

Model answer

M.Sc. Semester-III

Paper LZT 303(A): Neuroendocrinology and Non-classical Hormones

Q 1

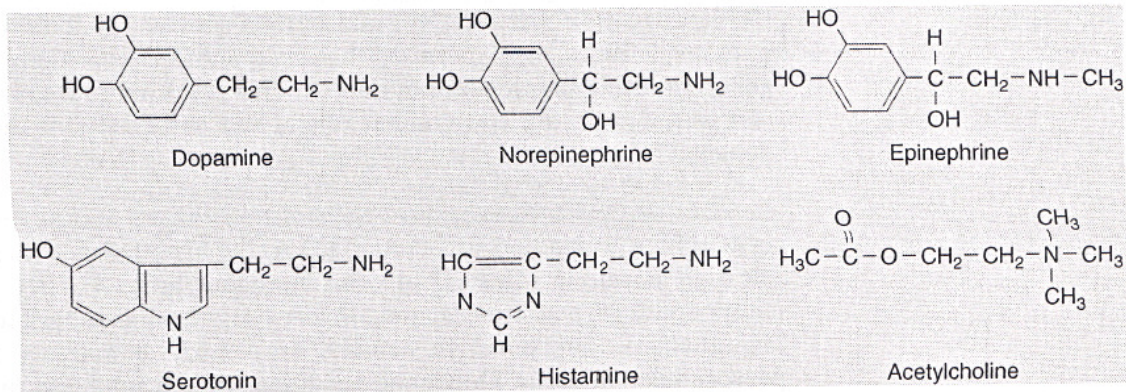
Answer

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|--------|---------|----------|--------|-------|
| (i) c | (ii) a | (iii) a | (iv) a | (v) c |
| (vi) c | (vii) a | (viii) a | (ix) b | (x) b |

Q 2

Answer

Pituitary responses are regulated by hypophysiotropic hormones. Brain is mainly composed mainly of neurons and supporting elements, other neurons within the brain are expected to regulate the hypophysiotropin-secreting neurons. These other neurons, in turn, are linked to yet other neuronal inputs such as sensory neurons that are receptive to endogenous (intrinsic) and exogenous (extrinsic) cues. Intrinsic and extrinsic stimuli received through sensory neurons are conducted through neuronal routes to the brain where this information may be inhibitory or stimulatory to hypophysiotropic hormone secretion. Conduction of sensory information is via neuronal elements, and each nerve must release a neurotransmitter to effect synaptic transmission. These neurohormones include the monoamine neurotransmitters and the amino acid neurotransmitter. There are well-defined aminergic (monoamine) pathways within the brain that are composed of serotonergic, dopaminergic, noradrenergic, and even epinephrine-containing neurons.



The monoamine neurotransmitters

Specific neurotransmitters regulate hypophysiotropin secretions

Control of CRH secretion

- A number of neurotransmitter are involved.
- Stress is a potent stimulus to ACTH secretion.
- One or more cholinergic pathways are involved.
- Stimulation of CNS cholinergic structures provokes ACTH release.
- Catecholamines induce release of both CRH and AVP.

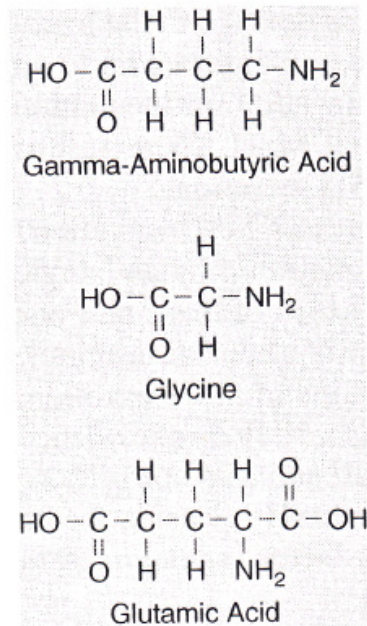
Control of PIF secretion

- Suckling is normal stimulus to PRL secretion.
- This involves the inhibition of dopaminergic neurons.
- A number of neurotransmitters are involved in control of PRL secretion.

- Nocturnal rise in PRL secretion involves activation of serotonergic neurons.
- GABA may also control PRL secretion.

Control of growth hormone secretion

- A variety of stimuli elevate GH secretion, through inhibition of somatostatin secretion or by an enhancement of GHRH secretion.
- Finding of SST receptors on GHRH-containing arcuate neurons signifies the concept of direct “cross-talk” between SST and GHRH neuronal system.
- This interaction may be a vital component in generation and maintenance of the ultradian rhythm of GH secretion.



The amino acid neurotransmitters

Control of GnRH secretion

- Several transmitters and neuropeptides participates in regulation of gonadotropin

secretion.

- At the level of hypothalamus, control of GnRH involves norepinephrine, GABA, glutamate, angiotensin II, neuropeptide T, neurotensin, and 5-hydroxytryptamine, as well as interleukins 1 and 2.
- Dopaminergic neurons are clearly stimulatory to GnRH release.
- Dopamine secretion itself is inhibited by enkephalinergic neurons.

Control of TRH secretion

- Apparently noradrenergic neurons stimulate TSH secretion by a stimulatory action on TRH-secreting neurons.
- Glucocorticoids excess inhibits thyroid function at a suprapituitary level.
- A complex array of other factors influence TRH production.

Q 3

Answer

- **Thyrotropin releasing hormone**

It is found in hypothalamus and cause release of TSH. The common occurrence of TRH outside the hypothalamus and its presence in extrahypothalamic regions of the nervous system of mammals, nonmammalian vertebrates and even invertebrates has led to the suggestion that TRH may also function as a neurotransmitter. The biological half life of TRH in blood is very short, apparently because peptidases in the blood rapidly inactivate TRH. TRH consists of simple peptide composed of three amino acids: glutamic acid, histidine and proline in equimolar ratios. The mode of TRH biosynthesis in the mammalian brain is by posttranslational cleavage of a larger precursor protein.

TRH also stimulates the release of PRL in human, cattle, sheep and rats. TRH stimulates

GH secretion in cattle and rats under certain specific conditions and in humans, with acromegaly or chronic renal insufficiency. The ability of TRH to stimulate TSH release, apparently, appears to be restricted to the homeotherms only. Thus, the ability of Thyrotropes to respond to TSH may be a recent evolutionary acquisition relative to that of the lactotropes, and that the TSH-releasing activity of the tripeptide may have emerged coincident with homeothermy. The thyrotropin-releasing hormone receptor (TRHR) is a G protein-coupled receptor which binds the tripeptide thyrotropin releasing hormone. The TRHR are found in the brain and when bound by TRH result act through phospholipase C to increase intracellular inositol trisphosphate.

- **Growth hormone releasing hormone**

Growth-hormone-releasing hormone (GHRH), also known as growth-hormone-releasing factor (GRF, GHRF) is a releasing hormone for growth hormone. It is a 44-amino acid peptide hormone produced in the arcuate nucleus of the hypothalamus.

GHRH first appears in the human hypothalamus between 18 and 29 weeks of gestation, which corresponds to the start of production of growth hormone and other somatotropes in fetuses.

Origin

GHRH is released from neurosecretory nerve terminals of these arcuate neurons, and is carried by the hypothalamo-hypophyseal portal system to the anterior pituitary gland, where it stimulates growth hormone (GH) secretion by stimulating the growth hormone-releasing hormone receptor. GHRH is released in a pulsatile manner, stimulating similar pulsatile release of GH. In addition, GHRH also promotes slow-wave sleep directly. Growth hormone is required for normal postnatal growth, bone growth, regulatory effects on protein, carbohydrate, and lipid metabolism.

Effect

GHRH stimulates GH production and release by binding to the GHRH Receptor (GHRHR) on cells in the anterior pituitary.

Receptor

The GHRHR is a member of the secretin family of G protein-coupled receptors. This protein is transmembranous with seven folds, and its molecular weight is approximately 44 kD.

Signal transduction

GHRH binding to GHRHR results in increased GH production mainly by the cAMP-dependent pathway, but also by the phospholipase C pathway (IP₃/DAG pathway), and other minor pathways.

The cAMP-dependent pathway is initiated by the binding of GHRH to its receptor, causing receptor conformation that activates G_s alpha subunit of the closely associated G-Protein complex on the intracellular side. This results in stimulation of membrane-bound adenylyl cyclase and increased intracellular cyclic adenosine monophosphate (cAMP). cAMP binds to and activates the regulatory subunits of protein kinase A (PKA), allowing the free catalytic subunits to translocate to the nucleus and phosphorylate the transcription factor cAMP response element-binding protein (CREB). Phosphorylated CREB, together with its coactivators, p300 and CREB-binding protein (CBP) enhances the transcription of GH by binding to CREs cAMP-response elements in the promoter region of the GH gene. It also increases transcription of the GHRHR gene, providing positive feedback.

In the phospholipase C pathway, GHRH stimulates phospholipase C (PLC) through the βγ-complex of heterotrimeric G-proteins. PLC activation produces both diacylglycerol (DAG) and inositol triphosphate (IP₃), the latter leading to release of intracellular Ca²⁺ from the endoplasmic reticulum, increasing cytosolic Ca²⁺ concentration, resulting in vesicle fusion and release of secretory vesicles containing premade growth hormone.

Some Ca^{2+} influx is also a direct action of cAMP, which is distinct from the usual *cAMP-dependent pathway* of activating *protein kinase A*.

Activation of GHRHRs by GHRH also conveys opening of Na^+ channels by phosphatidylinositol 4,5-bisphosphate, causing cell depolarization. The resultant change in the intracellular voltage opens a voltage-dependent calcium channel, resulting in vesicle fusion and release of GH.

- **Somatostatin**

Somatostatin (also known as growth hormone-inhibiting hormone (GHIH) or somatotropin release-inhibiting factor (SRIF)) or somatotropin release-inhibiting hormone is a peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G protein-coupled somatostatin receptors and inhibition of the release of numerous secondary hormones. Somatostatin regulates insulin and glucagon.

Somatostatin has two active forms produced by alternative cleavage of a single preproprotein: one of 14 amino acids, the other of 28 amino acids.

In all vertebrates, there exist six different somatostatin genes that have been named SS1, SS2, SS3, SS4, SS5, and SS6. The six different genes along with the five different somatostatin receptors allows somatostatin to possess a large range of functions. Humans have only one somatostatin gene, SST.

Production

Digestive system

Somatostatin is secreted in several locations in the digestive system:

- stomach
- intestine
- delta cells of the pancreas

Somatostatin will travel through the portal blood system, to the heart, then to systemic

circulation, where it will exert its digestive system effects. In the stomach, somatostatin acts on the acid-producing parietal cells via G-coupled receptor to reduce secretion. Somatostatin also indirectly decreases stomach acid production by preventing the release of other hormones, including gastrin, secretin and histamine which effectively slows down the digestive process.

Brain

Somatostatin is produced by neuroendocrine neurons of the ventro medial nucleus of the hypothalamus. These neurons project to the median eminence, where somatostatin is released from neurosecretory nerve endings into the hypothalamo-hypophysial system through neuron axons. Somatostatin is then carried to the anterior pituitary gland, where it inhibits the secretion of growth hormone from somatotrope cells. The somatostatin neurons in the periventricular nucleus mediate negative feedback effects of growth hormone on its own release; the somatostatin neurons respond to high circulating concentrations of growth hormone and somatomedins by increasing the release of somatostatin, so reducing the rate of secretion of growth hormone.

Somatostatin is also produced by several other populations that project centrally, i.e., to other areas of the brain, and somatostatin receptors are expressed at many different sites in the brain. In particular, there are populations of somatostatin neurons in the arcuate nucleus, the hippocampus, and the brainstem nucleus of the solitary tract

Somatostatin is classified as an inhibitory hormone, whose actions are spread to different parts of the body:

Anterior pituitary

In the anterior pituitary gland, the effects of somatostatin are:

- Inhibit the release of growth hormone (GH) (thus opposing the effects of Growth Hormone-Releasing Hormone(GHRH))
- Inhibit the release of thyroid-stimulating hormone (TSH)
- It is induced by low pH.
- Inhibit adenylyl cyclase in parietal cells.
- Inhibits the release of prolactin (PRL)

Gastrointestinal system

- Somatostatin is homologous with cortistatin and suppresses the release of gastrointestinal hormones
 - Gastrin
 - Cholecystokinin (CCK)
 - Secretin
 - Motilin
 - Vasoactive intestinal peptide (VIP)
 - Gastric inhibitory polypeptide (GIP)
 - Enteroglucagon
- Decrease rate of gastric emptying, and reduces smooth muscle contractions and blood flow within the intestine
- Suppresses the release of pancreatic hormones
 - Inhibits insulin release when somatostatin is released from delta cells of pancreas
 - Inhibits the release of glucagon
- Suppresses the exocrine secretory action of pancreas.

Q 4

Answer

Growth hormone (GH), also known as **somatotropin** or **somatropin**, is a peptide hormone that stimulates growth, cell reproduction and regeneration in humans and other animals. It is a type of mitogen which is specific only to certain kinds of cells. Growth hormone is a 191-amino acid, single-chain polypeptide that is synthesized, stored, and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland.

Effects of growth hormone on the tissues of the body can generally be described as anabolic (building up). Like most other protein hormones, GH acts by interacting with a specific receptor on the surface of cells.

Increased height during childhood is the most widely known effect of GH. Height appears to be stimulated by at least two mechanisms:

- Because polypeptide hormones are not fat-soluble, they cannot penetrate cell membranes. Thus, GH exerts some of its effects by binding to receptors on target cells, where it activates the MAPK/ERK pathway. Through this mechanism GH directly stimulates division and multiplication of chondrocytes of cartilage.
- GH also stimulates, through the JAK-STAT signaling pathway, the production of insulin-like growth factor 1 (IGF-1, formerly known as somatomedin), a hormone homologous to proinsulin. The liver is a major target organ of GH for this process and is the principal site of IGF-1 production. IGF-1 has growth-stimulating effects on a wide variety of tissues. Additional IGF-1 is generated within target tissues, making it what appears to be both an endocrine and an autocrine/paracrine hormone. IGF-1 also has stimulatory effects on osteoblast and chondrocyte activity to promote bone growth.

In addition to increasing height in children and adolescents, growth hormone has many other effects on the body:

- Increases calcium retention, and strengthens and increases the mineralization of bone
- Increases muscle mass through sarcomere hypertrophy
- Promotes lipolysis
- Increases protein synthesis
- Stimulates the growth of all internal organs excluding the brain
- Plays a role in homeostasis
- Reduces liver uptake of glucose
- Promotes gluconeogenesis in the liver
- Contributes to the maintenance and function of pancreatic islets
- Stimulates the immune system

- Increases deiodination of T4 to T3

Growth hormone and Somatomedins

Growth Hormones and Somatomedins

- The role of pituitary GH in control of growth is well established.
- GH causes growth of epiphyseal regions of long bones
- Growth of bone can be monitored by measuring the incorporation of sulfur (^{35}S) into epiphyseal cartilage.
- Experiments conducted showed that GH added to serum from hypophysectomized animals did not ~~help~~ ^{supply} addition of sulfur into bone.
- The conclusion was drawn that GH acts indirectly on bones by way of the production of a sulfation factor.
- The sulfation factor is now known to consist of several peptides ~~seen~~ referred to as somatomedins.
- Injected radiolabeled GH rapidly localizes to the liver rather than to the epiphysis of long bones.
- Somatomedins is generally used to refer to those growth factors found in plasma that are under control of GH, have insulin-like properties and promote the incorporation of sulfate into cartilage.
- Two substances isolated from plasma fulfil these criteria: IGF-I and IGF-II
- These peptides bear some structural relationship to proinsulin and, therefore, exhibit some affinity for insulin receptor.

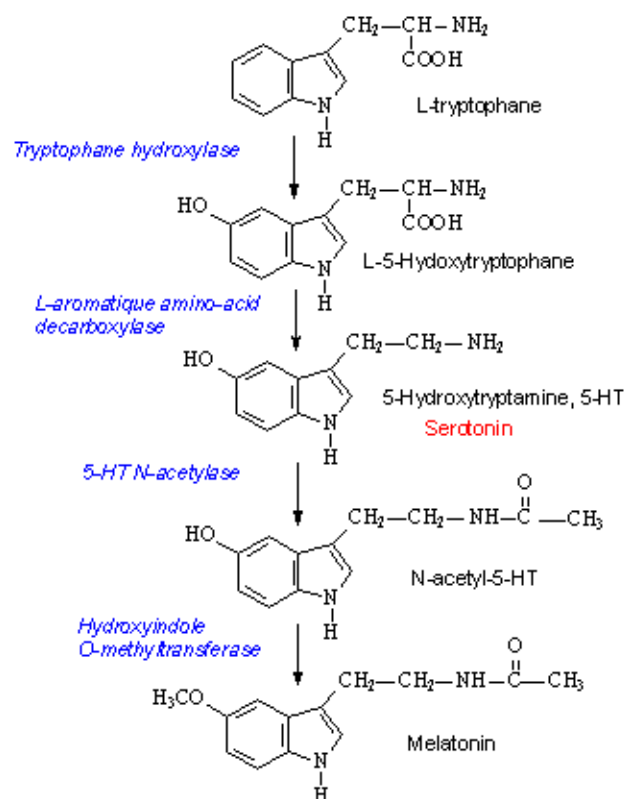
- IGFs are secreted by liver and by some other tissues in response to GH stimulation.
- Growth activity present in blood can not be neutralized by antibodies against insulin.
- IGF-I and IGF-II consists of 70 and 67 amino acid residues respectively.
- IGF-II is a three times more abundant in adult circulation than IGF-I.
- Insulin is more potent in stimulating metabolic effects in insulin target tissues than are IGF-I and IGF-II.
- On the other hand, insulin is a less potent stimulator of cell proliferation than these growth factors.
- From an evolutionary point of view, one precursor hormone of insulin and IGF-I must have existed that, millions of years ago, was responsible for both the acute regulation of metabolism and the growth.
- Later, a gene duplication must have taken place leading to the diversion of these two functions.
- Two receptors for IGFs are known.
- IGF-I receptors exhibits ligand dependent tyrosine kinase activity and autophosphorylation, causing phosphorylation of IRS which then activates PKB and MAPK pathways.
- IGF-I receptor has considerable structural and functional similarity to insulin receptor.
- The IGF-II receptor, in contrast, has a different structure. It has a higher affinity for IGF-II than IGF-I and does

Q 5

Answer

Transformation of tryptophan into serotonin involves two steps:

- Hydroxylation in 5-hydroxytryptophan catalyzed by tryptophan hydroxylase, which is the rate limiting enzyme of the synthesis. This enzyme requires for its activity the presence of tetrahydrobiopterine, oxygen, NADPH₂ and a metal, iron or copper.
- Decarboxylation of 5-hydroxytryptophan is catalyzed by L-aromatic amino acid decarboxylase with pyridoxal-phosphate as coenzyme.



In the brain, serotonin biosynthesis depends on the quantity of tryptophan which crosses the blood-brain barrier. Only free plasma tryptophan, i.e. unbound to albumin, penetrates into the brain; decrease of its free ratio reduces its penetration. Moreover, other amino acids are in competition with free tryptophan and limit its entry in the brain. Plasma cortisol, whose level is increased in depressed patients, decreases free L-tyrosine and free L-tryptophan concentrations in plasma, i.e. the forms which penetrate into the brain. Insulin, of which secretion is increased by

carbohydrates, has an opposite effect and decreases the concentration of the amino acids other than tryptophan.

Transformation of serotonin into melatonin, which should not be regarded as a degradation pathway because melatonin is also active, is carried out primarily in the pineal gland. It involves two steps:

- Acetylation of the amine group by N-acetyl transferase leading to N-acetyl-serotonin.
- Methylation of the OH group by 5- hydroxyindole-O-methyltransferase catalyzing the transfer of a methyl group from S-adenosyl-methionine to obtain acetyl-5-methoxytryptamine or melatonin.

The concentration of melatonin in the pineal gland presents circadian variations: it follows the variations of N-acetyl transferase activity, increasing during the night and decreasing during the day, darkness and light playing a regulatory role via catecholamines. Light inhibits melatonin biosynthesis.

Melatonin function

Melatonin secretion is related to the duration of darkness. The main function of melatonin is to mediate dark signals, with possible implications in the control of circadian rhythmicity and seasonality. The melatonin message, which is generated at night, is differently read in nocturnal animals and humans. In that sense, melatonin does not appear as the universal hormone of sleep. The role of melatonin for the seasonal changes in physiology and behaviour of various photoperiodic species has been extensively documented. For a long time, humans were claimed to be poorly sensitive to photoperiod variations, as no difference between the summer and winter melatonin duration was found in temperate zones. Studies conducted under appropriate natural or controlled laboratory conditions show that humans also exhibit changes in the daily profile of melatonin. It is proposed that the circadian pacemaker consists of two component oscillators. One is entrained to dusk and controls the onset of melatonin secretion, the other is entrained to dawn and controls the offset. The dusk and dawn entrained components of the circadian

pacemaker could be considered to control evening and morning transitions in melatonin secretion and to adjust the timing of these transitions in seasonal changes in day length.

Melatonin, the endogenous synchroniser

The time of melatonin secretion adjusts to the light/dark cycle. A general opinion is that melatonin, by providing the organism with the night information, could be an endogenous synchronizer able to stabilize circadian rhythms, to reinforce them and to maintain their mutual phase-relationship.

Antioxidant activity

Melatonin is a potent free radical scavenger. Melatonin directly scavenges the highly toxic hydroxyl radical and other oxygen centered radicals. Also, melatonin displays antioxidative properties: it increases the levels of several antioxidative enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase. On the other hand, melatonin inhibits the pro-oxidative enzyme nitric oxide synthase. Since considerable experimental evidence supports the idea that oxidative stress is a significant component of specific brain diseases, the ability of melatonin to protect against neurodegeneration has been tested in a multitude of models.

Immunity

Currently accumulated evidence shows that the pineal is able to play an important role in modulating the immune response. Melatonin can interact with specific membrane binding sites in cells from lymphoid organs. In addition, interactions between the pineal gland and the immune system are bidirectional since interleukins and cytokines affect melatonin synthesis and release.

Q 6

Answer

Neurotrophic Growth Factors -

Several peptides growth factors regulate the differentiation and growth of both the central and peripheral nervous systems.

NGF promotes survival of nervous system -

- Growth and differentiation of sensory and motor components of the peripheral nervous system are enhanced by factors released from peripheral target tissues.
- Certain mouse sarcoma induce hypertrophy of chick embryonic sensory and sympathetic ganglia.
- This neuronal hypertrophy depends on a humoral protein factor released by the transplanted tumors.
- This substance was named nerve growth factor (NGF).
- NGF is also present in snake venom and in submandibular (submaxillary) salivary glands of adult male mice.
- NGF is synthesized in tubular cells of submandibular gland and its production is androgen dependent.
- Salivary gland NGF levels in female mice rise during pregnancy and lactation when androgen levels are elevated.
- Testosterone given to female mice causes tubular cell hypertrophy and NGF rises markedly. In contrast, castration of male mice causes tubular atrophy and the salivary gland content of NGF falls dramatically.
- The findings that salivary gland synthesize NGF, that testosterone and thyroxine induce NGF synthesis and that NGF is ten fold higher in male were puzzling and unexplained.

- An alternative biological function for salivary NGF has now been hypothesized. Intraspecific fight in rodents results in massive NGF release into bloodstream.
- Since injections of NGF induce weight and size increase of adrenal glands and stimulates synthesis of tyrosine hydroxylase, it was suggested that salivary NGF is involved in defence and/or offence mechanisms.
- Peripheral sympathetic postganglionic neurons of the autonomic nervous system are the primary target cells for NGF.
- Injections of NGF antiserum in newborn mice or chick embryos cause almost complete destruction of sympathetic nervous system (immuno-sympathectomy).
- NGF antiserum administration to adult animals causes deleterious effects on sympathetic neurons that are reversible after antiserum treatment is stopped.
- Thus, it appears that an endogenous NGF is critical for sympathetic nervous system development, and NGF may function in the maintenance of sympathetic neurons throughout life.

Structure -

- Mouse salivary gland NGF is a molecular complex consisting of three types of polypeptide chains, designated α , β and γ .
- The β subunit is held together by noncovalent bonds.
- Only the β subunit possesses nerve growth promoting activity.
- NGF is synthesized as a precursor peptide, a proNGF.
- The individual β chains of NGF are then cleaved by the γ subunit, which is an endopeptidase.
- The α subunit may play a role in maintaining the functional integrity of NGF in the secretory granules.
- There is homology between NGF and proinsulin and there are also striking similarities between biological activities of two molecules. Both hormones bind initially with cell membrane, which results

- NGF is viewed as a hormone whose structural gene may have evolved from an ancestral proinsulin gene and whose mode of action on neurons might, therefore, be somewhat similar to that of insulin on its target tissues.
- To a limited extent, insulin and proinsulin can compete for the binding of NGF to its receptor.
- After an initial interaction with plasma membrane, NGF is internalized and, by retrograde axonal transport, carried to soma of cell.
- Binding of NGF is specific to target tissue surface receptor because motor neurons that can internalize and transport tetanus toxin fail to do so with NGF.
- Similarly, cholinergic parasympathetic nerves, which like motor neurons are refractory to the biological actions of NGF, do not transport NGF.
- NGF exerts a pleiotropic effect on nerve cells, which includes-
 - 1) Synthesis of tyrosine hydroxylase and dopamine β -hydroxylase.
 - 2) Stimulation of nerve fibre outgrowth
 - 3) Increase in activity of ornithine decarboxylase, a key enzyme in synthesis of polyamines in brain
 - 4) Functions in survival of peripheral sympathetic and spinal sensory neurons.
 - 5) It directs growing sympathetic nerve fibres towards their corresponding target tissues.

- There is hope that deterioration of cholinergic neurons in the brains of Alzheimer's patients might be ~~per~~ prevented or retarded by treatment with NGF, related growth factors, or drugs that mimic growth factor activity in the brain.

The NGF Family and its Receptors -

The gene family of neurotrophins includes -

- 1) Nerve Growth Factor (NGF)
- 2) Brain-derived neurotrophic factor (BDNF)
- 3) Neurotrophin-3 (NT-3)
- 4) Neurotrophin-4 (NT-4)

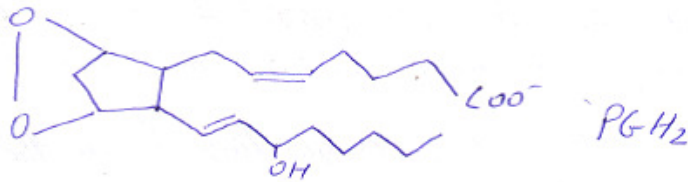
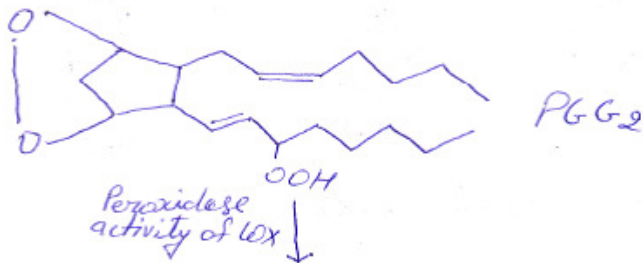
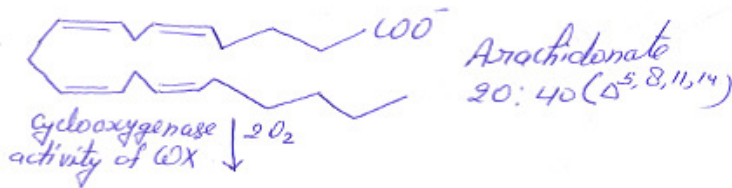
- Recently, neurotrophin-5 (NT-5), a possible mammalian homologue to NT-4 has been cloned in human, and rats.
- These factors regulate neuron survival, extent of their innervation to target tissues and their differentiation.
- These trophic factors are synthesized as precursor polypeptides that are subsequently cleaved to yield the mature neurotrophin.
- A 140-K tyrosine protein kinase encoded by proto-oncogene *trk* has been found to bind with high affinity and to evoke the cellular neurotrophic responses.
- Another protein encoded by *trk*-related gene *trk B* has been shown to bind BDNF.
- Recently, a third member of the *trk* family, *trk C*, has been cloned which binds with NT-3.

Q 7**Answer**

Eicosanoids are formed from 20-carbon polyunsaturated fatty acids. They act as short range messengers, affecting tissues near the cells that produce them. In response to hormone or other stimuli, phospholipase A₂ attacks membrane phospholipids releasing arachidonate 20:4. Enzymes of smooth ER then convert arachidonate into prostaglandins, beginning with the formation of PGH₂, the immediate precursor of many other prostaglandins and thromboxanes.

Phospholipid containing
arachidonate

Phospholipase A₂ ↓ → Lysophospholipid



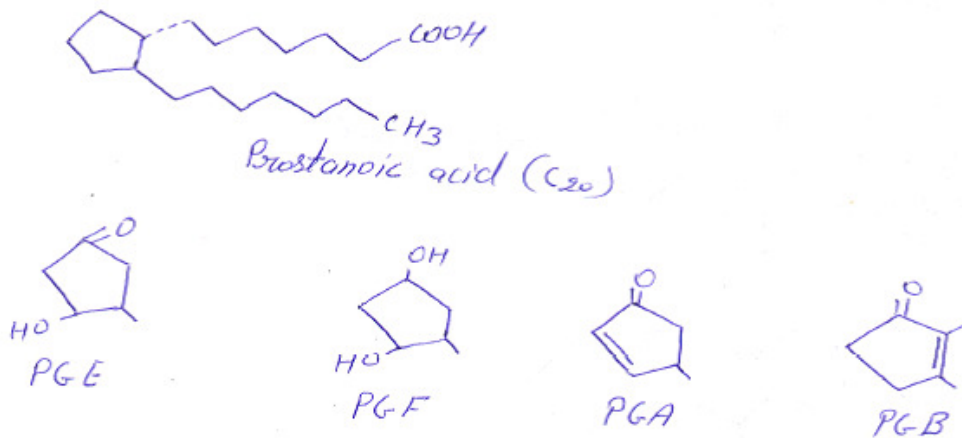
other prostaglandins Thromboxanes

Pathway from Arachidonate to prostaglandins and thromboxanes

Prostaglandins -

- Prostaglandins have been named based upon their source in man, the prostate gland.
- Over 16 prostaglandins have been elucidated.
- They all are related to the basic structure of prostanoic acid, and they can be separated into four classes (E).

- The most commonly occurring PGs are PGE₁, PGE₂ and PGF_{2α}.



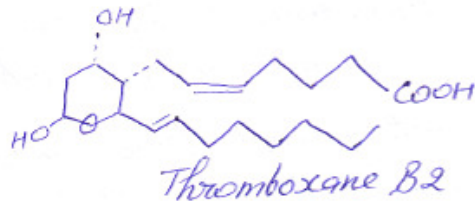
Structures of Prostaglandins

- PGs are not restricted to male genital tract, as they have been found in most tissues in both males and females.
- They contain a five-carbon ring originating from the chain of arachidonic acid.
- Two groups of prostaglandins were originally defined: PGE, for ether-soluble, and PGF, for phosphate buffer soluble.
- PGs act in many tissues by regulating the synthesis of cAMP.
- Some PGs stimulate contraction of smooth muscle of the uterus during menstruation and labor, while others affect blood flow to specific organs, the sleep-wake cycle, and responsiveness of certain tissues to hormones such as epinephrine and glucagon.
- Third group of PGs elevate body temperature and cause inflammation and pain.

- PGs also reduce progesterone synthesis by corpus luteum, induce ovulation and lactation in rat and may be involved in labor.
- PGs may be involved with inflammatory responses.

Thromboxane

- Thromboxane synthase present in platelets converts PGH_2 into thromboxane A_2 , from which other thromboxanes are derived.
- Thromboxanes induce constriction of blood vessels and platelet aggregation.
- They have a six-membered ring containing ether and act in the formation of blood clots and reduction of blood flow to the site of clot.



- The Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), aspirin, ibuprofen, acetaminophen, and meclizolam, for example have been shown to inhibit enzyme prostaglandin synthase which catalyzes early step in the pathway of from arachidonic to PGs and thromboxanes.

Q 8

Answer

Tumor Necrosis Factor -

- TNF refer a group of cytokines that can cause cell death (apoptosis).
- TNF α is the best known member of TNF family.
- It is monocyte derived cytokine cytotoxin that has been implicated in tumor progression, septic shock etc.
- The primary role of TNF α is the regulation of immune cells.
- TNF α is able to induce fever, apoptotic cell death, inflammation and to inhibit tumorigenesis and viral replication and response to sepsis via IL1 and IL6 producing cells, and implicated

Structure

- TNF is primarily produced as a 212-amino acid, long transmembrane protein arranged in stable homotrimers.
- From this membrane-integrated form the soluble homotrimeric cytokine (sTNF) is released via proteolytic cleavage.
- The secreted form of human TNF α weighs around 17-kD.
- Both the secreted and the membrane bound forms are biologically active.

Signalling

- Two receptors are known - ~~TNF~~ TNFR1 and TNFR2.
- TNFR1 is expressed in most tissues, and can be fully activated by both membrane bound and soluble trimeric form of TNF.
- TNFR2 is found only in cells of immune system, and respond to the membrane bound form of TNF homotrimer.
- Upon contact with their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between ^{TNF} monomers.
- This binding causes conformational change in receptor, leading to the dissociation of inhibitory proteins from intracellular domain.
- This dissociation enables the adapter protein to bind to intracellular domain, serving as a platform for subsequent protein binding.

- TNF was thought to be produced primarily by macrophages but it is produced also by a variety of cells including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissues, fibroblasts and neurons.
- Large amounts of TNF are released in response to LPS, other bacterial products and IL-1.
- It has a number of actions on various organ systems, generally together with IL-1 and IL-6:
 - On hypothalamus:
 - > stimulation of HPA axis by stimulating CRH
 - > Suppressing appetite
 - > Fever
 - On the liver: stimulating the acute phase response. It also induces insulin resistance by promoting serine-phosphorylation of IRS-1, which impairs insulin signaling.
 - It is a potent chemoattractant for neutrophils, and promotes the expression of adhesion molecules on endothelial cells, helping neutrophils migrate.
 - On macrophages: stimulates phagocytosis, and production of IL-1 and prostaglandin E₂ (PGE₂)
 - On other tissues: increasing insulin resistance.

TNF-beta -

- This factor is produced predominantly by mitogen-stimulated T-lymphocytes and leucocytes.
- The factor is also secreted by fibroblasts, myeloma cells, endothelial cells, epithelial cells and other transformed cell lines.
- Synthesis of TNF- β is stimulated by interferons and IL-2.
- It is an N-glycosylated protein of 171 long amino acids.
- It does not contain disulfide bond and forms heterodimer.
- TNF- α and TNF- β show 30% sequence homology.
- TNF- β binds to the same receptor as TNF- α .

Biological Activities -

- Acts on a variety of cells.
- In general TNF- α and TNF- β display similar spectra of biological activities in *in vitro* systems, although TNF- β is less potent.
- TNF- β induces the synthesis of GM-CSF, G-CSF, IL-1, collagenase and prostaglandin E₂ in fibroblasts.
- TNF- β is cytolytic for many tumor cells.
- TNF- β induces the terminal differentiation and synthesis of G-CSF.
- It is a mitogen for B lymphocytes.
- In neutrophils TNF- β induces the production of reactive oxygen species. It is also a chemoattractant for these cells.

- TNF- β inhibits the growth of osteoclasts and keratinocytes.
- It promotes proliferation of fibroblasts and is involved probably in processes of wound healing.
- Administration of TNF induces metabolic acidosis, decreases the partial pressure of CO_2 , induces the synthesis of stress hormones such as epinephrine, norepinephrine, and glucagon and also alters glucose metabolism.