COURSE CODE:	VBB 301
COURSE TITLE:	Biochemistry of Hormones & Disease
NUMBER OF UNITS:	2 Units
COURSE DURATION:	Two hours per week

COURSE DETAILS:

Course Coordinator:	**Dr (Mrs) Funmilola Clara Thomas** DVM., M.Sc.
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Other Lecturers:	**Dr Babatunde Samuel Okediran** B.Sc., DVM., M.Sc

COURSE CONTENT:

Molecular mechanisms of hormone action. Biochemistry of hormones of hypothalamus, anterior and posterior pituatuary, parathyroid, thyroid, pancreatic islets, adrenal, pineal(epiphysis) glands, ovaries, testis placenta,kidney, prostrate, gastrointestinal tract and the brain. Biochemical basis of disease. Biochemistry of some common tropical animal disease e.g infections bursal disease (IBD), trypanosomosis. Biochemical basis of ageing. Metabolic profiles of muscles, kidneys and liver.

COURSE REQUIREMENTS:

This is a compulsory course for all students of Veterinary Medicine. In view of this, Veterinary students are expected to participate in all the course activities and have a minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. V.K Malhotra. Biochemistry for students. Tenth edition. Jaypee Brothers Medical Publishers (p) ltd.
2. J.Jerry Kaneko., John W. Harvey., Michael L. Bruss. Clinical Biochemistry of Domestic Animals. Sixth edition. Academic Press.
3. Harvey Lodish., David Baltimore., Arnold Berk., S. Lawrence Zipursky., Paul Matsudaira and James Darnell. Molecular Cell Biology. Third Edition. By Scientific American Books, Inc.
4. David A. Bender., Kathleen M. Botham., Daryl K. Granner., Frederick W. Keeley., Peter A. Mayers., Robert K. Murray., Victor W. Rodwell., P. Anthony Weil.(2006). Harpers Illustrated Biochemistry. 27th Edition. Published By Mc Graw Hill

LECTURE NOTES

BIOCHEMISTRY OF AGING AND DISEASE.

AGING

Aging is the accumulation of irreversible processes of deterioration which follows the development of an organism. It is generally characterized by declining ability to respond to external or environmental stresses as a result of impaired adaptive and homoeostatic mechanisms. Aging is also known as **senescence.**

Physical/physiological changes in aging

- Vision and hearing decline
- Reduction in muscle strength and size
- Decreased flexibility of soft tissue, blood vessels, skin, joint cartilages that can result into arthritis etc
- Overall decline in body tone including intestinal motility, movement and decreased effectiveness of body organ functions
- Diminished sensitivity to triggers/stimulations
- Loss of number of functional cells in tissues and organs e.g. the brains loses some amount of neurons with age.
- Lowered metabolic activity, immune functions, heart, kidney, lungs, liver functions etc

- Graying of hair (occurs in animals too!)
- Some animals develop dull hair coat, brittle nails and had foot/hand pads
- Dental/gum diseases leading to teeth loss
- Bone marrow progressively gets replaced by fat.

THEORIES OF AGING

Many of these theories are interlinked, in the same complex way the biological processes of the body and the many factors affecting it are linked.

DNA and Genetic theory: closely related to this theory is also the programmed theory of aging this theory implies that aging is regulated by biological clocks operating throughout the life span; it focuses upon the encoded programming within the DNA. DNA is the blue-print of individual life obtained from our parents. It depends on changes in genes relating to the body repair, defense and maintenance mechanisms.

Evolution theory; here it is suggested that that longevity is a product of evolutionary forces e.g. body weight brain weight and flight e.t.c different species of animals have different life span, this provides evidence that longevity is genetically influenced. All adaptations that afford protection from predators and other hazards e.g. spines in porcupines justify greater developmental resources to build more durable animal and a longer maximum lifespan.

Neuroendocrine or hormonal Theory; this theory Suggests the role of specific hormones in the aging process, notably cortisol, a hormone known to increase as organisms age and plays crucial role in stress; estrogen and so on. Generally it is known that the hypothalamus loses it precision regulatory ability and the receptors which uptake individual hormones become less sensitive to them. Accordingly, with increasing age the secretion of many hormones declines and their effectiveness (compared unit to unit) is also reduced due to the receptors down-grading.

Telomere or Telomerase Theory of Aging. Telomeres (the sequences of *nucleic acids* extending from the ends of *chromosomes*), shorten every time a cell divides. This shortening of telomeres is believed to lead to cellular damage due to the inability of the cell to duplicate itself correctly. Each time a cell divides it duplicates itself a little worse than the time before, thus this eventually leads to cellular dysfunction, aging and indeed death. Telomerase is an enzyme that appears to repair and replace telomeres helping to re-regulate the clock that controls the life-span of dividing cells, it is found only in germ and cancer cells.

Mitochondria theory; this theory is closely related to the free radical theory but emphasis of damage by FRs is placed on the mitochondria which is the power house of the cell where most endogenous generation of FRs occur. Mitochondria are the only cellular organelles possessing

their own DNA, these DNA unlike nuclear DNA do not have protective heat shock and histone proteins, also lack DNA repair mechanisms, hence are liable to quick damage by FRs. The mitochondrial theory of AGING postulates that damage to mitDNA occur at a rate 10-20 times more than damage to nuclear DNA due to deficiencies in the oxidative phosphorylative pathway leading to loss of mitochondria functions (mitDNA code for the protein complexes of the electron transport chain) and imminently cellular function (due to insufficient production of energy). About 1-2% of oxygen leak from the respiratory chain to for reactive oxygen species.

Free radical theory; this theory of aging was developed by Denham Harman, free radicals (FR) are molecules that have one or more free electrons (unpaired electron) and is capable of existing independently, and this property makes it react with healthy molecules in a destructive way. Reactive species is a term used to describe FRs and other molecules that are easily converted to FRs and are powerful oxidizing agents. These compounds are found both intra and extracellularly and maybe produced endo and exogenously.

It is known that diet, lifestyle, drugs (e.g. tobacco and alcohol) and radiation etc., are all accelerators of free radical production within the body. However, there is also natural production of free-radicals within the body. This is the result of the production of energy, particularly from the mitochondria as a byproduct of oxidative metabolism. Other endogenous sources include phagocytic processes, prostaglandins, detoxification processes and so on.

Free radicals are known to attack long lived biopolymers in the body such as structural proteins, DNA, lipids (membranes of cells), prostaglandins etc. for instance attack on lipids in cell membrane can damage the membrane by disrupting fluidity and permeability, while lipid peroxidation (oxidative change caused by free radical on lipids) of mitochondrial membranes reduces electrical potential and the mitochondria's ability to generate energy through the electron transport chain. Also FR damage cause fragmentation of DNA, loss of function and structural integrity of proteins, disrupt protein synthesis etc

Oxidative stress is caused by FRs.

REDUCTION OF OXYGEN TO REACTIVE SPECIES

$O_2 + e + H^+$---------HO_2^* (hydroperoxyl radical)

HO_2^*---------$H^+ + {}^*O_2^-$ (Superoxide radical)

$O_2^- + 2H^+ + e$----------H_2O_2 (hydrogen peroxide)

$H_2O_2 + e$----------$OH^- + {}^\cdot OH$ (hydroxyl radical)

${}^\cdot OH + e + H^+$---------- H_2O

Lipid peroxidation.

$^{\cdot}OH + LH$--------$^{\cdot}L + H_2O$; hydroxyl radical reacts with lipid molecules (LH) in the membranes of cells to produce lipid molecule radical (alkyl= $^{\cdot}L$)

$^{\cdot}L + O_2$ ------$LOO^{\cdot}$; The lipid radical then reacts with oxygen to form lipid peroxides (lipid peroxyl radicals, lipid molecules containing paired oxygen groups)

$LOO^{\cdot} + LH$----------$LOOH + {}^{\cdot}L$.

The lipid hydroperoxides can promote a Fenton reaction;

$Fe^{++} + LOOH$----------$Fe^{+++} + {}^{\cdot}OL + H_2O$

The lipid alkoyl radical ($^{\cdot}OL$) is more reactive and damages more than the lipid peroxide radical ($LOO^{\cdot}$). However if two alkoyl, alky or peroxide radicals collide they nullify each other after creating a cross link between two lipids.

FACTORS INFLUENCING THE OCCURRENCE OF OXIDATIVE STRESS

Antioxidants; these group of compounds that delay or inhibit the occurrence of oxidative damage to target molecules by acting as replacement to such target cells, keeping formation of reactive species to a minimum, replacing and repairing damaged molecules, scavenging FRs, and binding metal ions required for the formation of highly reactive species e.g. Fe^{2+} , Cu^{+} etc. Antioxidants could be enzymes, minerals or compounds.

- Antioxidant enzymes found endogenously which play a crucial role in scavenging FRs these include superoxide dismutase (SOD), glutathione peroxidase and catalase. These enzymes are found in all cells
- SOD – catalyzes the reaction between 2 superoxide ions to produce H_2O_2 and triplet oxygen.
- Catalase catalyzes the formation of water and free oxygen from H_2O_2, it is present in membrane limited organelles called peroxisomes which contains other enzymes involved in degrading amino acids and fatty acids with the production of H_2O_2 as a byproduct.
- Glutathione peroxidase (GP) catalyses the reduction of H_2O_2 to water by using the antioxidant compound glutathione.
- Glutathione is a tripepetide and a major antioxidant in the non-lipid portion of cells. It exists as reduced glutathione GSH and oxidized GSSG. GP takes hydrogen molecules from glutathione and transfers to H_2O_2 to yield water.
- Vitamin E is the main F.R trap in the lipid bilayer of membranes.
- Vitamin C acts as an antioxidant in the non-lipid portion of cells and blood stream. Melatonin is a hormone produced by the pineal gland in decreasing quantities with age and it has been shown to be effective in protecting against $^{\cdot}OH$ molecules.
- Uric acid (produced from purine degradation) can also act as an antioxidant by binding to ion metal like Fe.

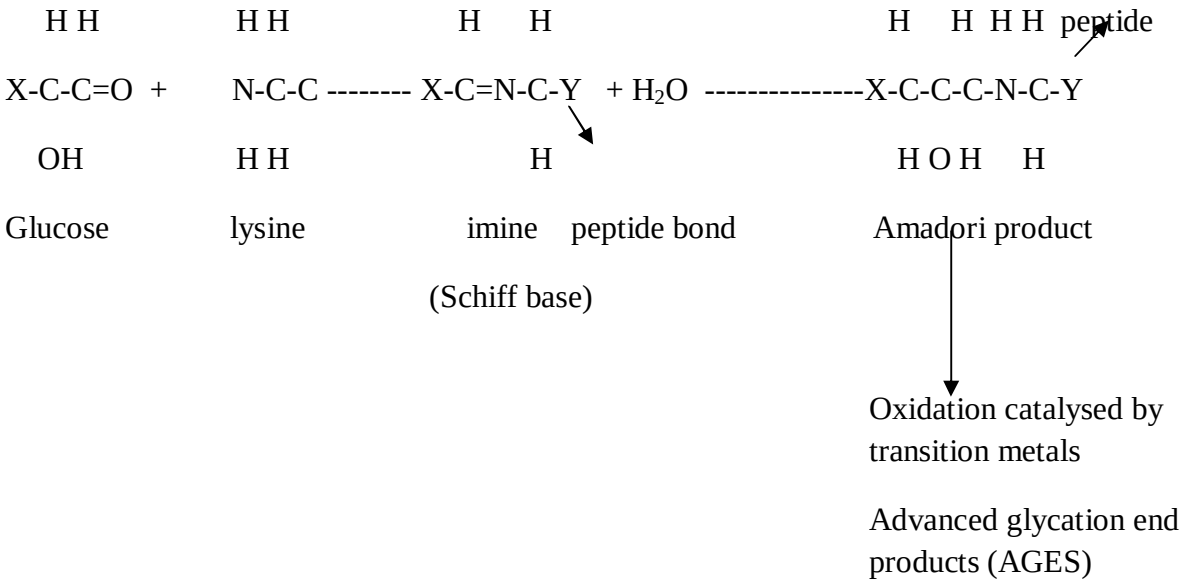
- A number of other compounds and chemicals notably found in plants e.g. lycopene, resveratrol, kolavirion etc has also been shown to have Free radical scavenging capabilities.

Increase in FRs or reactive species; this can be influenced by

- excessive activation of phagocytes which produce FRs that may impose oxidative stress on tissues
- toxins form the environment e.g. cigarette smoke known to stimulate FRs production
- products of detoxification of toxins include FRs
- increased oxygen concentration or tension
- caloric restriction has been shown to increase life span of yeast cells, drosophila, worms and rodents, it is hypothesized that caloric restriction slows and reduces the overall metabolism (energy production, electron transport chain) hence also reduced production of reactive oxygen species.

Glycation theory; glycation is the formation of double bond between the glucose aldehyde and the lysine groups of amino acids with the elimination of water. An end product AGES- advanced glycation end products - is formed. AGES in tissues increases the rate of FR production up to 50 times the rate of production in unglycated proteins. AGES attaches to LDL-cholesterol accelerates oxidation and subsequent atherosclerosis. It can also aggravate protein cross linking; AGES may also be ingested in food. These compounds are known universal symptoms of aging and can adversely affect skin, lings, muscles, blood vessels and organ function in general.

The damage of proteins by FRs and glycation is also called Maillards reaction.

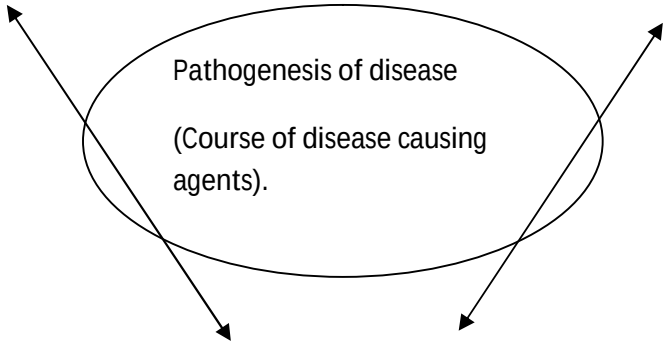


BIOCHEMISTRY OF DISEASE

Biochemical changes occur as the basis for occurrence of disease. An understanding of the physiological biochemistry of the organism forms a baseline in understanding biochemistry of diseases.

Summarily;

CELL INJURY ⟷ FREE RADICALS



TISSUE DAMAGE

Changes therefore in the body of organisms with disease occurs as a result of the basic mechanism above and due to the body's effort to contain these changes for examples cell death to remove non-functional cells (apoptosis) such situation occurs to red cells as diseased ones are rapidly removed from the circulation by spleen leading to anemia a common feature of many parasitic blood diseases e.g. trypanosomosis.

SOME CHANGES IN DISEASE AND BIOCHEMICAL BASES

1. Anemia- rapid breakdown of infected erythrocytes by the RES.
2. Hypoglycemia- excessive utilization of energy body cells to fight on going infections, and by the invading organisms to the detriment of host.
3. Hypergammaglobulinemia-increased synthesis of globulins to fight on-going invasion
4. Elevation of plasma enzymes- due to rapid tissue/cellular breakdown and release of contents into blood. The cell death is self-induced as protective mechanism; apoptosis
5. Damage to more cells as a result of rapid release of FRs from invading organisms, phagocytes etc

LIVER AND LIVER FUNCTIONS

Functions of the Liver

- Detoxification of endogenous and exogenous toxins
- Metabolism of CHOS, fats, and proteins
- Bile production
- Blood filtration
- Blood glucose regulation

Liver Function tests

The various liver function tests can be clarified broadly used on the major functions of the liver, including:-

1) Excretory functions

The liver is responsible for conjugating bilirubin, a production formed from the catabolism of hence to diglucuronide which is readily excreted in bile Bilirubin and other dyes like urobilinogens and sterobilinogen on be measured is blood (serum), and urine as important that for liver function. Bilirubin is estimated by Van Der Bergh reaction where diazotized suphanilic acid reacts with bilirubin to form a people colored complex-azobilirubin. For conjugated bilirubin the color change is produced immediately (direct), while for unconjugated color is produced only after addition of alcohol (indirect)

Only conjugated bilirubin is soluble in water and excretable wine, hence when there is obstructive jaundice, urine contains bilirubin as a means of excreting it from the body.

- Bromsulphthalein (BSP) test or sulphobromophthalein (organic anion)

When this dye is injected the hepatic cells conjugation it with glutathione although a significant faction is excreted unconjugated, when a single bolus dose to 50g/l is given, the retention of the dye after 45minutes in normal individuals (is less than 5% impairment of the liver cell function causes an increase in BSP retention.

Indocyacine green (ICG) is another dye also used.

2) Metabolic functions

The liver function tests are based on substances that are selectively metabolized by the liver e.g. galactose, ½ life of galactose in blood is about 10-15 minutes, but in defective liver is prolonged, antipyrine is rapidly and completely absorbed from the intestine and mostly metabolized by hepatic monoxygenase system, normal subject excrete 5-8% of this compound in their breathe on 2hrs while patients with cirrhosis excrete 2-3% and hepatitis 2-4%.

3) Synthetic functions liver

The liver functions in synthesis of almost all plasma proteins excepts Igs, so levels of plasma proteins may be arrested to determine the condition of the liver serum Albumin is appreciably reduced in all chronic liver disease but is not a good indicator of acute liver disease b/c of its long half-life. Haptoglobulin and transferrin are better indicators of acute liver changes. Prolong prothrombin time is used an indicator of poor prognosis in chronic liver disease. Others are alpha feto-protein which is a tumor marker, whole level is markedly increased is blood during hepatocellular damage.

4) Serum Enzymes

Amino transferases levels in serum are used to indicate liver disease as they are elevated usually in almost all liver disease. Alkaline phosphatase (ALP) whose synthesis is induced by bile duct obstruction, have elevated levels in serum cholestasis and hepatic carcinomas as compared to parenchymal liver disease. Gamma glutamyl transferase levels are also used and are sensitive to biliary tract disease (usually obstructions).

Others include 5-nucleotidase and leucine amino peptidase and in special circumstances glutathione-S-transferase. Others are Arginase, Sorbitol dehydrogenase (esply used in large animals as against ALT in small animals). All in small animals) Glutamate dehydrogenase, gamma glutamyl transpeptidase etc.

Jaundice

Definition: Yellowish coloration of tissues as a result of higher than normal concentrations of bilirubin in plasma.

Types

In Hemolytic jaundice unconjugated bilirubin is increased hence the Van der Bergh test is indirect position, while in obstructive jaundice, conjugated bilirubin is elevated and the test direct. In hepatocellular jaundice and biphasic reaction is observed because both conjugated and unconjugated bilirubin is seen.

Claim of Jaundice	**Type of Bilirubin**	**Causes**
Prehepatic/hemolytic	Unconjugated	Abnormal red cells, Abs drugs and toxins, thalassemias, Hemoglobinopathies, Gilbert's syndrome etc
Hepatic/hepatocellular	Conjugated/unconjugated	Viral hepatitis, toxic hepatitis, intrahepatic cholestasis
Post-hepatic/obstructive	Conjugated	Extra hepatic cholestasis, gall stories, tumors of bile duct, carcinoma of pancreas, Lymph Node enlargement etc

KIDNEY AND KIDNEY FUNCTION TESTS

The major function of the kidney is to excrete metabolic waste products and to maintain water, PH and electrolyte balance it also has endocrine functions of producing rennin, erythropoietin and calcitriol.

Kidney function tests can be broadly grouped into

1) Tests of glomerular filtration rate
2) Tests of tubular functions

Clearance Tests

Measurement of GFR is a useful index for assessment of severity of renal damage. Clearance is defined as the quantity of blood or plasma completely cleared of a substance per unit time and is expressed as milliner per minute. It estimates the amount of plasma that must lower formed true glomeruli per minutes not complete remove of that subtract to acct for its appearing in urine.

$$\text{Clearance} = \frac{\text{mg of substance excreted per minute}}{\text{Mg of substance per ml of plasma/serum}}$$

$$C = \frac{u \times V}{P}$$

U = concentration of substance in urine P

P = concentration of substance plasma

V = ml of urine excreted per minute

Inulin – is neither absorbed nor secreted by the tubules: - its clearance is a good measure of GFR.

Diodrast – chi-iodo-pyridone acetic acid, it is used is urinary tract x-ray, because

It is filtered and excreted.

Para amino hippurate (PAH) is also used as above, hence a measure of renal plasma flow.

Creatinine is a waste prdt formed from creatine PO_4, it has a continuous production much hardly fluctuates, hence its excretion is a good measure of GFR

Urea is partially reabsorbed, so GFR is slighting more than urea-clearance.

Tubular function

- measurement specific gravity (SG) – indicating osmolality
- concentration test
- ADH test

Muscle Action

- muscle comprise approximately 50% of the body (man)
- it is composed of long, multinucleated spindle shapes cells called myofibres
- These myofibres contain an array of specific contractile proteins and conductible membranes that give the muscle its excitable nature.
- Different types of mm exits- skeletal smooth cardiac muscles. These different mm tissues differ in myofibre constituents, vascular supply and nervous supply.
- Sarcolemma (plasmalemma of the skeletal myofibre) is the membrane of the muscle cells and is electrically excitable.
- Sarcolemma is also able to activate the contraction machinery located within the cells in response to signals it receives from the motor nerve, in contact with it.
- On this membrane surface (% mm cells0 are contained membrane spanning (transmembrane ion conducting pathways and channels gaits which regulate entry of Na+, K+, Ca2+ and + ions across the sarcolemma. There pathways and gates open selectively in response to ligands, transmitters, or changes in voltage and they close by intrinsic regulatory processes.

i) Voltage gated channels - have voltage sensing transmembrane domains and they are on essential for generation and modification of action potentials.

ii) Ligand gated ion channels – are essential for producing optimum myoplasmic calcium concentrations and establishing signal transduction pathway.

Motor End Plate

This is the neuromuscular junction, where there is a synapse, thus chemical transmission from the presynaptic axon terminal of a motor neuron (nerve) to the post gnaptic skeletal myofibre (muscle). The position of the NMJ on a mm fibre on vary among species, among different muscles an among fibres in a given muscle.

Axon Terminal (AT)

- The axon terminal rests on a 1o depression of sarcolemma called – primary cleft.
- The axon terminals contain nervous small vesicles that contain acetylcholine (ACH)
- ACH is a neurotransmitter, responsible for the excitation of skeletal myofibres.
- The space between the axon terminal the post synaptic sarcolemma comprises the synaptic cleft.
- the synaptic cleft is filled with basal lamina containing acetycholinesterase (AChE)
- When a nerve action potential arrives at the axon terminal there is activation (Opening) voltage gated calcium ion channels on the presynaptic membrane, hence influx of Ca2= into the AT.
- This Ca2+ influx results in a Ca2+ dependent exocytose of Ach –containing vesicles from presynaptic membrane. The Ach diffuses across the s/cleft to bind with Ach receptor on the muscle sarcolemma. AChR is an intergral transmembrane protein having 5 subunits.
- Muscle excitation is initiated by the reversible binding of Ach to the AChR, though a local depolarization of the post synaptic Mb, leading to the increased conductance of Na+ and K+ though the AChR cation-channel
- Meanwhile voltage gated K+ channels in the presynaptic Mb close the voltage gated Ca2+ channel back, and restore the resting Mb potential in the axon.
- Also Ach binding to AChR is transient, and α is abolished by diffusion of Ach away from the receptors α hydrolysis by AChE.
- The large conductance of Na+ α K= lead to a wave of depolarization (Normal resting Po in MM fibre is about – 95mV) exceeding a threshold (-50mV) to cause a muscle action potential (MAP).

- This MAP is propagated over the surface of the myofibril α into its depth via transverse (T) - tubules).
- At the T-tubules depths in the myofibres junctional complexes adjacent terminal cisternae of the sarcoplasmic reticulum (SR) are formed called 'triads'. It is at this triad (which occurs twice in a sarcomere) that calcium ions are released and lead to mechanical shortening of the myofibres as a result of the transmission of the MAP.
- The SR function in the uptake storage and release of Ca2+ to regulate the conclusion of Ca2+ in the mm sarcoplasm which bathes the myofilaments and other organelles in the mm cell.
- The concentration of Ca2+ in the SR is aided by the presence of a protein calsequestrin found in the lumen of the SR cisternae.

Muscle contraction

- The contractile constituents of the sarcomere include the thick (made up of myosin) and thin (actin) filaments the thick myofilaments possess lateral projections that from reactive sites with action-(cyclically annotate and dissociate during muscle contraction and relaxation).

  Myosin – Asymmetric protein with 2 identical heavy chains and 2 pairs of light chains. The composition of heavy chains within sarcomere varies among spps, muscles and muscles cells.

- Regulatory constituents of sarcomere include tropomyosin (a fibrous protein arranged along the length of the filaments) and Troponin (a globulin component TN-I (b) Tropomyosin-binding TN-I component and (c) Calcium binding component. There two proteins work in concert with calcium to regulate muscle contraction.
- Within the sarcomeres, the myofilaments are supported by complex cytoskeletal network of intermediate filaments in addition to a number of accessory proteins which help to maintain the alignment of myofilaments and sarcomere, adjacent myofibrils attach sarcomere of peripheral myofibrils to sarcolemma etc. these from the structural constituents of the sarcomere.
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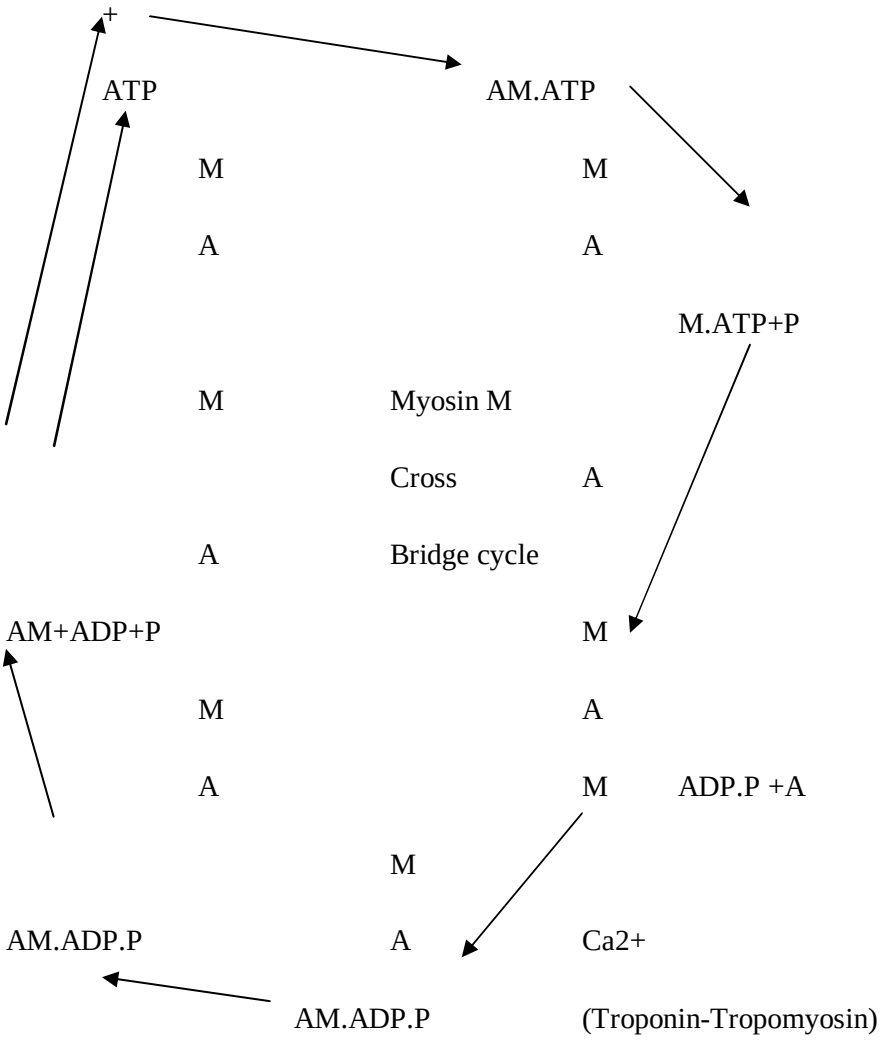
Energetics of muscle contraction (MC)

MC results from transformation of chemical energy to mechanical energy.



Force generation Step

A = action, M= myosin

ATP is hydrolyzed to ADP and inorganic Phosphate under the catalysis of myosin ATPase on the myosin head –leading chemically cyclical association and dissociation of the contractile proteins (Action and myosin) while mechanically its sees as shortening so sarcomere (as a result of sliding of overlapping arrays).

1) A.M connected at cross bridges of myosin

2) A.M: ATP 2 molecules of ATP bind to M molecule

3) A, M + ATP +P, A and M dissociate

4) ATP hydrolyses, when A is not associated with M=ADP +P

5) GH of M moves to a new location on A = A.M This recombination of A and M is under control so Troponin and tropomyosin in response to calcium concentration

6) The GH of M den includes to a 45^{o} angle of attachment

7) ADP + P den detaches, x ATP is reformed through rephoshorylation

Each sarcomere shortens approximately 12mm

Rigor Mortis

Is the rigid and stiff condition of skeletal muscles that develops after death. Here all ATP stores one utilized, hence dissociation of action and myosin does not occur, and the cycle is terminated w large no of A-M complexes 4med w myosin head set at 45^{0}.

NB: Cross bridges project from thick myofilaments and make contact with thin myofilaments

S2, S1 O Globular head. Myosin has both structural and enzymatic properties.

HORMONES

INTRODUCTION

Most glands of the body deliver their secretions by means of ducts. There are called exocrine glands.

There are few other glands that produce chemical substance that they directly secrete into the blood stream for transmission to various target tissues. These are ductless or endocrine glands. The secretions of endocrine glands are called as hormones.

DEFINITION OF HORMONES

It is a chemical substance which is produced in on part of the body, enters the circulation and is carried to distant target organs and tissues to modify their structures and functions.

SIMILARITIES OF HORMONES AND ENZYMES

- They act as body catalysts resembling enzymes in some aspect.
- They are required in small quantities.
- They are not used up during the reaction.

DISSIMILARITIES OF HORMONE AND ENZYME

- They are produced in an organ other than that in which they ultimately perform their action.
- They are secreted in blood prior to use.
- The circulating levels of hormones can give some indication of endocrine gland activity and target organ exposure. Because of the small amount of the hormones required, blood levels of the hormones are extremely low. In many cases it is ng/µg or MIU etc
- Structurally they are not always proteins. Few hormones are protein in nature, few are small peptides. Some hormones are derived from amino acids while some are steroid in nature.

The major hormone secreting glands are:

- Pituitary * Thyroid * Parathyroid * Adrenal * Pancreas
- Ovaries * Testes

Several other glandular tissues are considered to secrete hormones viz

- Juxtaglomeular cells of kidney: May produce the hormone erythropoietin which regulates erythrocytce maturation, erythropoiesis.
- Thymus: this produces a hormone that circulates from this organ to stem cells in lymphoid organ inducing them to become immunologically competent lymphocytes.
- Pineal gland: It produces a hormone that antagonizes the secretion or effects of ACTH. It also produces factors called glomerulotrophins that regulates the adrenal secretion of aldosterone

- Gastrointestinal tract: few hormones are also produced by certain specialized cells of GI tract and they are called GI hormones e.g. gastrin, secretin etc.

CLASSIFICATION OF HORMONES

According to Li the hormones can be classified chemically into three major groups.

- Steroid hormones: These are steroid in nature such as adrenocorticosteroid hormones, androgens, estrogens and progesterone
- Amino acid derivatives: These are derived from amino acid tyrosine e.g. epinephrine, norepinephrine and thyroid hormones.
- Peptides/protein hormones: These are either large proteins or small or medium size peptides e.g insulin, glucagon, parathormone, calcitonin, pituitary hormone etc.

Factors regulating Hormone Action

Action of a hormone at a target organ is regulated by four factors.

- Rate of synthesis and secretion: The hormone is stored in the endocrine gland.
- In some cases specific transport system in plasma
- Hormone-specific receptor in target cell membranes which differ from tissue to tissue
- Ultimate degradation of the hormone usually by the liver or kidneys

Mechanism of Action of Hormone

Although the physiological apparently secondary effects of most of the hormones have been rather completely known for a number of years, their primary biochemical mechanism of actions at a cellular/molecular level are also known in much details now. Many hormones serve as inducers or repressor in the genetically controlled synthesis of certain key cellular enzymes. Although the exact site of action of any hormone is still not well understood, the following mechanisms of actions of hormone have been proposed.

1. **Interaction with nuclear chromatin (Nuclear Action):**

   Steroid hormones act mostly by changing the transcription rate of specific genes in the nuclear DNA. The steroid hormone has a specific soluble, oligomeric receptor protein (mobile receptor) either in cytosol and or inside the nucleus. This brings about conformational changes and also changes in the surface of the receptor protein to favour

its binding to the nuclear chromatin attached to nuclear matrix. The receptor-steroid complex is translocated to the nuclear chromatin and binds to a steroid-recognizing receptor site called the hormone-responsive element (HRE) of a DNA strand on the upstream side of the promoter site for a specific steroid responsive gene. The consequence change in the intracellular concentration of mRNA alters the rate of synthesis of a structural, enzymatic carrier or receptor protein coded by it. This results in ultimate cellular effects. The receptor-steroid complex subsequently leaves the acceptor site as the free receptor and the steroid. In addition to regulating the transcription, some steroid hormones may also act as regulatory agents for post transcriptional processing stability and transport of specific mRNAs.

2. Membrane Receptor

As per the suggestion of Heller, certain molecules cannot enter target cells through membrane lipid bilayer. This is achieved by the specific receptor molecules present on the surface of the plasma membrane. Many hormones seen specifically involved in the transport of a variety of substance across cell membrane. In general these hormones specifically bind to the receptor on cell membrane. They cause rapid secondary metabolic changes in the tissue but have little effect on metabolic activity of membrane free preparations. Most protein hormones and catecholamines activate transport of membrane enzyme systems by direct binding to specific receptors on the membrane.

3. Stimulation of Enzyme Synthesis at the Ribosomal Level

Activity at the level of translation of information is carried by the mRNA on the ribosomes for the production of enzyme. Ribosomes taken from growth hormone treated animals have a modified capacity to synthesize protein in the presence of normal mRNA. Thus, in this case either increased production of new ribosomes or to create new population of more active or more selective ribosomes might be taking place.

4. CAMP and Hormone Action

3'-5'cAMP plays a unique role in the action of many protein hormones. Its level may be decreased or increased by hormonal action as the effect varies depending on the tissue. The hormones such as glucagons, catecholamines, PTH, etc. acts by influencing a change in intracellular cAMP concentration through the adenylate cyclase c-AMP system. The hormone binds to a specific membrane receptor. Different types of these receptors remain associated with wither G_s or G_i type of GTP-dependent trimeric nucleotide regulatory complexes of the membrane. Both G_s and G_i are made up of 3 subunits. G_s contain $\alpha_s\beta\gamma$ while G_i contains $\alpha_i\beta y$. Formation of the receptor hormone complex promotes the binding of GTP to the α subunit of

either G_s or G_i. When α_s GTP is released it binds to adenylate cyclase located on the cytoplasmic surface of the membrane and changes its conformation to activate it. However in some cells calmodulin-4Ca^{2+} is also required for activation. Adenylate cyclase catalyses the conversion of ATP to cAMP thus increased the intracellular concentration of the latter. On the other hand α_i-GTP inhibits adenylate cyclase by binding with it. This lowers the intracellular concentration of cAMP. The action of cAMP is mainly to activate some protein kinases allosterically.

Insulin can decrease hepatic cAMP in opposition to the increase caused by glucagon. Tissue levels of cyclic AMP can be influenced not only by hormone but also by nicotinic acid, imidazole, methylxanthine.

5. Role of Polyphosphoinositol and diacycglycerol in hormone action

Just like c-AMP other compounds such as 1,4,5- inositol triphosphate (ITP) and diacylglycerol (DAG) act as second messengers. This is especially found in case of vasopressin, TRH, GnRH, etc. These hormones activate the phospholipase c-polyphosphoinositol system to produce ITP and DAG by binding with the specific receptor protein on cell membrane, the hormone activates a trimeric nucleotide regulatory complex. The complex in turn activates phospholipase C on the inner surface of the membrane. ITP enhances the mobilization of ca^{2+} into the cytosol from intracellular ca^{2+} pool from mitochondria. Ca^{2+} then act as tertiary messenger. While DAG activates Ca^{2+} phosphatidyl-serine-dependent protein kinase c located on the inner surface of the membrane, by lowering its Km for $Ca^{2+.}$ This enzyme then phosphorylates specific enzymes and other proteins in the cytosol to modulate their activities.

6. Role of Calcium in Hormone Action

The action of most protein hormones is inhibited in absence of calcium even though ability to increase or decrease cAMP is comparatively unimpaired. This calcium may be more terminal signal for hormone action then cAMP. It is suggested that ionized calcium of the cytosol is the important signal. The source of this calcium may be intracellular fluid or it may arise from mobilization of intracellular tissue bound calcium. As mentioned, membrane receptor binding may be responsible for this. The hormone receptor binding may directly inhibit the Ca^{2+}-ATPase. It may also directly open up voltage-independent Ca^{2+} channels in the membrane to increase the diffusion of Ca^{2+} into the cell down its inward concentration gradient resulting in increased cytosolic Ca^{2+} concentration which then acts as a second messenger to affect cellular activities. The receptor-hormone complex may produce ITP which in turn can increase cytosolic Ca^{2+} concentration by enhancing the mobilization of Ca^{2+} from mitochondrial and endoplasmic reticular pool. Calcium is involved in the regulations of several enzymes such as phospholipase A2, Ca^{2+}- phosophatidylserine dependent protein kinases, guanylate cyclase, adenylate cyclase,

and glycogen synthetase. All these enzymes have special biochemical metabolic roles. Ca^{2+} also changes membrane permeability. Many of its effects are mediated through its binding to Ca^{2+}-dependent regulatory proteins like calmodulin and troponin.

7. Role of c-GMP in Hormone Action

Hormone such as insulin and growth hormone affect the guanylate cyclase c-GMP system. This will increase the intracellular concentration of c-GMP and activate c-GMP dependent protein kinase. The active c-GMP protein kinase would in turn bring about phosphorylation of specific cellular proteins to change their activities leading to relaxation of smooth muscle, vasodilation and other effect. It is likely that Ca^{2+} may act as a second messenger to activate guanylate cyclase and thereby increasing the concentration of c-GMP inside the cell.

8. Role of Phosphorylation of Tyrosine kinase

In fact a second messenger for insulin, growth hormone prolactin, oxytocin etc has not been identified so far. However, binding of them to their respective membrane receptors activates a specific protein kinase called tyrosine kinase which phosphorylates tyrosine residue of specific proteins. This may bring about some metabolic changes.

REGULATION OF HORMONE SECRETION

Hormones secretion is strictly under control of several mechanisms.

a. **Neuroendocrinal Control Mechanism**

Nerve impulses control some endocrine secretion. Cholinergic sympathetic fibers stimulate cathecholamine secretion from adrenal medulla. Centres in the midbrain, brainstem, hippocampus, etc can send nerve impulses which react with the hypothalamus through cholinergic and bioaminergic neurons. At the terminations of these neurons they release acetylcholine and biogenic amines to regulate the secretions of hypophysiotropic peptide hormones from hypothalamic peptidergic neurons. Some of the endocrine releases are controlled by either stimulatory or inhibitory hormones from a controlling gland, e.g corticosteroids are controlled by coticotropins and thyroid hormonrs are controlled by thyroptropin from anterior pituitary. The tropins are further regulation by hypothalamic releasing hormones.

b. **Feedback Control Mechanism**

It is due mainly to negative feedback that such control is brought about. When there is a high blood level of a target gland hormone. It may inhibit the secretion of the tropic hormone stimulating that gland. Adrenal cortex secretes a hormone called cortisol which brings about the inhibition of secretion of corticotrophin from anterior pituitary and corticotropin releasing hormones from the hypothalamus by a long-loop feedback. This leads to reduction in cortisol secretion.

c. **Endocrine Rhythms**

There are certain cyclic rhythmic associated with the secretion of hormone over a period of time. When there is a cyclic periodicity of 24 hrs, it is called as circadian rhythm. However, if it is more than 24hrs, it is named as infradian rhythm and when it is less than 24 hrs it is called as ultradian rhythm. Due to rhythm the highest and lowest concentration of corticotrophin is normally found in the morning and around midnight. Growth hormone and prolactin rise in the early hours of deep sleep. Cortisol peak is found between 4am and 8am. Endocrine rhythms results from cyclic activities of a biological clock in the limbic system supplemented by the diurnal light-dark and sleep activity cycles and mediated by the hypothalamus.

Pituitary Hormones

Control of secretion

Secretion of hormones from anterior pituitary are controlled by

- Nervous mechanism: by release of regulatory factors from hypothalamus
- Hormonal mechanism by feedback inhibition

Hypothaciamic Releasing Factors

Control of hormone secretion from pituitary is in part modulated by regulating factors or hormone from the hypothalamus. The median eminence of hypothalamus is connected directly to the pituitary stalk. Within this stalk is a portal system of blood vessels required to maintain normal secretary activity of the pituitary gland. The activities of the cells of the anterior lobe are controlled by the nerve cells of the hypothalamus which send axons to the capillary beds. The nerve endings liberate chemical substances, hypothalamic releasing factors or hormones. At present 10 discrete regulatory factors have been described that may affect the synthesis as well as secretion of specific pituitary hormone. They are:

	Hypothalamic Hormone or factor	**Abbreviation**
*	Corticotropin (ACTH) releasing hormone	CRH or CRF
*	Thyrotropin (TSH) releasing hormone	TRH or TRE
*	Follicle stimulating hormone (FSH)	FSH-RH or FSH-RF
*	Luteinizing hormone (LH) releasing hormone	LH-RH or LH-RF
*	Growth Hormone (GH)	GH-RH or GH-RF
	releasing hormone	GH-RF

*	Growth hormone release inhibiting	GH-RH or GIF
*	Prolaction (PL) release	PL-RIH or PL-RIF
*	Melanocyte stimulating hormones	mSH-RIH or
*	(MSH) release inhibiting hormone	MSH-RIF
*	Melanocyte stimulating hormone	MSH-RH or
*	(MSH) releasing hormone	MSH- RF

Hormones of the Anterior Pituitary

The hormones secreted by the anterior lobe of the pituitary gland are:

- Growth hormone and
- Pituitary tropic hormones such as protactin, gonadotropins (FSH and LH), thyrotropic hormones (TSH) and adrenocorticotropic hormones (ACTH)

Growth Hormone.(GH)

Growth Hormone (GH) or somatotropin (STH) was first isolated in sufficient quantity from cattle, now it has been prepared in crystalline form several species including man.

Chemistry

Growth hormone from all mammalian species consists of a single polypeptide with a molecular weight of about 21500. It consists of 191 amino acids. There are two disulfide bridges between the adjacent cysteine residue (52 and 165 and 183 189). Although there is a high degree of similarity in the amino acid sequences of human, bovine and porcine GH, only human GH or that of other primates is active in man. GH can bring about some of the actions of prolactin and human placental lactogen (HPL) due to amino acid homology.

Metabolic Role

Growth hormone has a variety of effects on different tissues. The hormones act slowly requiring from 1-2 hours to several days before it biological effects are detectable. This slow action and its stimulatory effects on RNA synthesis suggest that it is involved in protein synthesis. The

hormone acts by binding to specific membrane receptors on its target cells. But its exact mechanism of action and second messenger are not yet known.

1. **Protein Synthesis**

Growth hormone brings about positive nitrogen balance by retaining nitrogen. It stimulates overall protein synthesis with an associated retention of phosphorus probably by increasing tubular reabsorption. Blood amino acid and urea level are decreased. It facilitates the entry of amino acids into the cell. In addition, growth hormone facilitates protein synthesis in muscle tissue by a mechanism independent of its ability to provide amino acids. This protein synthesis carries on even if the amino acid transport is blocked.

- Growth hormones increases DNA and RNA synthesis
- It increases the synthesis of collagen

2. **Lipid Metabolism**

Growth hormone brings about lipolysis in a mild way by mobilizing fatty acids from adipose tissue by activating the hormone sensitive triacylglycerol lipase. Thus it increases circulating fatty acids.

3. **Carbohydrate Metabolism**

Growth hormone is a diabetogenic hormone, antagonizes the effect of insulin. Hypersecretion of GH can result in hyperglycemia, poor sugar tolerance and glycosuria. Growth hormone produces.

- Hyperglycaemia by increasing gluconeogenesis
- It reduces insulin sensitivity and thereby decreases the hypoglycaemic effect of insulin
- It brings about glycostatic effect, i.e increases liver glycogen it can also increase muscle and cardiac glycogen level probably by reducing glycolysis.

4. **Effect on Growth of Bones and Cartilages**

Growth hormone when secreted in abnormally high concentration prolongs the growth of epiphyseal cartilages to cause over growth of long bones. Acromegaly is found in adults. Hyposecretion causes stunted stature due to premature cessation of growth of the epiphyseal cartilages and consequently of long bones.

- The effect of growth hormone partly depends upon its calcium anabolic action. It promotes the retention of calcium and phosphate which helps in ossification and osteogenesis.

- It enhances the incorporation and hydroxylation of proline in the matrix collagen, incorporation of amines into glycosaminoglycans of cartilage, incorporation of sulphate into matric proteoglycans like chondroitin sulphates, the synthesis of DNA and RNA in chondrocytes.
- The growth effects are mediated by a peptide called insulin-like growth factor I (IGF-I) or somatomedin – C)

5. **Prolactin Action**

Growth hormone has a sequence homology with prolactin. Growth hormone binds to membrane receptors for prolactin and stimulates the growth and enlargement of mammary gland.

6. **Ion or Minerial metabolism**

It is observed that the intestinal absorption of calcium is increased by GH, since the bone growth and development is stimulated by growth hormone. Growth hormone retains Na, Ca, K, Mg, and PO_4^{3-}

PITUITARY TROPIC HORMONES

In addition to GH, anterior pituitary gland secretes some tropic hormones usually called as pituitary tropins.

A tropin or tropic hormones is the one which influences the activities of other endocrine gland, principally those involved in stress and reproduction. These are carried by the blood to other target gland. The pituitary tropins are under the positive and negative control of peptide factors from hypothalamus. Further the tropic hormones are usually subject to feedback inhibition at the pituitary or hypothalamic level by hormone product of the final target gland. Prolactin (mammotropin), TSH (thyrotropin), FSH and LH (Gonadotropins), ACTH (Corticotropin) are the tropic hormones secreted by the pituitary gland.

A. Prolaction: PRL or Leuteotropic Hormone (LTH)

This is a monomeric simple protein (Mw23, 000). It contains 199 amino acids with three –s-s-linkages. It is secreted by lactotroph α-cells of anterior pituitary and has sequence homology with growth hormone.

Metabolic Role

- The main function of PRL is to stimulate mammary growth and the secretion of milk. By acting through specific glycoprotein receptors on plasma membrane of mammary gland cells, it stimulates mRNA synthesis. This ultimately leads to enlargement of breast (udder) during pregnancy. This is called mammotropic action.
- The synthesis of milk proteins such as lactalbumin, and casein takes place after parturition such an effect is called lactogenic action.
- Estrogen, thyroid hormones and glucocorticoids increases the number of prolactin receptors on the mammary cell membrane.
- Progesterone has the opposite effect.

B. **Thyrotrophic Hormone or Thyroid Stimulating Hormone (TSH)**

This is produced by basophil cells of anterior pituitary and is glycoprotein in nature. Its molecular weight is approximately 30,000. This consists of α and β submits.

- The α - subunit of TSH, LH and HCG and FSH are nearly identical
- The biological specificity of thyrotropin must therefore be in β -subunit. The α-subunit consist of 92 amino acids while β-subunit has 112 amino acids. Both α and β have several disulfide bridges. It carbohydrate content is 21% and it αand β chains bears two and one oligosaccharide chains linked by N-glycosidic linkages to specific asparagine residues. The chains are synthesized separately by separate structural genes and later undergo post-translation modification and glycosylation separately.

Metabolic Role

There are glycoprotein receptors on the thyroid cells membrane which binds to the receptor binding site on β-subunit of TSH. The complex then activates adenylate cyclase which catalyzes the formation of c-AMP which acts as the second messenger for most TSH actions an follows:

- TSH stimulates the synthesis of thyroid hormones at all stages such as Iodine uptake, organification and coupling.
- It enhances the release of stored thyroid hormones.
- It increases DNA content, RNA and translation of proteins, cell size.
- It stimulates glycolysis, TCA Cycle, HMP and phospholipids synthesis. Stimulation of last two does not involve c-AMP.
- It activates adipose tissue lipase to enhance the release of fatty acids (lipolysis)

C. ADRENOCORTICOTROPIC HORMONE (ACTH) OR CORTICOTROPIN: It is a single polypeptide containing 39 amino acids in its structure with a molecular weight of 4500 two forms have been isolated, α-corticotropin and β-corticotropin. Biological activity of ACTH resides in the first 23 amino acids from N-terminal end. The sequence of these 23 amino acids in the peptide chain is the same in all species tested. The remaining biologically inactive 16 amino acid residue varies accordingly to sources. ACTH is synthesized as a part of precursor peptide of mol.wt of 31500 with 260 amino acids. ACTH contains sequence of amino acids common for LPH, MSH and the endorphins. The precursor molecule is synthesized as a glycoprotein called pro-opiomelanocortin peptide (POMC). Various proteolytic enzymes hydrolyze POMC to give different peptides. Thus POMC is broken down into

- ACTH
- β-lipotropin (LPH). β-LPH is further cleaved into γ-LPH and endophins.

METABOLIC ROLE

The principal actions of corticotrophin are exerted on the adrenal cortex and extra adrenal tissue. ACTH increases the synthesis of corticosteroids by the adrenal cortex and also stimulates their release form the gland. Profound changes in the adrenal structure, chemical composition and enzymatic activity are observed as a response to ACTH. Total protein synthesis is found to be increased. Thus, ACTH produces both a tropic effect on steroid production and tropic effect an adrenal tissue. It is observed that ACTH has specific receptors on cells of fasciculata which increases c-AMP levels in the cell. This activation is calcium dependent. This result in DNA content aid RNA is transcribed. This leads to proliferation of fasciculata cells and growth of adrenal cortex.

- ACTH also stimulates the synthesis and secretion of glucocorticoids.
- ACTH is found to increase the transfer of cholesterol from plasma lipoproteins into the fasciculata cells.
- The ACTH induces rise in c-AMP, bring about phosphorylation and activation of cholesterol esterase. The enzyme action ultimately makes a large pool of free cholesterol.
- It activates the rate limiting enzyme for conversion of cholesterol to pregnenolone.
- It activates dehydrogenases of HMP to increase the concentration of NADPH required for hydroxylation.
- By activating adenylate cyclase of adipose tissue, it increases intracellular c-AMP which in turn activates hormone sensitive lipase. This enzyme is involved in lipolysis which increases the level of free fatty acids.
- It leads to increase ketogenesis.

- Direct effects on carbohydrate metabolism include :
- Lowering of blood glucose
- Increase in glucose tolerance
- Deposition of glycogen in adipose tissue is increased, regarded as due to stimulation of insulin secretion.
- It has MSH activity due to homology in amino acid sequence.

D. PITUITARY GONADOTROPINS

These tropic hormones influence the function and maturation of the testes and ovary, and are of two types.

- Follicle stimulating hormone (FSH)
- Luteinizing hormone (LH)

Both of them are glycoproteins with sialic acid, hexose and hexosamine as the carbohydrate moiety. Molecular weight of FSH is 25000 and that of LH is 40000. FSH, LH are dimers of α and β-chains linked non covalently. The α-chain is identical for TSH, FSH and LH of the same species. The β-chain of human FSH and LH has respectively 118 and 112 amino acid residues. Each chain has several disulfide bridges. A large precursor protein molecule for α and β chains is synthesized separately in gonadotroph β-cells.

METABOLIC ROLE OF FSH

It brings about it action by specific receptor binding and c-AMP

In females:

- It promotes follicular growth
- Prepares the Graafian follicle for the action of LH
- Enhances the release of estrogen induced by LH

In males:

- It stimulates seminal tubule and testicular growth
- Plays an important role in maturation of spermatozoa.

Role of FSH in Spermatogenosis

The conversion of primary spermatocytes into secondary spermatocytes in the seminiferous tubules is stimulated by FSH. In absence of FSH spermatogenesis cannot proceed. However,

FSH by itself cannot cause complete formation of spermatozoa. For its completion testosterone is also required. Thus, FSH seems to initiate the proliferation process of spermatogenesis, and testosterone is apparently necessary for final maturation of spermatozoa. Since the testosterone is secreted under the influence of LH, both FSH and LH must be secreted for normal spermatogenesis.

Metabolic Role of LH

This hormone is also known as interstitial cells stimulating hormone (ICSH)

In females

- It causes the final maturation of Graafian follicle and stimulates ovulation
- Stimulates secretion of oestrogen by theca and granulosa cells.
- It helps in the formation and development of corpus luteum for luteinization of cells.
- In conjunction with luteotropic hormone (LTH) it is concerned with the production of estrogen and progesterone by the corpus luteum.
- In the ovary it can stimulate the non-germinal elements, which contain the interstitial cells to produce the androgens, androstenedione, dihydroepiandronstenedione (DHEA) and testosterone.

ACTION OF LH IN OVULATION (Ovulatory surge for LH): It is necessary for final follicular growth and ovulation. Without this hormone, even though large quantities of FSH are available the follicle will not progress to the stage of ovulation. LH acts synergistically with FSH to course rapid swelling of follicles shortly before ovulation. It is worth noting that especially large amount of LH called ovulatory surge is secreted by the pituitary during the day immediately preceding ovulation.

REGULATION OF TESTOSTERONE SECRETION BY LH: Testosterone is produced by the interstitial cells of leydig only when the testes are stimulated by LH from the pituitary gland, and the quantity of testosterone secreted varies approximately in proportion to the amount of LH available. Thus in males LH stimulate the development and functional activity of leydig cells (interstitial) and consequently testicular androgen.

ENDORPHINS AND ENCEPHALINS

Endorphins are a group of polypeptides which influence the transmission of nerve impulse. They are also known an opioides, because they bind to those receptors which bind opiates like morphine and play a role in pain perception. The opiodes first discovered were two penta-peptides in the brain and were named encephalin. They are of two types:

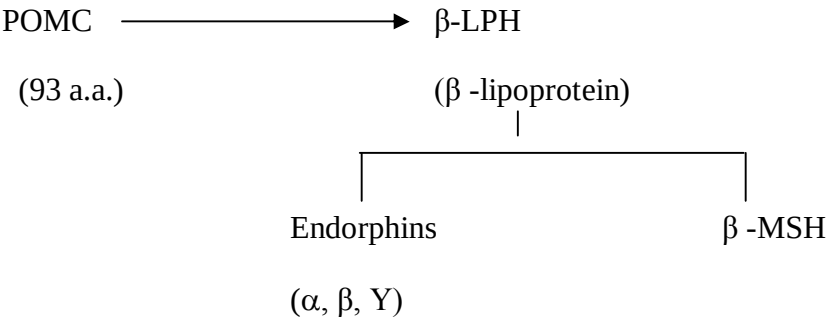
* Methionine - encephalin
* Leucine - encephalin

FORMATION OF ENDORPHINS

β – lipoprotein (β-LPH) is the precursor for endorphin, all the three type α, β and γ and also for β-MSH.

β- lipotprotein (β-LPH) is derived from the precursor molecule "Pro-opiomelanocortin peptide (POMC). It is a single chain polypeptide containing 93 amino acids.

γ-LPH containing 60 amino acids is a part of β-LPH



TYPES OF ENDORPHINS

There are three types of endorphinsα, β.y

- The sequence of 31 amino acids at the C-terminal of β-LPH, (Obtained from POMC) i.e amino acid 104 to 134 gives β endorphin
- α- endorphin (104 to 117 amino acid) containing 17 amino acids less than the β from the C terminal end.
- γ-endorphin (104 to 118) containing 16 amino acid less than the β from the C-terminal end.

FUNCTION

Endorphins bind to the same CNS receptors like the morphine opiates and they play a role in the endogenous control of pain perception. They have highs analgesic potency than morphine.

HORMONE OF MIDDLE LOBE OF PITUITARY

MELANOCYTE STIMULATING HORMONES

The hormones secreted by intermediate lobe or middle lobe of pituitary gland are called melanocyte stimulating hormones or MSH. POMC is the precursor molecule which is cleaved by proteases to give ACTH and β-lipotropin. The ACHT is further cleaved to β-MSH which has 13 amino acids. There is also α-MSH which is present in larger quantities. Amino acids 11-17 of β-MSH are common to both α-MSH and ACTH. MSH darkens the skin and is involved in skin pigmentation by deposition of melanin by melanocytes.

HORMONE OF POSTERIOR PITUITARY LOBE

The hormones have been isolated and characterized from extracts of posterior pituitary gland. They are

- Vasopressin (pitressin) or Arginine Vasopressin (ADH)
- Oxytocin

Both are small peptides containing nine amino acids. Oxytocin differs from vasopressin with respect to 3^{rd} and 8^{th} amino acid residues. Their biological activities depend on C-terminal glycinamide, the side chain amide group of glutamine and asparagine, the hydroxyphenyl group of tyrosine and the intra-chain –s-s-linkage between cysteine of 1^{st} and 6^{th} amino acid. Posterior pituitary hormones are synthesized in neuro-secretory neuron. They are stored in the pituitary in association with two proteins neurophysin I and II with molecular weight, of 19000 and 21000 respectively. The release of these two hormones is independent of each other.

METABOLIC ROLES OF VASOPRESSIN

1. Antidiuretic action: Antidiuretic effect is it main function. It reabsorbs water from the kidneys by distal tubules and collecting ducts. It is found to be mediated through formation of c-AMP. It is released due to rise in plasma osmolarity. This leads to formation of hypertonic urine having low volume, high specific gravity and high concentration of Na^+, Cl^-, PO_4^{3-} and urea. Halothane, colchicine and vinblastine inhibit antidiuretic effect of vasopressin.

CLINICAL IMPORTANCE

- Condition of *Diabetes insipidus* is described due to failure in secretion or action of vasopressin. It is characterised by very high volumes of urine output up to 20-30 litre per day with a low specific gravity and excessive thirst.
- In primary, central or neurohypophyseal diabetes insipidus, vasopression secretion is poor.
- In nephprogenic diabetes insipidus, kidney cannot respond to vasopressin due to renal damage. The damage is common in psychiatric patients on lithium therapy.
- Inappropriate vasopressin secretion is characterized by persistently hypertonic urine, progressive renal loss of Na^+ with low plasma levels of Na^+, symptoms of water intoxication like drowsiness, irritability, nausea, vomiting, convulsions, stupor and coma. It could be due to pulmonary infection and ectopic ADH secretion from lung tumor.
- Urea-retention effect: permeability of medullary collecting ducts to urea is increase by vasopressin. This leads to retention of urea and subsequently contributes to hypertonicity of the medullary interstitium. Urea retention effect can be reversed by phloretin.
- Pressor Effect: It stimulates the contraction of smooth muscles and this causes vasoconstriction by increasing cytosolic Ca^{2+} concentration.
- Glycogenolytic effect: By increasing intracellular calcium concentration.

METABOLIC ROLES OF OXYTOCIN

- Contraction of smooth muscle is the primary function of oxytocin. There are basically two effects, one on mammary glands called galactobolic effect and the other on uterus called as uterine effect.
- 1. **Galactobolic Effect**: This is released due to neuroendocrinal reflex such as sucking of nipples. By doing so it causes that contraction of myo-epithelial cells around mammary alveoli and ducts and the smooth muscle surrounding the mammary

milk sinuses. Estrogen increases the number of oxytocin receptors during pregnancy while progesterone decreases the dame and also inhibits the secretion of oxytocin.

- 2. **Uterine effects**: It is found to be elevated at full term pregnancy. It causes contraction of uterine muscle for childbirth. Estrogen enhance while progesterone decreases oxytocin receptors as well as its secretion. Oxytocin is also secreted during coitus by the female uterus which promotes the aspiration of semen into the uterus. This is also augmented by rise in estrogen in the follicular phases of menstrual cycle.

THYROID GLAND AND ITS HORMONES

Hormones produced by Thyroid gland

- Follicular cells: produces T4, T3 and reverse T3
- Parafollicular C-cells: produces calcitonin (hence also called thyrocalcitonin)

- **THYROID HORMONES**
- The principal hormones secreted by the follicular cells of thyroid are
- Thyroxine (T4)
- Tri-iodothyronine (T3)
- Reverse T3

CHEMISTRY OF THYROID HORMONE

- The hormones T4, T3 and reverse T3 are iodinated amino acid tyrosine. The iodine in thyroxine accounts for 80% of the organically bound iodine in thyroid venous blood. Small amounts of "reverse' tri-iodothyronine, monoiodotyrosine (MIT) and other compound are also liberated.

BIOSYNTHESIS OF THYROID HORMONE

- Two raw materials (substrates) required by thyroid gland to synthesize the thyroid hormone are:
- Thyroglobulin
- Iodine
- Thyroglobulin: Thyroid hormone are synthesized by the iodination of tyrosine residue of a large protein called thyroglobulin

CHEMISTRY OF THYROGLOBULIN

- Thyroglobulin is a dimeric of glycoprotein, 19s in type (a macroglobulin) with a molecular weight of 660,000
- The receptor tyrosine molecules are present in this macroglobulin protein, each molecule containing 115 tyrosine residues.
- Carbohydrates accounts for 8 to 10% of the weight of thyroglobulin and iodide for about 0.2 to 1.0% depending on the iodine content of the diet. The carbohydrates are N-acetyl glucosamine, mannose, glucose, galactose, fucose and sialic acid.
- About 70% of the iodide in thyroglobulin exists as inactive precursors monoiodo-tyrosine (MIT) and di-iodotyrosine (DIT) while 30% is in the iodothyronyl residues T4 and T3.
- When iodine supplies is sufficient, T4; T3 ratio is about 7:1. In iodine deficiency, the ratio decreases, including MIT/DIT ratio. T3 and T4 after being synthesized remains in the bound form until it is secreted. When they are secreted the peptide bonds are hydrolyzed and free T3 and T4 enter the thyroid cells, cross them and are discharged into the capillaries

THYROID ACINAR CELLS HAVE THREE FUNCTIONS

- They synthesize thyroglobulin and store as colloid in follicles
- They collect and transport iodine for synthesis of the hormones in the colloid
- They remove T3 and T4 from thyroglobulin secreting the hormones into the circulation.

TRANSPORT

Within the plasma, T4 and T3 are mostly transported almost entirely in association with two proteins, the so called thyroxine binding proteins" which act as specific carrier agents for the hormones.

Two main carrier proteins are:

- Thyroxine-binding globuline (TBG)
- Thyroxine binding prealbumin (TBPA)
- When large amount of T4 and T3 are present and the binding capacities of the above two specific carrier proteins are saturated, the hormones can be bound to serum albumin. Approximately about 0.05% of the circulating thyroxine is in the free unbound form. Free T3 and T4 are the metabolically active hormones in the plasma

MECHANISM OF ACTION OF THYROID HORMONE

- Thyroid hormones are transported into their target cells by a "carrier mediated" active transport system of the cell membrane. Target organs include; liver, kidneys, adipose tissue, cardiac, neurons, lymphocytes, etc.

1. Nuclear Action: T4 and T3 pass into the nucleus and bind directly to specific high affinity "nuclear receptors" which are histone chromatin proteins of specific genes. This receptor hormone binding increases the action of nuclear DNA dependent RNA polymerase increasing gene transcription, which in turn enhances m-RNA synthesis and induces synthesis of specific protein and enzyme.

1. Na^{+}-K^{+}- ATPase pump:

Thyroid hormone exerts most of metabolic effects by increasing 0_2-consumption. It has been suggested that much of the energy utilized by a cell is for driving the Na+-K+-ATPase pump. Thyroid hormones enhance the function of this pump by increasing the number of pump units, almost in all cells.

- **TRANSLATION OF PROTEINS**

Thyroid hormones may stimulate translation of proteins by directly increasing the binding of amino acid t-RNA complex to ribosome or by increasing the activity of peptidyl transferase or translocase enzymes.

METABOLIC ROLE OF THYROID HORMONES

1. ***Effect on protein metabolism***

* In hypothyroid children and in physiological doses, thyroid hormones when given in small doses, favour protein anabolism, leading to N-retention (positive N-balance) because they stimulate growth.

* Large, unphysiological doses of thyroxine, cause protein catabolism, leading to negative N-balance.

CLINICAL SIGNIFICANCE

- The catabolic response in skeletal muscle in cases of hyperthyroidism is sometimes so severe that muscle weakness is a prominent symptoms and creatinuria is marked, called thyrotoxic myopathy. The K^+ liberated during protein catabolism appears in urine and there is an increase in urinary hexosamine and uric acid excretion.
- Effect on bone proteins: Mobilization of bone proteins leads to hypercalcaemia and hypercalciuria with some degree of osteoporosis.
- Effect on skin: The skin normally contains a variety of proteins combined with polysaccharides hyaluronic acid and chondroitin sulphric acid.
- Clinical significance; in hypothyroidism, these complexes accumulate, promoting water retention, which produces characteristic puffiness of the skin, when thyroxine is administered, the proteins are mobilized and diuresis continues until the puffiness (myxoedema) is cleared.

- 2. **Effects on Carbohydrate metabolism**
- Net effect on carbohydrate metabolism:
- Increase in blood sugar (hyperglycaemia), and glycosuria
- Increase glucose utilization, and decreased glucose tolerance. Thyroid hormones are therefore, antagonistic to insulin

Thyroid hormone increase the rate of absorption of glucose from intestine

Decreased glucose tolerance may be contributed to also by acceleration of degradation of insulin.

Note: Diabetes mellitus is aggravated by coexisting thyrotoxicosis or by administration of thyroid hormone.

- Increased hepatic glycogenolysis, because they enhance the activity of Glucose-6-phosphatase
- In addition there is increased sensitivity to catecholamine; they potentiate the glycogenolytic effect of epinephrine by increasing the β-adrenergic receptors on hepatic cell membrane.
- Stimulate glycolysis as well as oxidative metabolism of glucose via TCA cycle and also increasing HMP shunt. Thyroxine increases the activity of G6PD enzyme in liver.
- Thyroid hormone causes a decrease of glycogen store in the liver and to a lesser extent, in the myocardium and skeletal muscle.
- At the same time, thyroid hormones increase hepatic gluconeogenesis by increasing the activities of pyruvate carboxylase and PEP carboxykinase.

3. Effect on Lipid Metabolism

- Increase lipolysis in adipose tissue thus increasing plasma FFA. This effect is rather indirect in the sense it increase sensitive, to catecholamine, by increasing the β-adrenergic receptor on adipose cell membrane.
- They may stimulate, at the same time lipogenesis by increasing the activities of malic enzymes, ATP citrate lyase and G6 PD.
- Cholesterol despite the fact that hepatic synthesis of cholesterol and phospholipids is depressed following thyroidectomy and is increased in thyrotoxicosis, the concentration of cholesterol and to lesser extent phospholipids in plasma is increased in hypothyroidism and decreased in hyperthyroidism.
  Decreased value in hyperthyroidism is explained as follows:

  Although thyroid hormones increase the rate of biosynthesis of cholesterol, they increase

- The rate of degradation
- Increase the formation of bile acids (cholic acid/ deoxycholic) acid and
- Increase biliary excretion, to a greater extent accounting for the lowered blood concentration.

Lipoproteins

The concentration of plasma lipoproteins of Sf 10-20 class (LDL) is frequently increased in hypothyroidism and decreased in thyrotoxicosis or following administration of thyroid hormones to normal subjects

2. Calorigenic Action

Thyroid hormones increase considerably O_2-consumption and oxygen coefficient of almost all metabolically active tissues.

Exceptions are Brain, testes, uterus lymphnodes, spleen and anterior pituitary. There is increase in heat production and BMR. These effects is due to**:**

- Induction of glycerol-3-P-dehydrogenase and other enzymes involved in mitochondrial oxidation.
- More important is increased activity and increased units of Na^+-K^+ ATPase pump.

It hydrolyzes ATP for transmembrane expression of Na^+, leading to enhanced heat production, O_2-consumption and oxidative phosphorylation.

5. Vitamins

- Administration of large amounts of thyroid hormones increases the requirement of certain members of vitamin B-complex (thiamine, pyridoxine, pantothenic acid) and for vitamin C. There are presumably related to the stimulation of oxidative and catabolic processes.
- Thyroxine is necessary for hepatic conversion of carotene to vitamin A and the accumulation of carotene in the blood stream in hypothyroidism is responsible for yellowish tint of the skin.

PARATHYROID GLANDS AND THEIR HORMONES

The parathyroid glands are intimately concerned with regulation of the concentration of

Ca and PO_4 ions in the blood plasma. This is accomplished by secretion of a hormone parathormone (PTH) by the chief cells, the net effect of which is:

- To increase the concentration of Ca and
- decrease the PO_4.

In addition to its effects on plasma ionized Ca via its action on bone, parathormone controls renal excretion of Ca and $P0_4$.

PARATHORMONE (PTH)

Chemistry: Parathormone is a linear polypeptide consisting of 84 amino acids. N-terminal amino acid is alanine and C-terminal is glutamine. Bovine PTH has Mwt of 9500. PTH from different spices differs only slightly in structure.

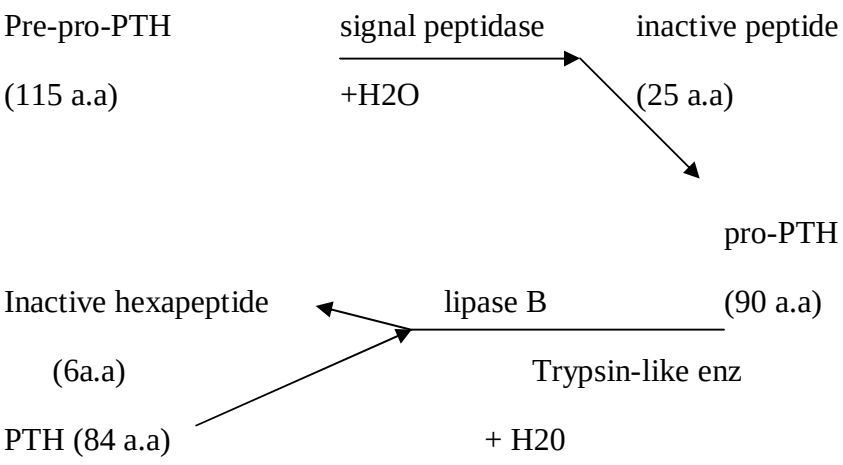
CORE OF ACTIVITY

Studies on the synthetic PTH indicate that the amino acid sequence 1 to 29 or possibly 1 to 34 from N-terminal end is essential for the physiologic actions of this hormone on both skeletal and renal tissues.

Methionine is important amino acid and necessary for calcium mobilization effect. The N-terminal end up to 34 amino acids possesses the receptor binding ability.

Biosynthesis

PTH is initially synthesized in chief cells as a pro-hormone.



- Pre-pro PTH: consisting of 115 amino acids is first formed in polysomes adhering on the rough ER membrane
- Pro-PTH: before the formation of Pre-pro PTH is completed its N-terminal end protrudes into the lumen of rough ER and a signal peptidase of rER membrane hydrolyzes the molecules to split off 25 a.a and thus pre-pro PTH is changed to pro-PTH having 90 amino acids.
- PTH: pro-PTH is transferred to rER lumen end moves to Golgi cisternae. A trypsin like enzymes called lipase B hydrolyses its N terminal amino acids and remove 6 amino acids rich in basic amino acids and thus converting pro-PTH to PTH.
  PTH thus formed is packaged and stored in secretory vesicles. Increased c-AMP concentration and a low Ca^{2+} level stimulates it release from secretory vesicles. On the other hand, a high concentration at Ca^{2+} stimulates the degradation of the stored PTH in secretory vesicles instead of it release.

MECHANISM OF ACTION

PTH increases serum Ca^{2+} level by acting on bones kidney and intestines.

a. Increasing cAMP level: PTH bind to specific receptor on the plasma membrane of bone cells, renal tubules cells, it activates the adenyl cyclase to form c-AMP in the cells. C-AMP acts as the “second messenger” which activate specific C-AMP dependent protein kinase which phosphorylate and thereby modulate the activities of specific proteins in the bone cell and kidney cells.

b. Role of Ca^{2+}: c-AMP also increases the Ca^{2+} concentration in these cells, which in turn may act as a messenger to modulate the activities of some intracellular proteins.

c. PH change in tissue: the hormone increase the amounts of both lactic and citric acid in the tissues and both of these acids may act to aid bone resorption.

METABOLIC ROLE OF PTH

The actions of PTH are reflected in the consequences of:

- its administration and
- removal of the parathyroid glands

A. The most conspicuous metabolic consequences of administration of PTH are:

- increase in serum Ca^{2+} concentration
- Decrease in serum inorganic PO_4 concentration.
- Increased urinary PO_4
- Removes Ca from bones particulars if dietary intake of Ca is inadequate.
- Increase in citrate content of blood plasma, kidney and bones
- Activates Vit. D in renal tissue by increasing the rate of conversion of 25-OH-Cholecalfiferol to 1, 25-di-OH-cholecalciferol, by stimulating α-1-hydroaylase enzymes
- Effect on Mg metabolism
  PTH has been reported to exert an influence on Mg metabolism. Primary hyperparathyroidism has been found to be associated with excessive urinary excretion of Mg and negative magnesium balance.

B. Actions on different Organs:

a. Action on kidneys: PTH acts through by increasing c-AMP. PTH binds to specific receptors on plasma membrane of renal cortical cells of both proximal and distal tubules and stimulates adenyl cyclase to produce c-AMP. c-AMP then is transported to apical/luminal part of the cell where it activated c-AMP dependent protein kinase, which phosphorylates specific proteins of the apical membrane to affect the several mineral transport across the membrane.

- PTH decreases the Trans-membrane transport and reabsorption of filtered Pi in both proximal and distal tubular cells and increases the urinary excretion of in organic phosphate (Posphaturia effect).
- Fall in serum PO_4 level leads to mobilization of PO_4 from bones, which also mobilizes Ca^{2+} along with it, resulting to hypercalcaemia.
- PTH stimulate α-1-hydroxylase enzyme located in mitochondria of proximal convoluted tubule cells, which converts 25-OH cholecalciferol to 1, 25-di-OH

cholecalciferol which in turn increases the intestinal and renal absorption of Ca^{2+} resulting to hypercalcaemia..

- PTH inhibits the transmembrane transport of K^+ and HCO_3 to decrease their reabsorption by renal tubules.
- PTH increases the transmembrane transport and reabsoption of filtered Ca^{2+} in the distal tubules resulting initially to decrease urinary excretion of Ca^{2+}. But later on, PTH induced hypercalceamia enhances the amount of filtered Ca^{2+} which increases the renal excretion.

b. Action on Bones

PTH binds to specific receptors present on membrane of osteoclasts, osteoblast and osteocytes and increases c-AMP level in these cells which act through c-AMP dependent protein kinases.

Following actions are seen.

- Osteoclastic activity: it stimulates the different action and maturation of precursors cells of osteoclasts to mature osteoclasts.
- Osteoclastic osteolysis: PTH stimulates the osteoclasts through "second messenger" c-AMP to increase the resorption of bones which enhances mobilization of Ca and P from bones.
- Osteocytic osteolysis: PTH also stimulates osteocytes which increase bone resorption thus mobilizing Ca^{2+} and Pi; there occurs enlargement of bones lacunae.
- Action on alkaline posphatase:

Alkaline phosphatase activity varies as per PTH concentration. At low concentrations, PTH stimulates the sulfation of cartilages and increases the number of osteoblast and alkaline phosphates activities of bone osteoblasts. At higher levels of physiological concentrations, PTH inhibits alkaline phosphatase activity and collagen synthesis in osteoblast and decreases the Ca^{2+} retaining capacity of bones. PTH induced rise in intracellular c-AMP in osteoclast and osteocytes leads to secretion of lysosomal hydrolases and collagenases which increase breakdown of collagen and MPS in bones matrices.

C. Action an intestinal mucosa

PTH does not act directly on intestinal mucosal cells as the cells do not possess the specific receptors for PTH. But it increases the absorption of Ca^{2+} and PO_4 through production 1, 25-dioH cholecalciferol (Calcitriol).

CALCITONIN

Calcitonin is a calcium regulating hormone. It is proved that calcitonin originates from special cells, called c-cells, parafollicular cells. C-cells constitute an endocrine system which are derived from neural crest and are found in thyroid, parathyroids and in thymus.

CHEMISTRY

Calcitonin is a single chain lipophilic polypeptide having a most 3600. As many as four separate active fractions have been isolated and they have been designated as α, β, γ and δ-calcitonin. Amino acids sequences of calcitonin have now been established. It contains 32 amino acids; N-terminal amino acid is cysteine, and C- terminal prolinamide. An inter chain disulphide bridge joins two cysteine residue between position I and 7. Low number of ionizable groups are present, 5 of 6 possible – COOH groups been amidated. Isoleucine and lysine are absent conspicuously from the molecule. There is high content of Aspartic acid and threonine.

MECHANISM OF ACTION

1. Role of c-AMP: Calcitonin binds to specific calcitonin receptors on the plasma membrane of bone osteoclasts and renal tubular epithelial cells, activates adenyl cyclase which increase c-AMP level which mediates the cellular effect of the hormone. This is the principal mechanism by which calcitonin acts.
2. Celluar Shift: It has been suggested that calcitonin may directly affect the relative distribution of bone cells. The hormone both in vitro and in vivo produced a celluar shift, in which the number of osteoclasts decreased.
3. PH change: Calcitonin may regulate PH at cellular level producing more alkaline medium which diminishes resorption.

METABOLIC ROLE

Calcitonin acts both on (a) bone (b) Kidneys. Indirectly, the effects on these two organ systems account for.

Hypocalcaemia and hypophosphataemia

a. **Action on Bones**

- Calcitonin inhibits the resorption of bone by osteoclasts and thereby reduced mobilization of calcium and inorganic PO_4 from bones into the blood.

- It also stimulates influx of phosphates in bone
- There is decrease in activites of lysosonal bydrolases, pyrophosphatases and alkaline phosphates in bones.
- Decrease in collagen metabolism and decreased excretion of urinary OH-proline
- Whether or not calcitonin promotes bone formation is uncertain and controversial. But it has been established that the hormone in addition to causing a decrease in number of osteoclasts, it increase osteoblsat cells, which are thought to be involved in bone laying.

b. **Action on kidneys**

- The hormone acts on the distal tubule and ascending limb of loop of Henle and decrease tubular reabsorption of both calcium and inorganic phosphate thus producing calcinuria and phosphaturia.
- The hormone inhibits α-1-hydroxylase and inhibits synthesis of 1-25-d-OH-D3 thus decreasing calcicum absorption from intestine.
- Both the above effects account for hypocalcaemia.

INSULIN

Insulin is a protein hormone, secreted by β-cells of islets of Langerhars of pancreas. It plays an important role in metabolism causing increased carbohydrate metabolism glycogenolysis/ and glycogen storage, FA synthesis/TG storage and amino acid uptake/ protein synthesis. Thus insulin is an important anabolic hormone which act on variety of issues. Major target tissues of insulin are the muscles, liver, adipose tissues and heart.

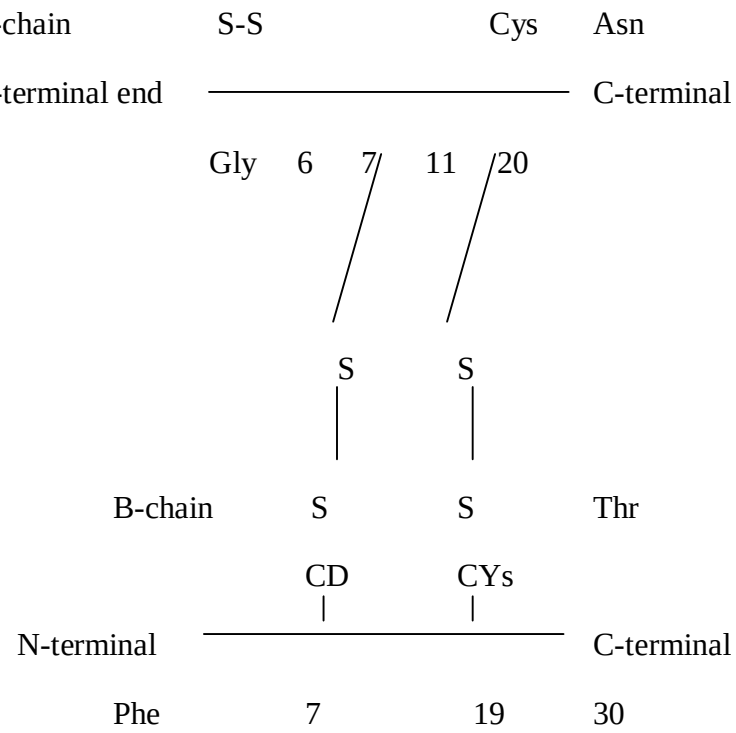
Note: RBC, GI tract epithelial cells and renal tubular epithelial cells are rather generally unresponsive to insulin.

CHEMISTRY

Insulin is a heterodimeric protein; it has been isolated from pancreas and prepared in crystalline form. For crystallization it requires zinc is also a constituent of stored insulin and normal pancreatic tissue is relatively rich in zinc. Insulin molecule is composed of two polypeptide chains, called A-chain and B-Chain, containing total of 51 amino acids. A-chains contains 21 amino acids and B-chain contains 30 amino acids. In A-chain, N-terminal amino acids is phenylalaine and C-terminal is threonine.

DISULFIDE BRIDGES

Both the chains are held together by two s-s-linkage. Cys 7 end Cys 20 of A chain are joined to Cys7 and Cys 19 of B chain respectively. In addition, the A-chain carries as intra-chain" s-s linkage between cyc 6 and cys 11.



INSULIN FROM OTHER SPECIES

Porcine insulin: Porcine insulin is similar to human insulin. It differs by only terminal amino acid No-30 of B-chain.

- In human: It is threonine
- In porcine: it is alanine in place of threonine. Removal of alanine (de-alaninated) retains the biological activity.

Note: De-alaninated insulin has been used in treatment of diabetes mellitus because of it low antigencity.

- Human insulin has been produced by recombinant DNA technology.

Important of S-S Bridges

Breaking of the disulfide bonds with alkali or reducing agents inactivate insulin. Digestion of insulin protein with proteolytic enzymes also inactivates the hormone; hence insulin cannot be given orally. Minimum calculated Mwt is 5734. Insulin can exist in different polymeric forms (dimmer, trimer etc) depending on pH, temperature and concentration.

BIOSYNTHESIS OF INSULIN

In biosynthesis of insulin, first "prepro-insulin" is formed which is converted to pro-insulin. The latter is finally converted to insulin.

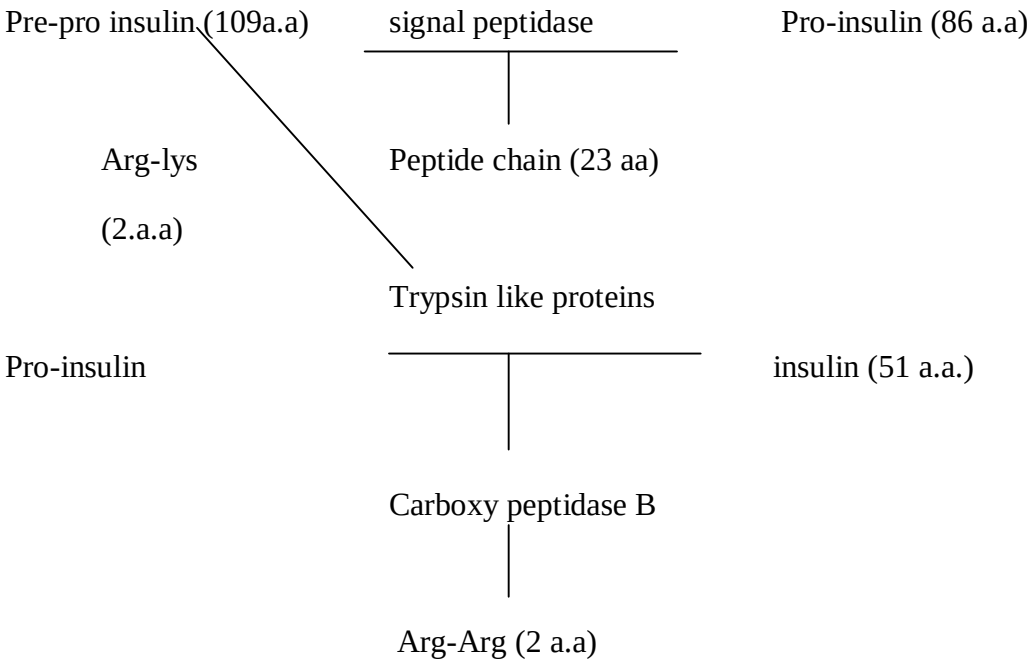
1. Synthesis of pre-pro insulin: Pre-pro insulin is syntheses in polysomes, attached to the membrane of rough endoplasmic reticulum in β-cell of islet of Langerhans. It is a polypeptide consisting of 109 amino acids, Mwt= 11,500
2. Conversion of pre-pro insulin to proinsulin

- Pre-proinsulin after synthesis is transferred to lumen of rER cisternae.
- A peptide chain consisting 23 amino acids in its N-terminal called leader sequence is split by an enzyme called signal peptidase present in the membrane of rER and pro-insulin is formed.
- Pro-insulin has 86 amino acids Mwt = 9000

3. Conversion of pro-insulin to insulin

Pro-insulin containing small vessicles are detached from ER and fuses with cisternae of Golgi apparatus.

- In the Golgi cisternae, Proinsulin is acted upon by a trypsin-like protease which hydrolyzes the peptide chain at two sites, so that an inactive connective C-peptide is liberated and two active peptide chain are left which forms the A and B chain.
- A carboxypeptidase B like enzymes splits the C-terminal peptide bonds in the two intermediates to release two C-terminal basic amino acids from each of them viz "Arg-63-lys 62" to form 'A' chains and "Arg 31-Arg 32" to form 'B' chain. C-peptide which is split off has 31 amino acids.
- Condensing vacuoles are pinched off from Golgi cisternae with equimolar amounts of insulin and C-peptide in their lumen. Insulin molecules form dimmers by hydrogen bonding between the peptide groups of phe 24 and tyr 26 residues of their B-chains. Gradually with increasing concentrations, condensing vacuoles change into secretory granules. In them insulin forms crystalloid forms of hexamers with two Zn^{2+} .C-peptides remain in the fluid surrounding the crystalloid granules.



Note: Pro-insulin is comparatively inactive biologically, but it can cross-react with antisera prepared against insulin.

- Plasma pro-insulin is not elevated in human diabetes or in normal after glucose stimulation, but it may be the predominant circulating form in some subjects with islet cell tumours.

CATABOLISM OF INSULIN

Insulin is very rapidly catabolised. Its plasma t½ is less than 3-5 minutes under normal conditions. Major organs where insulin is catabolised are liver, kidneys, and placenta.

About 50% of insulin is degraded in its single passage through the liver.

Mechanism

Two enzyme systems are involved for degradation of insulin

- Protease: an insulin-specific protease has been found in many tissues with highest concentration in liver and kidneys. The protease is-SH dependent and active at physiological PH.
- Second mechanism is more important. The enzyme is glutathione-insulin transhydrogenase (also called insulianse). This enzyme is found in higher concentration in liver and kidneys. Also present in skeletal muscles and placenta. This brings about reductive cleavage of the insulin molecule. Reduced glulathione (G-SH),

acting as a co-enzyme for the transhydrogenase, donates the H-atoms for the reduction and is itself thus converted to oxidized glutathione.

- After insulin is reductively changes, the A-chains and B-chains are further hydrolyzed by proteolysis.

INSULIN RECEPTORS

Insulin acts on target tissue by binding to specific insulin receptors, which are glycoproteins. The human insulin is found on chromosome 19. The insulin receptors are being constantly synthesized and degraded. Their t½ is 6-12 hrs only. It is synthesized as a single chain polypeptide pro-receptor" in the rER and is rapidly glycosylated in Golgi region. The "pro-receptor" has 1382 amino acids and most 190,000.

The pro-receptor is cleared to form mature "α" and "β" subunits ($\alpha_2\beta_2$) which is a heterodimer, linked by S-S bonds. Both subunits are extensively glycosylated and removal of sialic acid and galactose decreases insulin binding and insulin action. Insulin receptors are found in target cell membrane, up to 20,000 per cell.

Binding of insulin to the receptor, stimulates its, tyrosine kinase activity. Tyrosine kinase enzyme phosphorylates the phenolic –OH group of tyrosine residues in specific protein including that of a tyrosine in the β-chain of insulin receptor itself to modulate their activities, ATD + tryrosineprotein – ADP+ phosphor-tyrosine protein.

Regulation of insulin receptors

High blood insulin level decreases the number of insulin receptors on target cell membrane, probably through internalization of the insulin-receptor complex into the cell and thus decreases the insulin sensitivity of the target tissue.

MECHANISM OF ACTION OF INSULIN

When insulin binds to the specific receptor several events of actions take place.

- A conformational change of the receptor
- The receptor crosslink and form microaggregates
- The receptor complex is internalized and
- One or more signals is generated

But nature of the intracellular signal and intracellular second messenger" remains still uncertain and vague.

Various mechanisms have been proposed.

1. ***Role of c-AMP***: It is proposed that insulin promotes the phosphorylation of c-AMP phosphodiesterase. The active phosphodiesterase hydrolyses c-AMP and lowers the c-AMP level in the cells. The consequent fall in activities of c-AMP dependent protein kinase reduce phosphorylation of specific enzymes.

2. ***Role of c-GMP***: The insulin receptor binding may activate guanylate cyclase which forms c-GMP. Increased concentration of c-GMP act as second messenger" to activate c-GMP dependent protein kinase. These may phosphorylate some enzymes to modulate their activities

3. ***Role of protein phosphatase***: Insulin may act through the protein phosphates I which may dephosphorylate certain key enzymes thereby activating them. Best examples are the key enzyme glycogen synthase and pyruvate dehydrogenase complex. On the other hand inhibits phosphorylase enzyme and triacylglycerol lipase.

4. ***Action through tyrosine kinase***" Activity of β-subunit Receptor: The binding of insulin to its receptor enhances tyrosine kinase activity. Tyrosine kinase in turn phosphorylates pheolic-OH group of tyrosine residues of specific proteins leading to changes in enzyme activities.

5. **Role in mRNA translation**: Insulin is known to affect the activity or amount of at least more than 50 proteins in variety of tissue and many of these effects involves covalent modification. A role of insulin in the translation of mRNA has been proposed largely based on studies of ribosomal protein 6S, a component of the 40S ribosomal unit. Such a mechanism accounts for the general effect of insulin on protein synthesis in liver, heart muscle and skeletal muscles.

6. **Role on gene expression (Nuclear action):** insulin also affects the rate of transcription of specific genes, thereby regulates the synthesis of specific m-RNAs and thus changing the rate of synthesis of specific protein coded by them. e.g insulin decreases the transcription of gene involved in synthesis of the enzyme phosphoenol-pyrurate carboxy kinase (PEPCK), the key enzyme for gluconeogenesis. On the other hand insulin induce the synthesis of phosphofructokinase and pyruvate kinase required for glycolysis, by increasing the transcription of these genes.

METABOLIC ROLE OF INSULIN

A. Action on carbohydrate metabolism

Net effect is lowering of blood glucose level and

Increase glycogen store

The above is achieved by several mechanisms.

1. Increase glucose uptake:

- Insulin increases glucose uptake from extracellular fluid by the various tissues viz, muscles, adipose tissue, mammary glands, lens, etc.
- In adipose tissue and other extrahepatic tissues, insulin stimulates translocation of glucose transporters from their intracellular pool in Golgi cisternae to the plasma membrane where they participate as carrier in transportation of D-glucose and D-galactose across the membrane.
- Also in hepatocytes, insulin increases hepatic uptake of glucose (freely permeable to liver cells) it induces the synthesis of the enzyme glucokinase which simulataneously phosphorylates glucose, thereby lower intracellular concentration.

2. Increases glycolysis: increase utilization of glucose for providing energy which takes place in muscles, liver and many other tissues. Insulin enhances glycolysis because it induces the synthesis of key enzyme phosphofructokinase and also pyruvate kinase.
3. Increase conversion of pyruvate to acetyl -107 insulin increase aerobic oxidative decarboxylation of pyruvate to acetylcoa, because it causes dephosphorylation of pyruvate dehydrogenase complex which is thus converted to the form.
4. Stimulate glycogenesis: insulin stimulates glyogenesis in the liver and muscles by increasing dephosphorylation of the key and rate limiting enzyme, glycogen synthase, thus converting it to its active form. Insulin stimulates the protein phosphatase-1 directly, which brings about dephosphorylation.
5. Decrease Gluconeogenesis: Insulin reduces gluconeogenesis:

- By repressing the synthesis of the key rate limiting enzyme phosphoenol pyruvate carboxykinase (PEPCK) by decreasing the transcription rate of the gene.
- Also inhibits allosterically fructose-1 6-biphosphatase another key enzyme for gluconeogenesis.
- Insulin dephosphorylates fructose 2,6-biphosphatase so that it is converted to inactive form, which increases the concentration of fructose 2-6-biphosphate in the cell, which inturn allosterically inhibit fructose -1,6-biphosphatase.

6. Decrease glycogenolysis: insulin decreases glycogeneolysis.

- By dephosphorylating the key and rate limiting enzyme glycogen phosphorylase thus converting it to inactive form
- Also represses the enzyme glucose-6-phosphatase.

B. Action on lipid Metabolism

Net effects are lowering of free fatty acid level and increase in triglyceride store.

The above is achieved as follows:

1. Decrease lipohysis: Insulin decreases lipolysis in adipose tissue cells and consequently lower plasma FFA. Lipolysis is reduced due to

- Insulin activates phosphoprotein phosphatase which dephosphorylates the triacylgly cerolipase and thus converted to inactive form.
- At the same time, insulin activates phosphodiesterase which degrades c-AMP and prevent phosphorylation and reactivation of TG lipase

2. Increases fatty acid synthesis: Insulin increases the extramitochondrial denovo fatty acid synthesis by making available of more substrate acetyl CoA and also increasing the activity of acetylcoA carboxylase.

The above is done as follows:

- Insulin promotes dephosphorylation of pyruvate dehydrogenase complex and converts into active form so that more acetyl CoA is available from pyruvate
- Insulin induces the synthesis of ATP-citrate lyase to increase cleavage of citrate, so that more acetyl-CoA is available in cytosol.
- Insulin lowers the plasma FFA level, so prevent long chain acyl-CoA from inhibitory acetyl-CoA carboxylase.
- It induces the synthesis of acetyl-CoA carboxylase and fatty acid synthase, the cytosolic enzymes required for FA synthesis.
- Insulin activates acetyl-CoA carboxylase by dephosphorylation of the enzyme (Converting to active form).
- Provides more NADPH for the reductive steps in FA synthesis by stimulating HMP-shunt pathway.

3. Increase synthesis of TG: Insulin enhances TG synthesis in adipose tissues by:

- Proving more α-glycerol-P as glucose uptake and utilization is enhanced in adipocytes.
- Increased synthesis of FA provides the acyl CoA (FFA pool 1) required for TG synthesis.

- Insulin also induces the synthesis of lipoprotein lipase. This enzyme hydrolyzes TG of circulating chylomicrons and VLDL and releases FFA (FFA pool 2) which are taken up by the adipocytes and used for TG synthesis.

4 Decreases ketogenesis: As plasma FFA level is decreased less is oxidized by β-oxidation and less acetyl-CoA will be available for cholesterol synthesis and ketogenesis.

C. ACTION ON PROTEIN METABOLISM

Net effect is insulin promotes protein synthesis.

This is achieved as follows:

- Insulin increases amino acids uptake by the tissues by enhancing the rate of synthesis of membrane transporters for amino acids.
- Adequate supply of insulin is necessary for protein anabolic effect of growth hormone (permissive effect)
- Insulin increase protein synthesis by proving more amino acids in cells, by affecting gene transcription (nuclear level) by regulating specific m-RNA synthesis and affecting translation at ribosomal level.
- Regulation of ribosomal translation is done by two ways:
- Increase the synthesis of polyamines-required for ribosomal RNA synthesis, by increasing the synthesis of key and rate limiting enzyme ornithine decarboxylase.
- Secondly, insulin modulates ribosomal activity by causing phosphorylation of 6S ribosome (α component of 40S)

d. Action on mineral Metabolism

Decrease in concentration of K^+ and inorganic P in blood due to enhanced glycogenesis and phosphorylation of glucose.

e. Actions on growth and cell Duplication

Insulin stimulates growth in vivo and also cell proliferation in vitro. Cultural fibroblasts have been used most frequently in studies of cell proliferation. It has been found that insulin potentiates the ability of fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth and cell proliferation are seen in many tissues such as liver, mammary glands and adrenals and also in embryogenesis and tissue differentiation. There effects are largely due to stimulation of DNA replication, gene transcription, protein synthesis and modulation of various enzyme activities through phosphorylation dephosphorylation.

GLUCAGON HYPERGLYCAEMIC-GLYCOGENOLYTIC FACTOR

Glucagon is a hormone produced by α –cells of islet of Langerhan of pancreas and is an important hormone involved in

- Rapid mobilization of hepatic glycogen to give glucose by glycogenolysis and
- To a lesser extent FA from adipose tissue.

Thus it acts as a hormone required to mobilize metabolic substrates from storage depots.

CHEMISTRY

Glucagon has been purified and crystallized from pancreatic extracts and also the hormone has been synthesized. It is a polypeptide containing 29 amino acids. There are only 15 different amino acids in the molecule. Amino acid sequence has been determined, histidine is the N-terminal amino acid and threonine is the C-terminal. Mwt is approx 3485.

Unlike insulin

- It does not require zinc or other metals for it crystallization.
- Glucagons contains no cystine, proline, isoleucine but contains tyrosine, methionine and tryptophan

SYNTHESIS

It is synthesized first as a pro-hormone, proglucagon in α-cells. Lysosomal enzyme peptidase like carboxy-peptidase B and trypsin-like peptidase in α-cells hydrolyze pro-glucagon from both its N-terminal end and c-terminal end to yield glucagon and inactive peptides.

ENTERO-GLUCAGON OR GLUCAGON-LIKE IMMUNE REACTIVE FACTOR.

A glucagon-like immuno reactive factor (GLI) has been identified in gastric and duodenal mucosa. GLI is immunologically similar though not identical to the pancreatic hormone. Moreover, it is less active than pancreatic glucagons in stimulating adenyl cyclase and therefore cannot duplicate many of the function of pancreatic hormone. GLI is stimulated by absorbed glucose causing an apparent elevation of circulating pancreatic glucagons.

Recently, two different molecular fractions have been isolated:

- One having mol.wt =3500, has hyperglycaemic and glycogenolytic activity but far less potent than pancreatic glucagons.
- The other fraction, mol.wt=2000; devoid of the above activity.

Both have insulin releasing activity

MECHANISM OF ACTION

Glucagon binds to specific receptors on the plasma membranes of hepatocytes and adipocytes and activates adenyl cyclase to produce c-AMP in these cells, which is the principal "second messenger" and duplicates the functions of the hormone. C-AMP in turn activates c-AMP dependent protein kinases which further phosphorylates specific enzymes to increase/decrease their activities. C-AMP also induces synthesis of certain specific enzymes like glucose-6-phosphatases by increasing the transcription of their genes.

METABOLIC ROLE

1. Action on carbohydrate metabolism
   Net effect of the hormone is to increase the blood sugar level (hyperglycaemia). Hyperglycaemic effect is due to various causes.

- Glycogenolysis: glucagons increases glycogenolysis in liver. In muscles, it cannot bring about glycogenolysis as muscle cell membrane lacks the glucagons specific receptors glucagons also induces the synthesis of glucose-6-phosphatase enzyme.
- By increasing gluconeogenesis in liver: glucagon stimulates the conversion of lactic acid and glucogenic amino acids to form glucose.
- The increased hepatic c-AMP produced after glucagon action has been shown to increase protein kinases that catalyze nuclear histone phosphorylation in liver cell nucleus. This reaction inhibits the repressive effect normally exerted by histones on DNA and allows the initiation of a sequence of events leading to the synthesis off new enzyme proteins involved in gluconeogenesis. Thus, glucagons induces the synthesis of phosphoenol pyruvic carboxykinase, pyruvate carboxylase and fructose -1-6-biphosphatase enzyme, all key enzymes of gluconeogenesis.
- Also glucagon increases the pool of glucogenic amino acids in liver, so that they can be used for gluconeogenesis. This is achieved by increasing protein breakdown in liver and by reducing hepatic protein synthesis.

2. **On lipid metabolism**

- Lipolysis: In adipose tissue and also possibly in liver, glucagon increases the breakdown of TG to produce FFA and glycerol. FA undergo β-oxidation, increased breakdown may lead to ketone bodies formation and ketosis. Thyroid hormones help in the lipolytic action of glucagon, probably the hormones increases the number of glucagon specific receptors on adipocytes.
- Anti-lipogenic Action: Glucagon reduces F.A. synthesis. This is achieved in 2 ways:
- Increased lipolysis raise the concentration of FFA in blood. Long-chain acylCoA inhibits the rate limiting enzyme acetyl-CoA carboxylases.

- Increased c-AMP level in cells activates c-AMP dependent protein kinase which phosphorylates acetyl-CoA carboxylase. Phosphorylated form of the enzyme is inactive.

3. On protein metabolism

- Glucagon reduces protein synthesis by depressing incorporation of amino acids into peptide chains. This may be due to the inactivation of some ribosomal component by a protein kinase whose activity is enhanced by glucagon-induced rise in c-AMP.
- Glucagon also stimulates protein catabolism especially in liver thus increases the hepatic amino acid pool which is utilized for gluconeogenesis. Also increases urinary NPN and urea.

4. Action on Heart: Glucagon exerts a positive ionotropic effect on heart without producing increased myocardial irritability. Hence, use of glucagon in treatment of heart disease, viz in cardiac failure and cardiogenic shock.
   Advantage over non-epinephrine: glucagon increases the force of contraction, but does not produce any arrhythmias, tachycardia or increase in 0_2 consumption.

5. Calorigenic effect: glucagon increases heat production and rise in BMR. The calorigenic action is not due to hyperglycaemia perse but is probably due to increased hepatic deamination of amino acid, with thyroid hormones stimulating the utilization of deaminated residues. The calorigenic action requires the presence of thyroid and adrenocortical hormones and fails to occur in their absence.

6. **On mineral metabolism**:

   Potassium: glucagon increases K^+ release form the liver, an action which may be related to its glycogenolytic activity

   Calcium: Glucagon can increase the release of calcitonin from the thyroid.

SOMATOSTATIN

The peptide somatostatin (growth hormone release inhibiting factor) was first isolated from the hypothalamus and was implicated as a regulator of growth hormone secretion.

CHEMISTRY

It is a peptide consisting of 14 amino acids. There is an intrachain S-S linkage joining cysteine 3 and cysteine at position 14

Sources: there are three sources

- Hypothalamus
- Pancreas; somatostation is also secreted by δ-cells of islet of Langerhans of pancreas.

- GT tract: it is also produced by D-Cells of antral mucosa of stomach and also duodenal mucosa.

a. ***Hypothalamic somatostotin***

- Acts as a regulator of growth hormone secretion
- It inhibits growth hormone (GH) release
- It may also serve as a neurotransmitter

b. ***Pancreatic somatostatin***

- It inhibits both insulin and glucagon secretion and thus may serve as an intraislet regulator of secretion of these hormones. Thus act as intaorgan "synaptic transmitters" or neuromodulators.
- Somstostatin is secreted into the portal vein blood as a result of glucose or amino acid stimulus indicating extra-islet role
- Also directly inhibit secretion of both HCO_3^- and enzymes in pancreatic juice.

c. G.I. somatostatin

- Inhibit the secretions of gastrin, CCK and motilin.
- Also inhibits gastric acid secretion, secretion of Brunner's glands pancreatic HCO_3^- and enzymes secretions gastric emptying and gall bladder contraction.

  Since somatostatin can inhibit a variety of G.I functions (gastric emptying, G.T. motility), its major function may be to regulate nutritional influx at the level of GI tract.

ADRENAL STEROID HORMONES

Steroid Hormones Produced by Adrenal cortex about 50 steroids has been isolated from the adrenal cortex. But out of them only 7 (seven) are important and known to possess physiologic activity. They are all arrived from cholesterol which can be synthesized from active acetate and they contain the steroid nucleus, called cyclopentano perhydro phenanthrene nucleus. Seven important hormones are:

- 11-dehydro corticosterone (DOC)
- Cortisone
- Cortisol (17-OH cortisosterone)
- Aldosterone (mineralocorticoid)
- Androstenedione
- Dehydroepiandrosterone

Cortisol is the major free-circulating adrenocortical hormone (glucocorticoid) in human plasma.

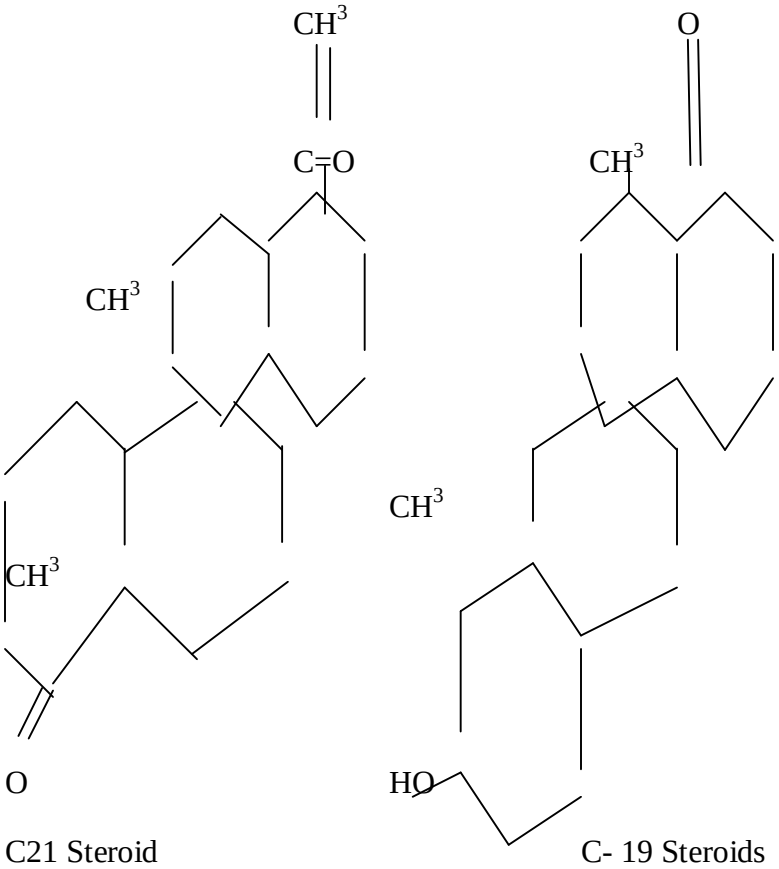
CLASSIFICATION

1. According to structure: Adrenocortical hormones are mainly of two structural types.

- C-21 steroids: those which have a two carbon side chain at position 17 of the D-ring and contain total 21 carbon atoms.
- C-19 steroids: Those which have on 0_2 atom or OH group at position 17 and contain 19 carbon atoms. Most of the C19 steroids have oxygen atom at position 17 and are therefore called as 17- oxosteroids (17-ketosteroids).
- Note: The C-21 steroids which have a –OH group at the position 17, in addition to the side chain are often called 17-OH corticoids or 17-OH corticosteroids

In general

- C- 19 steroids have androgenic activity and
- C-21 steroids have glucocorticoids and mineralocorticoids activity.



C21 Steroid C- 19 Steroids

According to function: Steriods are divided into three types according to function:

- Glucocorticoids: which primarily affect metabolism of carbohydrates, proteins and lipids and relatively minor effects on electrolytes and water metabolism e.g.cortisol, cortisone, corticosterone
- Mineralocorticoid are those which primarily affect the reabsorption of Na+ and excretion of K+ (mineral metabolism) and distribution of water in tissues e.g Aldosterone (chief mineralocorticoid). Others are cortisosterone, 11-deoxycortisol and 11-deoxycorticosterone
- Cortical sex hormones (Androgens and ertrogen) primarily affect secondary sex characters.

Relation of structure with functions:

1. Three structural features are essential for all known biological actions of the natural C21 adrenocortical hormones:

* a double bond of C4 and C5

* a ketonic group (C=O) at C3 and

* a ketonic group (C=O) at C20

2. Certain additional structural features have a profound effect upon the biological activity of these compounds:

* An-OH group at C21 enhance Na-retention and is required for activity in carbohydrate metabolism.

* The presence of 'O' either as –OH group or as O group, i.e hydroxyl or ketonic group of C11 is necessary for carbohydrate activity and decreases Na+ retention.

* An-OH group at C12 increases carbohydrate activity.

* A-CHO group at C18 necessary for mineral corticoid activity.

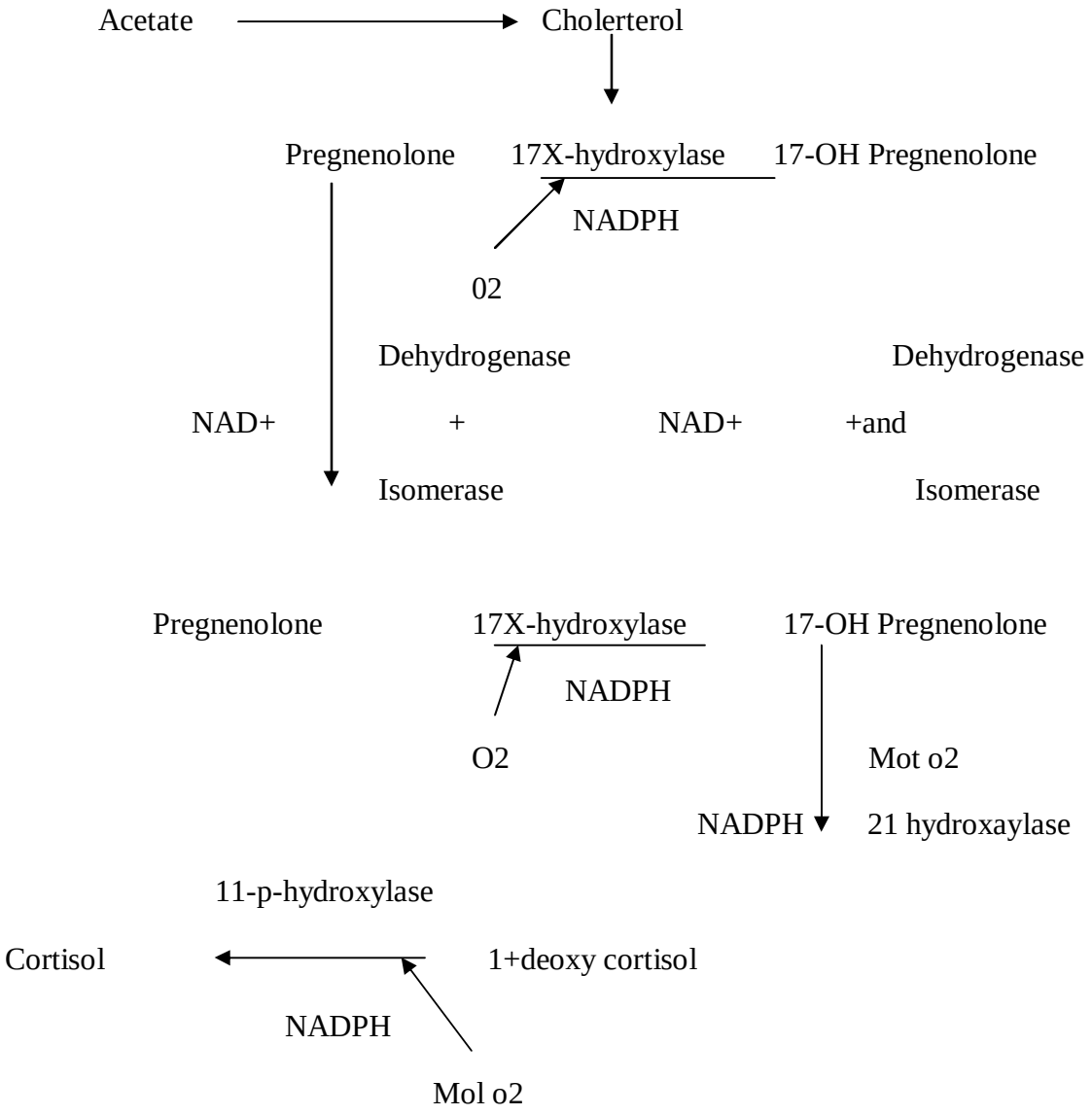
GLUCO CORTICOIDS

1. Biosynthesis of glucocorticoids:

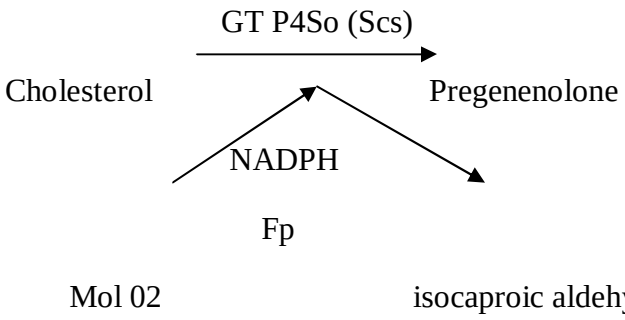
   Common pathway for all cortico-steroids:

   Corticosteroids are synthesized by a common pathway from cholesterol in the adrenal cortex.

In all the three zones of adrenal cortex,



- cholesterol is first changned to form pregneolone (common pathway) from this, free cholesterol is released in the cytosol from cholesterol esters of cytoplasmic lipid droplets and transferred into mitochondria. An enzyme called cytochrone-P- 450-side chain cleavage enzyme (P450 sce) prevent in inner mitochondrial membrane hydroxylates cholesterol at (22 and C20 (also called 20, 22-desmolase) and then cleaves the side chain to form pregnenolone and isocaproic aldehyde. The enzyme requires molecule O_2 and NADPH like all monoxygenases and also require FAD containing FP, and Fe_2S_2 protein (called adrenodoxin).



Glucocorticoid synthesis;

Glucocorticorids are synthesized in zona fasiculata cells.

ACTION OF ACTH ON CORTISOL FORMATION

ACTH stimulates the synthesis and secretion of glucocorticoids. It acts in several ways:

- Increase the availability of free cholesterol in fasciculata cells. This is achieved in two ways: through cyclic-AMP, activates the enzyme cholesteryl esterase which hydrolyzes cholesterol esters and increase free cholesterol in cells.
- Increase transfer of free cholesterol from plasma lipoproteins into fasciculata cells, probably by increasing lipoprotein receptors on plasma membrane of fasciculata cells.
- ACTH increases the conversion of cholesterol to pregnenolone, the rate limiting step.
- ACTH also stimulates the HMP_shunt pathway by increasing the activity of G-6-P D and phosphogluconate dehydrogenase. So that more NADPH is provided which is required for hydroxylation reactions.
- ACTH also increases the binding of cholesterol to mitochondrial cyt P450 necessary for hydroxylation reactions.

MECHANISM OF ACTION

All of the steroids act primarily at the level of cell nucleus (nuclear action) to increase m-RNA synthesis and increases protein synthesis.

- The first step occurs within minutes, which involves the binding of the steroids to a corresponding specific receptor protein present in cytosol.
- Glucocorticoids pass into target cells through plasma membrane and binds to specific glucocorticord receptor proteins present in cytosol.

The receptors occur in a wide variety of target tissues, viz liver, muscles, adipose tissue, lymphoid tissue, skin, bone, fibroblast etc.

Types of receptors: In humans, there are two types of receptor proteins.

α-form; containing approx 777 amino acids.

β- form having 742 amino acids.

Both differ in amino acid sequence in the c-terminal end. The receptor molecule has three distinct domains.

- A steroid binding domain near c-terminal
- A DNA binding domain near the middle of the molecule in c-terminal half ad
- A transcription activating domain near the N-terminal side.

A heat shock protein (hsp 90) binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor protein.

Glucocorticoids bind to the specific receptor in cytosol to steroid-binding site. This binding causes dissociation of the hsp 90 stabilizer and permits conversion to the active configuration.

The steroid-receptor complex enters the nuclear and binds by DNA-binding site to the "hormone responsive elements (HRE) of specific nuclear gene. This modulates the transcription rate of these genes, leading to increase synthesis of many proteins and enzymes and also to decreased synthesis of some proteins like corticotrophin.

METABOLIC ROLE OF GLUCOCORTICOIDS

1. Metabolic Actions:

Points to note:

- In general, glucocortidoids have anti-insulin effects
- Glucocorticoids are catabolic to peripheral tissues and anabolic to liver.

a. Effects on carbohydrate metabolism: overall effect increases blood glucose level (hyperglycaemia)

Mechanism of hypergly aemia

1. Decreases glucose uptake; and utilization in muscles, in adipocytes and lymphoid cell by inhibiting the membrane transport of glucose into these cells.

2. Enhancing gluconeogenesis in liver: induces the synthesis of key gluconeogenic enzymes such as pyruvate carboxylase, PEP carboxykinase, fructose 1, 6-diphosphatase and also glucose-6-phosphatase.

- By making available more of substrate required for gluconeogenesis. This is achieved by
- Increasing protein catabolism in extrahepatic tissue
- Decreasing incorporation of amino acids in protein in peripheral tissues.
- Also increasing synthesis of some key enzymes required for amino acid catabolism like alanine transaminase, tyrosine transaminase, tryptophan pyrollase.

3. Decreases glycolysis in peripheral tissues

- In liver: glucocorticoids are anabolic. It increases the glycogen store in liver. This is due to:
- Increases in gluconeogenesis from amino acid and glycerol
- Activates protein-phosphatase-1 which dephosphorylates and activates glycogen synthesis:
- Stimulate the synthesis of glycogen synthase also

b. Effect on lipid metabolism: Net effect increase FFA in plasma and also glycerol. Glycerol is utilized for gluconeogeneris in liver.
In adipocytes.

- Glucocorticoids increase lipolysis and liberates FFA and glycerol by activating hormone sensitive TG lipase.
- As glucocorticoid decrease the uptake of glucose in adipose tissue, there will be reduction in α-glycerol phosphate as a result esterification suffers, hence net flow of FFA in plasma increase.

c. ***Effect on protein metabolism***

- In peripheral extrahepatic tissues, cortisol is catabolic and increase protein breakdown, leading to increase amino acids availability in plasma.
  Reasons of increased catabolism

- Enhances synthesis of key enzymes of amino acid catabolism like transaminase, tyrosine transaminase
  Tryptophen pyrrolase etc

- Also there is decreased incorporation of amino acids in protein molecule
- In liver: cortisol is anabolic, it increases protein synthesis it increases;
- Hepatic uptake of amino acids

- Incorporation of amino acids into ribosomal proteins.
- Increased m-RNA formation and synthesis of proteins including plasma protein
- In liver, cortisol also enhances urea synthesis from amino acids. There is increased synthesis of enzymes necessary for urea cycle, e.g arginino succinate synthetase, arginase etc.

MINERALO CORTICOIDS

Mineralo corticoids are C21 steroids, which influence the metabolism of Na+ and K+. The chief mineralocorticoid is aldosterone. It is produced by zona glomerulosa of the adrenal cortex. Structurally, it bears –OH group at C11 and aldehyde (CHO) group at C18.

Other corticosteroids which have mineralocorticoid activity are:

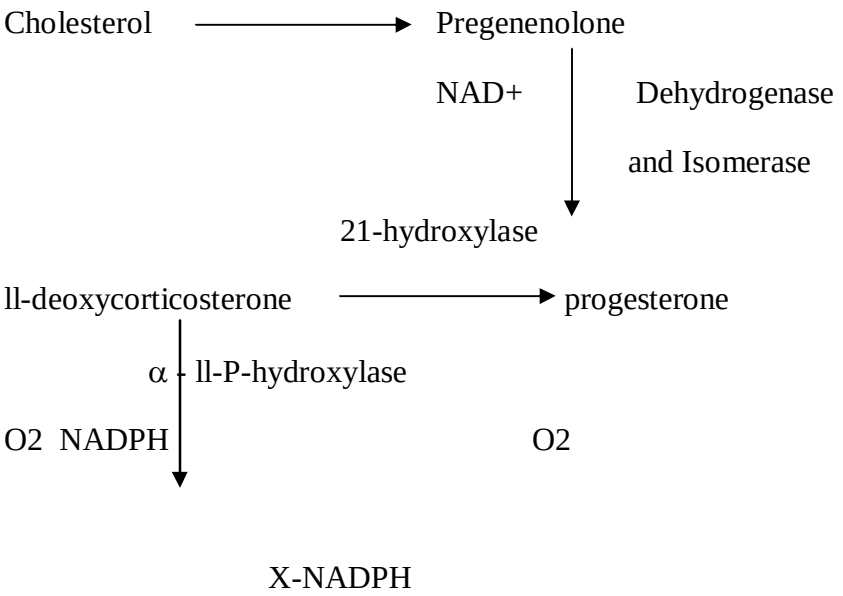
- Corticosterone. * II-deoxycortisol * II-deoxycorticosterone

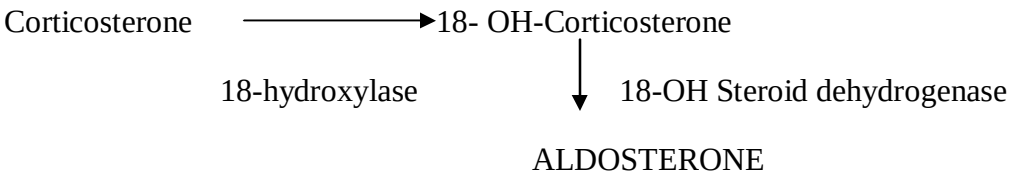
Il-deoxycorticosterone is secreted in minute quantities and has almost the same effects as aldosterone, but a protency only 1/30th that of aldosterone.

Biosynthesis

Mineralocorticoids are synthesized in zona glomerulosa cell only. They cannot be synthesized in other two layers of adrenal cortex. Only zona glomerulosa cells have the enzymes 18-hydroxylase and 18-hydroxysteroid dehydrogenase, which are lacking in other layers

Steps in synthesis:





MECHANISM OF ACTION

Mineralocorticoids enter the target cells through the plasma membranes and binds to a specific protein present in cytosol, and nucleosplasm, called mineralocorticoid receptors. They are present in epithelial cells of renal distal tubular cells and collecting ducts and also in gastro-intestinal mucosa, salivary gland duct and sweat ducts. The steroid receptor complex then enters the nucleus and binds to hormone responsive element of specific nuclear genes and increase the transcription rates of genes. Thus, aldosterone initiates an increase in mRNA synthesis, at the level of transcription of DNA. The induced mRNA stimulates protein synthesis at the ribosomal level.

METABOLIC ROLE OF ALDOSTERONE

a. Renal effects of Aldosterone

1. Effect on tubular reabsorption of sodium:

By far the most important effect of aldosterone and other mineralocorticoids is to increase the rate of tubular reabsorption of Na. Sodium is reabsorbed from the renal tubules along their entire extent. Aldosterone has a specially potent effect in the distal tubule, collecting tubule and at least a part of loop of Henle.

Note: Total lack of aldosterone secretion can cause loss of as much as 12 gram of Na in the urine in a day, an amount equal to $1/7^{th}$ of all the sodium in the body.

2. Effect on tubular rebsorption of chlorides:

Aldosterone also increase the reabsorption of Cl ions from the tubules. This probably occurs secondarily to the increased Na reabsorption. Absorption of positively changed Na+ causes an electrical potential gradient to develop between the lumen and outside of the tubules with positivity on the outside.

This positivity in turn attracts negatively charged diffusible amino through the membrane since Cl- are by far the most prevalent anion in the tubular fluid, the absorption of Cl increase.

3. Increased renal secretion of K+: as aldosterone causes increased tubular reabsorption of Na+ at the same time it also increase loss of K+ in the urine by the renal distal tubules and collecting ducts. This may result from the elimination of K+ in exchange of the reabsorbed Na+.

Clinical significance:

Hypokalaemia and muscle paralysis: the loss of K+ in urine decrease K+ in ECF resulting to hypokalaemia. Thus at the same time that Na+ and Cl become increased in ECF, there will be group decrease in K+. The low K+ concentration sometimes leads to muscle paralysis, this is caused by hyperpolarization of the nerve and muscle fiber membrane which prevents transmission of action potentials.

4. Effect an acid-base balance (Alkalosis): A large proportion of Na+ reabsorption from the tubules results from an exchange reaction on which H+ are secreted into the tubules to take place of Na+ that is reabsorption is enhanced, in response to aldosterone, the H+ concentration in the body fluids is reduced. For each Na+ reabsorption by the H+ exchange, one HCO3 enters the ECF which shifts the reaction to alkaline side. Thus increased secretion of aldosterone promotes alkalosis, whereas decreased secretion produced acidosis.

b. ***Effect of aldosterone on fluid volume***:

1. **Effect on ECF volume**:

Mineralocorticoids greatly increase the quantities of Na+, Cl and HCO3 in the ECF increasing the electrolyte concentration in ECF. These in turn increase water reabsorption from the tubules by:

- Stimulating the hypothalamic OH system and
- Creating an osmotic gradient across the tubular membrane. When the electrolytes are absorbed, carries water through the membrane in the wake of electrolyte absorption
- Also increased electrolyte concentration of ECF causes thirst, thereby making the persons to drink excessive amount of water

Hence the final result is an increase in ECF volume, sometimes enough to course generalized edema.

2. **Effect on blood volume**:

The plasma volume increases almost proportionally during the early part of increase in ECF volume. Hence one of the effects of increased aldosterone secretion is a mild to moderate increase in blood volume.

c. Effect of aldosterone on sweat glands, salivary glands and gastric mucosa:

The mineralocorticoids have almost the same effect on the sweat glands, salivary glands intestinal glands as on the renal tubules, greatly reducing the lows of Na+ and Cl in the glandular secretions. The effect on the sweat glands is important to conserve body salt in hot environment whereas; the effect on intestinal gland is probably of importance to prevent salt loss in the gastrointestinal excretory products.

RENIN ANGIOTENSIN SYSTEM

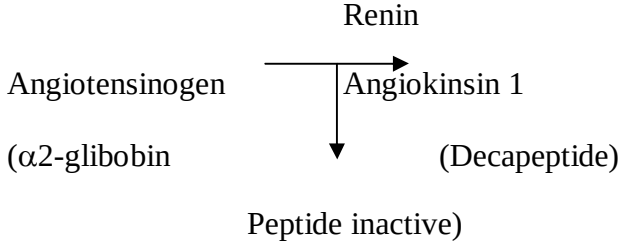
Juxtaglomerular (JG) cells: Afferent arteriole of nephron show cytoplasmic granules which contain an enzyme called Renin. A fall in sodium concentration, hypovolemia, hypotension and a fall in intra cellular Ca2+ stimulate the release of rennin from JG cells to the blood. Brady Kinin and glucagon also stimulate release of renin.

CHEMISTRY

Renin is a proteolytic enzyme mwt 35000 recently renin isoenzymes or renin like enzyme have been described in brain, placenta, and sub-maxillary duct and at the junction of uterine endometrium and myonetrium.

Action of Renin.

- Formation of Angiotensin I: Renin acts on a plasma substrate, and α 2-globulin, called angiotensinogen or Hypertensinogen, which is produced by the liver. The enzyme cleaves the leucyl-leucy bind between 10 and 11 positions from N-terminal end to produce angiotensin 1, a decapetide and a polypeptide having 7400 amino acids inactive.



This is the rate limiting step. Cortisol and estradiol enhance the reaction, probably by increasing hepatic synthesis of angiotensinogen.

Formation of Angiotensin II

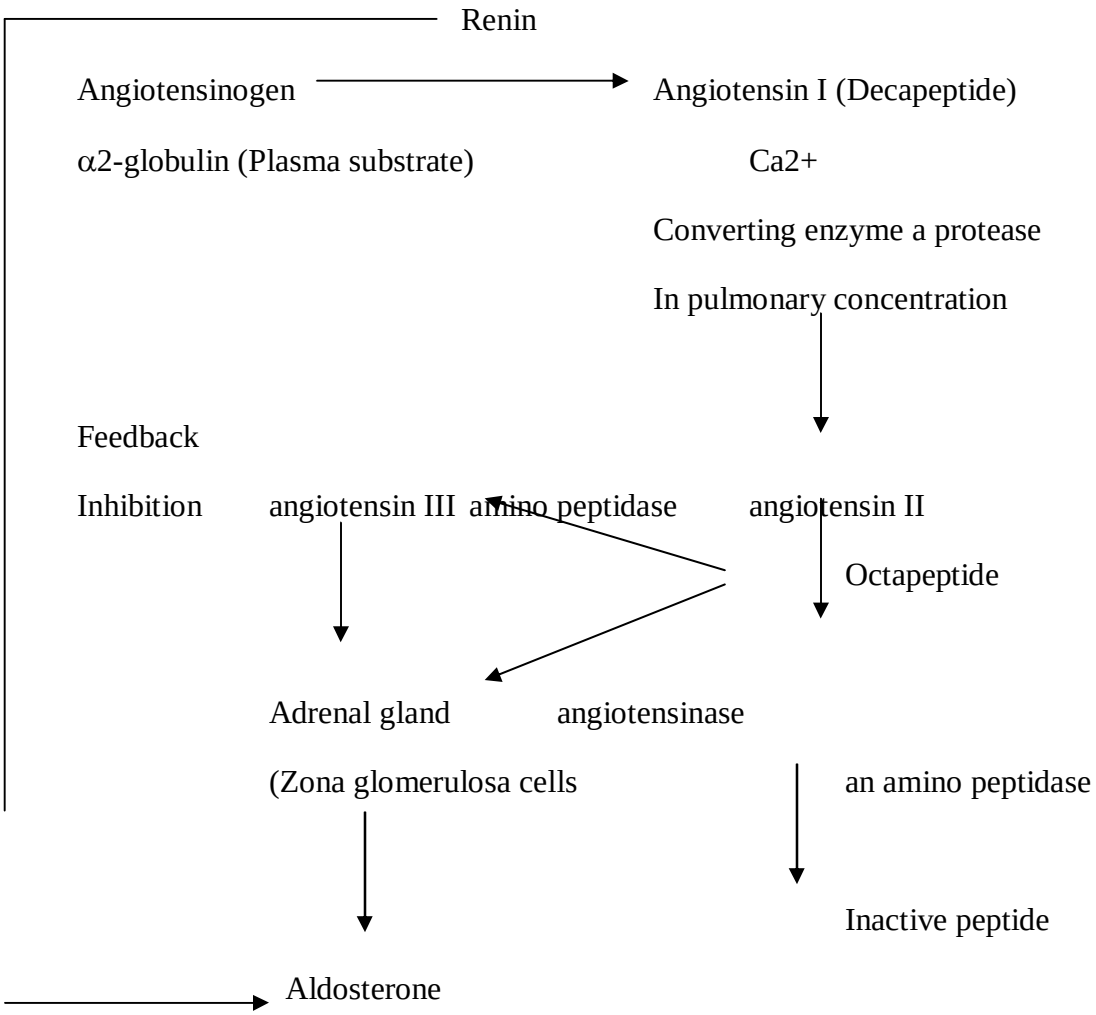
Angiotensin 1, a decapeptide, mot 1296, while circulatory, is acted upon by another enzyme, called converting enzyme (a protease) which occurs on the walls of small verses of living. The enzyme is Ca2+ dependent and it removes terminal histidyl-leucyl dipeptide in pulmonary

circulation forming angiotensin II a hexapeptide mwt 1046 and an inactive dipeptide. Angiotensin II is the active component which acts a zona glomerulosa cells to increase synthesis of aldosterone and increases rate of release of the hormone.

In activation of Angiotensin II

An enzyme angiotensinase II by hydrolysis.

Angiotensin III: Recently in rat heptapeptide angiotensin IV has been isolated. It is claimed to be also present in humans. Both heptapeptide (angiotensin II) are claimed to be equipotent in stimulating aldosterone secretion aldosterone can inhibit the enzyme renin by feedback inhibition so that angiotensin II formation is decreased.



Inactivation of Renin: In addition to feedback inhibition by aldosterone.

- Renin is also destroyed by a cephalin derivatives in plasma and
- Also inhibited by a lysophospholipid, liberated by the action of phospholipase A2.

Actions of Angiotensin II:

Principal action is angiotensin II stimulates aldosterone synthesis in zona glomerulosa cells and increases rate of secretion of aldosterone.

Mechanism of action and effects:

Angiotensin II binds with specific reception an membrane of zona glomerulosa cells and

- Enhance cytosolic concentration of Ca2+ in cells and
- Formation of inositol-1, 4, 5- triphosphate.

The above act as a second messenger and in turn:

a. enhances conversion of cholesterol to pregnenolone
b. and corticosterone to aldosterone by increasing the activity of 18-hydroxylase

Aldosterone thus formed and secreted:

- Increases the active tubular reabsorption of Na+ and
- Consequently passive reabsorption of Cl and water. Renal retention of water restores the falling ECF volume and helps in long term increase of arterial blood pressure.

Others actions of Angiotensin II.

- Stimulates contraction of smooth muscles on the walls of alimentary canal uterus, arteries arterioles but unlike catecholamine it reacts with a specific receptor in the cell membrane of smooth muscles leading to a rise in intracellular Ca+ which then promotes contraction of smooth muscle fibers.
- May raise arterial B.P by causing arteriolar construction and is thought to be responsible for hypertension associated with ischaemic kidney.
- Also stimulates V.M centre in the hind brain leading to refer rise in cardiac output, reflex arteriolar constriction and a rise in B.P
- May stimulate vasopressin secretion, indirectly causing water retention
- May also stimulate synthesis and release of PG in renal medulla.

On the other hand PG particularly PGE, may act against angiotensin II and reduce the renal vasoconstrictor and antidiuretic effect of angiotensin II.

ADRENAL MEDULLARY HORMONES

Chemistry

1. Two biologically active compounds have been isolated from the adrenal medulla and synthesized. They are

- Epinephrine (Adrenaline or Adrenin)
- Norepinephrine (Noradrenaline or Arterenol)

2. The naturally occurring forms are laevorotatory, the synthetic are racemic, the form being almost twice as active as the latter.

3. The above two hormones are called catecholamines and are closely related to tyrosine and synthesized in body from tyrosine .

Epinephrine is primarily synthesized and stored in adrenal medulla. Nor epinephrine is primarily synthesized in sympathetic nervous system and acts locally as neurotransmitter at the post synaptic cell. Norepinephrine is also synthesized and stored in adrenal medulla.

Biosynthesis

In adrenal pheochromocytes and renal cells, the synthesis of catecholamine is essentials same. Both are produced from the amino acid tyrosine.

STORAGE

- Epinephrine, norepinephrine and Dopamine are stored in the form of granules in the pheochromocytes of adrenal medulla.
- Norepinephrine only occurs in adrenergic nerve terminals as granules/ or vesicles 400 to 500 ‘A’ and diameter. And some is probably free in cytoplasm. Both the hormones are stored in the granules in the adrenal medulla and in adrenergic neurons as a complex containing ATP in the ratio of about 4 molecules of hormone; one molecule of ATP and in combination with several incompletely characterized proteins like chromogenin A and Chromomembrane B.

Clinical importance:

As catecholamines cannot penetrate blood brain barriers the norepinephrin in the brain must be synthesized within that tissue. L-DOPA, the precursors of catecholamines does penetrate the barrier, it is hence, used to increase brain catecholamine synthesis in Parkinson's disease.

Mechanism of Action

1. **Role of c-AMP**

Catecholamines on binding to β-receptors (β1and β2) activate adenyl cyclase which increases c-AMP level in the cells. Increased c-AMP activates c-AMP dependent protein kinases which phosphorylates specific protein/or enzymes and activated / inactivate them. β-receptor action is mediated through increased intracellular c-AMP level.

- Catecholamines on binding to α-receptors inhibit adenyl cyclase, thus decreasing the intracellular c-AMP level. α–receptor action is mediated through decreasing intracellular c-AMP level.

2. Role of Ca2+ and phosphor-inositides:

Catecholamines on binding with α1 receptor effect the formation of inositol 1, 4 ,5 triphosphate and diacylglycerol, and or intracellular Ca^{2+} these may act as second messenger to produce tissue response during α-effects.

METABOLIC ROLE OF CATHECHOLAMINES

a. Glycogenolysis

1. Liver epinephrine stimulates rapid breakdown of glycogen of liver (glycogenolysis) producing hyperglycaemia.

Action mediated by two ways:

- It's binding to β2 receptors on hepatic cell membrane by increasing c-AMP level.
- Also exerts it effect by binding to α1 receptors on hepatic cell membrane, which increases intracellular Ca2+ level which act as second messenger.

The effect of c-AMP increase in hepatic cells is similar to glucagon. But measurement of c-AMP levels after epinephrine and/or glycogen indicate that glucagon is by for the more active hormone in liver tissue. Nor epinephine has very little effect on blood glucose.

2. ***Muscle***: In muscle, epinephrine also causes breakdown of glycogen (glycogenolysis) by increasing c-AMP level (β-effect), but in this tissues it is more active than glucagon. Glucagon has very little effect or no effect due to lack of specific receptor. In exercising muscle, this can result in increased lactic acid formation, which passes to blood.

3. ***Heart muscle***: Increase c-AMP after epinephrine administration is seen in 2-4 seconds, the effect of epinephrine on cardiac output (lonotropic effect) is seen shortly after wards, whereas activation of phosphorylase is not detectable for 45 seconds.

4. ***Heart glycogen***: In vivo, actually epinephrine action can results in an increase in heat glycogen. This is probably secondary to the hormone action an adipose tissue causing adipose and increase FFA. Fatty acids as utilized as fuel. Increased glycogen is due to gluconeogenesis; the glucose is not utilized for energy and diverted to glycogen formation.

b. ***Lipolytic Action***: Both epinephrine and norepinephrine increase the breakdown of TG in adipose tissue by increasing c-AMP level (β effect). Net effect of lipolysis is rapid release of FFA and glycerol from adipose tissue to blood.

c. ***Glucogenic Action***: Epinephrine increase cyclic c-AMP which induces the synthesis of key enzymes pyruvate carboxylase, PEP carboxykinase and fructose -1,6-biphosphate. Increased FFA level in blood produced by lipolytic action can also activate hepatic gluconeogenesis.

d. ***Action on glucoses***: Epinephrine increase blood lactic acid level by promoting neither muscle glycolysis, nor epinephrine has very little effect on blood lactic acid.

e. ***Action on insulin Release***: Epinephrine has a direct inhibitory action on insulin release from β-cells of pancreas (α2-effect). Thus, in pancreases the α-adrenergic response to epinephrine predominates, c-AMP decrease and insulin release inhibited. However in the presence of α-blockers such as phentolamine (Regitine), the β-effect predominates and epinephrine cause increased c-AMP and increase insulin release.

f. ***Calorigenic Action***: Norepinephrine and epinephrine are almost equally potent in their calorigenic action. They produce a prompt rise in the metabolic rate which is independent of the liver,

* A smaller delayed rise which is abolished by hepatectomy and coincides with rise in blood lactic acid. The calorigenic action does not occur in the absence of the thyroid and adrenal cortex.

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