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 Test

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

- mm comprise approx 50% of body man
- 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 gaits open selectively in response to ligands, transmitters, or changes in voltage and they close by intrinsic regulatory processes.

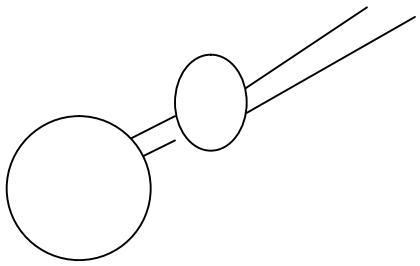
i) Voltage gated channels - have voltage sensing transmembrane domains and they are on essential for generation and modification of action potentials.
ii) Ligand gated ion channels – are essential for producing optimum myoplasmic calcium concentrations and establishing signal transduction pathway.

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

- 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 presyn mb, hence influx of Ca2= into the AT.



- This Ca2+ influx results in a Ca2+ dependent exocytose of Ach –containing vesicles from presyn. Mb. The Ach diffuses across the s/cleft to bind with Ach receptor on the muscle sarcolemma. AChR is an intergral transmembs protein having 5 subunits.
- Muscle excitation is initiated by the reversible binding of Ach to the AChR, though a local depolarization of the post syn Mb, leading to the increased conductance of Na+ and K+ though the AChR cation-channel
- Meanwhile voltage gated K+ channels in the presyn 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 posses lateral projections that from reactive sites with action-(cyclically annotate and dissociate during muscle contraction and relaxation).

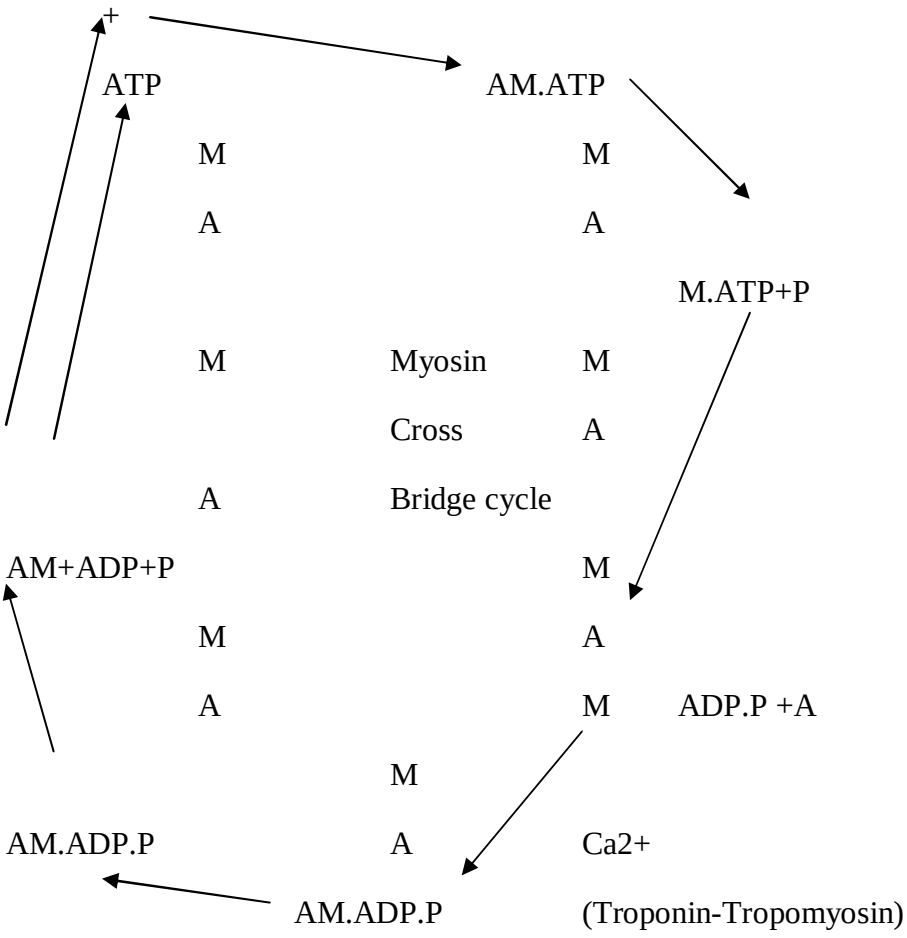
  Myosin – Asymmetric protein with 2 identical heavy chains and 2 pairs of light chains. The composition of heavy chains within sarcomere varies among spps, muscles and muscles cells.

- 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.

Energetics of mm contraction

M.C results from transformation of chemical energy to mech. Energy.

AM



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 following death. After death 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 45o.

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.