M.Sc IIIrd Semester Examination 2013

Zoology

Special Paper: Mammalian Reproductive Physiology and Endocrinology Paper : First

Neuroendocrinology and Non-classical Hormones

1. i) a ii) a iii) a iv) d v) a vi) a vii) a viii) d ix) c x) c

2. Gonadotrophin releasing hormone (GnRH) in vertebrate is a decapeptide hormone crucial for initiation and maintenance of reproductive function. The amino acid residue sequence of the mammalian form of the decapeptide is: pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. GnRH mediates the neural control of the synthesis and release of the gonadotrophin, FSH and LH. GnRH is derived from a GnRH prohormone consisting of the GnRH decapeptide and a larger GnRH associated peptide (GAP). The decapeptide and GAP are liberated from the prohormone by a series of proteolytic cleavages.

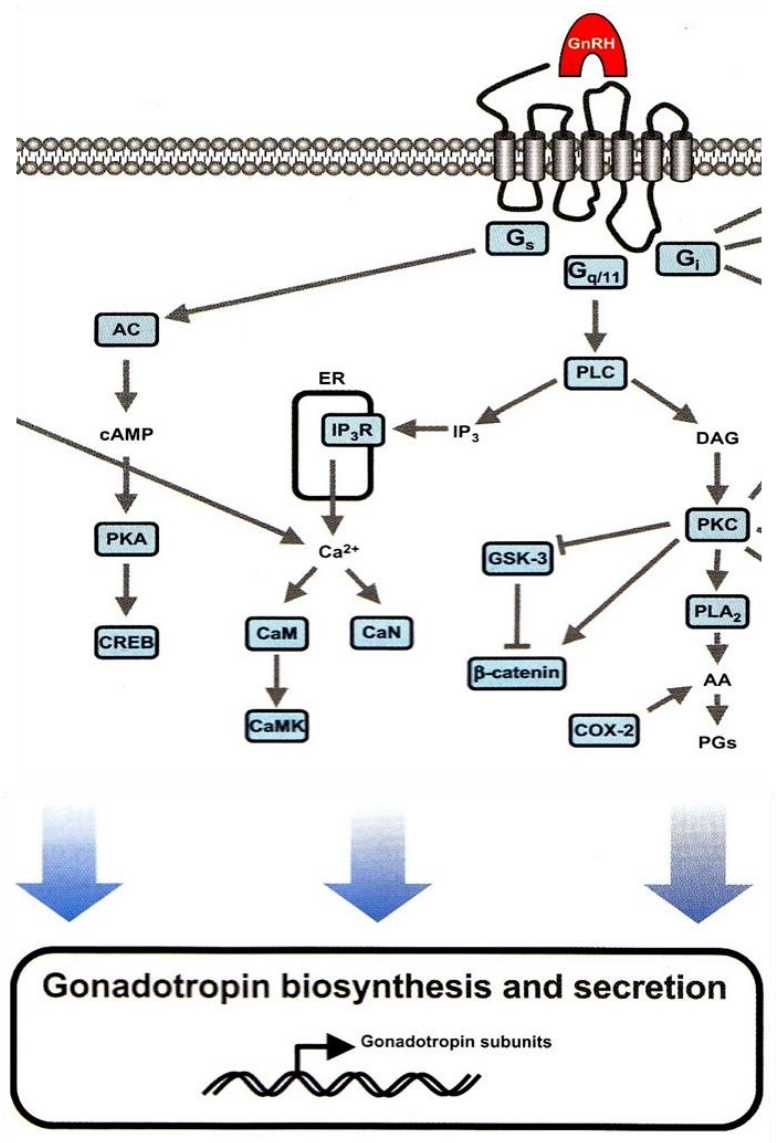
GnRH release is controlled by numerous stimulatory and inhibitory factors as well as factors with biphasic effects on GnRH neurons. After the isolation and sequencing of porcine and ovine GnRHs, it appeared that these peptides isolated from several classes of vertebrates showed multiple substitutions in their sequence when compared with pig or sheep GnRH. Until now, more than a dozen isoforms of GnRH sharing 10-50% amino acid identity has been found in vertebrates. The conservation of the length of these peptides, NH2 terminus and COOH terminus indicates that these features are critically important for receptor binding and activation. It is generally thought that most vertebrates possess at least two, and usually three, forms of GnRH which differ in their amino acid sequence, localizations and embryonic origins. The most ubiquitous is chicken GnRH II which is the evolutionary conserved member of the GnRH peptide family. It has been shown that the biological functions of GnRH I and GnRH II are different. Whereas GnRH I play a pivotal role in the regulation of reproduction by stimulating the pituitary release of LH and FSH, GnRH II participates mainly in the control of puberty, reproductive behavior, feeding and energy balance. Growing evidence shows that both GnRH I and GnRH II are potentially important autocrine/paracrine regulators in some extrapituitary compartments.

Mechanism of Action:

The actions of GnRHs are mediated by the GnRH receptors (GnRHR) which belong to a G protein-couple receptor (GPCR) subfamily. The GnRHR cDNA encodes a 327-328 amino acid

protein with seven putative membrane spanning domains characteristic for GPCR. Only one conventional GnRH receptor subtype (GnRH-IR) uniquely lacking a carboxyl-terminal tail has been found in mammals. The GnRH-IR lacks a typical intracellular carboxyl terminus, making it one of the smallest receptors with the seven-transmembrane segment motive.

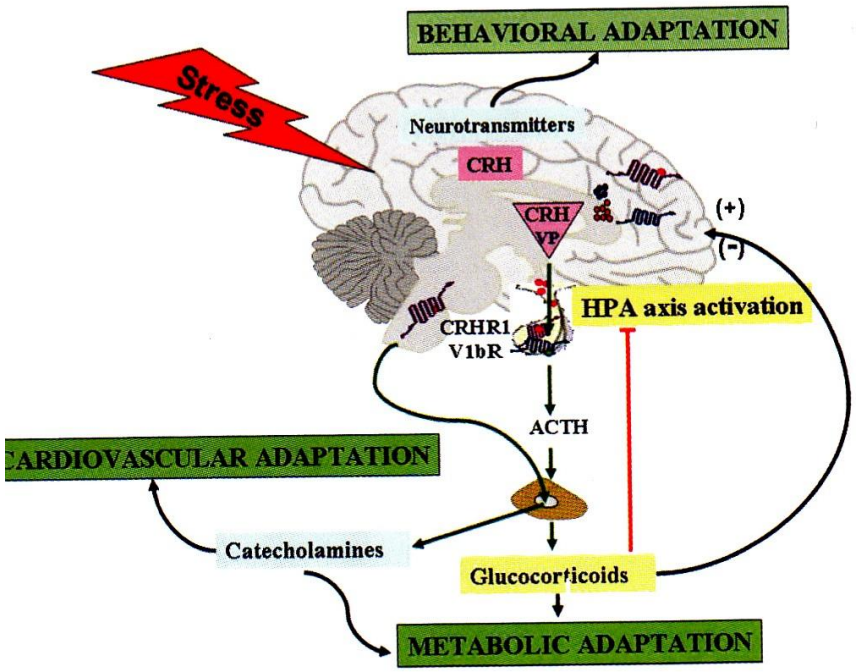
In gonadotropes, GnRH activates Phospholipase C via Gq/11, resulting in the hydrolysis of membrane bound phosphatidyl ionositol 4,5-bisphosphate (PIP2) to IP3 and DAG which mobilize intracellular calcium and activate protein kinase C respectively. These in turn stimulate the biosynthesis and secretion of gonadotrophins, LH and FSH.



Additionally, binding of the GnRH to the extracellular ligand binding domain of GPCR in plasma membrane of gonadotrope causes conformational changes in the transmembrane domain which leads to activation of GPCR. Intracellular domain of the GPCR is a multiprotein complex of

α, β and γ subunit that split into Gsα and βγ. The stimulatory Gα activate the membrane bound Adenyl cyclase to generate cyclic AMP from ATP. cAMP in turn activates the protein kinase A and ultimately lead to the activation of CREB and transcription of gonadotrophins, FSH and LH.

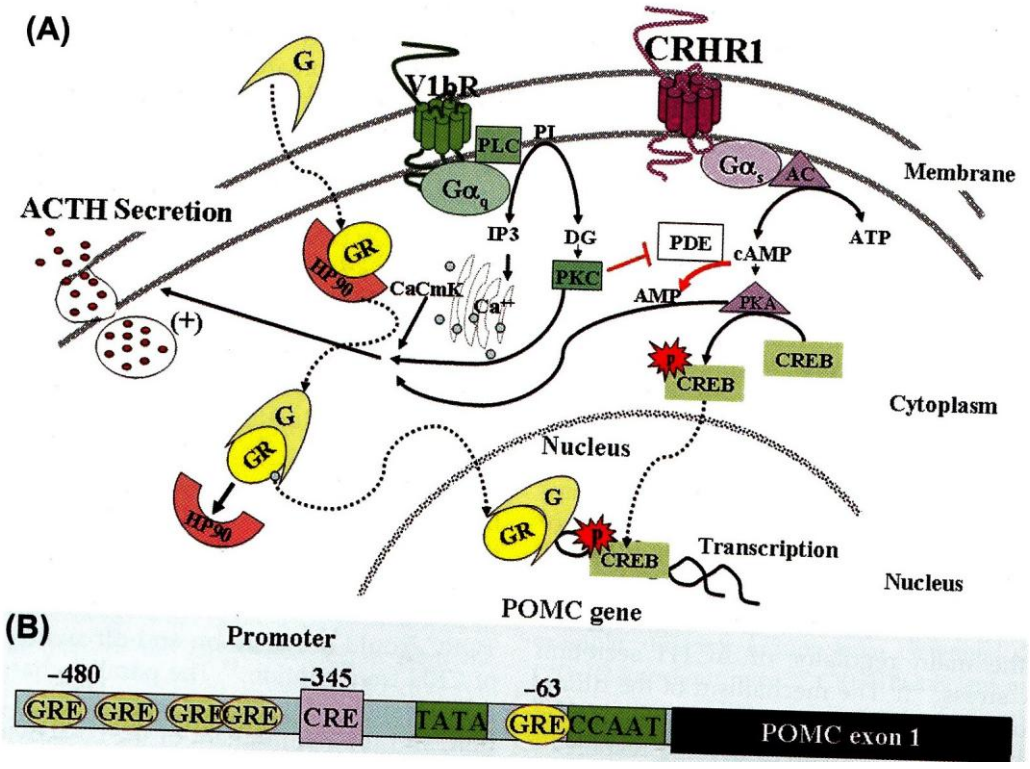
3. Living organisms are constantly challenged by external or internal challenges or stressors, which threaten homeostasis defined as constancy of the internal environment. Adaptation to stress and restoration of homeostasis requires the co-ordinated activation of complex neuroendocrine responses including behavior, endocrine and autonomic nervous systems. The major endocrine response to stress is activation of the hypothalamic pituitary adrenal axis leading to increases in circulating glucocorticoids, which are essential for the metabolic adaptation to stress. The neuroendocrine mechanisms controlling HPA axis activity during stress and the contribution of HPA axis activation to overall stress response.



Activation of the HPA axis involves stimulation of a specific group of neurons located in the dorsomedial parvocellular subdivision of the paraventricular nucleus (PVN) of the hypothalamus. These neurons synthesize corticotrophin releasing factor which are released into the pituitary portal circulation and reach corticotrope cells in the anterior pituitary gland where they stimulate the secretion of adrenocorticotropic hormone (ACTH) into the peripheral circulation. The main target of ACTH action is the zona fasciculate of the adrenal cortex where it stimulates the production and secretion of glucocorticoid.

Hypothalamic Regulation of ACTH by HPA Axis:

The paraventricular nucleus of the hypothalamus, site of production of hypophysiotropic CRH and VP can be divided into three main functional regions controlling distinct physiological effector of stress. First, parvocellular neurons in the dorsomedial and anterior divisions of the PVN release peptides to the pituitary portal circulation from axonal terminal in the external zone of the median eminence. CRH producing neurons responsible for the stimulation of ACTH secretion are located in this region. CRH exerts its effects in the pituitary corticotrope and other target cells by binding to plasma membrane receptors coupled to adenylate cyclase via guanyl nucleotide binding protein (G protein), specifically Gs. There are two major types of CRH receptors : Type 1 CRH receptors are located mainly in the brain and pituitary corticotropes and also in peripheral tissues including the reproductive organs and the immune system . type 2 receptor activate cAMP/protein kinase A dependent pathways upon binding to their ligand. Type 1 CRH receptor is the major player in the stress response and is essential for pituitary ACTH secretion and behavioral response to stress.



The Anterior Pituitary is an endocrine gland controlled by the hypothalamus in several fundamentally different fashions than is the posterior pituitary. None of the six major hormones released by the adenohypohysis are of hypothalamic origin, rather all are synthesized in cells embryonically derived from Rathke's pouch in the anterior pituitary itself and released directly into the blood stream. Releasing- and release-inhibiting hormones that are synthesized in the arcuate,

paraventricular, periventricular and supraoptic nuclei of the hypothalamus control anterior pituitary hormone secretion. Parvocellular neurons in these nuclei send their axons into the tubero-infundibular tract and terminate on a capillary bed of the superior hypophyseal arteries located around the base of the median eminence. A given parvocellular neuron may release one or more releasing factor into these capillaries that coalesce into 6 to 10 small straight veins that form the hypophysial-portal blood circulation which descends along the infundibular stalk and forms a second capillary plexus around the anterior pituitary. The releasing-hormones gain access to the five distinct types of target cells in the anterior pituitary from this plexus and stimulate anterior pituitary hormone release back into the capillary bed that then drains into the systemic circulation and transports the hormones to peripheral target tissues. The target tissues are stimulated to produce final mediator hormones that induce the physiological changes in peripheral tissues typical of each hormone.

4. **STRUCTURE OF THE PINEAL GLAND**

At first sight, the structure of the mammalian pineal is not very exciting. It lies at the exact centre of the brain behind the eyes and consists of only two major cell types, pinealocytes and immature astrocytes. Occasionally, the pinealocytes are arranged in follicles surrounding narrow or wide spaces. The gland is richly innervated by postganglionic sympathetic nerve fibres, most of which are found in the perivascular spaces of capillaries. In addition, pinealopetal fibres of central origin are present. Observed under the light microscope the pineal specific cells, the pinealocytes, lack prominent and differential staining properties. Special staining reagents such as silver impregnation are necessary to demonstrate their complete outlines. Then it becomes apparent that pinealocytes are nerve cell like, consisting of a perikaryon and an unknown number of cytoplasmic processes. The large, pale nucleus with its prominent nucleolus is also reminiscent of a large ganglion cell. As cytoplasmic basophilia is virtually non existent, there is no satisfactory explanation for the high metabolic activity of pinealocyte nuclei.

At the ultra structural level it is quite difficult to relate structure to function. In most mammalian species investigated the pinealocytes contain few granules that can be regarded as morphological correlated of secretory products, and the circadian behaviour of the few dense core vesicles present does not support the assumption that they contain melatonin. Dense core vesicles act as storage sites for serotonin. Pinealocyte perikarya house a prominent Golgi apparatus and relatively small amounts of smooth and rough endoplasmic reticulum. In some species cisternea of the endoplasmic reticulum contain flocculent material which may represent a form of secretory substances. Highly pleomorphic mitochondria tend to form clusters reminiscent of the ellipsoids of

inner segments of photoreceptor cells. That the pinealocytes are responsible for the conversion of serotonin to melatonin has been demonstrated histochemically by showing that it is these cells that contain serotonin and melatonin and not the astrocyte-like interstitial cells.

It is generally accepted that mammalian pinealocytes are phylogenetically derived from pineal photoreceptor cells. In lower vertebrates pineal photoreceptors are similar to retinal cones and show outer and inner segments as well as synapses with afferent pinealofugal nerve fibres. During phylogenesis the outer segments regress as do the pinealofugal nerve fibres, but the synaptic ribbons of the afferent synapses persist. In view of the phylogenetic regression and the current concept that it is the function of mammalian pinealocytes to synthesize melatonin and to release it into the systemic circulation, both the shape of the pinealocytes and the architecture of the gland are surprising.

Cell type	Description
Pinealocytes	The pinealocytes consist of a cell body with 4–6 processes emerging. They produce and secrete melatonin. The pinealocytes can be stained by special silver impregnation methods. Their cytoplasm is lightly basophilic. With special stains, pinealocytes exhibit lengthy, branched cytoplasmic processes that extend to the connective septa and its blood vessels.
Interstitial cells	Interstitial cells are located between the pinealocytes. They have elongated nuclei and a cytoplasm that is stained darker than that of the pinealocytes.
Perivascularphagocyte	Many capillaries are present in the gland, and perivascular phagocytes are located close to these blood vessels. The perivascular phagocytes are antigen presenting cells.

pinealneurons	In higher vertebrates neurons are located in the pineal gland. However, these are not present in rodents.
peptidergicneuron-like cells	In some species, neuronal-like peptidergic cells are present. These cells might have a paracrine regulatory function.

Human pinealocytes are equipped with long cytoplasmic processes. Comparable processes are present in all mammalian species investigated ultra structurally. When we compare these process bearing cells with the pineal photoreceptor cells of lower vertebrates it is difficult to envisage that mammalian pinealocytes represent regressed photoreceptor cells. Instead it appears that mammalian pinealocytes are highly differentiated cells similar to nerve cells, the process of which receive messages and pass on signals. A puzzling feature is that, although the possible morphological correlates of pineal secretory products are particularly prominent in terminal swellings of pinealocyte processes in many mammalian species, only a few terminals are close to blood vessels. Instead, the perivascular spaces are filled with large bundles of postganglionic sympathetic nerve fibres. In fact, according to quantitative studies in the rat, 91.1% of the nerve fibres have a perivascular location, the remainder lying between pinealocytes. It is enigmatic that sympathetic nerve fibres predominate in the perivascular spaces since the nervi conari reach the pineal gland independently of blood vessels, as separate nerves.

INNERVATION OF PINEALOCYTES

Sympathetic innervation

This type of innervation has been clearly defined both morphologically and functionally. The fibres originate in the superior cervical ganglia (SCG) of the sympathetic trunk, continue in the internal carotid nerve and enter the pineal gland as nervi conari. Their importance for the regulation fo melatonin synthesis has been demonstrated by biochemical studies after sympathectomy or electrical stimulation of the SCG, the latter leading to an approximately 50 fold increase of serotonin N-acetyltransferase (NAT) activity. A study of rat pinealocytes after electrical stimulation of the SCG that some pinealocytes did not appear to be influenced by SCG stimulation, a second group responded with enhanced electrical activity and in a third group electrical activity was depressed. In

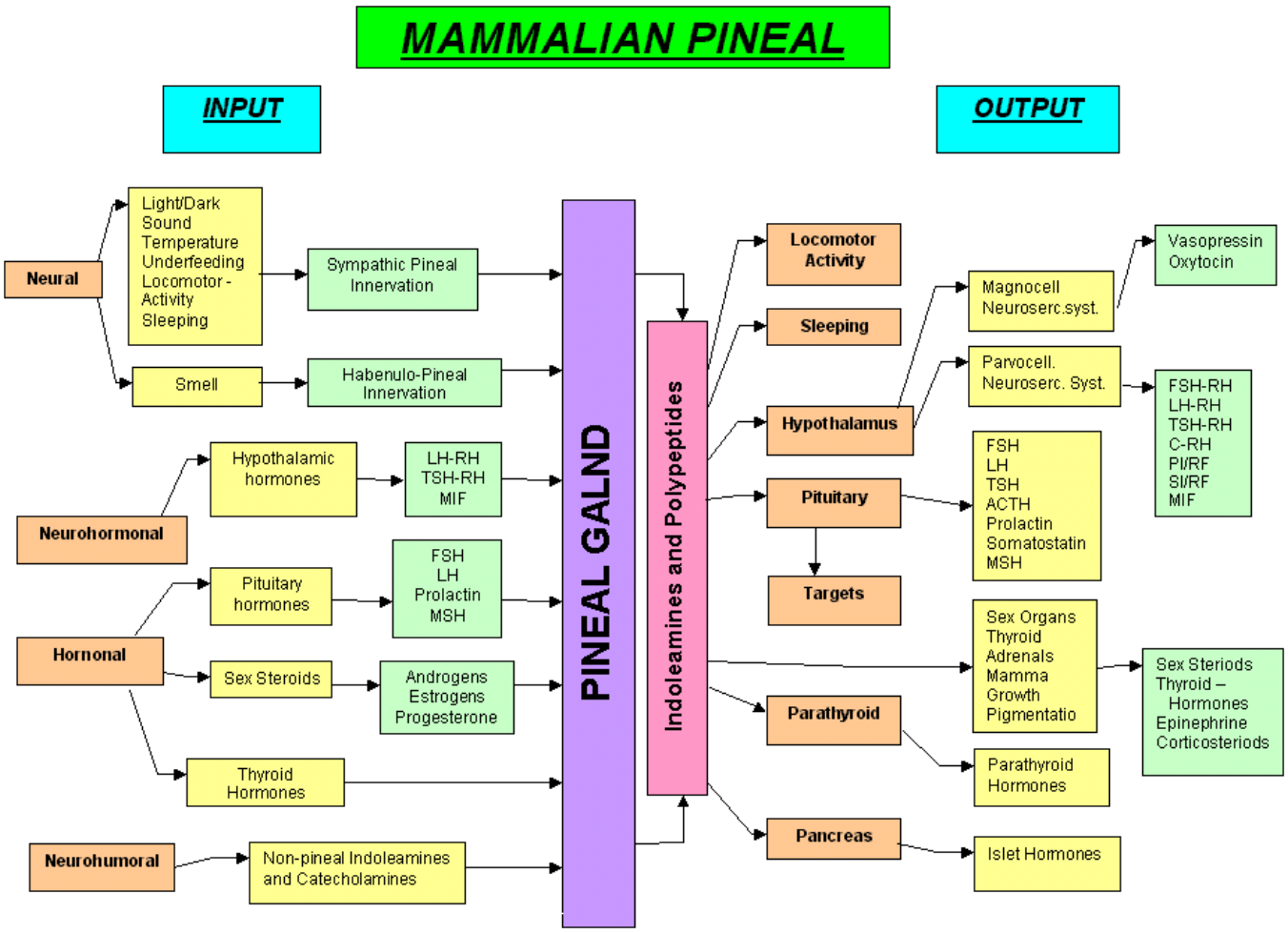
view of the continuing controversy about whether NAT of hydroxyindole O-methyltransferase (HIOMT) is the rate limiting enzyme for melatonin synthesis, and the lack of a clear-cut day / night rhythm of HIOMT, in contrast to NAT, it is relevant to recall that preganglionic electrical stimulation of sympathetic nerves resulted in an increase of pineal NAT activity but a decrease of HIOMT activity.

Central innervation

Nerve fibres reach the pineal gland via habenular and posterior commissures and now with modern neurobiological techniques available, it becomes apparent that these fibres are of functional importance. Lesion and horseradish-peroxidase studies have revealed that central pinealopetal nerves fibres originate in diverse brain regions including the habenular, paraventricular and suprachiasmatic nuclei as well as the preoptic area, amygdala, olfactory centres, lateral geniculate bodies and the sites of origin of the stria medullaris. The central fibres contain a variety of peptides such as oxytocin, vasopressin, luteinizing hormone-releasing hormone, vasoactive intestinal polypeptide. The fibres are unevenly distributed in the pineal gland, some lying in the periphery and others in the centre.

FUNCTION OF THE PINEAL GLAND

The mammalian pineal gland evolved from a well differentiated photoreceptive organ in lower vertebrates, a functional third eye. In human, pineal gland is a small pea size structure situated in the middle of the brain. The mean weight of the pineal in a woman is 173 mg which does not differ from the mean weight of 172 mg for a man. Several conditions and compounds arranged in four categories have been listed which are known to influence pineal function. Conditions of stress could affect the pineal either by way of the pineal sympathetic innervation of via the neurohumoral route. On the right or output side, indoleamines and polypeptides are mentioned. These are substances include melatonin which are known to be produced and generally secreted by the pineal.



5. The fibroblast growth factor (FGF) family of paracrine factors comprises nearly two dozen structurally related members, and the FGF genes can generate hundreds of protein isoforms by varying their RNA splicing or initiation codons in different tissues. There are at least 20 FF members, designated FGF-1 through FGF-20, but acidic FGF and basic FGF are names commonly used for FGF-1 and FGF-2, and keratinocyte growth factor (KGF) for FGF-7. Although FGF was originally named after its fibroblast mitogenicity, some FGFs do not induce fibroblast growth at all. Members of the FGF family generally share 30-50% amino acid sequence homology, have two conserved cysteine residues, and bind with high affinity to heparin. Several FGF members are oncogene products, e.g., FGF-3 (int-2), FGF-4 (hst-1, K-FGF), FGF-5 and FGF-6 (hst-2). FGFs interact with four distinct FGF receptors on cells of mesodermal, ectodermal and endodermal origin, eliciting changes in migration, morphology, function or proliferation. FGFs play several roles, including angiogenesis, wound healing, tissue regeneration, embryonic development, endocrine modulation and neurotrophic support.

- Fgfl protein is also known as acidic FGF and appears to be important during regeneration;

- Fgf2 is sometimes called basic FGF and is very important in blood vessel formation; and
- Fgf7 sometimes goes by the name of keratinocyte growth factor and is critical in skin development.
- Fgf8, is especially important during limb development and lens induction. Fgf8 is usually made by the optic vesicle that contacts the outer ectoderm of the head.
- Members FGF11, FGF12, FGF13, and FGF14, also known as FGF homologous factors 1-4 (FHF1-FHF4), have been shown to have distinct *functional* differences compared to the FGFs. Although these factors possess remarkably similar sequence homology, they do not bind FGFRs and are involved in intracellular processes unrelated to the FGFs. This group is also known as FGF.
- Human FGF18 is involved in cell development and morphogenesis in various tissues including cartilage.
- Human FGF20 was identified based on its homology to *Xenopus* FGF-20 (XFGF-20)
- FGF15 through FGF23 were described later and functions are still being characterized. FGF15 is the mouse ortholog of human FGF19 (there is no human FGF15) and, where their functions are shared, they are often described as FGF15/19 In contrast to the local activity of the other FGFs, FGF15/19, FGF21 and FGF23 have systemic effects

Although FGFs can often substitute for one another, the expression patterns of the FGFs and their receptors give them separate functions. In *Drosophila*, Breathless is an FGF protein. One member of this family, Fgf8, is especially important during limb development and lens induction. Fgf8 is usually made by the optic vesicle that contacts the outer ectoderm of the head. After contact with the outer ectoderm occurs, *fgf8* gene expression becomes concentrated in the region of the presumptive neural retina—the tissue directly opposed to the presumptive lens. Moreover, if Fgf8-containing beads are placed adjacent to head ectoderm, this ectopic Fgf8 will induce this ectoderm to produce ectopic lenses and to express the lens-associated transcription factor L-Maf. FGFs often work by activating a set of receptor tyrosine kinases called the fibroblast growth factor receptors (FGFRs). The Branchless protein is an FGF receptor in *Drosophila*. When an FGFR binds an FGF, the dormant kinase is activated and phosphorylates certain proteins within the responding cell. These proteins, once activated, can perform new functions. The RTK pathway was one of the first signal transduction pathways to unite various areas of developmental biology. Researchers studying *Drosophila* eyes, nematode vulvae, and human cancers found that they were all studying the same

genes. The pathway begins at the cell surface, where an RTK binds its specific ligand. The RTK spans the cell membrane, and when it binds its ligand, it undergoes a conformational change that enables it to dimerize with another RTK. This conformational change activates the latent kinase activity of each RTK, and these receptors phosphorylate each other on particular tyrosine residues. Thus, the binding of the ligand to the receptor causes the autophosphorylation of the cytoplasmic domain of the receptor.

The phosphorylated tyrosine on the receptor is then recognized by an adaptor protein. The adaptor protein serves as a bridge that links the phosphorylated RTK to a powerful intracellular signaling system. While binding to the phosphorylated RTK through one of its cytoplasmic domains, the adaptor protein also activates a G protein, such as Ras. Normally, the G protein is in an inactive, GDP bound state. The activated receptor stimulates the adaptor protein to activate the guanine nucleotide releasing factor (GNRP). This protein exchanges a phosphate from a GTP to transform the bound GDP into GTP. The GTPbound G protein is an active form that transmits the signal to the next molecule. After the signal is delivered, the GTP on the G protein is hydrolyzed back into GDP. This catalysis is greatly stimulated by the complexing of the Ras protein with the GTPase-activating protein (GAP), hi this way, the G protein is returned to its inactive state, where it can await further signaling. Without the GAP protein, Ras protein cannot catalyze GTP well, and so remains in its active configuration.

Mutations in the *RAS* gene account for a large proportion of cancerous human tumors and the mutations of *RAS* that make it oncogenic all inhibit the binding of the GAP protein. The active Ras G protein associates with a kinase called Raf. The G protein recruits the inactive Raf protein to the cell membrane, where it becomes active. The Raf protein is a kinase that activates the MEK protein by phosphorylating it. MEK is itself a kinase, which activates the ERK protein by phosphorylation. In turn, ERK is a kinase that enters the nucleus and phosphorylates certain transcription factors. The RTK pathway is critical in numerous developmental processes. Moreover, it can be activated by paracrine factors other than those of the FGF family. FGFs are potent regulators of cell proliferation, differentiation and function and are critically important in normal development, tissue maintenance and wound repair. FGFs are also linked with several pathological conditions

Acidic FGF (aFGF) and basic FGF (bFGF) are the prototypic FGF members named because of their different isoelectric points. They share a 55% homology in amino acid sequence and similar size, depending on translation extensions and truncations (15-18 kDa for aFGF and 16-24 kDa for bFGF). Neither aFGF nor bFGF genes include a secretory signal sequence and the principle mechanism of their release into extracellular fluid has not yet been resolved. Acidic FGF has high

expression levels in brain, retina, bone matrix and osteosarcomas. Basic FGF is found in a variety of tissues, including pituitary gland, neural tissue, adrenal cortex, corpus luteum, and placenta. Acidic and basic FGFs stimulate proliferation in all cells of mesodermal origin, and many cells of neuroectodermal, ectodermal, and endodermal origin. They are chemotactic and mitogenic for endothelial cells and induce the release of agents that break down basement membranes. These two FGFs appear to play significant roles in modulating such normal processes as angiogenesis, tissue repair, embryonic development, and neural function. They also appear to participate in some pathological conditions that involve excessive cell proliferation or angiogenesis, such as tumor production.

Functions

1. FGFs are multifunctional proteins with a wide variety of effects; they are most commonly mitogens but also have regulatory, morphological, and endocrine effects. They have been alternately referred to as "pluripotent" growth factors and as "promiscuous" growth factors due to their multiple actions on multiple cell types Promiscuous refers to the biochemistry and pharmacology concept of how a variety of molecules can bind to and elicit a response from single receptor. In the case of FGF, four receptor subtypes can be activated by more than twenty different FGF ligands. Thus the functions of FGFs in developmental processes include mesoderm induction, antero-posterior patterning, limb development, neural induction and neural development and in mature tissues/systems angiogenesis, keratinocyte organization, and wound healing processes.

2. FGF is critical during normal development of both vertebrates and invertebrates and any irregularities in their function leads to a range of developmental defects.

3. FGFs secreted by hypoblasts during avian gastrulation play a role in stimulating a Wnt signaling pathway that is involved in the differential movement of Koller's sickle cells during formation of theprimitive streak

4. One important function of FGF1 and FGF2 is the promotion of endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures. They thus promote angiogenesis, the growth of new blood vessels from the pre-existing vasculature. FGF1 and FGF2 are more potent angiogenic factors than vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF). FGF1 has been shown in clinical experimental studies to induce angiogenesis in the heart.

5. In addition to stimulating blood vessel growth, FGFs are important players in wound healing. FGF1 and FGF2 stimulate angiogenesis and the proliferation of fibroblasts that give rise

to granulation tissue, which fills up a wound space/cavity early in the wound-healing process. FGF7 and FGF10 (also known as Keratinocyte Growth Factors KGF and KGF2, respectively) stimulate the repair of injured skin and mucosal tissues by stimulating the proliferation, migration and differentiation of epithelial cells, and they have directchemotactic effects on tissue remodeling.

6. During development of the central nervous system, FGFs play important roles in neurogenesis, axon growth, and differentiation. FGFs are also important for maintenance of the adult brain. Thus, FGFs are major determinants of neuronal survival both during development and during adulthood. Adult neurogenesis within the hippocampus e.g. depends greatly on FGF-2. In addition, FGF-1 and FGF-2 seem to be involved in the regulation of synaptic plasticity and processes attributed to learning and memory, at least in the hippocampus

7. Most FGFs are secreted proteins that bind heparan sulfates and can, therefore, be caught up in the extracellular matrix of tissues that contain heparan sulfate proteoglycans. This local action of FGF proteins is classified as paracrine signalling, most commonly through the JAK-STAT signaling pathway or the Receptor tyrosine kinase (RTK) pathway.

8. Members of the FGF19 subfamily (FGF15, FGF19, FGF21, and FGF23) bind less tightly to heparan sulfates, and so can act in an endocrine fashion on far-away tissues, such as intestine, liver, kidney, adipose, and bone.

6. Immnunoinflammatory mediators are the chemical agents that behave as a hormone in mediating immune function when an organism exposed to a challenging condition with potenyial to activate the immune response. Most important among these immnuinflammatory hormones are the cytokines. Cytokines are low-molecular weight (less than 30 kDa) regulatory proteins or glycoproteins secreted in the body in response to a number of stimuli. Cytokines are secreted by white blood cells and various other cells The half-life of cytokines in the blood stream or other extra-cellular fluid is very short. Cytokines are referred to as **interleukins, a name** indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins 1–29 have been identified. Lymphokines , Monokines , Chemokines, IFN and TNF. Growth factors tend to be produced constitutively, whereas cytokines and hormones are secreted in response to discrete stimuli. Secretion is short-lived, generally ranging from a few hours to a few days. Unlike hormones, which generally act long range in an endocrine fashion, most cytokines act over a short distance in an autocrine or paracrine fashion. Most hormones are produced by specialized glands and tend to have a unique action on one or a few types of target cell. In contrast, cytokines are often produced by, and bind to, a variety of cells.

TABLE 1. Cells of Origin and Biological Properties of Interleukins

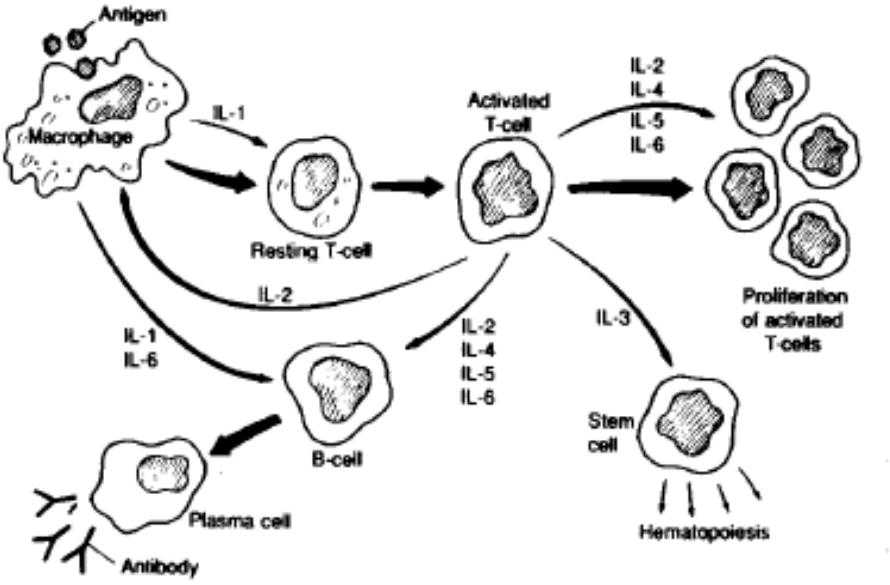
Cytokine	Size (kD)	Primary Source	Principal Biological Properties
Interleukin-1 (alpha and beta)	17	Macrophage	T-cell activation B-cell proliferation and maturation Inflammatory mediator
Interleukin-2	15	Activated T-lymphocytes	T-cell proliferation B-cell growth Macrophage cytotoxicity NK and LAK cell proliferation
Interleukin-3	28	Activated T-lymphocytes	Growth of multilineage bone marrow stem cells Mast cell-growth factor
Interleukin-4	15–20	Activated T-lymphocytes Bone marrow stromal cells	B-cell growth and differentiation Down regulation of IL-2 Fibroblast proliferation
Interleukin-5	40–50	Activated T-lymphocytes	B-cell growth and differentiation Eosinophil differentiation
Interleukin-6	26	Macrophage	Maturation of B cells Induction of hepatic acute phase response
Interleukin-7	25	Bone marrow stromal cells	B-cell growth and differentiation
Interleukin-8	10	Macrophage	Neutrophil chemotactic factor
Interleukin-9	40	Activated T-lymphocytes	Stimulates erythroid and mast cell development
Interleukin-10	16–20	Th2 lymphocytes	Inhibits Th1 cytokine synthesis Enhances B-cell survival Stimulates mast cell proliferation

Interleukin-1

Interleukin- 1, also known as endogenous pyrogen, is related functionally to TNF and IL-6 in that all three ar important mediators of both local and general inflammatory response. Mononuclear phagocyte production of IL- 1 is stimulated by bacteria and other microorganisms, endotoxins, inflammatory agents such as bile salts and urate crystals, plant lectins, and various lymphokine. Interleukin- 1 may induce synthesis of phospholipases, that in turn cause release of arachidonic acid from membrane phospholipids. Increased concentrations of prostaglandin E develop in the hypothalamic thermoregulatory center, triggering febrile response. Antipyretic agents that inhibit the cyclooxygenase enzyme reduce fever by decreasing prostaglandin **E** synthesis in the hypothalamus.

During an inflammatory response, IL- 1, TNF, and IL- 6 all induce synthesis and release of hepatic acute-phase Protein. In addition, IL- 1 stimulates endothelial cell proliferation and induces procoagulant increases osteoclast-mediated bone resorption, and promotes release of proteases and prostaglandin E**,** from chondrocytes and interleukin- 1 plays an important role in the pathogenesis of inflammatory diseases such as rheumatoid arthritis, osteoarthritis and infectious purpura of ~hi1dren.lR~e cently, IL-I has been isolated from synovial joint fluid of horses with osteoarthritis.The apparent antiinflammatory effect of certain polyunsaturated fatty acids may be attributed in part to their inhibitory effect on the production.

One of the most important activities of IL- 1 is activation T lymphocytes during antigen presentation. Antigen-specific activation of T cells depends on two distinct but interrelated macrophage signa. The first signal requires the macrophage to internalize, process, and present the antigen on its cell membrane, bound to MHC. The second signal depends on macrophage production of IL- 1 (and IL-6). Interleukin- 1 has two effects on T lymphocytes: (1) interleukin- 1 stimulates synthesis and secretion of interleukin-2 (IL-2) by T cells, and (2) IL-1 also induces synthesis of IL-2 receptors, which in turn allow the T cell to respond to IL-2 by undergoing clonal expansion in addition to IL-2, activated T lymphocytes produce interleukin-3 (IL-3, multicolony- stimulating factor), interleukin-4 (IL-4, B-cell stimulating factor l), interleukin-5 (IL-5, B-cell growth factor) and interleukin-6 (IL-6, B-cell stimulating factor2).



Interleukin-2

Interleukin-2 (IL-2), first described as T-cell growth factor, is a 15-kD glycoprotein purification of interleukin- 2. Important functions of IL-2 is its ability to stimulate clonal expansion of activated T-lymphocyte. Receptors for interleukin-2 are present in extremely low concentrations on resting T –cells. Once activated by antigen or mitogens in combination with IL- 1, IL-2 receptor expression increases markedly. Interleukin-2 binding to newly expressed IL-2 receptors initiates T-cell proliferation, antibodies to interleukin-2 or its receptor, or exposure to agents that block expression of IL-2 receptors, prevent T-cell proliferation. Immunoregulating functions also attributed to interleukin-2 include promoting B-cell proliferation and immunoglobulin production enhanncing macrophage cytotoxicity and supporting growth of cytotoxic T lymphocytes (CTL), and

natural killer (NK) cells, Cytotoxic T lymphocytes and NK cells are capable of killing virus-infected cells and some tumor cells.

Interleukin-3

Interleukin-3 (IL-3), also known as multi-colony stimulating factor, is one of four major hemopoietic growth factors. The other major growth factors are macrophage colony stimulating factor (M-CSF), granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF) Bone marrow progenitor cells were identified by the generation of discrete populations of cells that grew in colonies. Factors necessary to ensure colony formation and growth were therefore referred to as colony stimulating Factors individuals bone marrow cell lineages appear to respond preferentially or exclusively to a particular growth factor, depending on their direction of differentiation. Interleukin-3 is a 28 kD glycoprotein and has been derived from antigen or mitogen-activated T-cells. *In vitro*, IL-3 supports development of pluripotential stem cells, leading to the production of macrophages, neutrophils, eosinophils, megakaryocytes, erythroid cells and mast ce11s.Recent study suggests that IL-3 may initiate proliferation of hemopoietic cells via activation of cellular protein kinase C.

Interleukin-4

Interleukin-4 is a 15-20 kD glycoprotein secreted by activated T lymphocytes, mast cells and bone marrow stromal cells. Originally IL-4 was believed to function solely as a growth factor for activated B cells and was therefore called B-cell stimulatory factor.lthough an important promotor of B cell growth and differentiation, IL-4 also enhances T-cell growth, myeloid colony formation, eosinophil growth and differentiation, mast cell growth, production of IgG, and IgE and class I1 MHC antigen expression.

Interleukin-5

Interleukin-5, (B-cell growth factor 2, T-cell replacing factor, and eosinophil differentiation factor) is a recently identified 40-50 kD glycoprotein produced by activated T lymphocyte. *In vitro***,** murine recombinant IL-5 augments B-cell growth and differentiation, participates in T cell activation, induces generation of cytotoxic T-lymphocytes and increases production of IgM, IgG,, and IgA by B cells in the presence of bacterial lipopolysacharide. Interleukin-5 receptors are expressed by murine myeloma cell lines, but not by T cell lines, mastocytoma cell lines or macrophage tumor cell lines.

Interleukin-6

Interleukin-6, also called B-cell stimulating factor 2, T cell activation factor, beta, interferon, and hepatocyte stimulating factor, is a 26 kD glycoprotein secreted by blood monocytes, activated T lymphocytes, tissue macrophages and fibroblast. Macrophages are the primary source of IL-6. During inflammatory processes, macrophage-derived IL-6 mediates the final maturation of proliferating B cells into specialized immunoglobulin secreting plasma cells and participates with IL-1 and TNF in the induction of hepatic acute-phase response interleukin-6 plays an important role in regulating antibody production. Tumor-related hypergammaglobulinemia may be due to excessive IL-6 production by neoplastic cells. Interleukin- 6 has recently been determined to be a potent myeloma- cell growth factor in most patients with multiple myeloma. High concentrations of IL-6 have been detected in peritoneal fluid of cats with feline infectious peritonitis. Thus, inhibition of IL-6 activity may prove useful for treatment of multiple myeloma and other hypergammaglobulinemic syndromes.

Interleukin-7

Interleukin-7 is a 25 kD protein that was originally identified in conditioned media from murine and human bone marrow stromal cells, based on its ability to maintain growth of B-lymphocyte progenitor cells derived from long-term bone marrow **cultures. Inte**rleukin- 7 is thought to play an important role in differentiation and proliferation of B-cell precursor and appears to be an absolute requisite for the bone marrow stromal cell-dependent process of B-cell development.

Interleukin-8

Interleukin-8, also known as monocyte-derived neutrophi1 chemotactic factor (MDNCF) and neutrophil attractant/ activation protein (NAP-1), a 10 kD glycoprotein, was originally isolated from human peripheral blood monocytes. The IL-8 plays a key role of IL-8 in inducing neutrophil migration and activation. Monocytes-derived IL-1 and TNF responsible for neutrophil chemotaxis during inflammation, IL- l and TNF stimulate macrophage release of IL-8, which may explain the ability of IL-1 and TNF to induce neutrophilic infiltration. The chemotactic activity of IL8 is specific for neutrophils and is distinct from monocyte chemotactic activity present in crude supernatants from endotoxin-stimulated macrophage.

Interleukin-9

Interleukin 9, a T cell-derived cytokine, was originally described in the mouse as a factor capable of sustaining growth of a selective number of T helper cell clone in human cell lines, IL-9 appears to interact primarily with erythroid progenitor cells. *In vitro,* IL-9 has been shown to selectively support the growth of erythroid colony formation. Recently, 1L-9 was shown to enhance IL-3 induced proliferation of bone marrow-derived mast cells. Thus, early evidence indicates that

IL-9 is a T lymphocyte derived cytokine that may selectively stimulate erythroid and mast cell development in the hematopoetic system.

Interleukin-10

Immune responses to strong immunogens generally consist of either delayed type hypersensitivity (DTH) or antibody production and the two responses appear to be mutually exclusive. Recent evidence from studies in mice indicate that two functionally different subsets of T helper lymphocytes (Th), Thl and Th2, mediate these distinct immune responses. Activated Th 1 lymphocytes, which produce gamma-interferon, lymphotoxin, and IL- **2,** are most effective at stimulating cell-mediated immunity (and DTH).

Immunomodulation is emerging as an important therapeutic option in medicine today. Cytokine research has increased our understanding of immune system function and interrelatedness of various interleukins. As the biological functions of interleukins, and their roles in normal and abnormal physiology are determined, therapeutic
intervention will become a reality.

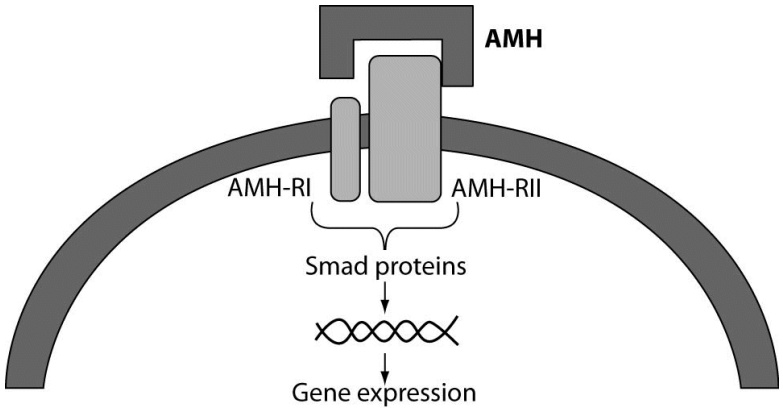
8. i) **ANTI-MULLERIAN HORMONE**

Anti-Müllerian hormone (AMH) is a 140-kDa dimeric glycoprotein hormone and belongs to the transforming growth factor-β (TGF-β) family. AMH is synthesized as a large precursor with a short signal sequence followed by the pre-prohormone that forms homodimers. Prior to secretion, the mature hormone undergoes glycosylation and dimerization to produce a 144-kDa dimer composed of identical disulphide-linked 72-kDa monomer subunits. Each monomer contains an N-terminal domain and a C-terminal domain; it is believed that N-terminal domain accentuates the activity of the C-terminal domain in which resides the bioactivity of the molecule. During cytoplasmic transit, between 5 and 20% of AMH is cleaved at a specific site between the N-terminal and the C-terminal domain of the 72-kDa monomer, to form two polypeptides of 58-kDa (pro region) and 12-kDa (mature region). These two parts of the molecule remain in non-covalent attachment. The human gene coding for AMH is located on the short arm of chromosome 19.

AMH signaling pathway

Target organs for AMH in males are Müllerian ducts and, in both sexes, gonads. As a member of the TGFβ family of growth factors, it is thought that AMH uses the signal transduction system that has been identified for the other factors of the family, notably TGFβ itself, activin and the bone morphogenetic proteins (BMPs). These factors signal through a serine–threonine kinase receptor complex consisting of ligand-specific type II receptors and more general type I receptors,

also known as activin receptor-like protein kinases (ALKs). An activated receptor complex phosphorylates and activates cytoplasmic Smad proteins that translocate to the nucleus and directly or indirectly affect gene expression (Figure 1). For AMH one type II receptor has been identified (AMHRII) and shown to be specific and necessary for AMH signaling. The gene coding for AMH receptor is located on the long arm of chromosome 12. The *AMHRII* gene is specifically expressed in the gonads and in the mesenchymal cells adjacent to the Müllerian ducts. Besides the exclusive AMHRII, three candidate AMH type I receptors have been identified to be involved in AMH-induced Müllerian duct regression. However, the relative contribution of these three type I receptors to AMH signaling in the ovary remains to be determined.



FUNCTIONS OF AMH

Prenatal stage

AMH plays fundamental role in fetal sex differentiation.

From birth to puberty

Serum AMH in male infants reflects the function of testes reliably and can also be used in cryptorchidic males as the initial laboratory test to assess the presence of the testes.

In girls, AMH circulating values are almost undetectable at birth with a slight increase within the first years of age, prior to puberty.

Puberty and adulthood

In boys, the inhibitory effect of testosterone prevails over FSH stimulation, resulting in down-regulation of AMH expression and the circulating levels rapidly decrease. The secretion of AMH reaches values in adult males and is maintained almost constant until the rest of men's life.

In girls, AMH is produced by ovaries and secreted into the bloodstream. It is first expressed in granulosa cells of the recruited primordial follicles and continues to be expressed in the growing follicles in the ovaries until they have reached the size of about 4-6 mm. At that differentiation state, usually one follicle is selected for dominance and its further growth is controlled by action of pituitary FSH.

Physiology and pathophysiology of AMH in males

- Anti-Müllerian hormone is the earliest Sertoli cell hormone secreted in males and, together with inhibin B and FSH, is an important indicator of Sertoli cell function.
- The role of AMH in ovarian reserve evaluation
- AMH as indicator of ovarian aging throughout reproductive life
- AMH as a marker of premature ovarian senescence
- AMH as a predictor of success in assisted reproduction techniques (ART)
- AMH as a marker of ovarian pathophysiology

ii) Activin

Activin was discovered in the 1980s as a gonadal protein that stimulated FSH release from pituitary gonadotropes and was thought of as a reproductive hormone. The **activin** protein complexes are both dimeric in structure. In each complex, the two monomers are linked to one another by a single disulfide bond. Both complexes are derived from the same family of related genes and proteins but differ in their subunit composition. Below is a list of the most common activin complexes and their subunit composition:

Activin---stimulates FSH secretion-----Activin A,

Activin AB,

Activin B

The alpha and beta subunits share approximately 25% sequence similarity, whereas the similarity between beta subunits is approximately 65%. In mammals, four beta subunits have been described, called activin β_A, activin β_B, activin β_C and activin β_E. Activin β_A and β_B are identical to the two beta subunits of inhibin. A fifth subunit, activin β_D, has been described in *Xenopus laevis*. Two activin β_A subunits give rise to activin A, one β_A, and one β_B subunit gives rise to activin AB,

and so on. Various, but not all theoretically possible, heterodimers have been described. The subunits are linked by a single covalent disulfide bond.

The β_C subunit is able to form activin heterodimers with β_A or β_B subunits but is unable to dimerize with inhibin α.

Activin is produced in the gonads, pituitary gland, placenta, and other organs:

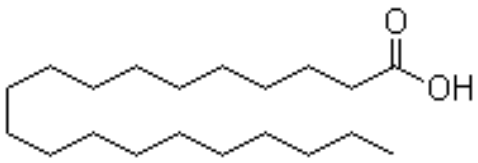
- In the ovarian follicle, activin increases FSH binding and FSH-induced aromatization. It participates in androgen synthesis enhancing LH action in the ovary and testis. In the male, activin enhances spermatogenesis.

- Activin is strongly expressed in wounded skin and overexpression of activin in epidermis of transgenic mice improves wound healing and enhances scar formation. Its action in wound repair and skin morphogenesis is through stimulation of and stromal cells in a dose-dependent manner.

- Activin also regulates the morphogenesis of branching organs such as the prostate, lung and especially kidney. Activin A increased the expression level of type-I collagen suggesting that activin A acts as a potent activator of fibroblasts

- Lack of activin during development results in neural developmental defects.

iii) Eicosanoids are a large group of autocoids with potent effects on virtually every tissue in the body. these agents are derived from metabolism of 20-carbon, unsaturated fatty acids (eicosanoic acids). The eicosanoids all have short plasma half-lives (typically 0.5—5 min). Most catabolism occurs in the lung. Metabolites are excreted in the urine.



The eicosanoids include:

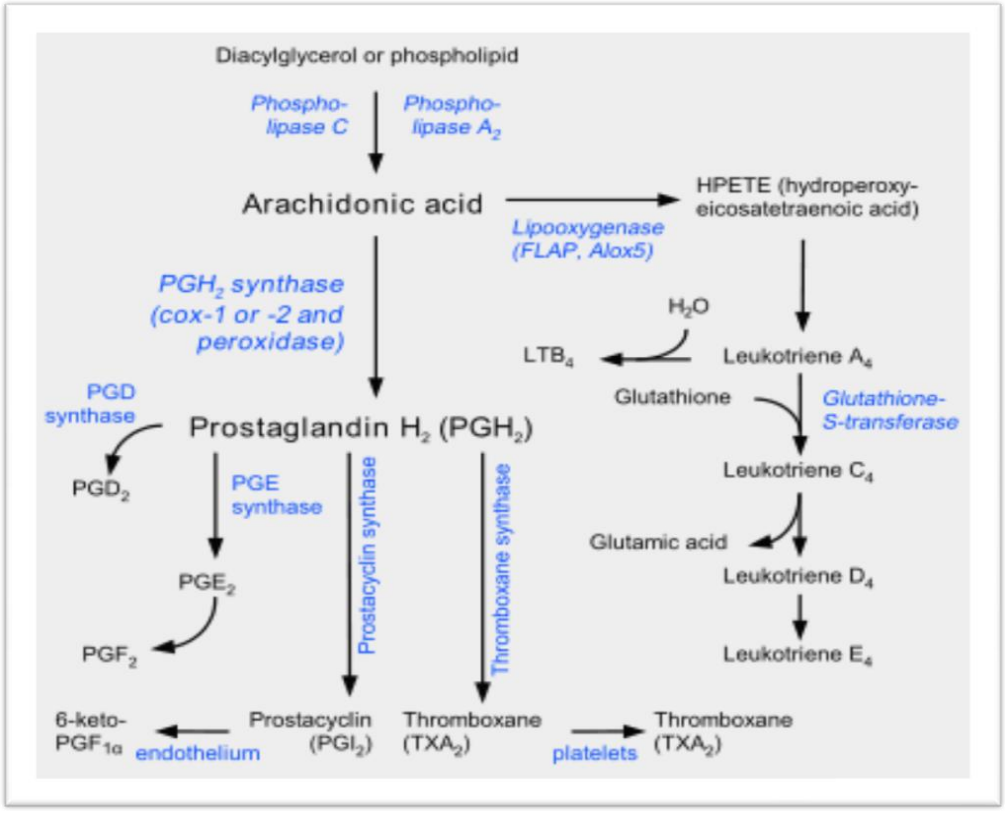
1. the prostaglandins
2. thromboxanes
3. leukotrienes

4. hydroperoxyeicosatetraenoic acids **(HPETEs)**
5. hydroxyeicosatetraenoic acids **(HETEs).**

Arachidonic acid, the most common precursor of the eicosanoids, is formed by two pathways. **Eicosanoids are synthesized by two pathways:The prostaglandin H synthase (COX, cyclooxygenase) pathway produces:**

A. thromboxane
B. the primary prostaglandins
C. prostacyclin (PGI_2)

T**hromboxane** is a member of the family of lipids known as eicosanoids. The two major thromboxanes are thromboxane A2 and thromboxane B2. The distinguishing feature of thromboxanes is a 6-membered ether-containing ring. Thromboxane is named for its role in clot formation (thrombosis).



Thromboxane synthesis occurs primarily in platelets. **Thromboxane A_2 (TXA_2)** is rapidly hydrated to the less active TXB_2.

Function of Thromboxane:

- Thromboxane is a vasoconstrictor and a potent hypertensive agent, and it facilitates platelet aggregation.
- It is in homeostatic balance in the circulatory system with prostacyclin, a related compound. The mechanism of secretion of thromboxanes from platelets is still unclear.
- They act in the formation of blood clots and reduce blood flow to the site of a clot.
- Thromboxane A_2 (TXA_2), produced by activated platelets, has prothrombotic properties, stimulating activation of new platelets as well as increasing platelet aggregation.
- Platelet aggregation is achieved by mediating expression of the glycoprotein complex GP IIb/IIIa in the cell membrane of platelets. Circulating fibrinogen binds these receptors on adjacent platelets, further strengthening the clot.