Class Notes/Study Material

M.Sc. – Semester II

Paper V – Endocrinology

Nonclassical hormones – Growth Factors

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Discoveries are always scholarly events

Minute and careful observation of unexpected result leads to a Discovery

Never neglect outcomes of any experiment

Sometimes, explanation of Failure gives rise to a new Discovery

Non-Classical Hormones

(Growth Factors)

Classical hormones are those which we know as chemical mediators (first messengers) secreted by specialised classical endocrine glands into blood streams for their action on distantly located target tissues or organs. Classical endocrine glands are pituitary, thyroid, parathyroid, thymus, pancreas, adrenal, testes and ovaries. Apart from these, brain, heart, liver, kidney and certain circulating blood elements also produce factors (peptides or steroid metabolites) that deserve the designation of hormones as they pour/secrete factors into blood. These organs secreting certain chemicals of endocrine importance are referred to as nonclassical endocrine organs as they are not exclusively meant for hormone production rather they have other important physiological roles in an animal. Therefore, factors produced by nonclassical endocrine organs are categorised as nonclassical hormones. In other words, **nonclassical hormones are the chemical mediators produced and secreted by nonclassical endocrine organs such as heart, liver, kidney etc, and are having auto-, para- or endocrine functions. In most of the places or instances, these are generally said to be 'factors'**. Some of the examples of nonclassical hormones are as follows:

Nonclassical Endocrine organs	Nonclassical Hormones*
Brain	Brain derived natriuretic peptides (BNP)
Heart	Atrial natriuretic peptides (ANP)
Liver, Fibroblasts, other tissues/organs	Insulin-like growth factor – I (IGF – I)
Kidney	Erythropoietin, Renin, 1,25 dihydroxyvitamin D
Platelets in circulating blood	Platelet-derived growth factors (PDGF), Transforming growth factor-β (TGF-β)
Macrophages, Lymphocytes	Cytokines, TGF-β, pro-opiomelanocortin (POMC)-derived peptides
Various cell/tissue sites	Epidermal growth factor (EGF), TGF-α, neuregulins, neurotrophins

(*These are now called factors)

Growth Factors

Cellular proliferation, differentiation, migration and cells survival/death are under the tight regulation of genes, expression/activities of which are in turn regulated by extracellular signals. These extracellular signals are provided by regulatory molecules which may act in paracrine, autocrine or endocrine fashion. Therefore, theoretically, any chemical mediator stimulating and regulating the cellular growth, proliferation and differentiation are categorised under growth factors. One such

chemical factor is pituitary growth hormone (GH) or somatotropin (STH). This pituitary GH is only one of a large family of protein growth factors that are secreted by one set of cells and promote growth of other set of cells. Many of the growth factors are also recognized as 'cytokines' – local protein hormones facilitating cell-to-cell communication to regulate growth, development and differentiation. Cytokines are the key players or regulators of haematopoiesis and immunological responses.

The difference between growth hormone and growth factor is indeed arbitrary and the reason behind their nomenclature is the order of their discovery. For the first time by Bayliss and Starling (1902-05), the name 'hormone' was given to those mediators which are secreted from one site/tissue into blood to regulate the response of distantly located tissue site. In this way, the first hormone discovered was named 'secretin' (gastrointestinal regulatory hormone secreted from initial portion of small intestine). After several years, pituitary somatotropin was known to endocrinologists for their role in growth of bone and general body. It was then experimentally realised that most of the growth promoting effects of STH were mediated through certain plasma factors, absence of which caused failure in STH effects. Such factors mediating the action of somatotropin were named as somatomedins. Now, these factors are recognised as insulin-like growth factors (IGFs) as their structure and sequence show high degree of similarity with those of insulin. Afterword, several other factors promoting and regulating the growth of various cells and tissue types have been recognised.

In nutshell, **growth factors are any group of protein/peptide that stimulate the growth of specific tissue or cell. Growth factors play an important role in promoting cell division and differentiation**. When investigators began studying the effects of biological substances on cells and tissues in culture, they discovered a group of peptide-hormone-like substances that were distinct from any previously known hormones. Because these substances were active in stimulating the growth of cells and tissues, they were called growth factors. Some growth factors are similar to hormones in that they can be secreted into the blood stream, which carries them to their target tissues. However, whereas the production of hormones is limited to glandular tissue, growth factors can be produced by many different types of tissue. Further, receptors of most of the growth factors belong to receptor tyrosine kinase family and activate one or more of the RAS/MAP kinase pathway, PI3 kinase/AKT pathway or PLCγ pathway.

Families of Growth Factors

There are multiple superfamilies of growth factors that contain multiple subfamilies of protein or peptides all with related primary structure (amino acid sequence). Classical growth factor superfamilies are as follows:

1. Insulin-like growth factors (IGFs)
2. Epidermal growth factors (EGFs)

3. Fibroblast growth factors (FGFs)
4. Nerve growth factors (NGFs)
5. Transforming growth factors (TGFs)
6. Platelet-derived growth factors (PDGFs)

Growth factors are ligands for transmembrane receptors. Each growth factor superfamily has a corresponding family of related receptors. There is high specificity with respect to receptor binding between superfamilies, but there are also cross reactivity among members of same family for same family of receptors. For instance, there are four FGF receptors for the 22 members of FGF superfamily.

1. Insulin-like Growth factors (IGFs)

IGFs are important growth factors promoting the growth and proliferation of general body cells, skeletal muscles and bones. The first growth factor discovered was insulin-like growth factors (IGF). These were recognised as mitogenic peptides isolated from plasma and were firstly named as somatomedins because of their role in mediating the growth promoting action of pituitary somatotropin. Previously, they were also known as 'sulphation factor' as these were firstly found to stimulate the incorporation of sulphur (in the form of sulphated polysaccharides) into the epiphyseal cartilage under the influence of GH.

They show high degree of structural similarity with insulin, that is why they are named as IGF. They also share some of the metabolic effects of insulin but are less active. On the other hand, they are much more active than insulin in promoting cellular growth, proliferation and differentiation.

Types of IGFs and structure: IGFs are of two types – IGF-I and IGF-II containing 70 and 67 amino acid residues, respectively. Polypeptide sequence is similar to proinsulin with a shorter connecting peptide of 12 residue and an extension of 8 residues at the A chain terminus.

Source of IGF secretion: IGF – I and IGF – II are synthesised and secreted from liver under the influence/stimulus of pituitary growth hormone (GH, somatotropin). Experimental evidences also suggest GH induced synthesis of IGF-I in proliferative epiphyseal chondrocytes of developing bones. IGF-II is three times more abundant in adults that IGF-I, yet its origin and functions are less well defined.

Functions:

1. IGFs are the hormones/factors that are similar in structure to insulin, and work with somatotropin to stimulate cellular growth, proliferation, differentiation and sometimes regeneration.

2. IGF-I, also known as 'somatomedin C' is principal mediator of pituitary growth hormone (somatotropin). Cell culture studies suggest that GH induces differentiation of cells, and IGF-I then induces a rapid proliferation of the newly differentiated cells. Homologous IGF-II may have similar function in fetal development. Therefore, GH activity in the body is dependent IGF-I, deficiency of which may cause insensitivity to GH actions on growth and repair of body.

3. Upon getting stimulus form GH, IGFs play important roles in growth and development of organism (as in the womb and throughout the life), strengthening of tissues, elongation of bones, improvement of bone density, muscle building, healing of injured areas of skin, bones, gut linings etc.

4. IGF-I is crucial in development and if it is not adequately present in growing child, it may result in short stature.

Pathophysiological Association of IGF: Because IGF-I stimulates growth and proliferation, it may have negative consequences during development and progression of tumour or cancer. It exerts powerful effect on each stage of cancer development. It increases cellular proliferation, angiogenesis and metastasis, and reduces cell death (apoptosis). It can also lead to resistance to chemotherapeutic agents.

IGF receptors: The functions of IGFs are mediated by two main classes of IGF receptors:

- IGF-I receptor exhibits ligand-dependent tyrosine kinase activity, and has considerable structural and functional similarity to the insulin receptor, but has higher affinity for IGF-I and IGF-II than for insulin.
- IGF-II receptor, in contrast, has different structure and is homologous with mannose-6-phosphate receptor. It has higher affinity for IGF-II than IGF-I, and does not bind with insulin.

Both IGF-I and II can bind to insulin receptor, although at low potency.

2. Epidermal Growth Factor (EGF)

Human epidermal (or epithelial) growth factor (EGF) was first recognised as **urogastrone** because of its resemblance of gastric inhibitory action to duodenal hormone enterogastrone. Because of their first observation in urine of pregnant women, this factor was named 'urogastrone'. It was then found that urine of most animals including human contained such factors.

EGF was first isolated from submandibular (or submaxillary) glands of mouse by **Cohen (1962)** during exploration of nerve growth stimulating factors from the same. After that, **Gregory (1975)** isolated urogastrone from human urine and studied its detailed structure. Later on, observations on its

similar biological functions and sequence homology to mouse submandibular EGF has led to the identification of urogastrone as human EGF.

Structure of EGF: Well characterised mouse EGF is a 53 amino acid peptide of low molecular weight (~6 kDa). It is quite resistance to heat as well as proteolytic digestion by trypsin, chymotrypsin and pepsin. This peptide growth factor has three disulphide bonds which are important for its biological activity. This factor is synthesised as high molecular weight prepro-EGF of 1217 amino acid residues. This prepro-EGF is also a source of seven related growth factors e.g. TGF-α, neuregulin, epiregulin etc.

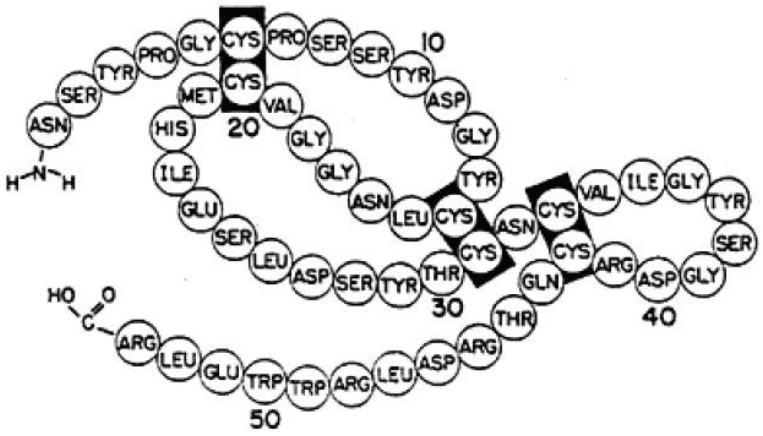

Figure 1. Structure of EGF (Ref: J. Biol. Chem. 1973, 248, p. 7670)

Sources of EGF: EGF is present in about all body fluid and secretions of man and animals. Major synthesis and secretion sites of EGF are submandibular salivary glands, Brunner's glands in the duodenum, and the renal medulla. Small amounts of this hormone is also synthesised and secreted by male reproductive organs, pancreas, bone marrow, sweat glands, mammary glands etc. Because of this reason, EGF is found in saliva, gastric juices, bile, breast milk, urine and seminal fluid. Its plasma concentration is too much lower than the tissue level because of its rapid clearance by liver.

Biological functions of EGF:

1. The first observed biological function of EGF was its ability to induce early eye opening and tooth eruption in neonatal mice due to enhanced epidermal growth and keratinization. EGF is a potent mitogen and has effects on epithelial, mesothelial and endothelial cell types.
2. Classical function of EGF as urogastrone is to inhibit the secretions of acid and pepsin from gastric mucosa. It is also having cytoprotective effect on gastric mucosa. Therefore, it is very important factor in stimulating the healing of gastric ulcers.
3. EGF also has trophic effect on gastrointestinal mucosa and it is appeared to be required for the development and maintenance of intestinal epithelium. It also promotes the production of brush-border enzymes in intestinal mucosa. (This action is particularly evidenced by the presence of

significant amount of EGF in lactating milk. This milk EGF reaches GI tract of infants where it has role in development of gastrointestinal epithelia)

4. Experimental evidences also support its wound healing capabilities. EGF promotes rapid wound healing in injured skin and cornea. (This wound healing potential of this factor might explain the benefits of licking behaviour of animals on wounds – high EGF concentration in saliva accelerates healing of injured areas on skin after rapid and frequent licking. Another advantage of licking the wounds is salivary antibacterial action which is exerted by the presence of lysozymes and IgA in saliva)
5. EGF also stimulates the synthesis of DNA, keratin, fibronectin, and collagen. It is also reported to stimulate prostaglandin biosynthesis, secretion of human chorionic gonadotropin (hCG) and bone resorption.
6. In fatal and neonatal life, EGF appears to play an important role in development of oral cavity, lungs, gastrointestinal tract, and eyelids.

EGF receptors (EGF-R): EGF receptor is large transmembrane glycoprotein of 170 kDa. EGF-R has four domains – an extracellular EGF binding domain/portion, a hydrophobic membrane spanning segment, a proximal ATP binding tyrosine kinase cytoplasmic domain, and terminal cytoplasmic portion containing three tyrosine residues (for autophosphorylation after EGF binding). Hence, EGF-R is a type of receptor tyrosine kinase (RTK) group.

3. Nerve Growth factors (NGFs)

NGFs, also called neurotrophins, are critical for the development and maintenance of central and peripheral nervous system. NGF was first discovered by Italian developmental biologist **Rita Levi-Montalcini** and Viktor Hamburger **in 1952**. They observed potent growth of chick nervous system when mice tumors were transplanted to chick embryos. Since this induced outgrowth of neurons in embryos did not require direct contact with those tumors, Rita Levi-Montalcini concluded that tumors released some nerve growth-promoting factors responsible for development of chick nervous system. After that, American Biochemist **Stanley Cohen** isolated and purified **first NGF** from snake venom and salivary gland extract which were observed to be its rich sources. Later on, Cohen and co-workers also discovered another rich source of NGF – mouse salivary gland. Cohen also identified and purified first epidermal growth factor (EGF) from mouse submandibular gland while searching for NGF. In 1986, **Rita Levi-Montalcini** and **Stanley Cohen** were jointly awarded **Nobel Prize in Physiology and Medicine** for their pioneer contributions for NGF and EGF, respectively.

NGF Family: The neurotrophins represent a family of structurally and functionally related, homodimeric proteins, including following members:

- Nerve growth factor (NGF)
- Brain-derived neurotrophic factor (BDNF)
- Neurotrophin-3 (NT-3)
- Neurotrophin-4/5 (NT-4/5)
- Neurotrophin-6

All these neurotrophins stimulate survival and differentiation of a range of target neurons by binding to cell surface receptors.

Structure of NGF: The mature NGF is derived from the preproNGF precursor (240–260 amino acids). It is a noncovalent homodimer with each monomer having a mass of 13 kDa or 118–129 amino acid residues. NGF is a member of cysteine knot family because of a double loop formed by two disulphide bonds, which is then penetrated by a third disulphide bond. Further, there is sequence homology between NGF and proinsulin molecule.

Sources of NGF: Neurotrophins are synthesised and secreted by the nerves. In the central nervous system (CNS), NGF is enormously produced in cerebral cortex, hippocampus and pituitary gland. These factors are also produced in significant amount in other areas including the basal ganglia, thalamus, spinal cord, and in the retina. NGF are also synthesised by cells of bone marrow involve in haematopoiesis of immune cells.

Snake venom and submandibular salivary glands of male mouse are also rich sources of NGF. In male mouse, this NGF synthesis and secretion is androgen dependent.

Biological functions of NGF: NGFs are neurotrophic factors promoting the growth, maintenance, proliferation, and survival of certain target sensory and motor neurons. Following functions are attributed to NGFs:

1. NGF is crucial factor for the development and phenotypic maintenance of neurons in peripheral nervous system, and for the functional integrity of cholinergic neurons in CNS. Therefore, NGF plays central role in the maintenance of attention, arousal, motivation, memory and consciousness.
2. NGF regulates noradrenergic nuclei of hypothalamus and brain stem, and thereby participates in the central regulation of autonomic response.
3. Neurotrophins also play important roles in the regulation of activities of immune cells and immune responses. NGFs regulate survival and differentiation of haematopoietic stem cells, granulocytes, lymphocytes and monocytes.
4. They also exert trophic action on keratinocytes, regulate hair growth and have role in wound healing.

NGF Receptors: NGF elicits its function by binding to two families of transmembrane receptors—the selective and specific TrkA tyrosine kinase receptor and the common p75 neurotrophin receptor. Other receptors are TrKB and TrKC receptors.

All Trk receptors are type I receptor tyrosine kinases. They have an extracellular ligand-binding domain, a single transmembrane domain and a cytoplasmic part with tyrosine kinase catalytic activity. The p75 receptor is a member of tumor necrosis factor (TNF) superfamily. p75 Receptors can modulate Trk receptor signaling, but they also signal independently of Trks. Depending on the developmental stage, cell type, whether they are unbound or ligand bound, and the type of ligand, p75 can activate apoptosis or survival, regulate Schwann cell migration, myelination, axonal growth, and regeneration.

4. Fibroblast Growth Factor (FGF)

Fibroblast growth factors (FGFs) constitute a large family of structurally related polypeptide growth factors that are found in the organisms ranging from nematodes to humans. These growth factors are characterised by their **heparin binding ability for proper functioning**. FGF is a representative growth factor which has **potential effects on the repair and regeneration of tissues by promoting growth and proliferation of fibroblast, and angiogenesis**. Members of this family also play crucial roles in embryonic development.

FGF study was recognised during the exploration of mitogenic activity of pituitary extracts by **Armelin in 1973**. Subsequently in 1974, **Gospodarowicz** isolated first fibroblast growth promoting factor from cow brain extract, and named it as FGF. In 1975, he isolated and purified two FGFs – acidic FGF (aFGF/FGF-1) and basic FGF (bFGF/FGF-2) from brain and pituitary extracts. Since then, at least 22 distinct FGfs have been isolated and identified.

FGF Family: FGF family includes acidic and basic FGFs, keratinocyte growth factors, and several other related peptides. There are different methods for classifying FGF family in invertebrates and vertebrates. By methods of phylogenetic analysis, human FGF gene family are divided into seven subfamilies:

1. FGF-1 subfamily – aFGF/FGF1 , bFGF/FGF2 (prototype members of FGF family)
2. FGF-4 subfamily – FGF4, FGF6, FGF5
3. FGF-7 subfamily – FGF3, FGF7 (keratin growth factor/KGF), FGF10, FGF22
4. FGF-8 subfamily – FGF8, FGF17, FGF18
5. FGF-9 subfamily – FGF9, FGF16, FGF20
6. FGF-11 subfamily – FGF11, FGF12, FGF13, FGF14
7. FGF-19 subfamily – FGF19, FGF21, FGF23

   There is no such FGF15 in human.

   Functionally, FGF can be classified into intracrine, paracrine and endocrine forms.

Structure of FGF: FGFs ranges in molecular weight from 17 to 34 kDa, while *Drosophila* FGF is of 84 kDa polypeptide. All members of FGF family share a conserved sequence of about 120 amino acids that show 16-65% sequence homology.

Sources of FGF: FGFs are expressed by wide variety of cells and tissues. Acidic FGF (FGF1) are predominantly produced in brain, ratina, bone matrix, whereas basic FGF (FGF2) is found in variety of tissues including pituitary gland, neural tissues, adrenal cortex, kidney, prostate, thymus, corpus luteum and placenta.

Biological functions of FGF: In general, FGFs are potent regulators of cellular proliferation, migration and differentiation. They are critically important in normal development, tissue repair and regeneration, neuronal maintenance, and angiogenesis. They have potent mitogenic ability towards cells of epithelial, mesenchymal and neuronal origin. For the proper biological functioning, FGFs interact and bind with heparin or heparin sulphate proteoglycan (HSPG). These interactions also stabilise the FGF molecules and protect them from proteolytic degradation.

Some of their specific functions are as follows:

1. FGFs are major proliferative factor for fibroblast and epithelial, endothelial and stem cells. They also stimulate preadipocytes which is important in adipogenesis.
2. FGFs are the potent stimulating factors for cell migration which is an important phenomenon during embryonic development and wound healing.
3. They also stimulate and regulate cellular differentiation during development and morphogenesis. FGFs are also key regulators of stem cell differentiation in adult tissues.
4. FGFs are principal angiogenesis stimulating factors which cause the development of new blood vessels during embryonic development, tissue repair during wound healing and inflammation. Therefore, they are also involved in rapid angiogenesis during tumor growth.

FGF Receptors: FGFs exert their effects by binding to high affinity tyrosine kinase FGF-receptors (FGFR) on the surface of target cells. After binding with receptor, intracellular signalling occurs predominantly through RAS/MAP kinase pathway. FGFR signalling also utilises PI3 kinase/AKT pathway or PLCγ pathway.

5. Platelet-derived Growth factors (PDGF)

PDGF are released from platelets during platelet aggregation in the process of blood clot formation at site of wound or vascular injury. It is well established component of serum based culture media for successful growth of cells such as fibroblast or other mesenchymal connective-tissue forming cells. PDGF was discovered as component released from platelets in culture media that promotes

growth of certain cell types e.g. smooth muscle cells (SMCs) and other connective tissues in culture (Ross et al., 1974; Kohler & Lipton, 1974).

Structure of PDGF: Human derived PDGF is a highly cationic (pI 9.8-10) glycoprotein of ~30 kDa. It is a heterodimer of two peptide chains – chain A (~16 kDa) and chain B (~14 kDa). Both the chains share ~60% sequence homology, and can dimerise to form three forms – PDGF-AA, PDGF-AB, and PDGF-BB. All three forms of PDGF are naturally occurring and biologically active forms. Intra- and inter-chain disulphide bonds stabilise three dimensional structure and biological functions of native PDGF molecule.

Sources of PDGF: Principal source of PDGF is circulating blood platelets. PDGF is stored in the **α-granules of platelets** that are released upon the exposure to thrombin, ADP and collagen. In addition, PDGF and PDGF-like molecules are also secreted from circulating platelets, monocytes/macrophages, and certain resident cells e.g. smooth muscle and endothelial cells.

Biological functions of PDGF: PDGF is considered a potent mitogen for connective-tissue-forming cells such as dermal fibroblast, arterial smooth muscle cells, and glial cells. It also induces growth and proliferation of some specialised cells e.g. epithelial cells and capillary endothelial cells. Therefore, PDGF play crucial role in wound healing and tissue regeneration. Some important biological functions and effects are as follows:

1. PDGF is released from activated platelets during platelet aggregation at wound site to induce the proliferation of arterial smooth muscle cells and capillary endothelial cells for vascular healing.
2. PDGF stimulates overall protein synthesis in target responsive cells.
3. PDGF released from platelets also attracts neutrophils and monocytes at the site of tissue injury, and activate them.
4. It is also a potent vasoconstrictor.
5. Under experimental conditions, PDGF has been shown to induce the synthesis and release of IGF-I (somatomedin C) like peptides from fibroblast and smooth muscle cells.

PDGF Receptors: Like the receptors for other growth factors, PDGF receptors are also transmembrane glycoproteins (receptor tyrosine kinase) with C-terminus tyrosine kinase activity.