Adrenocorticotropin (ACTH)

- Promoting steroidogenesis and stimulates cortisol secretion by the adrenal cortex.
- Promotes growth of adrenal cortex.
- Cushing's syndrome – due to excess production of ACTH from basophil pituitary tumors.

17.2.4 Follicle – Stimulating Hormone (FSH)

- **Females:** Stimulates growth and development of ovarian follicles, promotes secretion of estrogen by ovaries.
- **Males:** Essential for sperm production.
- Preparations are available for clinical use:
  - Urofollitropin (purified from of the urine of post menopausal women).
  - 2 recombinant forms: follitropin alpha and follitropin beta.

17.2.5 Leutinizing Hormone (LH)

- **Females:** Mainly triggers the ovulation, formation of corpus luteum in the ovary, and regulation of ovarian secretion of female sex hormones.
- **Males:** Stimulates the testes to secrete testosterone.
- Lutropin alfa, approved for use in combination with follitropin alfa for stimulation of follicular development in infertile women with profound LH deficiency.

17.2.6 Prolactin

- Lactotroph cells are responsible for the secretion of of prolactin.
- Its secretion is stimulated by estrogen.
- **Females:** stimulates breast development and milk production.
- **Males:** involved in testicular function.
- Prolactin secretion inhibited by dopamine agonists, which act in the pituitary to inhibit prolactin release, used in the treatment of hyperprolactinemia.

Prolactin Inhibitors:

Bromocriptine:

- It is an ergot derivative and a potent dopamine agonist.
- Act on D_2 receptor.
- Inhibit prolactin release.
- Increases growth hormone release in normal individuals.

17.3 POSTERIOR PITUITARY HORMONES

Oxytocin:

- It is synthesized in the hypothalamus and transported to the posterior pituitary.
- It is an effective uterine stimulant produces contraction and used intravenously to induce or reinforce labor.
- Induces the release of milk.
- Suckling sends a message to the hypothalamus via the nervous system to release oxytocin, which further stimulates the milk glands.

Clinical uses of Oxytocin:

- Induction of labor.
- Control of postpartum bleeding.

ADR:

- Fetal distress, placental abruption or uterine rupture, excessive fluid retention.

Vasopressin (Antidiuretic Hormone ADH):

- Synthesized in the hypothalamus and transported to the posterior pituitary.
- ADH is to increase water conservation by the kidney.
- High level of ADH secretion leads to reabsorption of water by kidney.
- ADH causes peripheral blood vessel constriction to help elevate blood pressure.

Clinical Uses:

- Diabetes insipidus.
- Nocturnal enuresis (by decreasing nocturnal urine production).

AE:

- Hyponatremia and seizures.

Synthetic ADH Drugs:

- Vasopressin, Desmopressin.

QUESTIONS

1. Explain the pharmacology of LH, FSH and prolactin.
2. Explain the pharmacology of growth hormone and their inhibitors.

■■■

Chapter ... 18

Thyroid Hormones - Analogues and their Inhibitors

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the thyroid hormones analogues and inhibitors, functions.*

18.1 INTRODUCTION

- Diseases of the thyroid gland are prevalent, and in this chapter we deal with drug therapy used to mitigate these disorders.
- We set the scene by briefly outlining the structure, regulation and physiology of the thyroid, and highlight the most common abnormalities of thyroid function.
- We then go on to consider the drugs that replace the thyroid hormones when these cease to function adequately, and the drugs that decrease thyroid function when this is excessive.

18.2 SYNTHESIS, STORAGE AND SECRETION OF THYROID HORMONES

- The thyroid gland secretes three main hormones: thyroxine (T_4), triiodothyronine (T_3) and *calcitonin*. T_4 and T_3 are critically important for normal growth and development and for energy metabolism.
- Calcitonin is involved in the control of plasma Ca^{2+} and is dealt with in. The term *thyroid hormone* will be used here solely to refer to T_4 and T_3.
- The functional unit of the thyroid is the follicle or acinus. Each follicle consists of a single layer of epithelial cells around a cavity, the follicle lumen, which is filled with a thick colloid containing thyroglobulin.
- Thyroglobulin is a large glycoprotein, each molecule of which contains about 115 tyrosine residues. It is synthesised, glycosylated and then secreted into the lumen of the follicle, where iodination of the tyrosine residues occurs.
- Surrounding the follicles is a dense capillary network, and the rate of blood flow through the gland is very high in comparison with other tissues. The main steps in the synthesis, storage and secretion of thyroid hormone are as follow:
  - Uptake of plasma iodide by the follicle cells.
  - Oxidation of iodide and iodination of tyrosine residues of thyroglobulin.
  - Secretion of thyroid hormone.

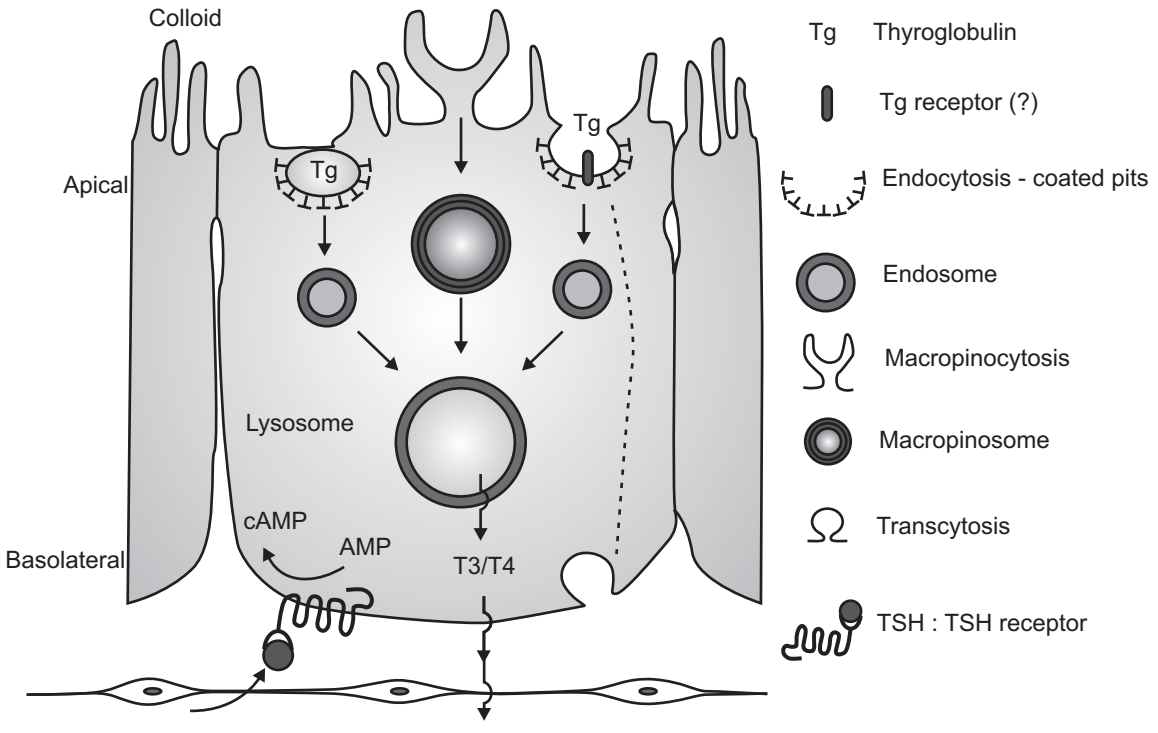


Fig. 18.1: Thyroid Hormones (T3/T4) - Synthesis, Storage and Secretion

18.3 REGULATION OF THYROID FUNCTION

- Thyrotrophin-releasing hormone (TRH), released from the hypothalamus in response to various stimuli, releases thyroid-stimulating hormone (TSH; thyrotrophin) from the anterior pituitary as does the synthetic tripeptide **protirelin** (pyroglutamyl-histidyl-proline amide), which is used in this way for diagnostic purposes.
- TSH acts on receptors on the membrane of thyroid follicle cells through a mechanism that involves cAMP and phosphatidylinositol 3-kinase. It controls all aspects of thyroid hormone synthesis, including:
  - The uptake of iodide by follicle cells, by stimulating transcription of the iodide transporter genes; this is the main mechanism by which it regulates thyroid function.
  - The synthesis and secretion of thyroglobulin.
  - The generation of H_2O_2 and the iodination of tyrosine.
  - The endocytosis and proteolysis of thyroglobulin.
  - The actual secretion of T_3 and T_4.
  - The blood flow through the gland.
- Thyroid-stimulating hormone also has a trophic action on the thyroid cells; it stimulates the transcription of the genes for thyroglobulin and thyroperoxidase, as well as the I^- transporters.

- The production of TSH is also regulated by a negative feedback effect of thyroid hormones on the anterior pituitary gland, T_3 being more active than T_4 in this respect.
- The peptide *somatostatin* also reduces basal TSH release.
- The control of the secretion of TSH thus depends on a balance between the actions of T_4 and TRH (and probably also somatostatin) on the pituitary, although even high concentrations of thyroid hormone do not totally inhibit TSH secretion.
- The other main factor influencing thyroid function is the plasma iodide concentration. About 100 nmol of T_4 is synthesised daily, necessitating uptake by the gland of approximately 500 nmol of iodide each day (equivalent to about 70 mg of iodine).
- A *reduced* iodine intake, with *reduced* plasma iodide concentration, will result in a decrease of hormone production and an increase in TSH secretion. An *increased* plasma iodide has the opposite effect, although this may be modified by other factors.
- The overall feedback mechanism responds to changes of iodide slowly over fairly long periods of days or weeks, because there is a large reserve capacity for the binding and uptake of iodide in the thyroid. The size and vascularity of the thyroid are reduced by an increase in plasma iodide.
- Diets deficient in iodine eventually result in a continuous excessive compensatory secretion of TSH, and eventually in an increase in vascularity and (sometimes gross) hypertrophy of the gland. 'Derbyshire neck' was the name given to this condition in a part of the UK where sources of dietary iodine were once scarce.

18.4 ACTIONS OF THE THYROID HORMONES

- The physiological actions of the thyroid hormones fall into two categories: those affecting metabolism and those affecting growth and development.
- The thyroid hormones produce a general increase in the metabolism of carbohydrates, fats and proteins, and regulate these processes in most tissues, T_3 being three to five times more active than T_4 in this respect.
- Although the thyroid hormones directly control the activity of some of the enzymes of carbohydrate metabolism, most effects are brought about in conjunction with other hormones, such as insulin, glucagon, the glucocorticoids and the catecholamines.
- There is an increase in oxygen consumption and heat production, which is manifested as an increase in the measured basal metabolic rate. This reflects action of these hormones on tissues such as heart, kidney, liver and muscle, although not on others, such as the gonads, brain or spleen.
- The calorigenic action is important as part of the response to a cold environment.
- Administration of thyroid hormone results in augmented cardiac rate and output, and increased tendency to dysrhythmias such as atrial fibrillation.

Effects on Growth and Development:

- The thyroid hormones have a critical effect on growth, partly by a direct action on cells, and also indirectly by influencing growth hormone production and potentiating its effects on its target tissues.
- The hormones are important for a normal response to parathormone and calcitonin as well as for skeletal development; they are also essential for normal growth and maturation of the central nervous system.

MOA:

- While there is some evidence for non-genomic actions these hormones act mainly through a mechanism dependent on occupation of a member of the nuclear receptor family, TR.
- Two distinct genes, TRα and TRβ, code for several receptor isoforms that have distinct functions. T_4 may be regarded as a prohormone, because when it enters the cell, it is first converted to T_3, which then binds with high affinity to a member of the TR family.
- This interaction is likely to take place in the nucleus, where TR isoforms generally act as a repressor of target genes.
- When T_3 is bound, the receptors change conformation, the corepressor complex is released and a coactivator complex is recruited, which then activates transcription- resulting in generation of mRNA and protein synthesis.

Transport and Metabolism:

- Both hormones are transported in the blood bound mainly to thyroxine-binding globulin (TBG). Plasma concentrations of these hormones can be measured by radioimmunoassay, and normally fall into the range 1×10^{-7} mol/l (T_4) and 2×10^{-9} mol/l for T_3.
- Both are eventually metabolised in their target tissues by deiodination, deamination, decarboxylation, and conjugation with glucuronic and sulfuric acids.
- The liver is a major site of metabolism, and the free and conjugated forms are excreted partly in the bile and partly in the urine. The metabolic clearance of T_3 is 20 times faster than that of T_4 (which is about 6 days).
- The long half-life of T_4 is a consequence of its strong binding to TBG.
- Abnormalities in the metabolism of these hormones may occur naturally or be induced by drugs or heavy metals, and this may give rise to a variety of (uncommon) clinical conditions such as the low T_3 syndrome.

Abnormalities of Thyroid Function:

- Thyroid disorders are among the most common endocrine diseases, and subclinical thyroid disease is particularly prevalent in the middle-aged and elderly.
- They are accompanied by many extrathyroidal symptoms, particularly in the heart and skin. One cause of organ dysfunction is thyroid cancer.

- Depending on where it is located, this can affect all aspects of glandular function including iodide uptake, TSH expression and thyroglobulin synthesis.
- Many other thyroid disorders have an autoimmune basis; the ultimate reason for this is not clear, although it may be linked to polymorphisms in the *PDS*, *TNF*-α; or other genes. Regardless of causation, there are two principal manifestations of the disease.

Hyperthyroidism (Thyrotoxicosis):

- In thyrotoxicosis, there is excessive activity of the thyroid hormones, resulting in a high metabolic rate, an increase in skin temperature and sweating, and a marked sensitivity to heat.
- Nervousness, tremor, tachycardia, heat sensitivity and increased appetite associated with loss of weight occur.
- There are several types of hyperthyroidism, but only two are common: diffuse toxic goitre (also called *Graves' disease* or *exophthalmic* goitre) and *toxic nodular goitre*.
- Diffuse toxic goitre is an organ-specific autoimmune disease caused by thyroid-stimulating immunoglobulins directed at the TSH receptor.
- Constitutively active mutations of the TRH receptor may also be involved. As it is indicated by the name, patients with exophthalmic goitre have protrusion of the eyeballs.
- The pathogenesis of this condition is not fully understood, but it is thought to be caused by the presence of TSH receptor-like proteins in orbital tissues.
- There is also an enhanced sensitivity to catecholamines.
- Toxic nodular goitre is caused by a benign neoplasm or adenoma, and may develop in patients with long-standing simple goitre.
- This condition does not usually have concomitant exophthalmos.
- The antidysrhythmic drug **amiodarone** is rich in iodine and can cause either hyperthyroidism or hypothyroidism.
- Some other iodine-containing drugs, such as **iopanoic acid** and its congeners, which are used as imaging agents used to visualise the gall bladder, may also interfere with thyroid function but may have some clinical utility in treating hyperthyroidism.

Simple, Non-Toxic Goitre:

- A dietary deficiency of iodine, if prolonged, causes a rise in plasma TRH and eventually an increase in the size of the gland.
- This condition is known as simple or non-toxic goitre. Another cause is ingestion of *goitrogens* (e.g. from cassava root).
- The enlarged thyroid usually manages to produce normal amounts of thyroid hormone, although if the iodine deficiency is very severe, hypothyroidism may supervene.

Hypothyroidism:

- A decreased activity of the thyroid results in hypothyroidism, and in severe cases *myxoedema*. Once again, this disease is immunological in origin, and the manifestations include low metabolic rate, slow speech, deep hoarse voice, lethargy, bradycardia, sensitivity to cold, and mental impairment.
- Patients also develop a characteristic thickening of the skin (caused by the subcutaneous deposition of glycosaminoglycans), which gives myxoedema its name.
- Hashimoto's thyroiditis, a chronic autoimmune disease in which there is an immune reaction against thyroglobulin or some other component of thyroid tissue, can lead to hypothyroidism and myxoedema.
- Therapy of thyroid tumours with **radioiodine** (see below) is another cause of hypothyroidism.
- Thyroid deficiency during development, caused by congenital absence or incomplete development of the thyroid, which is the most prevalent endocrine disorder in the newborn (1 in 3000-4000 births) causes cretinism, characterised by gross retardation of growth and mental deficiency.
- Pendred's syndrome, an autosomal recessive disorder caused by mutations in the PDS transporter gene, may cause goitre as well as deafness and other symptoms.

18.5 ANTI-THYROID DRUGS

- These are the drugs used as the treatment of hyperthyroidism. These drugs control the over production of thyroid hormones.

  Classification:

  1. **Goiterogens:**
     - **(a) Thio urea derivatives:** Thiourial, Methyl thiouracil, Propyl thio uracil, Methimazole and Carbimazole.
     - **(b)** Ionic Inhibition:
       Potassium thiocyanate, Potassium perchlorate.
  2. **Iodide:**
     Sodium Iodide, Potassium Iodide.
  3. **Radio active iodine:**
     $Iodine^{131}$, $Iodine^{125}$.
  4. **Beta-Adreno receptor blockers:**
     Propranalol, Timolol

Thiourea Derivatives:

Mechanism of action:

- Inhibits the oxidation of iodide to free iodine.
- Prevents combination of iodide with tyrosine.
- Prevent the coupling reaction in the biosynthesis of thyrosine.
- Inhibits peripheral conversion of T3 to T4.
- Brings down BMP of- Grave's disease and Thyrotoxicosis.

- Patients just can avoid operation by eraluse of this drugs.
- Methyl thiouracil is more toxic compared to methimazole and carbimazole.
- Methimazole is les toxic and safe drugs use in the treatment of thyrotoxicosis.

ADME:

- Well absorbed within 20-30 min after oral administration.
- 40-50% binds to plasma protein
- Only a fraction is metabolised in the body
- The rest is excreted in unchanged form.
- They cross placenta barrier and also excreted in milk.

Therapeutic Uses:

- Hyperthyroidism
- In the preparation of patient for thyroid surgery.
- Also use in the children, pregnancy and women with hyperthyroidism leads to Grave's disease.

Adverse Effect:

- Hypothyroidism, Goitre, skin rashes, arthralgia, agranulocitosis, leukopenia thrombocytopenia.

Propyl Thiouracil:

- Ideosyncracy, fever, transient- leukopenia, agranulocytosis.

Methimazole:

- Fever, bone marrow depression, leads to blood disorder.

Ionic Inhibitors:

MOA:

- These drugs competitively inhibits the trapping of iodine by the thyroid gland.
- Thus decreases biosynthesis of thyroid hormone.

Adverse Effect:

- Gastric irritation, fever, skin rashes, agranulocytosis.

Iodide:

- The iodide acts by decreasing the response of thyroid gland to TSH.
- Iodide inhibits the release of thyroid hormone and thus it is called thyroid constipation.
- Shrinkage of gland. Release of Iodine in the circulation is decreased and thus decreases the size of glands.

Lugal's Solution:

- Inhibits the release of thyroid hormones from the thyroid glands.

Other Action:

- The secretion of thyroid hormones is decreased.

- Reduction in basal metabolic rate.
- The glands become less vascular and firm.
- The acinar cell become small in size and collid content decrease.

Adverse Effect:

- Iodism: characterised by- skin rashes, Increase salivary secretion, Lacrimination, Acute hyper sensitivity reaction, Cutaneus haemorrhage, agioedema.

Therapeutic Uses:

- Pre operative to control hyperthyroidism in Grave's disease.
- Best control hyperthyroidism with propyl uracil, then iodine is given for 10 days before surgical operation.

Chronic Adverse Effect:

- Increase salivation
- Sourness of teeth and gums
- Swelling of eye lids
- Upper respiratory tract infection
- Inflammation of pharynx and larynx.

Radio Isotopes:

- Radio isotopes emites alpha and gamma rays which are having cytotoxic action on the thyroid gland and can be use for thyroid carcinoma.

Therapeutic Uses:

- Highly effective in the treatment of hypothyroidism.
- Especially in old patients where other hyperthyroidism drugs are contraindicated.
- To diagnose any thyroid disorders.
- Recurrent hyperthyroidism after any suitable anti thyroid drug therapy.

Side Effects:

- High incidence of delay hyperthyroidism.
- Patients after 30 years- chances of cancer and potential damage of offspring.

QUESTIONS

1. Write a note on synthesis, mechanism of action and uses of Thyroid hormones and their inhibitors.

■■■

Chapter ... 19

Hormone Regulating Plasma Calcium Level

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the management of calcium levels.*

19.1 CALCIUM

- Calcium is the fifth most common element in the body.

	Body content	Bone	Intracellular	Extracellular
Calcium	1300 g	99%	1%	0.1%

- Total plasma calcium = 2.5 mmol/L.

Absorption:

- Milk, Cheese, Egg-yolk, Fish, Beans, and Lentils are rich source of calcium.
- The mechanisms behind the intestinal absorption of calcium are:
  - **Active:** It is a saturable, transcellular process which involves calbindin (calcium-binding protein) – regulated by the active form of vitamin D.
  - **Passive:** It is a non-saturable, paracellular low efficiency process, which is not affected by calcium status or parathyroid hormone.
- Both processes occur throughout the small intestine.

Functions:

- Major structural element in the bones and teeth.
- Essential for several physiological processes such as neuromuscular transmission, smooth, skeletal, and cardiac muscle contractions, nerve function, and cell division and movement.
- Co-factor in Blood coagulation.
- Plays an important role in the action of other intracellular messengers e.g. cyclic adenosine monophosphate (cAMP) and Inositol-triphosphate, which are responsible for mediating the cellular response to various hormones including epinephrine, glucagon, ADH, and secretin.
- Release of neurotransmiters and hormones.

- There are three main hormones involved in the homeostatic regulation of calcium:
  - Parathornone PTH
  - Vitamin D
  - Calcitonin

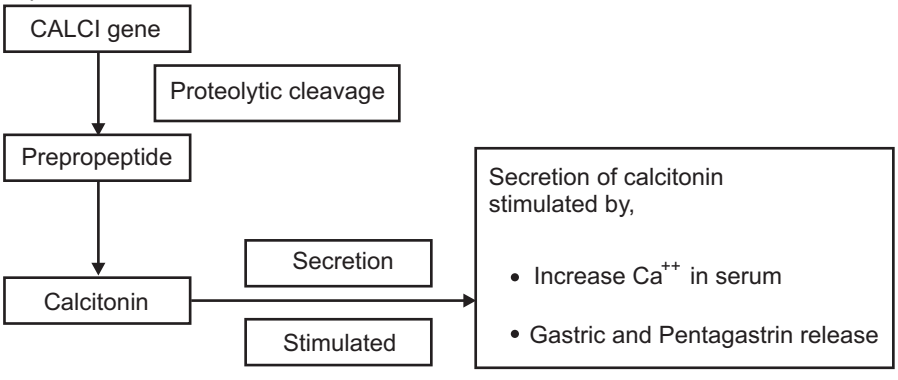
  Acting at 3 target organs: intestine, bone and kidneys.

19.2 CALCITONIN (THYROCALCITONIN)

- Hormone secreted by thyroid gland.
- It is a polypeptide hormone produced by parafollicular cells of thyroid gland. Also known as c-cells.
- Major stimulus for calcitonin secretion is increased plasma Ca^{2+} concentration.

19.2.1 Synthesis

- Calcitonin is formed by proteolytic cleavage of prepropeptide (product of CALCI gene).



19.2.2 Pharmacological Action

- Helps in metabolism of calcium and phosphorous.
- Calcitonin lowers blood calcium through following four methods.
  - Inhibit calcium absorption in intestine.
  - Inhibit osteoclast activity in bones.
  - Stimulate osteoblastic activity in bones.
  - Inhibit renal tubular cell reabsorption of calcium.
- It also inhibits the phosphate reabsorption by the kidney tubules.

19.2.3 Mechanism

- Calcitonin is bound with calcitonin receptor. Caicitonin receptor are present in osteoblast cells, kidney and different regions of the brain. Calcitonin receptor is a G-protein coupled receptor which stimulates adenyl cyclase ------cAMP generated (increase cAMP), which regulates Ca^{++} metabolism and phosphate metabolism.

Uses:

- Calcitonin is used therapeutically for the treatment of hypercalcemia.
- Treatment of mania giving subcutaneous injection of calcitonin for decreasing irritability, euphoria, hyper activity.
- Calcitonin mainly act as a supporting drug for the treatment of **bipolar diseases.**
- It is an important tool for diagnosing thyroid cancer. (if an increase calcitonin in blood – chances of thyroid cancer).

19.3 PARATHORMONE (PTH)

- Polypeptide containing 84 amino acids residues.
- Secreted by the chief cells in the 4 parathyroid glands.
- In hypocalcaemia, parathyroid hormone secretion is stimulated.
- In hypercalcaemia, secretion is inhibited, and the calcium is deposited in the bones.

PTH increases serum calcium levels through:

- Increasing bone resorption by activating osteoclastic activity.
- Increasing renal calcium reabsorption by the distal renal tubules.
- Increasing renal phosphate excretion by decreasing tubule phosphate reabsorption.
- Increasing the synthesis of 1, 25-dihydroxy vitamin D (also called calcitriol) by increasing the activity of alpha-hydroxylase enzyme in the kidney.

19.3.1 Mechanism

If there is an increase in extracellular calcium concentration

↓

Ca^{2+} binds to the receptor and activates phospholipase C

↓

Which results increased levels of IP3/Ca^{2+}, leading to the inhibition of PTH secretion.

↓

When extracellular Ca^{2+} is decreased, there is decreased Ca^{2+} binding to the receptor, which stimulates PTH secretion

19.3.2 Pharmacological Action

- **PTH mainly** acts upon bone, kidney and intestine.

Action on bone

- **PTH promotes** bone resorption (osteoclast action) and increase calcium concentration.

Action on kidney

- **PTH** is highly responsible for reabsorption of Ca, Mg in distal tubule and thick ascending loop of henle and decreases the reabsorption of phosphate.

Action on intestine:

- Enhance the absorption of Ca^{2+} in intestine by increasing Vitamin-D level.

Stimulators for Parathyroid Hormone Secretion:

- If there is a decrease in serum Ca^{++} level.
- Mild decrease in serum Mg.
- Increase in serum phosphate level.

19.4 VITAMINE-D

- It is a fat soluble vitamin. It is very important for intestinal absorption of Ca^{2+}, F, Mg^{2+} and phosphate. Vitamin D_3 (cholecalciferol), Vitamine D_2 (Ergocalciferole) are obtained from diet.

19.4.1 Pharmacological Action

Bone Health:

- Important for growth of bone.
- It increases the Ca^{2+} and phosphate reabsorption.
- Used in the treatment of osteoporosis.
- Older people with osteoporosis taking Vit. D with calcium to prevent hip fracture.

Kidney:

- It increases the Ca and phosphate reabsorption in kidney.

Intestine:

- Increases the calcium and phosphate reabsorption.
- Synthesis of calbindin D-28K.

Cardio Vascular System:

- Slightly reduces the risk of stroke and myocardial infraction. But it does not have any activity upon B.P. regulation.

Immune System:

- It mainly activates innate immunity, act against viral infection. Used for the treatment of autoimmune diseases.

Pregnancy:

- If mother having less Vit. D in blood, which leads to type IV DM (gestational D.M.). Mother should consume Vit. D supplements.

Common diseases related to Vitamin D:

1. **Rickets:** Due to insufficient amounts of calcium and phosphate to mineralize the growing bones leading to growth failure and skeletal deformities in children.
2. **Osteomalacia:** Due to inadequate levels of phosphate, calcium and Vit. D (impairment of bone metabolism) in older people.

MOA:

Vitamin D

(blood stream)

↓

Calcidiol

↓

Calcitriol

(Kidney biologically active)

↓

Vitamin D binding protein

↓

Produce the Effect

- Under the UV radiation mainly animal synthesizes Vit. D from 7-dehydrocholesterol.

Biological Activity

- Maintenance of Ca^{2+} balance by promoting Ca^{2+} absorption in the intestine.
- Promote the bone resorption by increasing osteoclast cells.
- Allowing the proper functioning of parathyroid hormone to maintain serum Ca^{2+} level.

QUESTIONS

1. Explain the pharmacology of parathormone and calcitonin.
2. Write a note on pharmacology and therapeutic uses of Vitamin-D.

■■■

Chapter ... 20

Insulin, Oral Hypoglycemic Agents and Glucagon

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the management of diabetes.*

20.1 DIABETES MELLITUS

- **Diabetes mellitus (DM)** is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycemia.
- Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis.
- When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which, in turn, results in dehydration, thirst and increased drinking (polydipsia).
- Insulin deficiency causes wasting through increased breakdown and reduced synthesis of proteins.
- **Diabetic ketoacidosis** is an acute emergency. It develops in the absence of insulin because of accelerated breakdown of fat to acetyl-CoA, which, in the absence of aerobic carbohydrate metabolism, is converted to acetoacetate and β-hydroxy-butyrate (which cause acidosis) and acetone (a ketone).
- Various complications develop as a consequence of the metabolic derangements in diabetes, often over many years. Many of these are the result of disease of blood vessels, either large (macrovascular disease) or small (microangiopathy).
- Dysfunction of vascular endothelium is an early and critical event in the development of vascular complications. Oxygen-derived free radicals, protein kinase C and non-enzymic products of glucose and albumin (called advanced glycation end products) have been implicated.
- *Macrovascular disease* consists of accelerated atheroma and its thrombotic complications which are commoner and more severe in diabetic patients. *Microangiopathy* is a distinctive feature of diabetes mellitus and particularly affects the retina, kidney and peripheral nerves.
- Diabetes mellitus is the commonest cause of chronic renal failure, which itself represents a huge and rapidly increasing problem, the costs of which to society as well as to individual patients are staggering.

- Coexistent hypertension promotes progressive renal damage, and treatment of hypertension slows the progression of diabetic nephropathy and reduces myocardial infarction.
- *Angiotensin-converting enzyme inhibitors* or *angiotensin receptor antagonists* are more effective in preventing diabetic nephropathy than other antihypertensive drugs, perhaps because they prevent fibro proliferative actions of angiotensin II and aldosterone.
- Diabetic neuropathy is associated with accumulation of osmotically active metabolites of glucose, produced by the action of aldose reductase, but *aldose reductase inhibitors* have been disappointing as therapeutic drugs.
- It is lifelong metabolic disorder characterized by high blood sugar level
  Symptoms are: Hyperglycemia, Blurred vision, Fatigue, Polyurea, Polydypsia, Polyphagia.

Blood sugar levels chart

Sr. No.	Blood sugar level	Conditions	Units
1.	Normal	Fasting	60-100 mg/dL
2.		Post prandial (After food)	160-180 mg/dL
3.	Pre-diabetic	Fasting	100-125 mg/dL
4.		Post prandial (After food)	180-200 mg/dL
5.	Diabetic	Fasting	More than 125 mg/dL
6.		Post prandial (After food)	More than 200 mg/dL

There are two main types of Diabetes Mellitus:

Type 1 Diabetes:

- Previously known as insulin-dependent diabetes mellitus-IDDM-or juvenile-onset diabetes.
- Generally this occurs to the patients below 20 years of age.
- Failure to produce Insulin.
- 15-20% of patients suffering from type 1 diabetes mellitus.
- In type 1 diabetes, there is an absolute deficiency of insulin resulting from autoimmune destruction of β cells. Without insulin treatment, such patients will ultimately die with diabetic ketoacidosis.
- *Type 1* diabetic patients are usually young (children or adolescents) and not obese when they first develop symptoms. There is an inherited predisposition, with a 10-fold increased incidence in first-degree relatives of an index case, and strong associations with particular histocompatibility antigens (HLA types).

Type 2 Diabetes:

- Previously known as non-insulin-dependent diabetes mellitus-NIDDM-or maturity-onset diabetes.
- Generally this occurs to the patients over 40 years of age.
- Failure to utilise Insulin.
- 80-85% of patients suffering from type 2 diabetes mellitus.
- *Type 2* diabetes is accompanied both by insulin resistance (which precedes overt disease) and by impaired insulin secretion, each of which are important in its pathogenesis. Such patients are often obese and usually present in adult life, the incidence rising progressively with age as β-cell function declines.
- Treatment is initially dietary, although oral hypoglycaemic drugs usually become necessary, and about one-third of patients ultimately require insulin. Prospective studies have demonstrated a relentless deterioration in diabetic control over the years.

Type 3 Diabetes:

- These types of diabetes occurs due to other cause like chronic therapy with some dugs (Thiazide Urea, Glucocorticoids, Diazoxide, Growth Hormone) or disease induced (Pancreatitis).

Type 4 Diabetes:

- This is also called gestational diabetes.
- 4-5% of patients suffering from type 4 diabetes.
- Increased blood sugar level than normal generally occurs during third trimester and after post partum period.
- Placental hormone promotes insulin resistance.

20.2 TREATMENT OF DIABETES MELLITUS

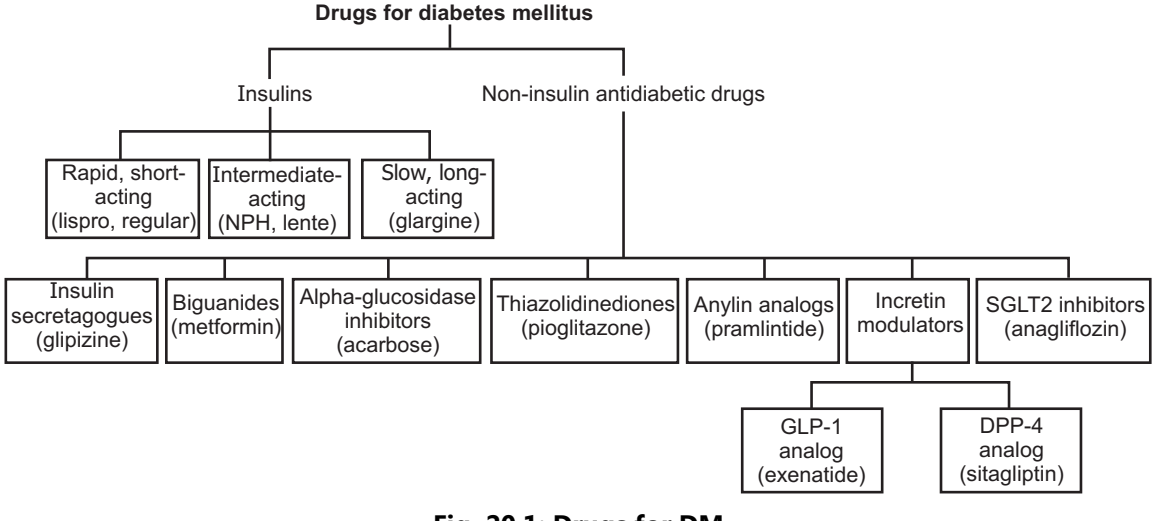


Fig. 20.1: Drugs for DM

20.3 CLASSIFICATION

Anti-diabetic Drugs:

- **Oral Hypoglycemic Drugs:**
  - **Sulfonyl urea Derivatives:**
  - **First Generation:** Tolbutamide, Chlorpropamide, Tolazomide, Acetohexamide.
  - **Second Generation:** Glibenclamide, Glipizide, Gliclazide.
- **Anti Hyperglycemic Drugs:**
  - **Bioguanide:** Phenformin, Metformin.
  - **Thiazolidine:**Troglitazone, Coglitazone, Aeuglitazone, Pioglitazone.
  - **α- Glucosidase Inhibitors:** Acarbose, Guargum, Miglitol.
- **Parenteral Anti-diabetic Drugs:** Insulin.

20.3.1 Oral Hypoglycemic Drugs

Sulfonyl Urea Derivatives:

Sulfonylurea:

- The sulfonylureas were developed following the chance observation that a sulfonamide derivative (used to treat typhoid) caused hypoglycemia.
- Numerous sulfonylureas are available. The first used therapeutically were **tolbutamide** and **chlorpropamide**. Chlorpropamide has a long duration of action and a substantial fraction is excreted in the urine. Consequently, it can cause severe hypoglycemia, especially in elderly patients in whom renal function declines inevitably but insidiously.
- It causes flushing after alcohol because of a disulfiram-like effect, and has an action like that of antidiuretic hormone on the distal nephron, giving rise to hyponatraemia and water intoxication. Tolbutamide, however, remains useful.
- So-called second-generation sulfonylureas (e.g. **glibenclamide**, **glipizide** are more potent (on a milligram basis), but their maximum hypoglycemic effect is not greater and control of blood glucose not better than with tolbutamide.
- These drugs all contain the sulfonylurea moiety and act in the same way, but different substitutions result in differences in pharmacokinetics and hence in duration of action.

MOA:

- The principal action of sulfonylureas is on β cells, stimulating insulin secretion and thus reducing blood glucose.
- High-affinity receptors for sulfonylureas are present on the K_{ATP} channels in β-cell plasma membranes, and the binding of various sulfonylureas parallels their potency in stimulating insulin release.
- These drugs reduce the permeability of K^+ by competitively blocking sulfonyly urea receptors present on ATP sensitive K^+ (K_{ATP}) channels.

- The inhibition of K^+ channels causes opening of voltage gated Ca^{2+} channels, which leads to increased influx of Ca^{2+} and thus stimulate insulin secretion from β cells of pancreas.
- Reduced blood glucose level.
- They also suppress glucagon level which indirectly contributes to their hypoglycemic effects.

ADME:

- Sulfonylureas are well absorbed after oral administration, and most reach peak plasma concentrations within 2-4 hours. Glipizide absorption delayed by presence of food. The duration of action varies.
- All bind strongly to plasma (90-98%). Plasma protein bindings are less for first generation drugs in compare to second generation. Metabolized in liver and kidney.
- Most sulfonylureas (or their active metabolites) are excreted in the urine, so their action is increased in the elderly and in patients with renal disease.
- Most sulfonylureas cross the placenta and enter breast milk; as a result, use of sulfonylureas is contraindicated in pregnancy and in breast feeding when diet and, if necessary, insulin is used.

Therapeutic uses:

- In type 2 diabetes mellitus.
- Surgery during diabetes.
- In diabetes coma.

ADR:

- Hypoglycemia which may lead to renal and hepatic impairment.
- Weight gain, Fluid retention and edema.
- Photosensitivity, Skin Rashes, Blood dyscrasis, Cholestic jaundice.
- Flatulence.
- It exhibits disulfiram like action with alcohol due to inhibition of alcohol dehydrogenase causing accumulation of aldehyde dehydrogenase leading to headache, nausea, vomiting and sweating.

20.3.2 Anti Hyperglycemic Drugs

Bioguanide:

MOA:

- They increase glucose uptake and utilization in skeletal muscle (thereby reducing insulin resistance).
- Reduce hepatic and renal glucose gluconeogenesis, which reduced hepatic glucose outputs.
- Slowing down the glucose absorption from GIT, which increases availability of glucose for its conversion to lactate by entrecotes.
- It also promotes insulin binding to its receptor.

- Reduced in plasma glucagon levels.
- It does not depend upon functional state of β cells of pancreas and can also be given to obese person as it does not cause weight gain.
- While preventing hyperglycemia, does not cause hypoglycemia.
- Besides decreased elevated levels of glucose it also decreases low-density and very low-density lipoproteins (LDL and VLDL, respectively).
- All above action leads to Antidiabetic effects.

ADME:

- Well absorbs, distributed well throughout the body, metabolized in liver and excreted in unchanged form by kidney.
- Metformin has a half-life of about 3 hours and is excreted unchanged in the urine.

Therapeutic use:

- In type 2 diabetes mellitus.
- In obese diabetes mellitus, as it does not stimulate appetite.
- Surgery during diabetes.
- In diabetes coma.

ADR:

- Nausea, Metallic tase, Anorexia, Flatulence, Diarrhoea, Gastrointestinal disturbances.
- Long-term use may interfere with absorption of vitamin $B_{12.}$
- Lactic acidosis is a rare but potentially fatal toxic effect, and metformin should not be given to patients with renal or hepatic disease, hypoxic pulmonary disease, heart failure or shock.
- Such patients are predisposed to lactic acidosis because of reduced drug elimination or reduced tissue oxygenation. It should also be avoided in other situations that predispose to lactic acidosis, and is contraindicated in pregnancy.

Thiazolidine:

MOA:

- Thiazolidine diones bind to a nuclear receptor called the peroxisome proliferator-activated receptor-γ (PPARγ), which is complexed with retinoid X receptor (RXR).
- PPARγ occurs mainly in adipose tissue, but also in muscle and liver.
- It causes differentiation of adipocytes (this contributes to the unwanted effect of weight gain), increases lipogenesis and enhances uptake of fatty acids and glucose.
- It also promotes amiloride-sensitive sodium ion reabsorption in renal collecting ducts, so cause fluid retention.
- Thiazolidinediones cause the PPARγ-RXR complex to bind to DNA, promoting transcription of several genes with products that are important in insulin signalling.
- These include lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid-binding protein, Glut-4, phosphoenolpyruvate carboxykinase, malic enzyme and others.

- It remains something of a mystery that glucose homeostasis should be so responsive to drugs that bind to receptors found mainly in fat cells.
- It also causes reset of the glucose-fatty acid (Randle) cycle by the reduction in circulating free fatty acids.

ADME:

- Both rosiglitazone and pioglitazone are rapidly and nearly completely absorbed, with time to peak plasma concentration of less than 2 hours.
- Both are highly (> 99%) bound to plasma proteins, both are metabolized in liver.
- Rosiglitazone is metabolised by CYP2C8 to weakly active metabolites, pioglitazone mainly by a CYP2C isozyme and CYP3A4 to active metabolites. The metabolites of rosiglitazone are eliminated mainly in urine, and those of pioglitazone mainly in bile.

Therapeutic use:

- In type 2 diabetes mellitus.
- In obese diabetes mellitus.
- Surgery during diabetes.
- Diabetes mellitus along with bioguanides or sulfonyl urea.

ADR:

- Weight gain, fluid retention, edema, subcutaneous accumulation of fats, hemo-dilution leading to reduce in hemoglobin concentration, Hepatotoxicity.

α-Glucosidase Inhibitors:

MOA:

- **Acarbose**, an inhibitor of intestinal α-glucosidase, is used in type 2 patients whose diabetes is inadequately controlled by diet with or without other agents.
- It delays carbohydrate absorption, reducing the postprandial increase in blood glucose.

Carbohydrate (Polysaccharides)

↓ α glucosidase inhibitors

Inhibits Monosaccharide synthesis

↓

Slow down absorption of monosaccharides from GIT → Anti-diabetic action

ADME:

- Well absorbed, distributed well throughout the body, metabolized in liver and excreted in unchanged form by kidney.

ADR:

- Flatulence, Diarrhea, Abdominal pain, hypoglycemia (with sulfonly urea).

20.4 INSULIN

- Insulin was the first protein for which an amino acid sequence was determined (by Sanger's group in Cambridge in 1955).
- It consists of two peptide chains (A and B, of 21 and 30 amino acid residues, respectively).

20.4.1 Synthesis and Secretion

- Like other peptide hormones, insulin is synthesised as a precursor (preproinsulin) in the rough endoplasmic reticulum.
- Preproinsulin is transported to the Golgi apparatus, where it undergoes proteolytic cleavage first to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide.
- Insulin and C-peptide are stored in granules in B cells, and are normally cosecreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin.
- The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration. β-cells respond both to the absolute glucose concentration and to the rate of change of blood glucose.
- Other stimuli to insulin release include amino acids (particularly arginine and leucine), fatty acids, the parasympathetic nervous system, peptide hormones for the gut and drugs that act on sulfonylurea receptors.
- There is a steady basal release of insulin and also a response to an increase in blood glucose. This response has two phases: an initial rapid phase reflecting release of stored hormone, and a slower, delayed phase reflecting both continued release of stored hormone and new synthesis.

Pre- pro-insulin (110 Amino acid)

↓ Endopeptidasae

Pro-insulin (86 Amino acid)

↓ **Protease in golgi apparatus**

Insulin (51 Amino acid, Chain A-21 and Chain B- 30 amino acids)

- **Pro- insulin** (86 Amino acid, Chain A-21, Chain B- 30 and Chain C- contains 35 amino acids). Chain A and B are black in colour and chain C is yellow in colour.
- Human pancreas stores up to 8 mg of insulin which is equivalent to 220 units of insulin.

MOA:

- Insulin binds to a specific receptor on the surface of its target cells. The receptor is a large transmembrane glycoprotein complex belonging to the kinase-linked type 3 receptor superfamily and consisting of two α and two β subunits.

When Insulin binds to α subunits of outer surface of the cells cause:

- Aggregation and internalization of Insulin receptors along with Insulin in vesicles, resulting in down-regulation, and activation of Tyrosine kinase activity in β subunits.
- Result autophosphorylation of tyrosine kinase residue present on the cytoplasmic protein called Insuin receptor substrate I and Insulin receptor substrate II.
- Which result in cascade of phosphorylation and dephosphorylation reaction.
- Result stimulation or inhibition of enzyme system involved in rapid metabolism action of Insulin.
- Certain second messenger such as IP_3 and DAG system activated and subsequent activation of Phospholipase-C.

Result different actions like:

- Insulin stimulates glucose transport across the cell membrane by ATP dependent transportation of glucose transporter-4 (GLUT-4), so uptake and utilization of glucose by skeletal muscle is increased.
- Inhibits gluconeogenesis.
- Inhibits gylcogenolysis.
- Stimulate gylcogenesis.
- Stimulate glucogenesis.
- Stimulate storage of glycogen, fat and proteins.

- All these action leads to decrease blood sugar level, Antidiabetic action.

20.4.2 Pharmacological Action

Effect of Insulin on Carbohydrate Metabolism:

In Liver:

- Insulin influences glucose metabolism in most tissues, especially the liver, where it inhibits glycogenolysis (glycogen breakdown).
- It also inhibits gluconeogenesis (synthesis of glucose from non-carbohydrate sources) while stimulating glycogen synthesis.
- It also increases glucose utilization (glycolysis), but the overall effect is to increase hepatic glycogen stores.

In Muscles:

- In muscle, unlike liver, uptake of glucose is slow and is the rate-limiting step in carbohydrate metabolism.
- The main effects of insulin are to increase facilitated transport of glucose via a transporter called GLUT-4, and to stimulate glycogen synthesis and glycolysis.
- Insulin increases glucose uptake by GLUT-4 in adipose tissue as well as in muscle, enhancing glucose metabolism.
- One of the main end products of glucose metabolism in adipose tissue is glycerol, which is esterified with fatty acids to form triglycerides, thereby affecting fat metabolism.

In adipose Tissue:

- Insulin increases synthesis of fatty acid and triglyceride in adipose tissue and in liver.
- It inhibits lipolysis, partly via dephosphorylation (and hence inactivation) of lipases.
- It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagon by opposing their actions on adenylate cyclase.

Effect of Insulin on Protein Metabolism:

In Liver:

- It inhibits oxidation of amino acids in the liver.
- It also decreases breakdown of protein in the liver.

In Muscles:

- Insulin stimulates uptake of amino acids into muscle.
- Insulin increases protein synthesis.

Effect of Insulin on Fat Metabolism:

In Liver:

- Insulin increases Lipid synthesis (Lipogenesis)

In Adipose Tissue:

- It stimulates fatty acids synthesis and tri glycerides formation
- Insulin inhibits Lipolysis

Other Metabolic Effects:

- Insulin increase transport of K^+ , Ca^{2+} and Phosphate.
- Insulin stimulates vascular endothelial lipoprotein lipase activity and thus stimulates clearance of VLDL.

ADME:

- Being high molecular weight polypeptide insulin rapidly degraded in GIT if administered orally.
- Insulin can be administered by IV in emergency condition, by IM, it absorbs more rapidly.
- Hence it is administered by SC, in which rate of administration is slow and sustained action can be achieved.
- It is well absorbed and distributed well, metabolized in liver by insulinase enzyme and excreted in urine.

Therapeutic Uses:

- Patients with type-I and type-II diabetes mellitus.
- For gestational Diabets mellitus.
- For emergency treatment of diabetic ketoacidosis.
- In acute alcoholism, Insulin and glucose given to hasten metabolism of alcohol in liver.

ADR:

- Hypoglycemia, Lipodystrophy (Site of injectio).
- Allergic manifestation- Urticaria, Angeioedema.
- Blurred vision, obesity, nervous disorders, Very rarely anaphylaxis.

Management of Diabetic Coma:

- Glucose 5% by IV infusion.
- Glucagon 1 mg by IM route to raise sugar level.
- Diazoxide to treat resistance hypoglycemia, it cause release of Adrenaline and nor adrenaline which increases blood sugar level.
- Insulin preparation.

Conventional (standard) preparation of insulin

Types	Appearance	Onset (h)	Peak (h)	Duration (h)	Can be fixed with
Short Acting					
Regular (crystalline solution) insulin	Clear	0.5	2-4	6-8	All Preparation
Prompt I. Tine suspension (Amorphous/semi lent)	Cloudy	1	3-6	12-16	Regular Lente preparation
Intermediate Acting					
Insulin tn. Suspension or Lente (ultra:semi;7:3)	Cloudy	1-2	2-4	8-10	Regular/semi Lente
Neutral protamine Hagedorn NPH OR isophan insulin tine.	Cloudy	1-2	3-6	8-10	Regular or crystalline
Long Acting					
Extended I tn suspension (crystalline form or ultra-Lente)	Cloudy	4-6	14-18	24-36	Regular and semi Lente
Protamine tine insulin (PTI)	Cloudy	4-6	14-18	24-36	Regular
Ultra-Short Acting					
Insulin lispro	Clear solution	10-20	1-2	3-4	With all time of insulin
Insulin Aspart	Clear solution	do	do	do	do

20.5 GLUCAGON

Glucagon is a single-chain polypeptide of 21 amino acid residues.

20.5.1 Synthesis and Secretion

- Glucagon is synthesised mainly in the α cell of the islets, but also in the upper gastrointestinal tract.
- It has considerable structural homology with other gastrointestinal tract hormones, including secretin, vasoactive intestinal peptide and GIP. Endocrine pancreas and blood glucose.
- Islets of Langerhans secrete insulin from B (or β) cells, glucagon from α cells and somatostatin from D/ delta cells.
- Many factors stimulate insulin secretion, but the main one is blood glucose.
- Insulin has essential metabolic actions as a fuel storage hormone and also affects cell growth and differentiation. It decreases blood glucose by:
    - Increasing glucose uptake into muscle and fat via GLUT-4.
    - Increasing glycogen synthesis.
    - Decreasing gluconeogenesis.
    - Decreasing glycogen breakdown.
    - Glucagon is a fuel-mobilising hormone, stimulating gluconeogenesis and glycogenolysis, also lipolysis and proteolysis. It increases blood sugar and also increases the force of contraction of the heart.
- One of the main physiological stimuli to glucagon secretion is the concentration of amino acids, in particular L-arginine, in plasma.
- Therefore an increase in secretion follows ingestion of a high-protein meal, but compared with insulin there is relatively little change in plasma glucagon concentrations throughout the day.
- Glucagon secretion is stimulated by low and inhibited by high concentrations of glucose and fatty acids in the plasma.
- Sympathetic nerve activity and circulating adrenaline stimulate glucagon release via β adrenoceptors.
- Parasympathetic nerve activity also increases secretion, whereas somatostatin, released from D/delta cells adjacent to the glucagon-secreting α cells in the periphery of the islets, inhibits glucagon release.

Physiological Actions:

- Glucagon increases blood glucose and causes breakdown of fat and protein. It acts on specific G-protein-coupled receptors to stimulate adenylate cyclase, and consequently its actions are somewhat similar to β adrenoceptor-mediated actions of adrenaline.

- Unlike adrenaline, however, its metabolic effects are more pronounced than its cardiovascular actions. Glucagon is proportionately more active on liver, while the metabolic actions of adrenaline are more pronounced on muscle and fat.
- Glucagon stimulates glycogen breakdown and gluconeogenesis, and inhibits glycogen synthesis and glucose oxidation. Its metabolic actions on target tissues are thus the opposite of those of insulin.
- Glucagon increases the rate and force of contraction of the heart, although less markedly than adrenaline.

Therapeutic uses:

- **Glucagon** can be given intramuscularly or subcutaneously as well as intravenously.
- Treatment of hypoglycaemia in unconscious patients (who cannot drink), unlike intravenous glucose, it can be administered by non-medical personnel (e.g. spouses or ambulance crew).
- It is useful if obtaining intravenous access is difficult.
- Treatment of acute cardiac failure precipitated by β-adrenoceptor antagonists.

20.5.2 Control of Blood Glucose

- Glucose is the obligatory source of energy for the brain, and physiological control of blood glucose reflects the need to maintain adequate fuel supplies in the face of intermittent food intake and variable metabolic demands.
- More fuel is made available by feeding than is immediately required and excess calories are stored as glycogen or fat.
- During fasting, these energy stores need to be mobilised in a regulated manner.
- The most important regulatory hormone is insulin, the actions of which are described above. Increased blood sugar stimulates insulin secretion, whereas reduced blood sugar reduces insulin secretion.
- Hypoglycaemia, caused by excessive insulin, not only reduces insulin secretion but also elicits secretion of an array of 'counter-regulatory' hormones, including glucagon, adrenaline, glucocorticoids and growth hormone, all of which increase blood glucose.
- Their main effects on glucose uptake and carbohydrate metabolism are summarised and contrasted with those of insulin.

20.5.2.1 Somatostatin

- Somatostatin is secreted by the D/delta cells of the islets.
- It is also generated in the hypothalamus, where it acts to inhibit the release of growth hormone. In the islet, it inhibits release of insulin and of glucagon.
- **Octreotide** is a long-acting analogue of somatostatin.
- It inhibits release of a number of hormones, and is used clinically to relieve symptoms from several uncommon gastroenteropancreatic endocrine tumours, and for treatment of acromegaly (the endocrine disorder caused by a functioning tumour of cells that secrete growth hormone from the anterior pituitary.

20.5.2.2 Amylin (Islet Amyloid Polypeptide)

- The term *amyloid* refers to amorphous protein deposits in different tissues that occur in a variety of diseases, including several neurodegenerative conditions.
- Amyloid deposits occur in the pancreas of patients with diabetes mellitus, although it is not known if this is functionally important. The major component of pancreatic amyloid is a 37-amino acid residue peptide known as islet amyloid polypeptide or amylin.
- This is stored with insulin in secretory granules in β-cells and is cosecreted with insulin. Amylin delays gastric emptying. Supraphysiological concentrations stimulate the breakdown of glycogen to lactate in striated muscle.
- Amylin also inhibits insulin secretion. It is structurally related to calcitonin and has weak calcitonin-like actions on calcium metabolism and osteoclast activity.
- It is also about 50% identical with calcitonin gene-related peptide (CGRP) and large intravenous doses cause vasodilatation, presumably by an action on CGRP receptors.
- Whether amylin has a role in the physiological control of glucose metabolism is controversial, but there is interest in the therapeutic potential of amylin agonists (such as **pramlintide,** an analogue with three proline substitutions that reduce its tendency to aggregate into insoluble fibrils.

QUESTIONS

1. Explain the pharmacological action of insulin.
2. Write a note on insulin preparation.
3. Write the mechanism of action of sulfonyl ureas and biguanides.
4. Discuss the pharmacology of oral Hypoglycemic agents.

■■■

Chapter ... 21

ACTH and Corticosteroids

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the pharmacology of ACTH.*

21.1 ACTH (ADENOCORTICOTROPIC HORMONE/CORTICOTROPIN)

- It is a 35 amino acid containing polypeptide produced by anterior pituitary gland.

Physiological Functions:

- **ACTH** promote steroidogenesis.
- By stimulating cAMP formation in cortical cell ACTH rapidly increases the availability of cholesterol for conversion to pregnenolone.
- Hypothalamus regulate ACTH release from pituitary through corticotrophin-releasing hormone **(CRH).**
- Excess production of ACTH leading to Cushing's syndrome.

21.2 CORTICOSTEROIDS

- Steroidal hormones produced in the adrenal cortex mainly it is involved in the regulation of physiological activity, such as
  Immune response
  Carbohydrate metabolism
  Regulation of inflammation
  Protein metabolism
  Regulation of blood electrolyte level.

Corticosteroids are of two types:

1. Glucocorticoid
2. Mineralocorticoid

21.2.1 Glucocorticoid

- Dexamethazone
- Prednisolone
- Fludrocortisone

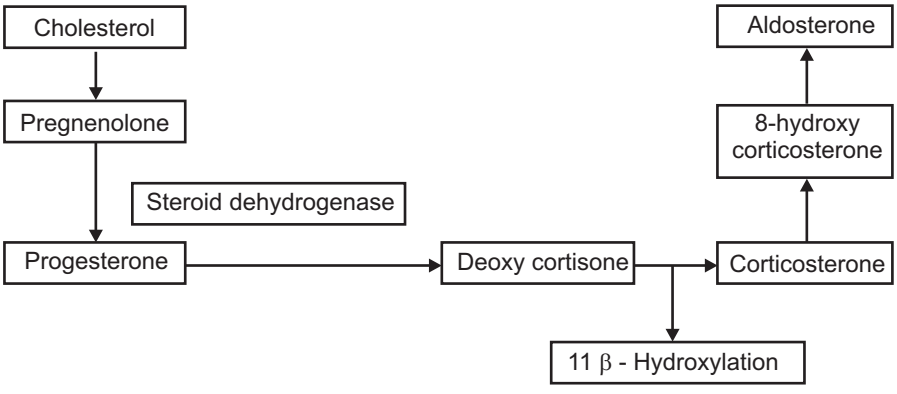
Function

- Glucocorticoids are used in the treatment of joint pain and inflammation, dermatitis, allergic reaction, asthma, Hepatitis, ulcerative colitis.
- Used in the treatment of Addison's disease.
- Topical formulations are used for skin infection, asthma and laryngitis.

Side Effects:

- Anxiety, depression, psychosis.
- Hyperglycemia.
- Osteoporosis.
- Retinopathy.

Biosynthesis



Pharmacological Action:

Carbohydrate and Protein Metabolism:

- It promote glycogen deposition in liver.
- It promote gluconeogenesis.
- Induce protein break down.
- Increases uric acid excretion.

Fat Metabolism:

- Glucocorticoids induce lipolysis due to glucagon, growth hormone, adrenaline and thyroxine.
- Break down of triglyceride enhanced by cAMP.
- Looses fat which deposited over face, neck and shoulder producing '**moon face', 'fish mouth' and 'buffalo hump'**.

Calcium Metabolism:

- It enhances the renal excretion of calcium.
- Results loss of Ca^{++} from bone, leading to negative calcium balance.

Cardiovascular System:

- Maintain myocardial contractility.
- It producing minor hypertension.

Skeletal Muscle:

- Enhances muscular activity.
- Excess glucocorticoid action leads to muscle wasting and myopathy.

CNS:

- Produces euphoria, insomnia, anxiety

Stomach:

- It increases gastric acid and pepsin secretion.

Blood and Lymphoid Tissue:

- Destruction of lymphoid tissue.
- Increasing the no. of RBC by inhibiting haemolysis.
- Decrease the no. of circulating lymphocytes, monocytes, basophil, eosinophils.

Effect on Immune Response:

- Blocks the synthesis of cytokines.
- Block the action of cytokines.
- Increasing the expression of gene coding for enzyme that degrade inflammatory mediators.

Hydrocortisone (Cortisol):

- Mainly used for the treatment of ulcerative cholitis and hormone replacement therapy.

Prednisolone:

- It is 4 times more potent than hydrocortisone and more selective. Act as anti-inflammatory, anti allergic and used for the treatment of autoimmune disease.

Dexamethasone:

- Potent and selective glucocorticoid. It is used for allergic and inflammatory condition, which is long acting.

21.2.2 Mineralocorticoid

- Aldosterone is the prototype which produces mineralocorticoid effects.
- By acting on the distal tubule aldosterone enhances the absorption of Na^+.
- A similar effect occurs in colon, sweat gland and salivary gland.
- Deficiency of mineralocorticoid action leads to hyponatremia, hyperkalaemia, acidosis.
- Hyperaldosterinism results positive Na^+ balance, increased plasma Na, hypokalaemia, alkalosis.

ACTH Inhibitors:

- **Aminoglutethemide:** Which stop/inhibit the conversion of cholesterol to pregnelone. Used in the treatment of adenocortical cancer.
- **Metyrapone:** 11 beta-hydroxylase enzyme inhibitor – used in Cushing's syndrome and test of pituitary efficiency.
- **Mifepristone:** Progesterone antagonist.
- **Ketoconazole:** Inhibit the synthesis of all hormones in the testes and adrenal cortex, used in the treatment of Cushing's syndrome and hirsutism in female.

QUESTIONS

1. Write a note on androgens and anabolic steroids.

■■■

Unit V

Chapter ... 22

Oral Contraceptives

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

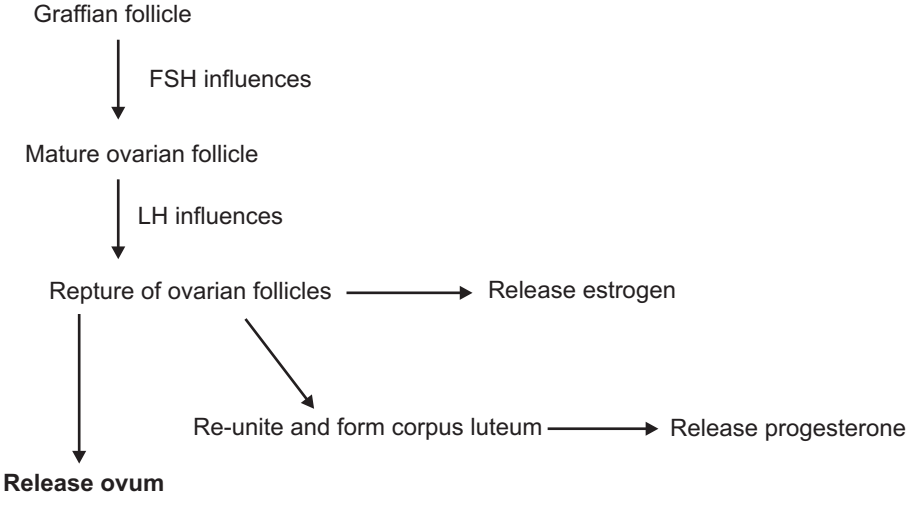
- *Describe the MOA of oral Contraceptives*

22.1 INTRODUCTION

- Contraceptives are the drugs which prevent contraception since they control fertility; they are also called anti fertility drugs.

22.2 GENERAL PHYSIOLOGY OF HUMAN REPRODUCTION

- Ovulation is the key event in human reproductive cycle. It usually occurs on the 14^{th} ± 2 days of 28 days cycle. During this cycle following changes occurs:



Release Ovum:

- This released ovum enters into the fallopian tube and gets fertilized by the sperm.
- The fertilized ovum gets converted into blastocyst.
- This reaches the uterine cavity and gets implanted by 21^{st} to 23^{rd} day of the cycle.
- The implantation completed by 35^{th} day.
- Oral contraceptives are the hormonal preparation/pills use to prevent conception, fertilized ovum.
- During pregnancy ovarian hormonal levels are very high and they prevent ovulations.

- So ovulation can be prevented either by estrogen/progesterone/both.
- So when ovulation is prevented, pregnancy is automatically prevented.
- Oral contraceptives contain estrogen/progesterone/both.
- Oral contraceptive pills are prescribed medications used to prevent pregnancy.
- Also used to reduce menstrual cramps and anemia.

22.3 ORAL CONTRACEPTIVE

- The combined oral contraceptive pill is extremely effective, at least in the absence of intercurrent illness and of treatment with potentially interacting drugs .
- The oestrogen in most combined preparations (second-generation pills) is **ethinylestradiol**, although a few preparations contain **mestranol** instead.
- The progestogen may be **norethisterone**, **levonorgestrel**, **ethynodiol**, or-in 'third-generation' pills-**desogestrel** or **gestodene**, which are more potent, have less androgenic action and cause less change in lipoprotein metabolism, but which probably cause a greater risk of thromboembolism than do second-generation preparations.
- The oestrogen content is generally 20-50µg of ethinylestradiol or its equivalent, and a preparation is chosen with the lowest oestrogen and progestogen content that is well tolerated and gives good cycle control in the individual woman.
- This combined pill is taken for 21 consecutive days followed by 7 pill-free days, which causes a withdrawal bleed.
- Normal cycles of menstruation usually commence fairly soon after discontinuing treatment, and permanent loss of fertility (which may be a result of early menopause rather than a long-term consequence of the contraceptive pill) is rare.

MOA:

- Oestrogen inhibits secretion of FSH via negative feedback on the anterior pituitary, and thus suppresses development of the ovarian follicle.
- Progestogen inhibits secretion of LH and thus prevents ovulation; it also makes the cervical mucus less suitable for the passage of sperm.
- These also modify the cervical mucosa, secretion become thick and slowing down sperm penetration and prevent implantation.
- Oestrogen and Progestogen act in concert to alter the endometrium in such a way as to discourage implantation.
- They may also interfere with the coordinated contractions of cervix, uterus and fallopian tubes that facilitate fertilization and implantation.
- Potential unwanted and beneficial effects of the combined pill.

Common ADR:

- Weight gain, owing to fluid retention or an anabolic effect, or both.
- Mild nausea, flushing, dizziness, depression or irritability.
- Skin changes (e.g. acne and/or an increase in pigmentation).

- Amenorrhoea of variable duration on cessation of taking the pill.
- Mastalgia, migraine, chlosma, Increased vaginal secretion, Hypertension.
- Venous thrombo embolism, Decreased HDL, Bleeding irregularities.

22.3.1 Types of Oral Contraceptive

1. Combined pills
2. Progesterone only pills
3. Post coital (emergency pills)
4. Long acting progesterone only pills
5. Mini pills
6. Male pills

1. Combined Pills:

- They are daily medications containing two hormones, estrogen and progesterone.
- Estrogens- ethinylestradiol, mestranol
- Progesterone- levonorgestrol, norethisterone, ethinodiol
- Is taken for 21 days (from 5^{th} day to 21^{st} of the 28 days cycle) a month and the last 7 days are pill free days.
- Menstruation starts after medication is over.

Benefits:

- Decreases menstrual symptoms like irregular periods and inter menstrual bleeding.
- Decrease in benign breast diseases, uterine fibroids, functional cysts.
- Hypertension and increased risk of breast cancer.

ADR:

- Weight gain.
- Nausea, flushing, dizziness.
- Skin changes like acne or pigmentation.
- Amenhorrea.

Indications where estrogen must be avoided:

- History of blood clot disorders.
- History of stroke or heart attack.
- Severe hypertension.
- Diabetes that relates to blood vessel disorders.
- Poorly controlled diabetes.
- Severe headaches (migraine).
- Breast cancer.
- Liver cancer.

2. Progesterone only Pills:

- This pill is also called mini pills.

- This pill is given monthly once and it contains norgesterol.
- Used in cases where estrogen is contraindicated.
- Reasons are venous thrombosis, old age, smoking, Increased Blood pressure.
- Contraceptive effect is not so good as in combined pills.
- Mechanism of action is by causing alteration in the cervical mucus, making it inhospitable to sperms.
- Adverse reactions include irregular bleeding, weight gain and hair loss.

3. Post Coital Emergency Pills:

- Taken after unprotected intercourse to avoid pregnancy.
- Mostly contains Levonorgestrol or levonorgestrol along with estrogen (di ethyl stilbestrol).
- Must be taken within 72 hours of unsafe intercourse and reduces risk of pregnancy by 75%.

Acts by:

- Blocking ovulation.
- Altering mucus in cervix.
- Changing endometrium.

ADR:

- Nausea and vomiting so taken with domperidone.
- Dizziness.
- Fatigue.
- Headache.
- Breast tenderness.
- Bleeding between periods or heavier menstrual bleeding.
- Lower abdominal pain or cramps.

4. Long acting Progesterone only pills:

- Used for a duration of 2-5 months.
- Mostly progesterone alone used or Medroxyprogesterone used.
- Medroxy progesterone given intra muscular.
- Progesterone implanted subcutaneously in biodegradable capsules.
- Is also given as intrauterine device that can show action up to 5 years.
- Effective and safe.
- Adverse effects include irregular bleeding and headache.

5. Sequential Pill:

- Ethinyl estradiol is given from 5^{th} day to 20^{th} day and then combination of estrogen and progesterone given from 21^{st} to 25^{th} day of 28 days menstrual cycle.

ADR:

- Weight gain.
- Nausea, Vomiting, dizziness.
- Skin changes like acne or pigmentation.

6. Mini Pill:

- These pills contain only progesterone and given once in a month.
- This contain norgesterol

ADR:

- Nausea and vomiting so taken with domperidone.
- Dizziness.
- Fatigue.
- Headache.
- Breast tenderness.
- Bleeding between periods or heavier menstrual bleeding.
- Lower abdominal pain or cramps.

7. Male Pill:

- Male contraceptives or male are methods of preventing pregnancy that primarily involve the male physiology.
- Most commonly used male contraceptive methods are condoms, withdrawal method or vasectomy.
- Pills are rarely available for men.
- Gossypol, an extract of cotton, has been studied as a male contraceptive pill. It decreases sperm production; however this is permanent in 20% of people.

22.4 OESTROGENS

22.4.1 Introduction

- Oestrogens are synthesised by the ovary and placenta, and in small amounts by the testis and adrenal cortex.
- As for other steroids, the starting substance for oestrogen synthesis is cholesterol. The immediate precursors to the oestrogens are androgenic substances-androstenedione or testosterone.
- There are three main endogenous oestrogens in humans: *oestradiol*, *oestrone* and *oestriol*. Oestradiol is the most potent and is the principal oestrogen secreted by the ovary.
- The mass ovary contains ovarian follicle, the follicles are about 40,000 in number they are formed during foetal life.
- About 400 of them developed into adult life, the rest get degenerated, but all the follicels are lost at menopause.

- Ovulation occurs due to the rupture of ovarian follicle , this is stimulated by LH of anterior pituitary.
- After discharging the ovum, the rest of the follicle in the ovary form the corpus luteum.
- Estrogen are produced by the developing follicle , progesterore are produced by corpus lutem.

22.4.2 Physiological Roles

- Effects of exogenous oestrogen depend on the state of sexual maturity when the oestrogen is administered.
- In primary hypogonadism: oestrogen stimulates development of secondary sexual characteristics and accelerates growth.
- Puberty changes in female such as appearance of pubic and auxillary hair, development of breast.
- Development and growth of Vagina, uterus, fallopian tube and ovaries. Growth of uterus during pregnancy.
- The development of uterus endomatrium during proliferative stage of menstrual cycle depend on the secretions of estrogen from the ovaries.
- Stimulates protein and fat metabolism and growth of skeletal muscles.
- Libido is inspaired and metabolic changes like, Na^+ and water retension.
- In adults with primary amenorrhoea: oestrogen, given cyclically with a progestogen, induces an artificial cycle.
- In sexually mature women: oestrogen (with a progestogen) is contraceptive.
- At or after the menopause: oestrogen replacement prevents menopausal symptoms and bone loss. Oestrogens have several metabolic actions, including mineralocorticoid (retention of salt and water) and mild anabolic actions.
- They increase plasma concentrations of high-density lipoproteins, a potentially beneficial effect that may contribute to the relatively low risk of atheromatous disease in premenopausal women compared with men of the same age.
- Oestrogens increase the coagulability of blood, and increase the risk of thromboembolism. This effect is dose-related.

MOA:

- As with other steroids, oestrogen binds to type 4 nuclear receptors. There are at least two types of oestrogen receptor, termed *ER*α; and *ER*β, the roles of which are currently being investigated using mice in which the gene coding one or other of these has been 'knocked out' .
- Binding is followed by interaction of the resultant complexes with nuclear sites and subsequent genomic effects-either gene transcription (i.e. DNA-directed RNA and protein synthesis) or gene repression (inhibition of transcription).

- In addition to these 'classic' intracellular receptors, some oestrogen effects, in particular its rapid vascular actions, may be initiated by interaction with membrane receptors.
- Acute vasodilatation caused by 17-β-oestradiol is mediated by nitric oxide, and a plant-derived (*phyto-*) oestrogen called **genistein** (which is selective for ERβ, as well as having quite distinct effects from inhibition of protein kinase C) is as potent as 17-β-oestradiol in this regard.
- Oestrogen receptor modulators (receptor-selective oestrogen agonists or antagonists) are mentioned briefly immediately below this section.

22.4.3 Classifications

- **Natural estrogen:** Estradiaol, Estrone, Estriol
- **Semisynthetic estrogens:** Ethinyl estradiaol, Mestranol, Quinestrol
- **Synthetic estrogens:** Diethylstillbestrol, Methallenosstrol, Chlorotrianisene
- **Non steroidal agents with estrogenic activity:** Hexestrol, Dienestrol, Benzestrol, Methallenoestril.

22.4.4 Preparations

- Many preparations (oral, transdermal, intramuscular, implantable and topical) of oestrogens are available for a wide range of indications.
- These preparations include natural (e.g. **estradiol**, **estriol**) and synthetic (e.g. **mestranol**, **ethinylestradiol**, **stilbestrol**) oestrogens.
- Oestrogens are presented either as single agents or combined with progestogen.

Anti-oestrogens

- Replacement therapy for:
  - Primary ovarian failure (e.g. Turner's syndrome).
  - Secondary ovarian failure (menopause) for flushing, vaginal dryness and to preserve bone mass.
  - Contraception.
  - Prostate and breast cancer (these uses have largely been superseded by other hormonal manipulations)
  - To treat oestrogen-sensitive breast cancer (tamoxifen).
  - To induce ovulation (clomiphene) in treating infertility.

ADME:

- Natural as well as synthetic oestrogens are well absorbed in the gastrointestinal tract, but after absorption the natural oestrogens are rapidly metabolised in the liver, whereas synthetic oestrogens are degraded less rapidly.
- There is a variable amount of enterohepatic cycling, which forms the basis for drug interaction, because broad-spectrum antibiotic use alters bowel flora and can thereby render oral contraception ineffective. Most oestrogens are readily absorbed from skin and mucous membranes.

- They may be given topically in the vagina as creams or pessaries for local effect. In the plasma, natural oestrogens are bound to albumin and to a sex steroid-binding globulin.
- Natural oestrogens are excreted in the urine as glucuronides and sulfates.

Therapeutic Uses:

- To induces menses in primary and secondary amenorrhoea.
- To reduced disturbing menopausal syndrome.
- Post menopausal osteoporosis.
- Acne and Hirsutism.
- Oral contraceptives.

ADR:

- Tenderness in the breasts, nausea, vomiting, anorexia, retention of salt and water with resultant oedema, and increased risk of thromboembolism.
- Used intermittently for postmenopausal replacement therapy, oestrogens cause menstruation-like bleeding.
- Oestrogen causes endometrial hyperplasia unless given cyclically with a progestogen. When administered to males, oestrogens result in feminisation.
- Oestrogen administration to pregnant women can cause genital abnormalities in their offspring. Carcinoma of the vagina was more common in young women whose mothers were given stilbestrol in early pregnancy in a misguided attempt to prevent miscarriage.

Oestrogen Receptor Modulator:

- **Raloxifene**, a 'selective oestrogen receptor modulator', has antioestrogenic effects on breast and uterus but oestrogenic effects on bone, lipid metabolism and blood coagulation.
- It is used for prevention and treatment of postmenopausal osteoporosis and reduces the incidence of oestrogen receptor-positive breast cancer, although its role in therapy of breast cancer is undefined. Unlike oestrogen, it does not prevent menopausal flushes.

Antioestrogens:

- Antioestrogens compete with natural oestrogens for receptors in target organs.
- **Tamoxifen** has antioestrogenic action on mammary tissue but oestrogenic actions on plasma lipids, endometrium and bone.
- It produces mild oestrogen-like adverse effects consistent with partial agonist activity.
- The tamoxifen-oestrogen receptor complex does not readily dissociate, so there is interference with the recycling of receptors.

- Tamoxifen up-regulates transforming growth factor-β, decreased function of which is associated with the progression of malignancy, and which has a role in controlling the balance between bone-producing osteoblasts and bone-resorbing osteoclasts.
- **Clomiphene** inhibits oestrogen binding in the anterior pituitary, so preventing the normal modulation by negative feedback and causing increased secretion of GnRH and gonadotrophins.
- This results in a marked stimulation and enlargement of the ovaries and increased oestrogen secretion.
- The main effect of their antioestrogen action in the pituitary is that they induce ovulation. It is used in treating infertility caused by lack of ovulation.
- Twins are common, but multiple pregnancy is unusual.

22.5 PROGESTERONE

22.5.1 Introduction

- It is secreted from corpus luteum from the healing scar of the ovary after ovulation.
- It is synthesized in placenta, adrenal and testes
- It is secreted during the last month of pregnancy. It is an important intermediate in the synthesis of steroids
- Progesterone inhibits ovulation, advance pregnancy, stabilies uterus and enlargement of breast.
- The natural progestational hormone (*progestogen*) is progesterone . This is secreted by the corpus luteum in the second part of the menstrual cycle, and by the placenta during pregnancy. Small amounts are also secreted by testis and adrenal cortex.

MOA:

- Progestogens act, as do other steroid hormones, on nuclear receptors. The density of progesterone receptors is controlled by oestrogens.

22.5.2 Classifications

- **Natural:** Progesterone.
- **Derivatives of progesterone:** Hydroxyprogesterone, Dehydrogesterone, Methoxy-progesterone.
- **Derivatives of testosterone:** Ethisterone, Dimethisterone.
- **Derivatives of 19-nor testosterone:** Norethisterone, Norethynodrel, Norgestrol, Lynesterol.

Preparations:

- There are two main groups of progestogens.
- The naturally occurring hormone and its derivatives (e.g. **hydroxyprogesterone**, **medroxyprogesterone**, **dyhydrogesterone**). Progesterone itself is virtually inactive orally, because after absorption it is metabolised in the liver, and hepatic extraction is nearly complete.

- Other preparations are available for oral administration, intramuscular injection, or administration via the vagina or rectum.
- Testosterone derivatives (e.g. **norethisterone**, **norgestrel** and **ethynodiol**) can be given orally. The first two have some androgenic activity and are metabolised to give oestrogenic products.
- Newer progestogens used in contraception include **desogestrel** and **gestodene**; they may have less adverse effects on lipids than ethynodiol and may be considered for women who experience side effects such as acne, depression or breakthrough bleeding with the older drugs.
- However, these newer drugs have been associated with higher risks of venous thromboembolic disease.

22.5.3 Physiological Actions

- Premenstrual stimulation of estrogens and preparations of the endomatrium for menstrual cycle. Menstrual occurs when progesterone level falls.
- Pregnancy is sustained because of progesterone secretion.
- It neutralizes Oxytocin of pituitary and protect pregnancy by preventing uterine contraction.
- During pregnancy menstruation is inhibiting and development of breast are due to progesterone.
- Birth passage is relaxed by progesterone and so its widen to facilitate birth of baby.
- Protein metabolism is decreased with progesterone.
- Estrogen-progesterone in combination may be synergestic or opposite, competitive depend upon the stage of in the sex life of a women.
- Progesterone acts, in turn, on oestrogen-primed endometrium, stimulating the *secretory phase* of the cycle, which renders the endometrium suitable for the implantation of a fertilised ovum. During this phase, cervical mucus becomes more viscous, less alkaline, less copious and in general less welcoming for sperm.
- Progesterone exerts negative feedback on hypothalamus and pituitary, decreasing the release of LH.
- It also has a thermogenic effect, causing a rise in body temperature of about 0.5°C at ovulation, which is maintained until the end of the cycle.
- If implantation of the ovum does not occur, progesterone secretion stops, triggering menstruation.
- If implantation does occur, the corpus luteum continues to secrete progesterone, which, by its effect on the hypothalamus and anterior pituitary, prevents further ovulation.
- The chorion (an antecedent of the placenta) secretes *human chorionic gonadotrophin* (*HCG*), which maintains the lining of the womb during pregnancy.

- For reasons that are not physiologically obvious, HCG has an additional pharmacological action in stimulating ovulation.
- As pregnancy proceeds, the placenta develops further hormonal functions and secretes a gamut of hormone variants (often with post-translational modifications), including *gonadotrophins* as well as *progesterone* and *oestrogens*.
- Progesterone secreted during pregnancy controls the development of the secretary alveoli in the mammary gland, while oestrogen stimulates the lactiferous ducts.
- After parturition, oestrogens, along with *prolactin* are responsible for stimulating and maintaining lactation, whereas high doses of exogenous oestrogen suppress this.
- Progesterone controls the later secretory phase, and has negative feedback effects on both hypothalamus and anterior pituitary.
- If a fertilized ovum is implanted, the corpus luteum continues to secrete progesterone.
- After implantation, human chorionic gonadotrophin from the chorion becomes important, and later in pregnancy progesterone and other hormones are secreted by the placenta.

ADME:

- Injected progesterone is bound to albumin, not to the sex steroid-binding globulin. Some is stored in adipose tissue.
- It is metabolized in the liver, and the products, pregnanolone and pregnanediol, are conjugated with glucuronic acid and excreted in the urine.

Therapeutic uses:

- To prevent the threatened operation in the first three month of pregnancy
- Recession of endomatrium cancer occurs if large dose of progesterone is administered
- To control bleeding during and after delivery
- Use as replacement therapy during irregular bleeding.
- As contraceptives:
  - With oestrogen in *combined oral contraceptive pill*.
  - As injectable or implantable progesterone-only contraception.
  - As part of an intrauterine contraceptive system.
  - Progesterone with high dose of estrogen use as contraceptives.
  - As progesterone-only contraceptive pill.
- Use in cystic fibrosis.
- If high dose of progesterone with lower dose of estrogen administered in first week of menstrual cycle it help in conceive.
- It is a pill of contraception, a pill to conceive, depend upon time of administration.
- In *endometrial carcinoma*; use in breast and renal cancer has declined.

ADR:

- Nausea, breast discomfort, headache, fatigue, mental depression and rarely liver damage.
- Weak androgenic actions, acne, fluid retention, weight change, depression, change in libido, breast discomfort, premenstrual symptoms, irregular menstrual cycles and breakthrough bleeding.
- There is an increased incidence of thromboembolism.
- Poorly validated uses have included various menstrual disorders.

22.5.4 Progestogens and Antiprogestogens

- Medical termination of pregnancy: **mifepristone** (partial agonist) combined with a prostaglandin (e.g. **gemeprost**).
- The endogenous hormone is progesterone. Examples of synthetic drugs are the progesterone derivative **medroxyprogesterone** and the testosterone derivative **norethisterone**.
- Mechanism of action involves intracellular receptor/altered gene expression, as for other steroid hormones. Oestrogen stimulates synthesis of progesterone receptors, whereas progesterone inhibits synthesis of oestrogen receptors.
- Main therapeutic uses are in oral contraception and oestrogen replacement regimens, and to treat endometriosis.
- The antiprogestogen **mifepristone**, in combination with prostaglandin analogues, is an effective medical alternative to surgical termination of early pregnancy.

22.5.5 Antiprogestogens

- Mifepristone is a partial agonist at progesterone receptors. It sensitises the uterus to the action of prostaglandins. It is given orally and has a plasma half-life of 21 hours.
- Mifepristone is used, in combination with a prostaglandin (e.g. gemeprost; see below), as a medical alternative to surgical termination of pregnancy.

22.6 ANDROGENS AND ANABOLIC STEROIDS

22.6.1 Introduction

- Androgen is the hormones that control the building up of proteins and male secondary sex characteristics.
- Natural androgen is testosterone that is secreted mainly from the testis, adrenal cortex and also in ovary.
- Testosterone is the main natural androgen. It is synthesized mainly by the interstitial cells of the testis, and in smaller amounts by the ovaries and adrenal cortex.
- Androgen is formed in the foetal testis under the influence of maternal gonado-tropin. It causes descent of the testis, later no androgen forms until puberty
- At puberty the hypophysial cells stimulate the laydig cells to produced androgen. It leads to the development of testis and secondary sexual characters

- Adrenal production of androgens is under the control of adreno corticotrophic hormone (corticotrophin). As for other steroid hormones, cholesterol is the starting substance. Dehydroepiandrosterone and androstenedione are important intermediates. They are released from the gonads and the adrenal cortex, and converted to testosterone in the liver.

22.6.2 Physiological Actions

- In general, the effects of exogenous androgens are the same as those of testosterone, and depend on the age and sex of the recipient.
- If administered to boys at the age of puberty, there is rapid development of secondary sexual characteristics maturation of the reproductive organs like prostrate, seminal vesicles and the external genitals is also stimulated.
- Life and fertility of spermatozoa is maintained
- Development of secondary sexual characters like appearance of moustache, beard, pubic and auxiliary hair etc.
- Growth of larynx, thickening of vocal cord and loudness of voice.
- The anabolic effects can be accompanied by retention of salt and water.
- The skin thickens and may darken, and sebaceous glands become more active (which can result in acne).
- Bony structure becomes more heavy and strong because of the stimulation by anabolic fraction of the testosterone.
- Muscular development is more in male compare to female because of the stimulation by anabolic fraction of the testosterone, which increased protein metabolism.
- Blood volumes and RBCs are more in male compared to female because of the stimulation by anabolic fraction of the testosterone.
- Water percentage is more in male since anabolic fraction stimulates Na^+ and water retention.
- High temperature inhibits testicular activity. Libido is inspired in male.
- Physiological and behavioral changes like feeling of well-being and an increase in physical vigour, and may increase libido. Whether they are responsible for sexual behaviour as such is controversial, as is their contribution to aggressive behaviour.
- Administration of 'male' doses to women results in masculinisation, but lower doses (e.g. 300µg/day testosterone patches) restore plasma testosterone to normal female concentrations and improve sexual dysfunction in women following ovariectomy, without adverse effects.

22.6.3 Mechanism of Action

- In most target cells, testosterone works through an active metabolite, *dihydrotestosterone*, to which it is converted locally by a 5α-reductase enzyme.

- In contrast, testosterone itself causes virilisation of the genital tract in the male embryo and regulates LH/ICSH production in anterior pituitary cells.
- Testosterone and dihydrotestosterone modify gene transcription by interacting with intracellular receptors.

ADME:

- Its well absorbed when given by oral route, undergoes metabolism in liver and hence ineffective therapeutically.

Therapeutic uses:

- Rejuvinate testis and in impotance to increased testicular secretion
- It prevents body atrophy in old people.
- Use in severe trauma and prolonged illness.
- To overcome osteoporosis in old people.
- In women testosterone can be used to produced symptomatic relief from breast cancer
- It is also used to check uterine bleeding in menorrhagia.

ADR:

- Liver damage, decreased release of gonadotropin hormones, increased salt and water retention leads to edema.
- In female- produced acne, masculisation.

Preparations:

- Testosterone itself can be given by subcutaneous implantation or by transdermal patches.
- Various esters (e.g. enanthate and proprionate) are given by intramuscular depot injection. Testosterone undecanoate and mesterolone can be given orally.

22.7 ANABOLIC STEROIDS

- Androgens can be modified chemically to alter the balance of anabolic and other effects. Such 'anabolic steroids' (e.g. **nandrolone**) increase protein synthesis and muscle development, but clinical use (e.g. in debilitating disease) has been disappointing.
- They are used in the therapy of aplastic anaemia, and (notoriously) abused by some athletes. Unwanted effects are described above, under *Androgens*. In addition, cholestatic jaundice, liver tumours and increased risk of coronary heart disease are recognised adverse effects of high-dose anabolic steroids.
- Androgens and the hormonal control of the male reproductive system.
- Gonadotrophin-releasing hormone from the hypothalamus acts on the anterior pituitary to release both follicle-stimulating hormone, which stimulates gametogenesis, and luteinising hormone (also called interstitial cell-stimulating hormone), which stimulates androgen secretion.
- The endogenous hormone is testosterone; intramuscular depot injections of testosterone esters are used for replacement therapy.

- Mechanism of action is via intracellular receptors.
- Effects depend on age/sex, and include development of male secondary sexual characteristics in prepubertal boys and masculinisation in women.

22.7.1 Classification

- **Derivatives of testosterone:** Nondrolone phenyl propionate, Nandrolone decanloate
- **Derivatives of methyl testosterore:** Oxymetholone, Oxondrolone, Stanozolol, Methly testosterone.

22.7.2 Pharmacological Actions

- **Protein metabolism:** They promote protein metabolism. This manifests as increased in muscle mass and body weight.
- **Anti catabolic effects:**
  The catabolic effects of glucocorticoids are counter − acted and a positive nitrogen balance is produced.
- **Miscellaneous:** Progestational activity and decreased in bone resorption which prevents osteoporesis.

22.7.3 Therapeutic Uses

- In chronic illness to accelerate rebuilding of tissues.
- To promote growth in hypogonaldal children and pituitary dwarfs.
- Breast cancer in female.
- Androgens (**testosterone** preparations) as hormone replacement in:
  - Male hypogonadism due to pituitary or testicular disease
  - Hyposexuality following ovariectomy (e.g. 300µg/day patches).

Antiandrogens:

- Both *oestrogens* and *progestogens* have antiandrogen activity, oestrogens mainly by inhibiting gonadotrophin secretion and progestogens by competing with androgens in target organs. **Cyproterone** is a derivative of progesterone and has weak progestational activity. It is a partial agonist at androgen receptors, competing with dihydrotestosterone for receptors in androgen-sensitive target tissues.
- Through its effect in the hypothalamus, it depresses the synthesis of gonado-trophins. It is used as an adjunct in the treatment of prostatic cancer during initiation of GnRH treatment.
- It is also used in the therapy of precocious puberty in males, and of masculinisation and acne in women.
- It also has a central nervous system effect, decreasing libido, and has been used to treat hypersexuality in male sexual offenders.
- **Flutamide** is a non-steroidal antiandrogen used with GnRH in the treatment of prostate cancer.
- Drugs can have antiandrogen action by inhibiting synthetic enzymes.

- **Finasteride** inhibits the enzyme (5α-reductase) that converts testosterone to dihydrotestosterone, which has greater affinity than testosterone for androgen receptors in the prostate gland.
- Finasteride is well absorbed after oral administration, has a half-life of about 7 hours, and is excreted in the urine and faeces.
- It is used to treat benign prostatic hyperplasia, although α_1-adrenoceptor antagonists, **terazosin** or **tamsulosin**, are more effective (working by the entirely different mechanism of relaxing smooth muscle in the capsule of the prostate gland). Surgery is the preferred option (especially by surgeons).

ADME:

- If given orally, testosterone is rapidly metabolised in the liver. It is therefore usually injected.
- Virtually all testosterone in the circulation is bound to plasma protein-mainly to the sex steroid-binding globulin.
- The elimination half-life of free testosterone is short (10-20 minutes). It is inactivated in the liver by conversion to androstenedione.
- This has weak androgenic activity in its own right and can be reconverted to testosterone, although approximately 90% of testosterone is eliminated as metabolites rather than the parent compound.
- Synthetic androgens are less rapidly metabolized, and some are excreted in the urine unchanged.

Therapeutic uses:

- Antiandrogens (e.g. **flutamide, cyproterone**) are used as part of the treatment of prostatic cancer.
- 5α-Reductase inhibitors (e.g. **finasteride**) are used in benign prostatic hypertrophy.

ADR:

- Cholestic jaundice, Liver damage, Sodium and water retention on prolonged use.
- Unwanted effects of androgens include eventual decrease of gonadotrophin release, with resultant infertility, and salt and water retention leading to oedema. Adenocarcinoma of the liver has been reported.
- Androgens impair growth in children (via premature fusion of epiphyses), cause acne, and lead to masculinisation in girls.
- Adverse effects of testosterone, replacement and monitoring for these are reviewed by Rhoden & Morgentaler.

QUESTION

1. Write a note on androgens and anabolic sterioids.

■■■

Chapter ... 23

Drugs Acting on the Uterus

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the MOA of Oxytoin.*

23.1 INTRODUCTION

- Drugs acting on uterus are uterine stimulants and uterine relaxants.
- These drugs directly can affect endomatrium amd myomatrium. Important drugs affecting endomatrium and myomatrium are estrogens and progesterone.
- Myomatrium receives both sympathetic and parasympathetic innervations. So autonomic drugs can affect motility.
- Drugs affecting directly and indirectly moderately affect the normal and gestational status of uterus.

23.2 UTERINE STIMULANTS

- These drugs increased uterine motility so they are called Ecbolics/ Abortifacients/ Oxytocics.

23.2.1 Oxytocin

- Oxytocin is a powerful hormone. Oxytocin's level increases when, we hug, or kiss someone. It plays roles especially in sexual reproduction, the most common situations are before and after childbirth.
- It is released in large amounts after distension of the cervix and uterus during labor, facilitating birth, maternal bonding, and after stimulation of the nipples, breast-feeding.
- This hormone affects orgasm, social recognition, pair bonding, anxiety, and breastfeeding. That's why sometimes it is called the "love hormone".
- Oxytocin is produced in the hypothalamus. The myoepithelial cells of the breast, which surround the alveoli of the mammary gland, and the smooth muscle cells of the uterus.
- Oxytocin is controlled by a positive feedback mechanism where release of the hormone causes an action which stimulates more of its own release. When

contraction of the uterus starts, for example, oxytocin is released which stimulates more contractions and more oxytocin to be released. In this way, contractions increase in intensity and frequency.

- There is also a positive feedback involved in the milk-ejection reflex. When a baby sucks at the breast of its mother, the stimulation leads to oxytocin secretion into the blood which then causes milk to be let down into the breast. Oxytocin is also released into the brain to help stimulate further oxytocin secretion. These processes are self-limiting; production of the hormone is stopped after the baby is delivered or when the baby stops feeding.
- Oxytocin is a mammalian hormone that has many functions, the most notable having to do with pregnant or lactating mammals. In this capacity, some of the hormone's main function are preparing a female's body for childbirth.
- Oxytocin as feed inhibitor, maintaining homeostasis In consummatory behavior.
- The structure of the hormone is very similar to that of vasopressin, also a nonapeptide with a sulfur bridge, whose sequence differs from oxytocin by two amino acids.

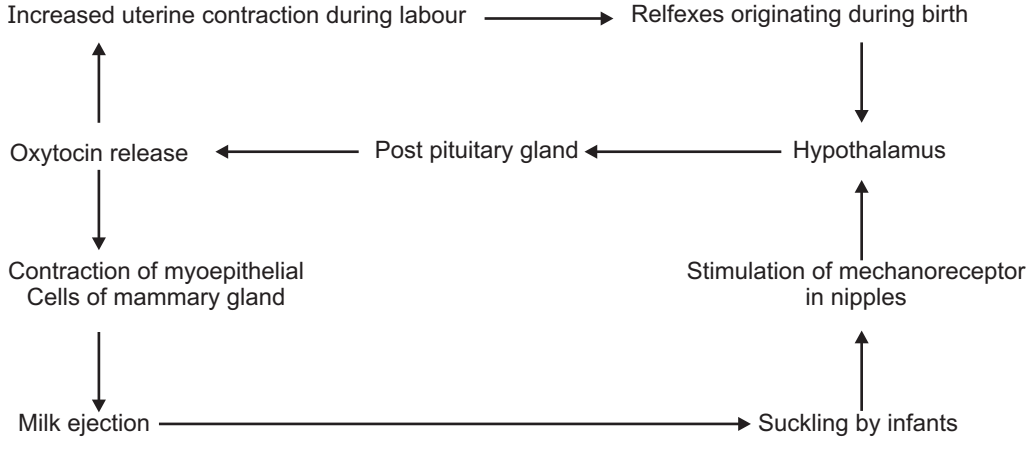
Classifications:

- **Posterior Pituitary Hormone:** Oxytocin.
- **Ergot Alkaloids:** Ergometrine, Methyl Ergometrine.
- **Prostaglandin:** PGE_2, $PGF_{2\alpha}$, Misoprostol.
- **Miscellaneous:** Quinine, Ethacridine.

Oxytocin means: OXYS-Quick; TOKOS-Child Birth.

- It is a nonapeptide hormone derived from paraventricular nucleus of hypothalamus and release from posterior pituitary gland along with ADH.

MOA:



Flowchart of Mechanism and Pharmacological actions of Oxytocin and factors controlling Oxytocin release:

- Oxytocin acts on Oxytocin receptors which is mediated by:
  - It acts through IP_3 /DAG system and increased influx of Ca^{2+} release, which cause contraction.
  - Caused depolarization.
  - Increased PG synthesis and release by endomatrium which causes contraction.
  - All the above actions together causes Uterine contraction.

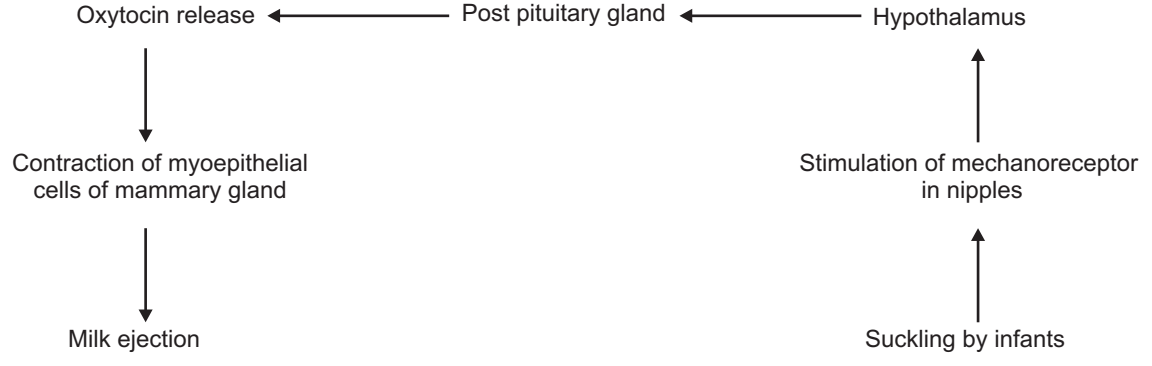
Pharmacological Actions:

Uterus:

- Oxytocin increases the force and frequency of uterine contraction.
- Basal tone of uterus is also increased at high dose.
- At low dose there is relaxation in between the contraction.
- Increased uterus contraction is due to heightened electrical activity of myomatrium.

Breast:

- It cause contraction of myo epithelial cells surrounding mammary gland, leads to milk ejection.



CVS:

- Promotes Oxytocin release, which leads to vasodilatation and decreased BP.

Kidney:

- High dose decreases urine output.

ADME:

- Being peptide is inactivated by oral route of administration. So it is administered by parenteral/intranasal route. Rapidly degraded in liver and kidney by oxytinase enzyme and excreted in urine.

Therapeutic activity:

- Induction of labour.
- In uterine inertia.
- Postpartum hemorrhage.

- Cesearean section.
- Abortion.
- Breast engorment and for milk ejection.

ADR:

- Due to powerful contraction during delivery it may cause damage of soft tissue of mother and baby.
- Asphyxia and Death.

23.2.2 Ergometrine

MOA:

- It acts through IP_3 /DAG system and increased influx of Ca^{2+} release, which cause contraction.
- Caused depolarization.
- Increased PG synthesis and release by endomatrium which caused contraction.
- All the above actions together caused uterine contraction.

Pharmacological Actions:

Uterus:

- It increases the force and frequency of uterine contraction.
- Basal tone of uterus is also increased at high dose.
- At low dose there is relaxation in between the contraction.
- Increased uterus contraction is due to heightened electrical activity of myomatrium.

CVS:

- Weaker vasoconstrictor, cause insignificant raise in BP.

GIT:

- At high dose increased GIT peristalysis.

ADME:

- Well absorbed and distributed well, Metabolised in liver and excreted in urine.

Therapeutic Uses:

- Management of third stage of pregnancy.
- To control and prevent post partum hemorrhage.
- To ensure normal ovulation.

ADR:

- Due to powerful contraction during delivery it may cause damage of soft tissue of mother and baby.

23.3 UTERINE RELAXANTS

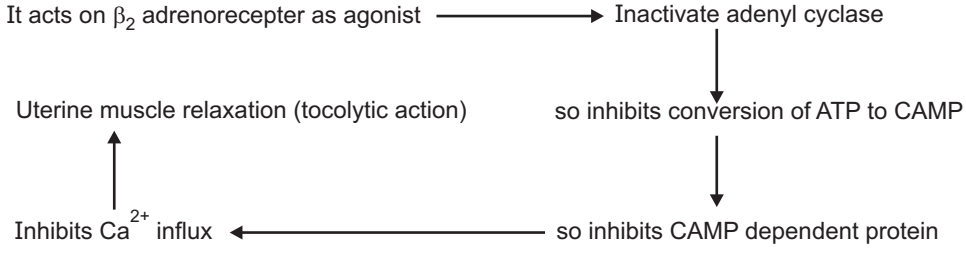
- The primary role of uterine relaxants is to prevent pre-mature delivery or labour that starts before the end of 37th week of gestation.
- Premature labour can be developed spontaneously or may follow genetically pre-disposed early rupture of foetal membrane.
- Tocolytics agents either acts directly to suppress myoepitheliam smooth muscles contraction by decreasing intracellular Ca^{2+} concentrations and reducing the efflux of Ca^{2+} on muscles contraction.
- Or they may act indirectly by inhibiting the synthesis or release or receptor actions of PG or Oxytocin.
- These drugs also used to delay labour, arrest threatened abortion or to treat dysmenorrheal.
- Suppression of labour also important to allow foetus to mature to initiate glucocorticoids therapy to mother, so that lungs of new born baby get sufficient time for maturation and neonatal respiration distress can be reduced.
- Tocolytics agents use for this pupose are β adrenorecepter agonists, magnesium sulphate, Ca^{2+} channel blocker, hydroxyl progesterone, PG synthesis inhibitors and Oxytocin receptor antagonists.

Classifications:

- Selective β adrenoreceptor agonists- Ritodrine, Salbutamol, Terbutaline, Orciprenaline, Isoxsuprine.
- Calcium Channel blocker: Verapamil, Nifedipine, Nicardipine, Amlodipine.

Ritrodine:

MOA:



Therapeutic uses:

- Uterine relaxants.

ADR:

- Hypotension, Bradycardia, Arrthymia, Pulmonary edema, Hypokalamia, Hyperglyemia.

Calcium Channel Blockers:

MOA:

- Calcium Channel Blockers blocks L-Type of voltage gated calcium channel in myomatrium.

↓

Inhibits Calcium influx

↓

Uterine muscle relaxation (Tocolytic action)

Therapeutic uses:

- Uterine relaxants.

ADR:

- Hypotension, Bradycardia, Foetal hypoxia.

Magnesium Sulphate:

MOA:

- It directly uncouple the excatation coupling and inhibits cellular action potential.
- Cause relaxation of uterine muscles, tocolytic action.
- It is preferred over β_2 agonists if the patients is having cardiac and hyperthyroidism problem.

Therapeutic uses:

- Uterine relaxants.

ADR:

- Feeling of warmth, sweating, flushing, dry mouth, nausea, headache, palpitation.

QUESTIONS

1. Explain the regulation and action of female sex hormones.
2. Adverse effect and uses of oxytocin.

■■■

Chapter ... 24

Bio-Assays General Aspects

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the principle and methods of bioassay.*

24.1 INTRODUCTION

- Bioassays are procedures by which the potency or the nature of the substance is estimated by studying its effects on living matter. The basic principle of such assays is to compare how much of a sample being tested produces the same biological effect as a given quantity of a standard preparation. They are generally carried out by using either intact animals; as in the case of bio-assay of insulin by using rabbits or mice, bio-assay of digitalis by using guinea pigs or pigeons; or isolated animal organs or tissues as in the case of bio-assay of histamine by using guinea pig ileum, bio-assay of acetyl choline by using frog's rectus abdominus muscle preparation.
- Bio-assay can be defined as a procedure for determining the quantitative relationship between the dose of a drug and the magnitude of biological response it evokes; or the determination of the potency or concentration of a biologically active drug of physical, chemical or biological origin by using a biological indicator. The biological indicator could be a whole animal like frog, mouse, rat, guinea pig, cat, dog etc., or part of an animal like isolated heart, strip of stomach, uterus, ileum, jejunum, colon, diaphragm, rectus abdominus muscle etc., or blood cells or micro-organisms. Unlike the physical and chemical methods, the bioassays are always comparative. The effect of the test substance is compared with that of the reference standard.

24.2 RESERVATIONS OF BIOASSAYS

- Bioassay procedures are generally employed:
  - When a chemical assay for the substance is not available or the substance gets inactivated by interacting with the chemicals as the case with hormones.
  - When the quantity of the sample is too small.
  - When the bioassay is more sensitive than the chemical assay.
  - To estimate the concentrations of active principles present in the tissue extracts such as the endogenous mediators like Ach, 5-HT, PGs etc.

- To measure the drug toxicity.
- To measure the pharmacological activity of a new or chemically unidentified substance.

- Bioassays are also essential in the development of new drugs. In the pre-clinical assessment of a new compound, the biological activity is compared with that of a known (standard) compound using appropriate test systems. In such studies the tests must be simple, reproducible and economical. Biological assessment of a new compound generally consists of carrying out a battery of such assays and based on these tests, constructing a profile of activity. Clinical testing of drugs is guided by such profile of activity generated in animals.

24.3 USE OF STANDARDS

- Bioassays are designed to measure relative potency of two preparations usually a standard and an unknown. Use of a standard substance for comparison also helps in solving problems arising out of biological variations. The observed response of the unknown would be always relative to the effect that is produced by a standard substance. The standard substance is a pure substance and in official bioassays it refers to Pharmacopeial standards. In case of hormones, biological products and vaccines it is often necessary to establish the standard response of the standard substance against which unknown samples are calibrated.

24.4 PRINCIPLES

- Burn and Dale enunciated certain principles for conducting bioassays. These include,
  - All bioassays must be comparative and compared against a standard drug or preparation. This is to overcome errors due to biological variation.
  - The standard and the new drug should be identical to each other, so that their dose-response curves will have the same slope and would be parallel to each other, i.e., the potency ratio would be constant all along the response levels.
  - The method for comparing the unknown and the standard should preferably test the therapeutic property of the drug. Ideally, an analgesic is tested for analgesic activity and an anti-convulsant for anti-convulsant activity. However, this is not always convenient or possible to do so. For e.g., For estimating digitaloid drugs, the cardiac arrest in pigeon or guinea pig is used as the end point; which is the toxic effect of the drug but not the therapeutic effect.
  - The method should eliminate all possible errors and allow an estimation of the error due to biological variation in different animals/persons at any one time and in the same animal/person at different times. Precautions should be taken to minimize errors due to biological variations. These include the selection of suitable animals/preparations and also the selection of the experimental conditions.
  - The results of the test should be subject to statistical analysis to minimize errors due to biological variation.

24.5 BIOLOGICAL VARIATION

- Biological variation means that no two-test preparations can be expected to give identical results and that the same preparation at some other point of time may be expected to react differently. In all biological methods, the interactions between the chemicals and the biological systems are observed. One of the characteristics of the biological systems is that they are continually changing and therefore are never the same. A number of factors have been found which alter the responsiveness of the isolated biological systems as well as the whole animals. These include,
  - Environment
  - Temperature
  - Diet
  - Solvent
  - Species
  - Strain
  - Sex
  - Age
  - Weight
  - Season and
  - Inexperience.

24.6 CLASSIFICATION

- Bioassays may be broadly classified into 2 types depending upon the type of responses recorded, i.e.,
  - Quantal
  - Graded

(A) Quantal Bioassays:

- The quantal bioassay is an all or none phenomenon. In this form of bioassay at least 2 groups of animals are employed. The response is either positive or negative, i.e., there is no intermediate response. For e.g.,
  - Insulin induced hypoglycemic convulsive reaction, i.e., an animal receiving insulin either show convulsions or does not;
  - The animal receiving a dose of a drug, as in toxicity studies, either dies or does not die.
- This method of bioassay is not very accurate, but can be employed for the following cases:
  - Comparison of threshold responses.
  - Comparison of ED_{50} or LD_{50}.

$$\text{Concentration of test substance} = \frac{\text{Threshold dose of Std.}}{\text{Threshold dose of test}} \times \text{Concentration of Std.}$$

$$\text{Concentration of test substance} = \frac{ED_{50}/LD_{50} \text{ of Std.}}{ED_{50}/LD_{50} \text{ of Test}} \times \text{Concentration of Std.}$$

(B) Graded Bioassays:

- Graded responses are those that are measured by continuous variables such as weight, body temperature, blood glucose level, blood pressure, the number and strength of contractions of heart, respiratory rate, the extent to which an isolated tissue contracts or relaxes etc., The graded responses may be assessed by using either the whole animal or a part of the animal.
- The graded bioassays are based on the observations that there is a proportionate increase in the observed response with a subsequent increase in the concentration or dose. Then the test responses are compared with that of the standard. The parameters employed in such bioassays are based on the nature of the effect of the substance that is expected to produce. For e.g., contraction of a smooth muscle preparation (guinea pig ileum) for assaying histamine; the study of blood pressure response in case of adrenaline.
- This type of assay gives almost identical results. The choice of the assay depends upon,
  - Precision of assay demands.
  - Quantity of the sample available.
  - Availability of experimental animals.
- The various methods of graded bioassays are,

1. Matching Dose Bioassay:

- It is the simplest form of all graded bioassays and involves no calculations. In this type of bioassay, the response of the standard substance is recorded first and then the response of the test substance is tried to match with that of the standard by a trial and error process, until they produce equal effects. It is also called as the *analytical dilution assay* as the assay involves the determination of the factor by which the test substance is either diluted or concentrated in order to produce a response that is equal to that of a known amount of the standard drug. A corresponding concentration of the test substance is then calculated.
  - This assay is generally employed when the ample amount of sample is available. Since the assay does not involve the recording of CRC, the sensitivity of the preparation is not taken into consideration.

Advantages:

- The assay does not depend on the assumption of a dose response relationship.

Disadvantages:

- Purely subjective method.
- Inefficient as preliminary effects are not utilized in final assessment.
- Lot of experimental errors, which cannot be determined.
- A crude method and not the exact method of determining the potency of a drug.
- Precision and reliability are poor.

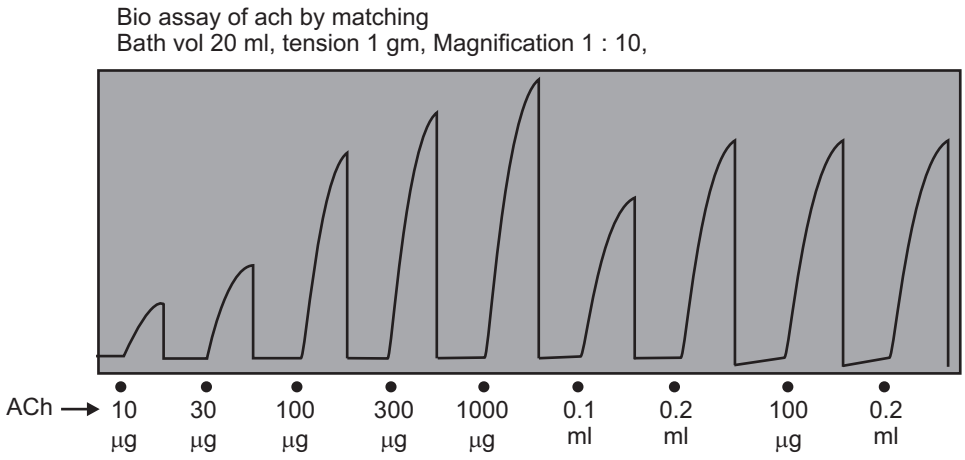


Fig. 24.1: Bioassay of Ach by matching method

2. Bracketting Dose Bioassay:

- It is also a simple assay procedure, which is employed when the test sample is very small. In this method, the response due to a constant dose of the test substance is bracketed between the greater and the smaller responses due to varying doses of standard substance that provides the closest bracket. Initially, two responses of the standard substance are taken. The doses are adjusted such that one is giving response of approximately 20% and the other 70% of the maximum. The response of unknown, which lies in between the two responses of standard doses, is taken. The panel is repeated by increasing or decreasing the doses of the standard till all three equal responses are obtained/ a closest bracket is provided for the test response by the two standard responses.
- In the end, the responses due to the double doses of the standard and the test are taken which should be equal. Concentration of the test sample can be determined as follows:

$$\text{Concentration of unknown} = \frac{\text{Dose of Standard}}{\text{Dose ot Test}} \times \text{Concentration of Standard}$$

- This method has following limitations:
  - It occupies a larger area of the drum as far as tracings are concerned.
  - There are chances of errors that one cannot determine.
  - It does not give any idea of dose-response relationship.
  - The precision and reliability of this assay procedure is poor.

  However, this method is particularly useful when the sensitivity of the preparation is not stable.

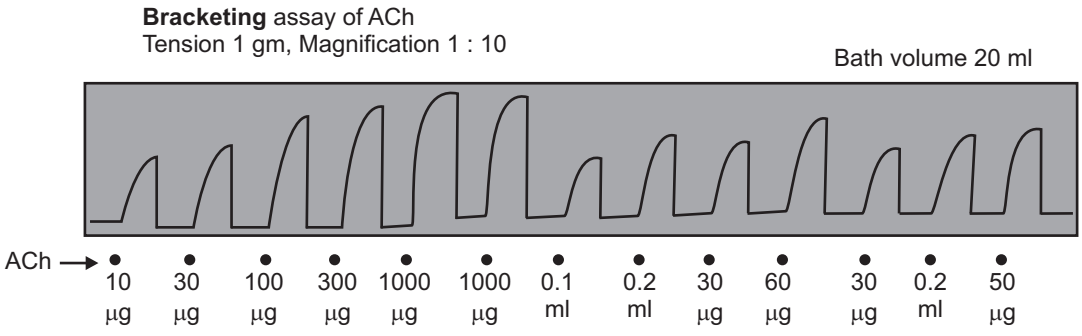


Fig. 24.2: Assay of Arch by bracketing method

3. Interpolation Method:

- This method is based on the assumption of dose-response relationship. At first, the concentration response curve due to graded doses of a standard substance followed by the dose response curve of the test substance is recorded. Then two standard doses and one test dose are selected from the respective DRCs such that they lie on the linear portion of the DRC. The test dose is selected in such a way that its response is greater than that of smaller dose of standard and is lesser than that of larger dose of the standard. Bioassay is then carried out with the selected standard and test doses in 2-3 cycles. The height of contraction of all the standard and test doses in the bioassay is measured. Then, a log DRC is plotted with the mean values of the standard responses in the bioassay and the dose of the standard producing the same response as produced by the test sample is directly read from the graph and the concentration of the test sample is determined.
- It is a simple method and chances of errors are less if the sensitivity of the preparation is not changed. The precision and reliability of the assay is much better as compared to the earlier methods as the sensitivity of the preparation is assessed prior to testing the unknown sample.

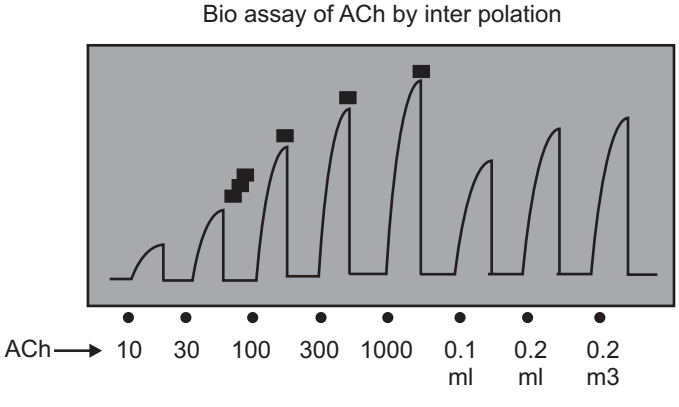


Fig. 24.3

4. Multiple Point Bioassays:

- The various types of multiple point bioassays, which are also based on dose-response relationship, are:
  - 2-point bioassay
  - 3-point bioassay
  - 4-point bioassay
  - 6-point bioassay
  - 8-point bioassay
- In these bioassays, the responses are repeated several times and the mean of each is taken. Thus, chances of error are minimized in these methods. The sequence of responses is recorded as per the Latin square method of randomization in order to avoid any bias.

(A) 2-Point Bioassay:

- Any one dose that produces from minimal to maximal response of the standard and the test substances are taken from the DRC and then their % of response is calculated.

$$\text{Concentration of Test Substance} = \frac{\%\ \text{Response of Standard}}{\%\ \text{Response of Test}} \times \text{Concentration of Standard}$$

However, this is not used practically.

(B) 3-Point Bioassay:

- This method is also based on the assumption of dose-response relationship. At first, the concentration response curve due to graded doses of a standard substance followed by the dose response curve of the test substance is recorded. Then, two standard doses and one test dose are selected from the respective DRCs such that they lie on the straightest and steepest part of the DRC. The test dose is selected in such a way that its response is greater than that of smaller dose of standard and is lesser than that of larger dose of the standard.
- Bioassay is then carried out with the chosen standard and the test doses in 3 successive cycles as per the Latin square design. The responses are recorded in the order of
  - S_1, S_2, T;
  - S_2, T, S_1 and
  - T, S_1, S_2.
- The height of contraction of all the standard and test doses in the bioassay is measured. Then, a log DRC is plotted with the mean values of the standard responses in the bioassay and the dose of the standard producing the same

response as produced by the test sample is directly read from the graph and the concentration of the test sample is determined. The concentration of the unknown can also be determined mathematically as follows:

$$\text{Concentration of unknown} = \frac{n_2}{T} \times \text{antilog}\left[\frac{T\text{-}S_1}{S_2 - S_1} \times \log \frac{n_2}{n_1}\right] C_s$$

Where

n_1 = Lower Standard dose

n_2 = Higher Standard dose

t = Test dose

S_1 = Response of n_1

S_2 = Response of n_2

T = Response of t

Cs = Concentration of Standard.

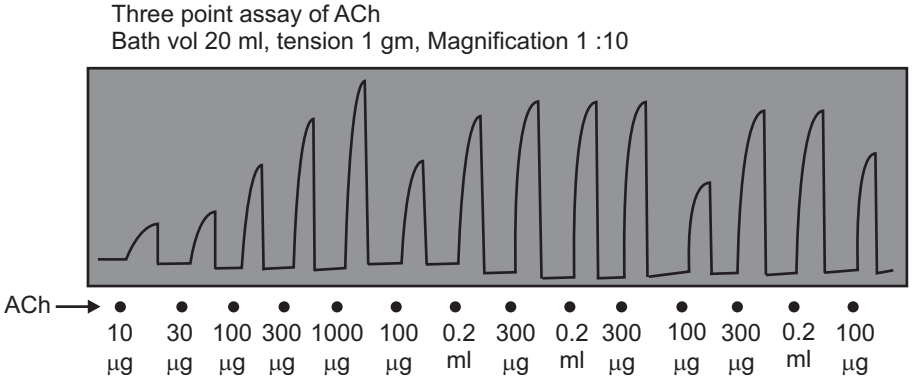


Fig. 24.4

(C) 4-Point Bioassay:

- This method is almost similar to the 3-point bioassay, but in this method 2 doses of the standard and the 2 doses of the test are used. The selection of the standard doses should be such that they lie on the linear portion of the CRC and also the ratio between the smaller to greater dose should be preferably 1:2. The selection of the test doses is done by hit and trial method so that the responses fall on the linear part of the curve. By employing the Latin square design, the responses of the chosen standard and the test doses is recorded in 4 successive cycles, in the order of
  - S_1, S_2, T_1, T_2;
  - S_2, T_1, T_2, S_1;
  - T_1, T_2, S_1, S_2 and
  - T_2, S_1, S_2, T_1.

- The height of contraction of all the standard and the test doses in the bioassay is measured and their mean values are calculated. The concentration of the test sample can be determined by graphical method as well as mathematically by employing the following formula:

$$\text{Concentration of unknown} = \frac{n_1}{t_1} \times \text{antilog}\left[\frac{(S_1 + S_2) - (T_1 + T_2)}{(S_2 + T_2) - (S_1 + T_1)}\right] \times \log \frac{n_2}{n_1}$$

Where,

n_1 = Lower Standard dose

n_2 = Higher Standard dose

t_1 = Lower Test dose

t_2 = Higher Test dose

S_1 = Response of n_1

S_2 = Response of n_2

T_1 = Response of t_1

T_2 = Response of t_2

- The precision, reliability and reproducibility of this assay method is very high. Hence, it is the most commonly used bioassay for the estimation of the concentration of active substances present in the biological fluids.

Advantages:

- A chemical assay finds out only the amount of active substance present in a given sample where as the bioassay measures the actual biological activity of the active substance.
- Bioassay measures small traces of compound too.
- Bioassay can establish the biological activity of a substance even when its chemical identity is not known.
- Sensitivity of a bioassay is greater than a chemical assay.
- Chemically unstable drugs can be conveniently assayed by a bioassay.
- The active and the inactive isomers present in a racemic mixture can be easily distinguished by a bioassay.

Disadvantages:

- Complicated set up.
- Expensive.
- Time consuming.
- Too laborious.
- Requires skilled labor.
- The effect observed in animals may not be observed in humans.
- The quantitative accuracy of a bioassay usually falls considerably below that attainable with most chemical assays.

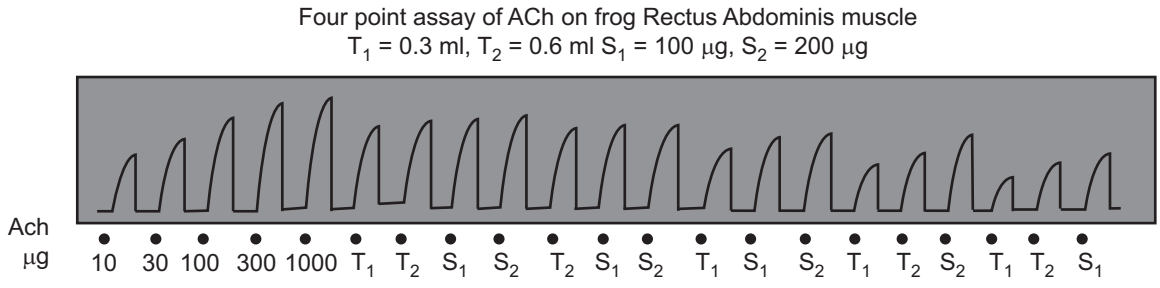


Fig. 24.5

Applications:

- Drugs, which are primarily of natural origin, are usually assayed by biological methods.
- When there is no suitable chemical assay is available for certain drugs such as insulin, oxytocin etc., then bioassay is the only choice to estimate them.
- Bioassay is the only choice of analysis for some substances when the chemical assay is not a valid indication of their biological activity.
- Bioassays are useful to standardize those drugs that are composed of a complex mixture of substances of varying structure and activity. E.g., Digitalis.
- Bioassays are helpful when the purification of the crude drug sufficient for the performance of a chemical assay is not possible or practical. E.g., Vitamin D from irradiated oils.
- Bioassays are employed to find out the LD_{50} and ED_{50} of a drug under investigation.

QUESTIONS

1. Write the principles and applications of bioassay.
2. Define bioassay. Write the principle and applications of interpolation bioassay.

■■■

Chapter ... 25

Bioassays

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to:

- *Describe the biological assay of insulin, oxytocin, vasopressin, ACTH, d-tubocurarine, digitalis, histamine and 5-HT.*

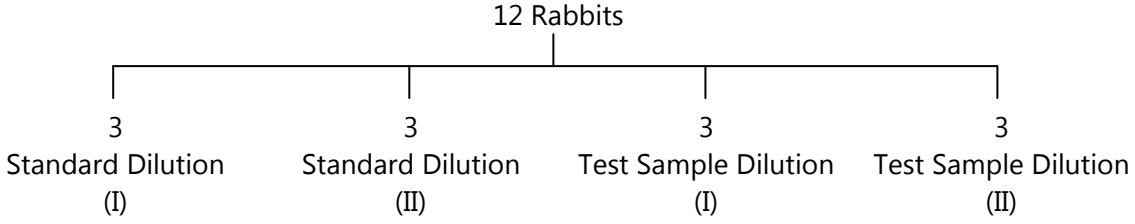
25.1 BIOLOGICAL ASSAY OF INSULIN

- **Standard Preparation and Unit:** It is pure, dry and crystalline insulin. One unit contains 0.04082 mg. This unit is specified by Ministry of Health, Government of India and is equivalent to international unit.
- **Preparation of Standard Solution:** Accurately weigh 20 units of insulin and dissolve it in normal saline. Acidify it with HCl to pH 2.5. Add 0.5% phenol as preservative. Add 1.4% to 1.8% glycerin. Final volume should contain 20 units/ml. Store the solution in a cool place and use it within six months.
- **Preparation of Test Sample Solution:** The solution of the test sample is prepared in the same way as the standard solution.

25.1.1 Rabbit Method

- **Selection of Rabbits:** They should be healthy, weighing about 1800-3000 gm. They should then be maintained on uniform diet but are fasted for 18 hrs. before assay. Water is withdrawn during the experiment.
- **Standard and Sample Dilutions:** These are freshly prepared by diluting with normal NaCl solution so as to contain 1 unit/ml. and 2 units/ml.
- **Doses:** The dose which can produce suitable fall in blood sugar level is calculated for the standard.
- **Principle:** The potency of a test sample is estimated by comparing the hypoglycemic effect of the sample with that of the std. preparation of insulin.
- **Experimental Procedure:** Animals are divided into 4 groups of 3 rabbits each. The rabbits are then put into an animal holder. They should be handled with care to avoid excitement.
- **First part of the Test:** A sample of blood is taken from the marginal ear vein of each rabbit. Presence of reducing sugar is estimated per 100 ml. of blood by a suitable chemical method. This concentration is called 'Initial Blood Sugar Level'.

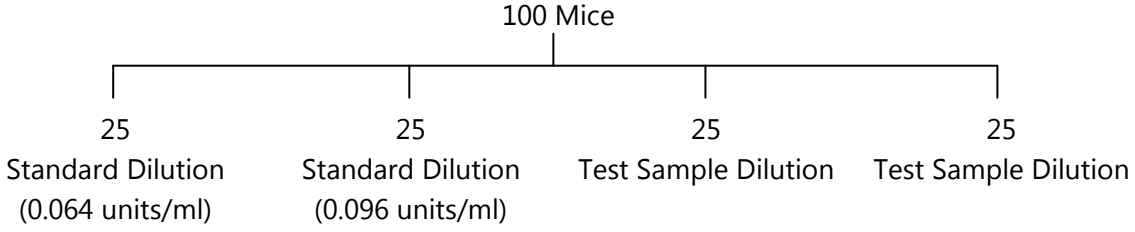
- The four groups of rabbits are then given sc. injections of insulin as follows:
- Any other suitable method can also be used.



- From each rabbit, a sample of blood is withdrawn up to 5 hrs. at the interval of 1 hr. each. Blood sugar is determined again. This is known as 'Final Blood Sugar Level'.
- Second part of the test (Cross over test): The same animals are used for the second part. The experiment can be carried out after one week.
- Again they are fasted and initial blood sugar is determined. The grouping is reversed, that is to say, those animals which received the standard are given the test and those which received the test are now given the standard. Those animals which received the less dose of the standard are given the higher dose of the test sample and vice-versa. This test is known as 'Twin Cross Over Test'.

25.1.2 Mouse Method

- Mice show characteristic convulsions after s.c. injection of insulin at elevated temperatures. The percentage convulsions produced by the test and standard preparations are compared.
- **Experimental Procedure:** Minimum 100 mice weighing between 18-22gm of the same strain are used. They should be maintained on constant diet. They should be fasted 18 hrs. prior to the experiment.
- **Standard and Sample Dilutions:** Dilutions are prepared with sterile saline solution, so as to contain 0.064 units/ml. (std dilution I) and 0.096 untis/ml. (std. dilution II). Similarly, test sample solutions are also prepared.
- Mice are divided into four groups each containing 25 mice and insulin is injected s.c. as follows:



- Mice are put in an air incubator at 33^{o}C and observed for one and a half hr. The mice which convulse or die are taken out of the incubator and observed. These mice usually convulse severely but failure of the animal to upright itself when placed on its back, should as well be considered as convulsion.

25.1.3 Rat Diaphragm Method

- Sprague Dawley rats weighing 70–100 g are used. The animals are sacrificed during anesthesia and the diaphragms still attached to the rib cages are carefully removed, released from the rib cages and adhering connective and fat tissues, washed in PBS, spread out and divided into two equal pieces as described by Müller and coworkers (1994). For assaying the effects of insulin/compounds/drugs, the hemidiaphragms are incubated in KRH buffer gassed with carbogen (95% O_2/5% CO_2) in the presence of 5 mM glucose.

Epididymal fat pad of rats:

- Insulin-like activity can be measured by the uptake of glucose into fat cells. Adipose tissue from the epididymal fat pad of rats has been found to very suitable.
- The difference of glucose concentration in the medium after incubation of pieces of epididymal rat adipose tissue or measured oxygen consumption in Warburg vessels, Radiolabelled 14C glucose, the 14CO2 is trapped and counted.
- The concentration is determined by immuno-assay.

25.2 BIOLOGICAL ASSAY OF OXYTOCIN

- **Principle:** The potency of oxytocin is determined by comparing its activity with that of the Standard Preparation of oxytocin under the conditions of a suitable method of assay.
- **Standard Preparation:** The Standard Preparation is the 4th International Standard for Oxytocin, established in 1978, consisting of freeze-dried synthetic oxytocin peptide with human albumin and citric acid (supplied in ampoules containing 12.5 Units), or another suitable preparation the potency of which has been determined in relation to the International standard.

Method A:

- **By depression of the blood pressure in chicken:** Anaesthetise a young healthy adult cockerel weighing 1.2 to 2.3 kg with an anaesthetic that will maintain a 18 prolonged and constant high blood pressure. Expose the gluteus primus muscle in one thigh and cut and retract it to reveal the popliteal artery and crural vein. Cannulate the popliteal artery and record the blood pressure on a suitable recorder calibrated for use over a linear range. Cannulate the crural or brachial vein. Immediately before use prepare a solution of the Standard Preparation in saline solution so that the volume to be injected is between 0.1 ml and 0.5 ml. Record the blood pressure responses to the injection into the cannulated vein of two doses of this solution; the doses should be such as to produce clearly discriminated, precipitous, submaximal decreases in blood pressure; the required doses normally lie between 20 and 100 milli Units. The interval between injections should be constant and lie between 3 and 10 minutes depending on the rate at which the blood pressure returns to normal. Immediately before use dilute the preparation

being examined with saline solution so as to obtain responses similar to those obtained with the Standard Preparation. The ratio between the two doses of the preparation being examined should be the same as that between the two doses of the Standard Preparation and this ratio should be kept constant throughout the assay. The two doses of the Standard Preparation and the two doses of the preparation being examined should be given according to a randomised block or a Latin square design and at least six responses to each should be recorded. If the animal rapidly becomes insensitive to the repeated injections of the solutions another animal must be used. Measure all the responses and calculate the result of the assay by standard statistical methods.

Method B:

- **By contraction of the rat uterus:** Inject 100 mg of oestradiol benzoate intramuscularly into a female rat weighing 120 to 200 g 18 to 24 hours before the assay. Immediately before the assay confirm by vaginal smear that the rat is in oestrus or preoestrus. Kill the rat and suspend one horn of the uterus in a bath containing a solution of the following composition:
  - Composition (% w/v)
  - Sodium chloride 0.662
  - Potassium chloride 0.045
  - Calcium chloride 0.007
  - Sodium bicarbonate 0.256
  - Disodium hydrogen phosphate 0.029
  - Sodium dihydrogen phosphate 0.003
  - Magnesium chloride 0.010
  - Dextrose 0.050
- Maintain the bath at a temperature of 32°C or at some other suitable temperature at which spontaneous contractions of the uterus are abolished and the preparation maintains its sensitivity. Oxygenate the solution with a mixture of 95% of oxygen and 5% of carbon dioxide and record the contractions of the muscle using a suitable instrument giving a linear response (for example an isotonic lever with a load not exceeding 2 g). Record the contractions produced by the addition to the bath of two doses of the Standard Preparation suitably diluted with the above solution. The doses should be such as to produce clearly discriminated, submaximal contractions; the required doses normally lie between 10 and 50 micro Units per ml of bath liquid. When maximal contraction has been reached, replace the bath liquid by a fresh solution. The doses should be added at regular intervals of 3 to 5 minutes depending upon the rate of recovery of the muscle.

- Dilute the preparation being examined so as to obtain responses on the addition of two doses similar to those obtained with the Standard Preparation. The ratio between the two doses of the preparation being examined should be the same as that between the two doses of the Standard Preparation and this ratio should be kept constant throughout the assay.
- The two doses of Standard Preparation and the two doses of the preparation being examined should be given according to a randomized block or a Latin square design and at least six responses to each should be recorded.
- Measure all the responses and calculate the result of the assay by standard statistical methods.

Method C:

- **By measurement of milk-ejection pressure in a lactating rat:** Select a lactating rat, in the third to twenty-first day after parturition and weighing about 300 g, separate it from the litter and 30 to 60 minutes later anaesthetise (for example, by the intraperitoneal injection of a solution of Pentobarbitone Sodium). Tie the rat to an operating table, maintained at 37℃ by its hind legs leaving the front legs free. Cannulate the trachea with a short polyethylene tube of internal diameter about 2.5 mm in such a manner so as to ensure a free airway; apply artificial respiration only if necessary. Cannulate an external jugular or femoral vein with a polyethylene tube of internal diameter about 0.4 mm which is filled with saline solution and closed with a pin. Shave the skin surrounding the inguinal and abdominal teats and excise the tip of one teat, preferably the lower inguinal teat. Insert a polyethylene tube of internal diameter about 0.3 mm and external diameter about 0.6 mm, to a depth sufficient to obtain appropriate measurement of pressure (3 to 10 mm depth), into the primary teat duct which opens onto the cut surface and tie firmly in place with a ligature. Connect this cannula with a suitable strain gauge transducer (such as that used for recording arterial blood pressure in the rat) and fill the whole system with a 3.8% w/v solution of sodium citrate or saline solution containing 50 Units of heparin sodium per ml to prevent clotting of milk. After cannulation, inject a small volume (0.05 to 0.2 ml) of this solution into the teat duct through the transducer to clear the milk from the tip of the cannula. (This procedure may be repeated during the assay should obstruction arise from milk ejected into the cannula). Clamp the strain gauge so that a slight tension is applied to the teat and its natural alignment is preserved and connect the gauge to a potentiometric recorder adjusted to give full-scale deflection for an increase in milk-ejection pressure of about 5.3 kPa. Inject all solutions through the venous cannula using a 1-ml syringe graduated in 0.01 ml and wash them in with 0.2 ml of saline solution. Prepare a solution of the Standard Preparation and a solution of the preparation being examined in saline solution so that the volume to be injected is between 0.1 ml and 0.4 ml. Choose two doses of the Standard Preparation such that the increase in milk-ejection pressure is about 1.35 kPa for the lower dose and about 2.7 kPa for the higher dose. As an initial

proximation, a lower dose of between 0.1 and 0.4 milliUnit and an upper dose of 1.5 to 2 times this amount may be tried. Choose two doses of the preparation being examined with the same inter-dose ratio, matching the effects of the doses of the Standard Preparation as closely as possible. Inject the four doses (two doses of the Standard Preparation and two doses of the preparation being examined) at intervals of 3 to 5 minutes. The two doses of Standard Preparation and the two doses of the preparation being examined should be given according to a randomised lock or a Latin square design and at least four responses to each should be recorded. Measure all the responses and calculate the result of the assay by standard statistical methods.

25.3 BIOASSAY OF DIGITALIS

- **Principle:** Potency of the test sample is compared with that of the standard preparation by determining the action on the cardiac muscle. Any other equivalent method, which gives results similar to those obtained by this method as also valid.
- **Standard Preparation and Units**: The standard preparation is a mixture of dried and powdered digitalis leaves (1 unit = 76 mg.)
- **Preparation of Extracts:** Exact amount of the powder is extracted with dehydrated alcohol in a continuous extraction apparatus for six hours. The final extract should contain 10 ml. (5 ml. alcohol + 5 ml. water) per 10 g. of digitalis powder. It should be stored in between 5˚C and –5˚C.

1. Guinea–pig Method (End point method):

- Standard and test sample extracts are diluted with normal saline in such a way that 1 g of digitalis powder is diluted to 80 ml. A guinea pig is anaesthetized with a suitable anaesthetic. It is dissected on the operation table.
- The jugular vein is traced out by removing adhering tissues and cannulated by means of venous cannula. A pin is inserted in the heart, such that it gets inserted in the apex of the heart. In this way, we can observe the heart beats by up and down movements of the pin.
- The injection is continued through venous cannula until the heart is arrested in systole. The amount of extract required to produce this effect is taken as the lethal dose of the extract.
- Another set of 19 animals of the same species are used for this experiment and the average lethal dose is determined.
- It is not necessary to determine the lethal dose of the std. during each time of the experiment. But it should be occasionally checked.
- The lethal dose of the test sample is determined in a similar way using minimum 6 guinea–pigs of the same strain.
- The potency of the test sample is calculated in relation to that of the std. preparation by dividing the average lethal dose of the sample to the test and expressed as units per gram.

2. Pigeon Method:

- Minimum 6 pigeons are used for testing each sample. They should be free from gross evidence of disease or emaciation.
- The weight of the heaviest pigeon should not exceed twice the weight of the lightest pigeon. Food is withheld 16-28 hours before the experiment. Pigeons are divided on the basis of their sex, weight and breed, into two groups.
- They are anaesthetized with anaesthetic ether. One side of the wing is dissected and the alar vein is cannulated by means of a venous cannula. Dilutions are made with normal saline. Average lethal dose of each sample is determined; results are tabulated and calculated as per guinea pig method.
- The lethal dose per kg. of body weight is determined for each pigeon. The potency of the test sample is determined by dividing the mean lethal dose of standard by the mean lethal dose of the test sample.
- In pigeons, stoppage of heart is associated with a characteristic vomiting response called 'emesis'. The milk from the crop sac of pigeons is being ejected out. This may be taken as the end point response of digitalis.

25.4 BIOASSAY OF D-TUBOCURARINE

25.4.1 Rabbit Head-drop Method

- **Principle:** d-Tubocararine hydrochloride is injected into the marginal vein of a rabbit's ear till the rabbit's neck muscles are relaxed such that the animal cannot hold its head up. The total amount of test sample required to produce the endpoint is compared with the total amount of the standard sample required to produce similar endpoint.
- **Selection of Rabbits:** Rabbits weighing 2 kg. are used. Animals should be free from disease, obtained from a healthy colony and should be accustomed with the experimental procedure.
- **Experimental Procedure:** Each rabbit is placed in a holder with its head protruding outside. The head should be freely movable. Minimum 8 rabbits are used. They are divided into two groups each containing 4 rabbits. First group will receive standard sample and the second group will receive the sample under test. d-Tubocurarine solution is injected at a constant speed by infusion apparatus through the marginal vein.
  (i) i.v. inj. of d-tubocurarine.
  (ii) Head drop after injection. Injection should be given at a rate of 0.4 ml/min and should take about 10 min. Infusion is continued till the rabbit will not be in a position to hold its head erect or there will be no response by focusing light on the eyes and the neck gets elongated and toneless.

- Suitable dose of d-tubocurarine is 0.012% w/v in saline. Rabbits recover immediately from the effect of curarization. During the expt. there is a possibility or respiratory embarrassment which is treated by injecting neostigmine methyl sulphate (0.05 mg.) and atropine sulphate immediately through the marginal ear vein.
- Cross-over test is carried out to minimise biological error due to animal variation. Those rabbits which received the standard sample on the first day will be given test sample on the second day of expt. and vice versa. Mean dose which produces head drop of the test sample is compared with the mean dose of standard preparation.

25.4.2 Frog's Rectus Abdominis muscle Preparation

- A frog is pithed and laid on its back on a cork covered board to which it is pinned. The skin covering the abdomen is cut away and the rectus abdominis muscle of one side is dissected from the pelvic girdle to its insertion in the cartilage of the pectoral girdle. The muscle is then pinned to the cork by four pins to keep its normal length while a thread is sewn through each end. It is then mounted in the organ bath containing frog's Ringer solution which contains:
- NaCl, 6.5gm.; KCl, 0.29 gm.; $CaCl_2$, 0.24 gm.; $NaHCO_3$, 0.4 gm.; glucose, 1.5 gm. and distilled water 2000 ml.
- Oxygenation is carried out to keep the tissue alive. The muscle is stabilized for 30-45 min. in order to get critical quantitative response. The responses are recorded using isotonic frontal writing lever with 1 G. tension.
- Two similar contractions with the same concentration of acetylcholine are obtained. Three doses of the standard sample and one intermediate dose of the test sample are selected and the reduction in height of contraction induced by acetylcholine is noted down.
- Acetylcholine contraction is recorded on slow moving drum for 90 sec. d-Tubocurarine is allowed to act for 30 sec. The percentage reduction at each dose levels is calculated and log dose response curve of the standard drug is plotted. A linear response will be obtained. The potency of test sample is calculated from the standard curve.

25.5 BIOASSAY OF HISTAMINE

Bioassay of Histamine using guinea-pig Ileum:

Drugs:

- Standard histamine solution (St.) (10 µg/ml)
- Test histamine solution (T)

Procedure:

- A clean strip of the ileum is placed in freshly prepared Tyrode's solution. A small segment (3cm) of ileum is cut; a thread is passed through the lumen & the wall at each end of the segment with the aid of a fine needle.

- One end of the segment is tied securely to the aeration tube & transferred to organ bath (already filled with Tyrode's solution & bubbled with gas). The other end of the ileum is attached to the transducer; the tension of the thread is adjusted.
- The baseline record of contraction on the physiograph and the sensitivity are adjusted before the addition of drugs.
- The normal tone of ileum is recorded for 1 min. then different volumes of St. histamine are started to be added. The contact time for the each histamine volume is 30 seconds. At the end of each cycle, the tissue is washed two times with the Tyrode's solution each time the tissue is allowed in contact with the solution for 30 seconds.(Cycle time= 2.5min, contact time for agonist(30 sec.) + normal record(1min.)+ washing twice(30 sec.))
- Different doses of St. histamine solution are added (0.05ml, 0.1ml, 0.2ml, 0.3ml &0.4ml) to chose two proper doses and bracketing the test in between them. The amount of standard which produce responses matching those of the dose of test is tried and attempt is made to reduce the limits as far as possible.
- Changing in St. histamine doses, followed by the dose of test, is continued till the test dose was bracketed between 2 doses of St. histamine given nearly the same response of the test.

25.6 BIOASSAY OF VASOPRESSIN

Definition:

- Bioassay is defined as estimation of the concentration or potency of a substance by measuring its biological response in a living system.

Principle:

- Potency of vasopressin injection is determined by comparing test activity with that of standard preparation of vasopressin.

Standard Preparation:

- It is a dried acetone extract of posterior lobes of pituitary gland of oxen or any other suitable preparation.
- Standard unit: Specific pressor activity corresponding to that yielded by 0.0005gm of standard preparation (20units/ml).

Procedure:

- **Animal:** Albino rat of 300g weight.
- Anaesthetize it by S.C. injection of Ethyl carbamate.
- After 40-60 min., cannulate the trachea with polyethylene tube of 2.5 mm external diameter.
- Dissect carotid artery for cannulation.
- Cannulate femoral vein close to inguinal ligament by the following process:

- Retract abdominal muscles to expose the inguinal ligament and superficial prudental vein to one side.
- Dissect femoral vein towards inguinal ligament from corresponding artery.
- Tie a short polyethylene cannula (1 mm external diameter) into femoral vein by two ligatures, joined by short piece of rubber tubing to 1 ml burette with an attached thistle funnel containing saline solution.
- Fix a wet cotton swab & tie to cover the incision and cannula .
- Inject 200U heparin in saline solution/100 g body weight.
- Connect carotid artery cannula with mercury manometer (2-3mm internal diameter).
- Inject all solutions through venous cannula by 1 ml syringe.
- A suitable hypotensive agent is given into tail vein to produce a constant basal pressure of 50 torr.
- Dilute standard & test preparations such that volume to be injected is between 0.1-0.5 ml.
- Choose 2 doses of standard so that lower dose produces 30 torr B.P. & higher produces 50 torr B.P.
- i.e., ratio of doses should be 3:5.
- Select test doses according to standard doses.
- Doses are added at intervals of 3-5 min. in a random order.
- Record rise in B.P. in response to each dose..

Method 2:

- Anaesthetize healthy cat with volatile anaesthetic agent.
- Insert a tracheal tube for artificial respiration.
- Expose spinal cord from behind by removing second cervical vertebrae.
- Destroy brain by passing suitable instrument through foramen magnum.
- Start artificial respiration through tracheal tube & leave animal for an hour to remove anaesthetic effect.
- Cannulate carotid artery for B.P. measurement & femoral vein for injection of drug solutions.
- Maintain normal B.P. at 50-100 torr.
- Select 2 doses of test & standard, inject 0.05-0.1 units at 30 min. interval.
- Record maximum rise in B.P. in response to each dose.

25.7 BIOASSAY OF ACTH

Official preparation

- Corticotropin injection: is a sterile solution, in a suitable diluents, of the polypeptide from the pituitary glands of mammals. Potency range should be 80.0 – 120.0% of cartiotropin units.

Purpose and Rationale:

- This is a historical assay method.
- Administration of pituitary gland ACTH decrease the ascorbic acid present in the adrenals.
- The depletion of adrenal ascorbic acid is a function of the dose of ACTH administered.
- This relationship has been used for a quantitative assay of ACTH.

Solution:

- Five units of test or standard dissolved in 0.25 ml of 0.5% phenol solution and diluted with 8.1 ml of 15% gelatin solution (Now 0.5 ml contains 300 mU ACTH). (Solution A)
- Three ml of solution A diluted with 6 ml of gelatin solution. Now concentration reduced to 100 mU ACTH/0.5 ml) (Solution B).
- Again 3 ml of solution B diluted with 6 ml of gelatin solution, the resulting solution contains 33 mUACTH/0.5 ml.

Procedure:

- Male wistar rat (100-200 g) are hypophysectomized (pituitary gland removed by surgery) one day prior to the test.
- For one test with 3 dose of test preparation and standard.
- Number of hypophysectomized rats required: at leat 36 (preferably 60).
- The hypophysectomized rats are randomly distributed in to six groups. Each rat receives subcutaneous 0.5 ml of the various concentration of test or standard.
- Three hours after injection, the animals are anesthetized and both adrenal and removed, freed from extraneous tissue and weighed. The rats are sacrificed and the skull opened to verify completeness of hypophysectomy.
- The adrenal are homogenized in glass tubes contains 200 mg pure sand and 8.0 ml of 4% trichloroacetic acid and the ascorbic acid determined.
- The potency ratio including confidence limits is calculated with the 3+3 point assay.

Estimation of Ascorbic Acid:

- Preparation of 1 mg/ml conc. Of ascorbic acid in 4% TCA (stock) solution A.
- Use solution A to prepare 0.2% of ascorbic acid in 4% TCA (solution B).
- Use solution B to prepare 0.02% of ascorbic acid in 4% TCA (solution B).
- The calibration curve is established at a wave length of 540 mm using the solutions without ascorbic acid as blank.

25.8 BIOASSAY OF 5HT

Objective: To record the concentration response curve of 5 HT using isolated rat fundus strip preparation.

Principle:

- The basic principle of bioassay is to compare the test substance with the Standard preparation of the same and to find out how much test substance is required to produce the same biological effect, as produced by the standard.
- Rat fundus is a very sensitive tissue for the study of the action of several naturally occurring substances like 5HT, Histamine, Acetyl Choline and Bradykinin.
- Unlike the intestinal smooth muscle this preparation is slow contracting and slow relaxing serotonin.
- Rat fundus preparation is generally employed for the bioassay of serotonin.
- The fundus is grey in colour and therefore, easily identified from pyloric part.
- A zig-zag preparation of the fundal strip is prepared so as to expose maximum portion of the tissue to drug.
- The tissue is sensitive to 1 ng/ml of serotonin.

Procedure:

1. Sacrifice the rat by a blow on the head and carotid bleeding.
2. Cut open the abdomen and expose the stomach.
3. Identify the fundus of the stomach, incise it from the junction of pyloric part and put it in the dish containing Krebs solution.
4. Incise the fundus from the lesser curvature and open it longitudinally. Give alternate Zig-Zag cuts to make a fundal strip preparation, tie both the ends with thread and mount in the organ bath containing Krebs solution at 37˚C. Aerate the tissue.
5. Apply 1g load and allow the preparation to equilibrate for 30 min. using frontal writing lever with 10-12 magnification record the contraction due to increasing concentration of serotonin. Since the muscle contracts slowly and relaxes slowly, a contact time of 90sec and 5 min time cycle is followed for proper recording of the concentration response curve.
6. Label and fix the tracing. Plot the concentration response curve.

QUESTION

1. Explain the principle and procedure behind bioassay of oxytocin and digitalis.

■■■

Multiple Choice Questions

Unit I	**Pharmacology of Drugs Acting on Cardio Vascular System**
Topic	**(a) Introduction to Hemodynamic and Electrophysiology of Heart**
1.	Cardiac output is equal to _____ (a) Stroke volume X Heart Rate (b) Blood pressure X Preload (c) Stroke volume X After load (d) Blood pressure X Heart rate
2.	Systolic pressure in Aorta _____ (a) 120 mm Hg (b) 80 mm Hg (c) 100 mm Hg (d) 200 mm Hg
3.	During Diastolic repolarization _____ (a) Na^+ in (b) Ca^{2+} in (c) K^+ out (d) Ca^{2+} out
Answer	**1. (a), 2. (a), 3. (b)**
Topic	**(b) Drugs used in Congestive Heart Failure**
1.	Most common symptom of heart failure _____ (a) Peripheral edema (b) angina (c) pulmonary hypertension (d) dyspnea
2.	Cardiac glycosides; principle mechanism of action _____ (a) block β adrenergic receptor (b) activate myocardial leukotrienes (c) inhibits Na/K ATPase (d) inhibit calcium transport
3.	Initial drugs for management of mild to moderate heart failure _____ (a) parenteral inotropic drugs, e.g. dobutamine (b) hydralazine (c) furosemide (d) Captopril
4.	Most important cardiac effect produced by digoxin relative to management of CHF _____ (a) increased rate (b) decreased AV conduction (c) peripheral vasodilation (d) shifted the force velocity curve upward
Answers	**1. (d), 2. (c), 3. (d), 4(d)**
Topic	**(c) Anti-hypertensive Drugs**
1.	Mechanism of action prazosin (a) alpha-receptor blocker (b) beta-receptor blocker (c) phosphodiesterase inhibitors (d) calcium channel blocker
2.	Increases stroke volume (a) hypothyroidism (b) peripheral vasodilators (c) aortic regurgitation (d) tachycardia

contd. …

3.	Primary or idiopathic hypertension _____ (a) caused by Cushing's syndrome (b) may be due to pheochromocytoma (c) caused by hyperaldosteronism (d) >90% of cases
4.	Essential hypertension _____ (a) beta adrenergic receptor up regulation (b) increased endothelium-mediated vascular relaxation (c) increased vasoconstrictive tone (d) reduced sympathetic activation
Answers	**1. (a), 2. (c), 3. (d), 4(c)**
Topic	**(d) Anti-anginal Drugs / (e) Anti-Arrhythmic Drugs**
1.	Potassium channel opener _____ (a) Nicorandil (b) Diltiazem (c) GTN (d) Isosorbide dinitrate
2.	In the heart "fast" response is mediated by this ion _____ (a) sodium (b) calcium (c) magnesium (d) potassium
3.	Major depolarizing current is carried by this ion in vascular smooth muscle _____ (a) sodium (b) calcium (c) magnesium
4.	Better drug class for management of variant angina _____ (a) beta-blockers (b) calcium channel blockers (c) equally appropriate
5.	Depolarization in the specialized conduction system, the SA and AV nodal tissue, is mediated by this ion movement _____ (a) sodium (b) calcium (c) potassium (d) chloride
6.	Sequence of cardiac purkinje fiber ion channel activation _____ (a) calcium, potassium, sodium (b) potassium, calcium, sodium (c) sodium, calcium, potassium
7.	Factors predisposing to delayed after depolarization (DAD) _____ (a) excessive adrenergic activity (b) low intracellular potassium (c) bradycardia (d) all the above
8.	Most common cardiac conduction abnormality leading to arrhythmia _____ (a) delayed after depoiarization (b) early after depolarization (c) reentry (d) each mechanism equally likely
Answers	**1. (a), 2. (a), 3. (b), 4(b), 5. (b), 6 (c), 7 (a), 8 (c)**

contd. …

Topic	**(f) Anti-hyperlipidemic Drugs**
1.	Most of the statins are absorbed following oral administration in the range of 40% - 75%. This statin however, is almost entirely absorbed. (a) Lovastatin (b) Rosuvastatin (c) Simvastatin (d) Fluvastatin
2.	Constipation and bloating sensation – most common complaints of this antilipidemic drug _____ (a) pravastatin (pravachol) (b) niacin (Vitamin B3) (c) gemfibrozil (Lopid) (d) cholestyramine (Questran, Questran Light)
3.	Very large polymeric cationic exchange resins _____ (a) niacin (b) colestipol (Colestid) (c) pravastatin (Pravachol) (d) clofibrate (Abitrate, Atromid-SA)
4.	This statin has been approved for children eight years or older for use in the presence of heterozygous familial hypercholesterolemia. (a) Pitavastatin (b) Atorvastatin (c) Lovastatin (d) Simvastatin
5.	The common and serious side effect associated with niacin administration is _____ (a) Renal toxicity (b) Blood discrasiasis (c) Hepatotoxicity (d) Pulmonary fibrosis
Answers	**1. (d), 2. (d), 3. (b), 4. (a), 5. (c)**

Unit II	**1. Pharmacology of Drugs Acting on Cardio Vascular System**
	(a) Drug used in the Therapy of Shock
1.	Ionotrope are _____ (a) an agent that changes myocardial contractility. (b) an agent that increases blood pressure (c) an agent that changes heart rate (d) an agent that increases cardiac conduction velocity.
2.	Dopamine is a vasopressor agent used in the treatment of _____ (a) cardiogenic and septic shock (b) Hypovolemic shock (c) Anaphylayic shock (d) Neurogenic shock
3.	Nature's vasopressor is (a) Epinephrine (b) Dopamine (c) Dobutamine (d) Vasopressin
Answers	**1. (a), 2. (a), 3. (a)**
	(b) Hematinics, Coagulants and Anticoagulants
1.	Spontaneous arresting of bleeding from blood vessel _____ (a) Vasospasm (b) Hemostasis (c) Fibrinolysis (d) Coagulation

contd. …

2.	Major adverse effect of heparin _____ (a) osteoporosis (b) alopecia (c) bleeding (d) spontaneous fractures
3.	Folic acid used in the treatment of (a) iron deficiency anemia (b) aplastic anemia (c) megaloblastic anemia (d) hemolytic anemia
4.	Iron Dextran (Imferon) is _____ iron preparation (a) oral (b) parenteral (c) sublingual (d) subcutaneous
5.	Excess vitamine B_{12} is stored in (a) spleen (b) bone marrow (c) intestine (d) liver
Answers	**1. (c), 2. (c), 3. (c), 4. (b), 5. (d)**
Topic	**(c) Fibrinolytics and Anti-Platelet Drugs**
1.	Platelet sticking to exposed damage blood vessel collagen _____ (a) vasospasm (b) platelet aggregation (c) fibrinolysis (d) platelet adhesion
2.	Inhibitor of fibrinolysis (a) t PA (b) urokinase (c) streptokinase (d) aminocaproic acid
3.	Complex of purified human plasminogen and bacterial streptokinase (a) alteplase (b) reteplase (c) anistreplase (d) dicumarol
4.	Aspirin irreversibly inhibit this enzyme, preventing thromboxane A_2 synthesis, (a) adenylyl cyclase (b) guanylyl cyclase (c) cyclooxygenase (d) streptokinase
Answers	**1. (d), 2. (d), 3. (c), 4(c)**
Topic	**(d) Plasma Volume Expanders**
1.	Contraindications to plasma expanders, except _____ (a) anaemia (b) diuresis (c) Pulmonary edema (d) allergy
2.	Human albumin is obtained from, (a) pooled human plasma (b) sugar beat (c) intestinal mucosa (d) bone marrow
3.	Dextran 70 expands plasma volume for nearly ----- (a) 24hrs (b) 48 hrs (c) 12 hrs (d) 3hrs
Answers	**1. (b), 2. (a), 3. (a)**

contd. …

Unit II	**2. Pharmacology of Drugs Acting on Urinary System**
	(a) Diuretics
1.	Diuretic least likely to produce hypokalemia _____ (a) ethacrynic acid (b) furosemide (c) triamterene
2.	Osmotic diuretic (a) furosemide (b) bumetanide (c) mannitol (d) thiazides
3.	Diuretics primarily acting on the thick ascending limb of the loop of Henle _____ (a) chlorothiazide (b) bumetanide (c) mannitol (d) triamterene
4.	Diuretic that may be used to treat epilepsy, acute mountain sickness and open-angle glaucoma _____ (a) chlorothiazide (b) acetazolamide (c) metolazone (d) bumetanide
5.	Predominate renal location of carbonic acid anhydrase _____ (a) cytoplasm of the epithelial cells (b) basolateral membrane (c) luminal membrane of the proximal tubule (d) collecting duct
Answers	**1. (c), 2. (c), 3. (b), 4. (b), 5. (c)**
Topic	**(b) Anti-diuretics**
1.	Agent(s) used to treat central diabetes incipidus. (a) vasopressin (b) desmopressin (c) oxytocin (d) dopamine
2.	This/these agents interfere with antidiuretic hormone (ADH/AVP) biological activity. (a) Democlocycline & Lithium (b) serotonin (c) sodium (d) ACTH
Answers	**1. (a), 2. (a)**

UNIT III	**1. Autocoids and Related Drugs**
	(a) Introduction to Autacoids and Classification
1.	Autocoids are all except, (a) Inflammatory mediators (b) derived from membrane phospholipids (c) act like local hormones (d) Neuronal transmitters
Answers	**1. (d)**

contd. …

Topic	**(b) Histamine, 5-HT and their Antagonists**
1.	H2 blocker most likely to inhibit P450 drug metabolizing system _____ (a) ranitidine (b) cimetidine (c) famotidine (d) nizatidine
2.	Example of second generation antihistamine _____ (a) cyclizine (b) diphenhydramine (c) astemizole (d) cyproheptadine
3.	The most important pathophysiologic mechanism of mast cell and basophil histamine release _____ (a) chemical (b) mechanical cell injury (c) Immunologic
4.	Serotonin is formed from _____ (a) tyrosine (b) L-tryptophan (c) glutamine (d) glutamic acid
5.	Most of the serotonin in the body its found in _____ (a) mast cell (b) cardiac cell (c) enterochromaffin cells (d) liver cell
Answers	**1. (b), 2. (c), 3. (c), 4. (b), 5. (c)**
Topic	**(c) Prostaglandins, Thromboxanes and Leukotrienes**
1.	Mediator of chronic inflammation (for example rheumatoid arthritis) (a) histamine (b) serotonin (c) interleukin 1 (d) bradykinin
2.	Most nonsteroidal anti-inflammatory drugs _____ (a) inhibit PG biosynthesis (b) weak organic basae (c) probably increases production of free radicals (d) mainly associated with guanyl cyclase interaction
Answers	**1. (c), 2. (a)**
Topic	**(d) Angiotensin, Bradykinin and Substance P** **(e) Non-Steroidal Anti-Inflammatory Agents**
1.	Mediator in acute inflammation _____ pain (a) histamine (b) serotonin (c) leukotrienes (d) bradykinin
2.	Angiotensin I is produced by the action of ----- (a) rennin (b) kinin (c) streptokinase (d) urokinase
3.	Substance P is (a) tachykinin neuropeptide (b) organic nitrate (c) monopeptide (d) dipeptide
Answers	**1. (d), 2. (a), 3. (a)**

contd. …

Topic	**(f) Anti-gout Drugs**
1.	Site(s) of action: probenecide (a) Loop of Henle (b) proximal tubule – middle segment (c) collecting duct (d) JG cell
2.	Probable direct effect of colchicines (mechanism of action) (a) direct membrane stabilization (b) bind to intracellular tubulin – preventing/reducing microtubule formation. (c) decreases purine synthesis directly. (d) prevent IL-1 release dire tly.
3.	Important sources of uric acid _____ (a) aminoacid, carbon dioxide (b) diet (c) lipids (d) organic nitrates
4.	Probably the initial step in acute gouty arthritis attack _____ (a) polymorphonuclear leukocytes migration into the joint (b) increased numbers of mononuclear phagocytes (macrophages) (c) urate crystals phagocytosis of synoviocytes (d) prostaglandin, lysosomal & IL 1 release.
Answers	**1. (b), 2. (b), 3. (a), 4. (c)**
Topic	**(g) Antirheumatic Drugs**
1.	Synovial fluid of patients wtth RA contains _____ (a) TNFα, IL-1 (b) streptokinine (c) methyl transferases (d) glutamine
2.	ADRs of Azathioprine is all except _____ (a) bone marrow suppression (b) hepatic toxicity (c) Lymphomas (d) anaemia
3.	Infliximab is a ------ monoclonal antibody (a) IgG1 (b) IgM (c) IgA (d) Ig H
4.	ANAKINRA is _____ (a) Recombinant human IL-1 receptor antagonist. (b) Antihistamine. (c) lipids (d) proteins
Answers	**1. (a), 2. (d), 3. (a), 4. (a)**

contd. …

UNIT IV	**1. Pharmacology of drugs acting on endocrine system**
Topic	**(a) Basic concepts in Endocrine Pharmacology.** **(b) Anterior Pituitary Hormones - Analogues and their Inhibitors**
1.	Hormones are all except (a) chemical substance/ chemical messengers (b) synthesized by endogenous glands. (c) secreted directly in to the blood circulation. (d) organic compound
2.	Growth hormone release-inhibiting hormone **(GHRIH)** _____ (a) oxytocin (b) ACTH (c) prolactin (d) Somatostatin
3.	Secretion of thyroxine (T4) and triiodothyronine (T3) stimulated by _____ (a) TSH (b) FSH (c) GH (d) LH
4.	The important function of prolactin is _____ (a) growth and development (b) triggers the ovulation (c) stimulates breast development and milk production. (d) Essential for sperm production
5.	Bromocriptine act as _____ (a) prolactin inhibitor (b) GH inhibitor (c) ADH (d) oxiolytics
Answers	**1. (d), 2. (d), 3. (a), 4. (c), 5. (a)**
Topic	**(c) Thyroid hormones – Analogues and their Inhibitors**
1.	Thyroid hormone more likely to be completely absorbed following oral administration _____ (a) thyroxine (b) triiodothyronine (c) both equally absorbed (d) neither absorbed orally, requires parenteral administration
2.	More potent thyroid hormone _____ (a) T4 (b) T3 (c) equal potent
3.	CNS: lethargy; neuropathy (a) thyrotoxicosis (b) hypothyroidism
4.	Inhibition of pituitary TSH release _____ (a) L-DOPA (b) somatostatin (c) dopamine (d) all the above
5.	Inhibition of thyroid hormone synthesis or release _____ (a) amiodarone (b) lithium (c) iodide (d) all the above
Answers	**1. (b), 2. (b), 3. (b), 4. (d), 5. (d)**

contd. …

Topic	**(d) Hormones regulating plasma calcium level- Parathormone, Calcitonin and Vitamin-D**
1.	3 main hormones involved in the homeostatic regulation of calcium _____ (a) PTH,Vitamin D,Calcitonin (b) FSH, LH, PTH (c) GH, LH, calcitonin (d) Vitamine D, PTH, FSH
2.	Calcitonin lowers blood caicium through 4 methods, except _____ (a) Inhibit calcium absorption in intestine. (b) Inhibit osteoclast activity in bones. (c) Stimulate osteoblastic activity in bones. (d) stimulate renal tubular cell reabsorption of calcium.
3.	PTH is decreases the reabsorption of (a) Ca (b) Mg (c) phosphate (d) Na
4.	Vitamine D3 is _____ (a) cholecalciferol (b) Ergocalciferole (c) cholesterol
Answers	**1. (a), 2. (d), 3. (c), 4. (a)**
Topic	**(e) Insulin, Oral Hypoglycemic Agents and Glucagon**
1.	Secretory product associated with pancreatic alpha cell _____ (a) insulin (b) proglucagon (c) somatostatin (d) pancreatic polypeptide
2.	Type I diabetes _____ (a) most often present in adults (b) anabolic metabolic disorder (c) circulating insulin is virtually absent (d) glucagon levels are low
3.	Characteristics of type II diabetes _____ (a) absence of endogenous insulin (b) tissue super sensitivity to insulin (c) most patients are obese (d) ketoacidosis
4.	Most common complication of insulin therapy _____ (a) hypotension (b) retinopathy (c) hypoglycemia (d) gallstones
5.	Biguanides --- oral hypoglycemic drugs _____ (a) Tolbutamide (b) acarbose (c) Metformin (d) troglitazone
Answers	**1. (b), 2. (c), 3. (c), 4. (c), 5(c)**

contd. …

Topic	**(f) ACTH and Corticosteroids**
1.	Prednisone is an eg for _____ (a) glucocorticoid (b) mineralocorticoid (c) organic nitrates (d) kinins
2.	Hydrocortisone also known as _____ (a) cortisol (b) neuropeptide (c) interleukins (d) polypeptide
3.	Eg for ACTH inhibitor _____ (a) dexamethasone (b) Mifepristone (c) cortisol (d) Progesterone
4.	Examples of short-to medium acting glucocorticoid _____ (a) fluprednisolone (b) prednisolone (c) dexamethasone (d) fludrocortisones
5.	Long acting glucocorticoid _____ (a) cortisone (b) fluprednisolone (c) betamethasone (d) Progesterone
Answers	**1. (a), 2. (a), 3. (b), 4. (b), 5. (c)**

UNIT V	**1. Pharmacology of Drugs Acting on Endocrine System**
Topic	**(a) Androgens and Anabolic Steroids**
1.	Androgens are also called as _____ (a) female hormone (b) lactating hormone (c) pregnancy hormone (d) male hormones
2.	Biologically active metabolite of testosterone _____ (a) dihydrotestosterone (b) dexamethasone (c) Progesterone (d) fludrocortisone
3.	The most common causes of high testosterone levels in women are, all except _____ (a) hirsutism (b) polycystic ovary syndrome (c) congenital adrenal hyperplasia (d) cushing's syndrome
Answers	**1. (d), 2. (a), 3. (d)**
Topic	**(b) Estrogens, progesterone and oral contraceptives**
1.	Estrogen effects on FSH release (at this stage of the cycle) _____ (a) inhibition (b) stimulation
2.	Considered serious adverse effects associated with contraceptive use _____ (a) cancer (b) depression (c) myocardial infraction (d) all the above
3.	Changes in liver function associated with contraceptive hormone administration _____ (a) altered drug excretion; changes in drug metabolism. (b) reduced cholelithiasis (c) facilitated clearance of sulfobromophthalein (d) increased bile flow

contd. …

4.	Which hormone known as pregnancy hormone _____ (a) FSH (b) prolactin (c) LH (d) progesterone
Answers	**1. (a), 2. (d), 3. (a), 4. (d)**
	(c) Drugs acting on the uterus
1.	Posterior pituitary hormone which act as uterine stimulant _____ (a) oxytocin (b) vasopressin (c) prolactin (d) ACTH
2.	Ergot alkaloids which act as uterine stimulant _____ (a) senna (b) cascara (c) Ergometrine (d) cinnarazine
3.	Anti progestin which act as uterine stimulant _____ (a) mifepristone (b) misoprostol (c) quinine (d) ethacridine
4.	Adverse effect of oxytocics _____ (a) uterine rupture (b) allergy (c) heart attack (d) MI
Answers	**1. (a), 2. (c), 3. (a), 4. (a)**
2. Bioassay	**(a) Principles and applications of bioassay** **(b) Types of bioassay**
1.	Principles of bioassay _____ (a) compare the test substance with international standards preparation of the same. (b) compare the potency of two different test substances.
2.	Quantal assay also known as (a) interpolation bioassay (b) multiple point assay (c) all or none response assay (d) graded assay
3.	Examples of graded bioassay are all except, (a) bracketing (b) interpolation (c) multiple point (d) quantal
Answers	**1. (a), 2. (c), 3. (d)**
C.	**Bioassay of insulin, oxytocin, vasopressin, ACTH, d-tubocurarine, digitalis, histamine and 5-HT**
1.	Rabbit method is an example for (a) bioassay of insulin (b) bioassay of digitalis (c) bioassay of histamine (d) bioassay of 5-HT
2.	Examples for bioassay of digitalis (a) guinea-pig method (b) Rabbit method (c) Rabbit head drop method (d) Rat diaphragm method
Answers	**1. (a), 2. (a)**

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