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.

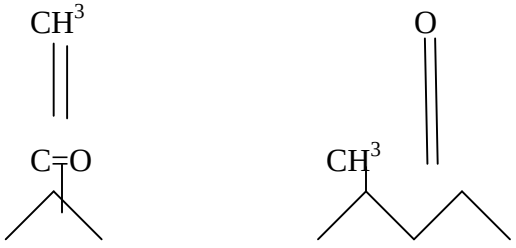
CLASSIFICATION

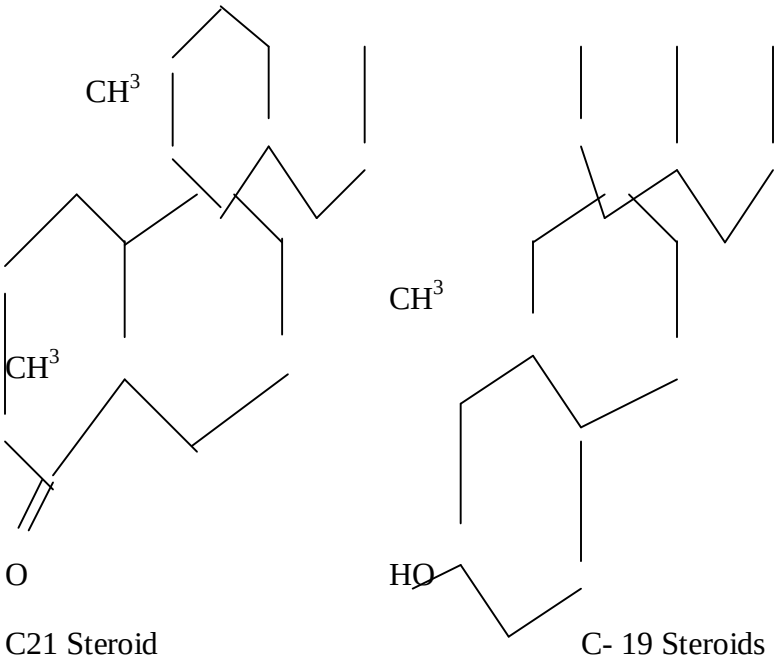
1. According to structure: Adrenocortical hormones are mainly of two structural types.

- 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.

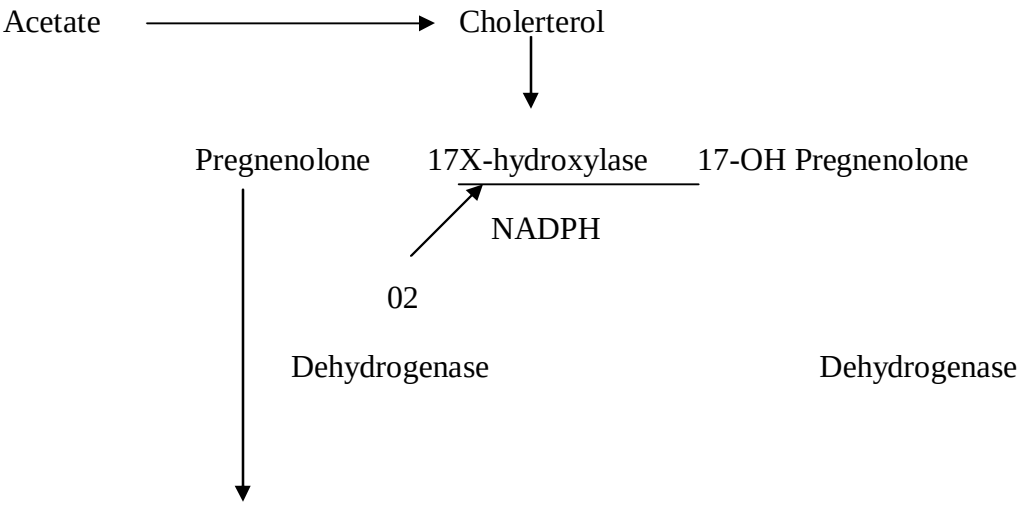
GLUCO CORTICOIDS

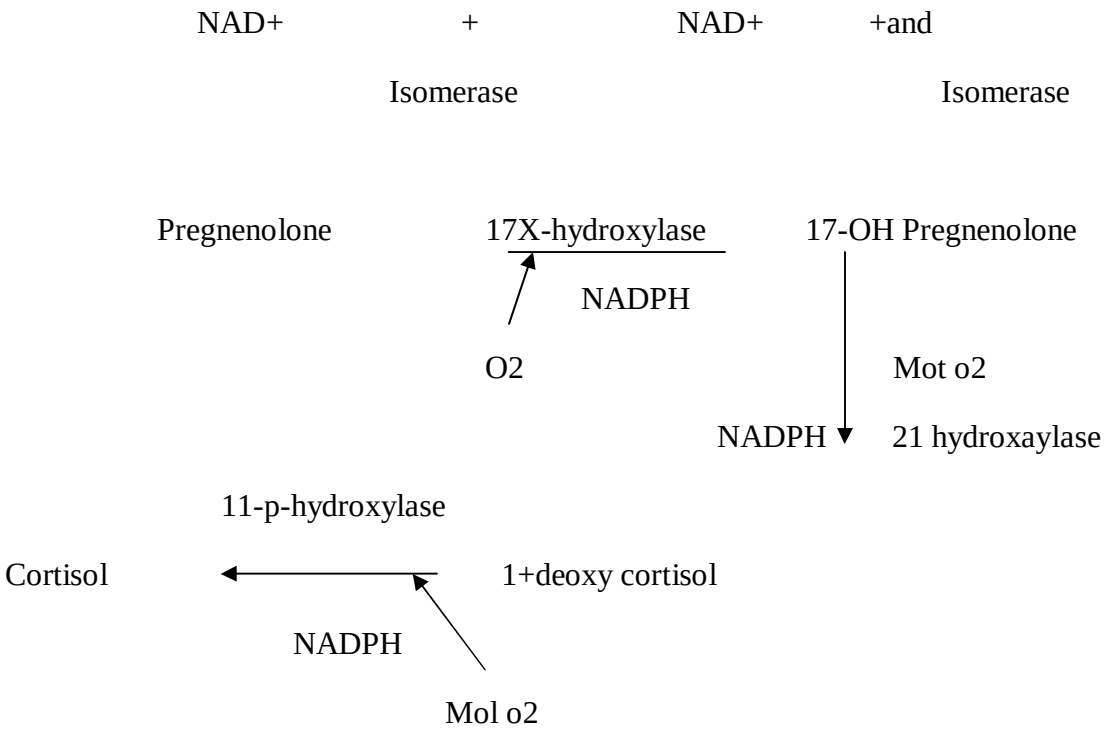
1. Biosynthesis of glucocorticoids:

   Common pathway for all cortico-steroids:

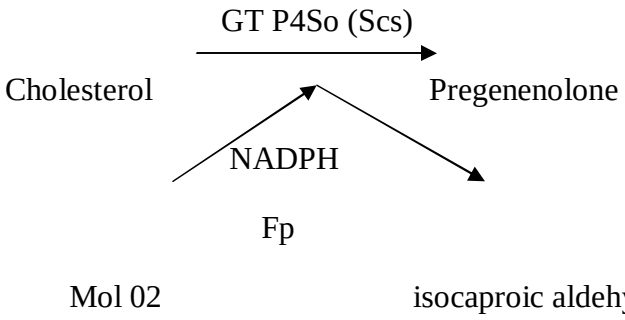
   Corticosteroids are synthesized by a common pathway from cholesterol in the adrenal cortex.

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

a. 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:

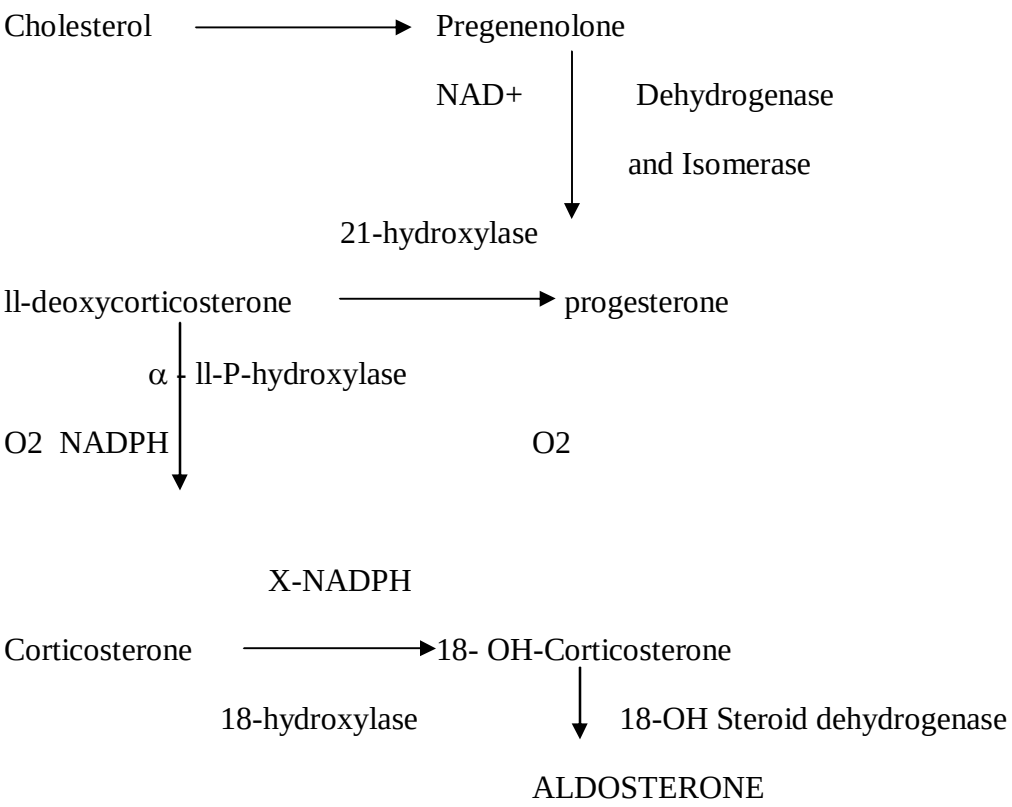
- Corticosterone. * II-deoxycortisol * II-deoxycorticosterone

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 oedema

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

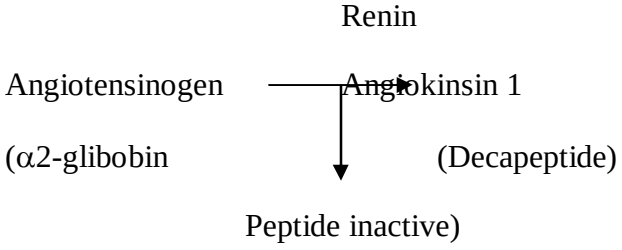
Juxtaglomerular (JG) cells: Afferent arteriole of nephron show cytoplasmic granules which contain an enzyme called Renin. A fall in sodium concentration, hypovolemia, hypotension and a fall in intra cellular Ca2+ stimulate the release of rennin from JG cells to the blood. Brady Kinin and glucagon also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

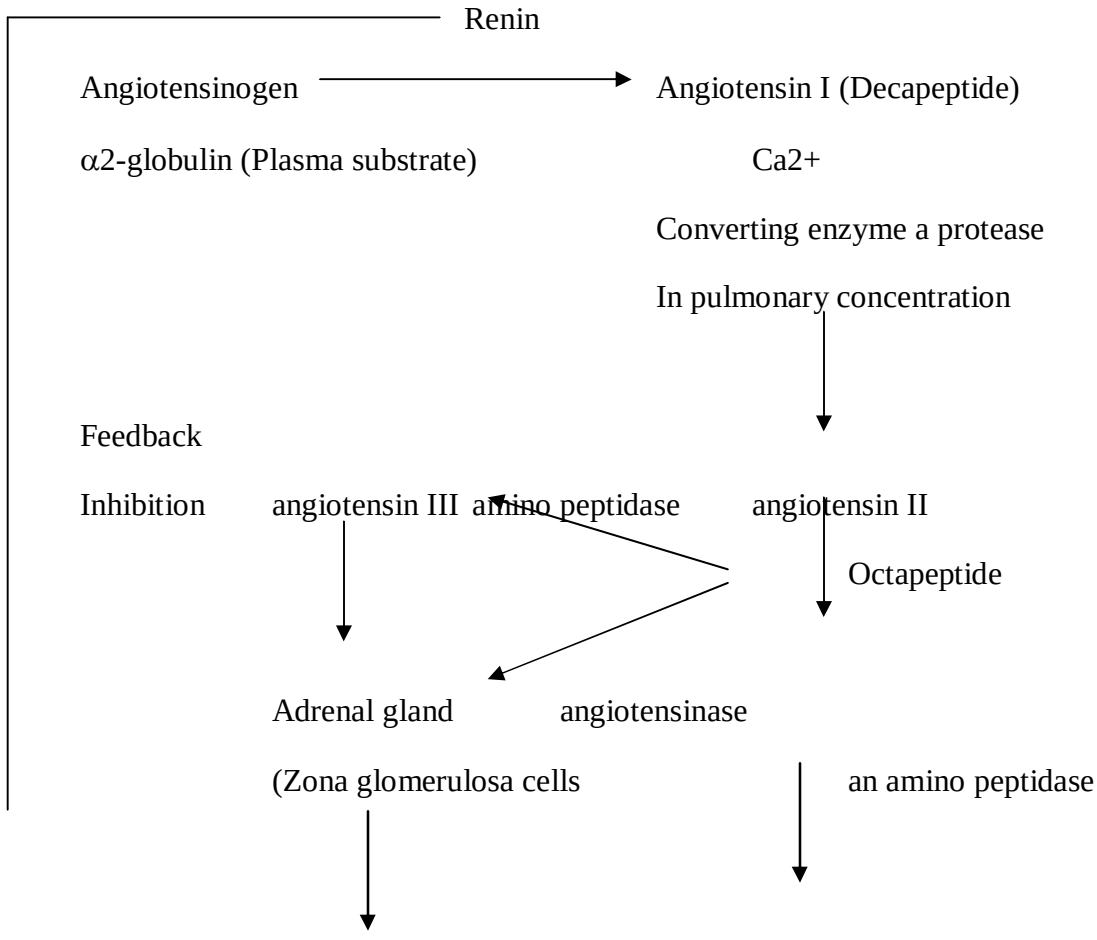
Formation of Angiotensin II

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.

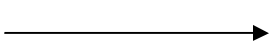
In activation of Angiotensin II

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.



Inactive peptide



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.

REFERENCES

1. Krstie, R.V. Ultra structure of mammalian cells, Springer-Verlag, Heidelberg, Germany, 1979.
2. Ernster, L. and Schatz, G. Mitochondria: a historical review. J. Cell Biol. **91,** 227 (S) - 235 (S), 1981.
3. Rothman, J.E. The compartmental organization of golgi body. Sci. Am. **253(3),** 84-95, 1985.
4. Duive. Microbodies in livings cells. Sci. Am. **248(5),** 52-62, 1983.
5. Bainton, D.L. The discovery of lysosomes. J. Cell Biol. **91,** 665-675, 1981.
6. Zimmerman, R.A. Ins and outs of ribosome. Nature **376,** 391-392, 1995.
7. Birchmeicr, W. Cytoskeleton structure and function. Trends Biochem. Sci. **9,** 192-195, 1984.
8. Murray, A.W. and Kirschner, M.C. What controls cell cycle. Sci. Am. **264 (3),** 34-41, 1991.
9. Collins, M.K.L. and Rivas, A.L. The control of apoptosis in mammalian cells. Trends Biochem Sci. **18,** 307-309, 1993.
10. Printon, P. Puzzan, T. and Rizzuto, R. The golgi apparatus is an inositol-1, 4, 5-triphosphate Ca2+ store with functional properties distinct from those of endoplasmic reticulum. EMBO. J. **17,** 5298-5308, 1998.
11. Nayasawa, M. Kanzaki, M. Vinoy. Morishita, Y. and Kojima, Y. Identification of novel chloride channel expressed in the endoplasmic reticulum, golgi apparatus and nucleus. J. Biol. Chem. **276,** 20413-20418, 2001.
12. Tinacirman *et al*. Selective disruption of lysosomes in the HeLa cells triggers apoptosis mediated by cleavage of Bid by multiple papain like lysosomol cathepsins. J. Biol. Chem. **279,** 3578-3587, 2004.

1. Krstie, R.V. Ultra structure of mammalian cells, Springer-Verlag, Heidelberg, Germany, 1979.
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6. Zimmerman, R.A. Ins and outs of ribosome. Nature **376,** 391-392, 1995.
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8. Murray, A.W. and Kirschner, M.C. What controls cell cycle. Sci. Am. **264 (3),** 34-41,

1991.
9. Collins, M.K.L. and Rivas, A.L. The control of apoptosis in mammalian cells. Trends Biochem Sci. **18,** 307-309, 1993.
10. Printon, P. Puzzan, T. and Rizzuto, R. The golgi apparatus is an inositol-1, 4, 5-triphosphate Ca2+ store with functional properties distinct from those of endoplasmic reticulum. EMBO. J. **17,** 5298-5308, 1998.
11. Nayasawa, M. Kanzaki, M. Vinoy. Morishita, Y. and Kojima, Y. Identification of novel chloride channel expressed in the endoplasmic reticulum, golgi apparatus and nucleus. J. Biol. Chem. **276,** 20413-20418, 2001.
12. Tinacirman *et al*. Selective disruption of lysosomes in the HeLa cells triggers apoptosis mediated by cleavage of Bid by multiple papain like lysosomol cathepsins. J. Biol. Chem. **279,** 3578-3587, 2004.
13. Ferri, K.F. and Kroemer, G. Organelle specific initiation of cell death pathways. Nature Cell Biology. **3,** E255-E263, 2001.
14. Karbowski, M. and Youle, R.J. Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death and Differentiation. **10,** 870-880, 2003.
15. Franklin, H.M. The way of the cell:Molecules, Organisms and order of life. Oxford University Press, 2003.
16. Cohen, R.M. and Roth, K.S. Biochemistry and disease: bridging basic science and clinical practice. Williams and Wilkins, 1996.
17. Dolman, N.J. *et al.* Stable golgi-mitochondria complexes and formation of golgi Ca2+ gradiants in pancreatic acinar cells. J. Biol. Chem. **280**, IS794-99, 2005.
18.Hartman, S.C. Purines and pyrimidines in metabolic pathways, Greenberg (Ed.). Vol. Academic Press, New York, 1970.
19.Holley, R.W. The nucleotide sequence of nucleic acids, Sci. Am. **214**, 30, 1966.
20.Hutchinson, D.W. Nucleotides and coenzymes. J. Wiley, New York, 1964.
21.Jost, J.P. and Ricken Berg, H.V. Cyclic AMP. Ann. Rev. Biochem. **40**, 741, 1971.
22.Zemeenick, P.C. Diadenosine tetra phophate. Its role in cellular metabolism Anal. Biochem. **134**, 1-10, 1983.
23.Naim, M., Seifert, R. Numberg, M. Grunbaum, L. and Schultz, G. Some taste substances are direct activators of G-proteins. Biochem. J. **297**, 451-454, 1994.
24.Joanne, S. Ingwell, ATP and the heart, Kluwar academic publisher, 2002.
25.Keneeth Alan Jacobson. Purines in cellular signalling: targets for new drugs. Springer Verlag, NY, 1990.
26.Amir pelleg. Effect of extracellular adenosine and ATP on cardiomyocytes. Vol.6. Landes Bioscience, 1999.
27.Geoffrey Burnstock. (Ed.). Cardiovascular biology of purines, Vol. 209, Kluwer Academic Publisher, 1998.
28.Dimple, H.Bhatt *et al.* cAMP induced repair of zebra fish spinal circuits. Science. **305**, 254-258, 2004.

29.Noji, H. *et al*. Purine but not pyrimidine nucleotides support rotation of Fo-ATPase, J. Biol. Chem. **276**, 25480-25486, 2001.
30. Boyer, P.D. Ed. The Enzymes. Vol. 3, 3rd ed. Academic Press, New York, 1971.
31.Cornish-Bowden, A. and Wherton, C.W. Enzyme Kinetics. IRL Press, Oxford, 1988.
32.Kraut, J. How Do Enzymes Work ? Science **242**, 533-540, 1988.
33.Segel, I.H. Enzyme Kinetics. Wiley, New York, 1975.
34.Wei, L. Clauser, E. Alhene-Gelas, F. and Corvol, P. The Two Homologous Domains of Angiotensin Converting Enzyme Interact Differently with Competitive Inhibitors. J. Biol. Chem. **267**, 13398-13405, 1992.
35.Purich, D.L. Ed. Methods in Enzymology. Vol. 63 and 64, Academic Press, New York, 1979 and 1980.
36.Cohen, P. The Role of Protein Phosphorylation in Neural and Hormonal Control of Cellular Activity. Nature **296**, 613-620, 1982.
37.Kantowitz, E.R. and Lipscomb, W.N. E. Coli Aspartate Trans Carbamoylase, the Relation Between Structure and Function. Science **241**, 669, 1988.
38.Georgiou, G. and Dewitt, N. Enzyme Beauty. Nature Biotechnology **17**, 1161-1162, 1999.
39.Hosfield, C. *et al*. Crystal Structure of Calpain Reveals Structural Basis for Ca2+ Dependent Protease Activity and a Novel Mode of Enzyme Activation. The EMBO J. **18**, 6880-6889, 1999.
40.Xiao, Y. *et al*. Plugging into Enzymes: Nanowiring of Redox Enzyme by Gold Nanoparticles. Science **299**, 1877-1881, 2003.
41.Stevens, S.Y. *et al.* Delineation of the Allosteric Mechanism of Cytidylyl Transferase Exhibiting Negative Co-operativity. Nature Structural Biology **8**, 947-952, 2001.
42.Eisenmesser, E.Z. *et al.* Enzyme Dynamics During Catalysis. Science **295**, 1520-1523, 2003.
43.Eisenthal, R. Enzyme Assays: A Practical Approach. Oxford University Press, 2002.
44.A.G. Maragoni. Enzyme Kinetics. A Modern Approach. Wiley, New York, 2002.
45.Zollner, H. Hand Book of Enzyme Inhibitors. 2nd ed., VCH Publishers, New York, 1993.
46.Natesh, R. *et al*. Crystal Structure of Human Angiotensin Converting Enzyme-Lisinopril Complex, Nature **421**, 551-554, 2003.
47.Fuchs, S. *et al*. Role of N-terminal Catalytic Domain of Angiotensin Converting Enzyme Investigated by Targeted Inactivation in Mice. J. Biol. Chem. **279**, 15946-15953, 2004.
48.Dun McElheny, *et al.* Defining role of active site of fluctuations in dihydrofolate reductase catalysis. Proc. Nafd. Acad. Sci. USA. **102**, 5032-5035, 2005.
49.Green stein, J.P. and Winitz, M. Chemistry of amino acids. Wiley, New York, 1961.
50.Meister, A. Biochemistry of amino acids Academic Press, New York, 1965.
51.Davies, J.S. Amino acids and peptides. Chapman and Hall, 1985.
52.Weinstein, B. Ed. Chemistry and biochemistry of amino acids, peptides and proteins. Vol. 4. Mercel and Dekkar, New York, 1977.
53.Meister, A. and Anderson, M.E. Glutathione. Ann Rev. Biochem. **52**, 711-760, 1983.
54.Erdos, E.G. Johnson, A.R. and Boyden, N.J. Hydrolysis of enkaphalin by peptidyl dipeptidase. Biochem Pharmacol. **27,** 843-848, 1978.

55.Sandgreen, S. *et al.* The human antimicrobial peptide LL. 37 transfers extracellular plasmid DNA to nuclear compartment of mammalian cells via lipid raft and proteoglycan dependent endocytosis. J. Biol. Chem. **279,** 17951-17956, 2004.
56.Pierre Jolle. S.D-Amino acids in sequences of secreted peptides of multicellular organisms. Kluwer Academic Publishers, 1998.
57.Huang, L. *et al.* Novel peptide inhibitors of angiotensin converting enzyme. J. Biol. Chem. **278,** 15532-15540, 2003.
58. Borras, C. *et al.* Glutathione regulates telomerase activity in fibroblasts. J. Biol. Chem. June, 2004.
59.Korsinozky, M.L.J. *et al.* Solution structure by 1H NMR of the novel cyclic trypsin inhibitor from sunflower. J. Mol. Biol. **311,** 579-591, 2001.
60.Burrett, G.C. and Elmore, D.T. Amino acids and peptides, Cambridge University Press, 1998.
61.Doonan, S. Peptides and proteins. Wiley, New York, 2003.
62.Miquel, V.P. *et al.* Structural dissection of a highly knotted peptide reveals minimal motifs with antimicrobial activity. J. Biol. Chem. **280**, 1661-1668, 2005.