Summary

What are hormones?

body-synthesized chemicals which are secreted in *low amounts*

Hormones function in:

- They help maintain homeostasis

- Mediate responses to external stimuli

- Play roles in growth and development

One hormone → several cell types

One cell type → several hormones

One hormone → several effects

The definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, regardless of the action.

Factors affect the concentration of the hormone at the target cell:

- The rate of synthesis and secretion of the hormone
- The proximity of the target cell to the hormone source (dilution)
- The Kd of the hormone
- receptor complex
- The rate of conversion of inactive form to the fully active form
- The rate of clearance from the plasma

Factors affecting the target cell response:

- The number, relative activity, and state of occupancy of receptors
- The metabolism (activation / inactivation) of the hormone in the target cell
- The presence of factors within target cell necessary for the response
- Up- or down-regulation of the receptors upon interaction with ligand
- Post-receptor desensitization of the cell

Hormone-receptor binding should be:

- Should be specific: displaceable by agonist or antagonist
- Should be saturable
- Should occur within the concentration range provided

And that is possible by having high affinity between the receptor and the hormone.

Association constant Ka

Dissociation constant Kd

Ka = [H-R] / {[H] X [R]}

Kd = {[H] X [R]} / [H-R]

20X dissociation constant is enough to saturate the receptor

Kd values for many hormone range from 10^{-9} to 10^{-11} M

Endocrine System and Nervous System

The endocrine system and the nervous system act individually and together to regulate the human body

Receptors

They are proteins. Proteins can undergo structural conformational change after binding to specific molecules. That is why receptors are not sugars or lipids.

The domain is a part of the protein that is responsible for a special function.

All receptors have at least twofunctional domains, which are:

- Recognition domain (binding domain): binds the ligand
- Coupling or signal transduction domain

Coupling occurs in two general ways:

- Changing the activity of an enzyme (Polypeptide&catecholamines) on the plasma membrane
- Direct (steroids, retinoids, and thyroid hormones) in the interior of the cell

Steroid, thyroid, and retinoid hormone receptors, for example, have hormone binding site, DNA binding site, co-regulator proteins binding site and cellular trafficking proteins binding site.

Receptor–effector coupling provides the first step in amplification.

Signal Amplification

If amplification did not exist to happen, the needed hormonal concentration would become high, and that does not happen in nature, because:

4- Hormonal synthesis would be energy-costy
5- Harder regulation
6- Low affinity; low specificity

<u>Signal amplification can occur by:</u>

- The production of high amounts of small intracellular molecules (ex. cAMP).

- After binding, the activated receptor performs its function, which can be a kinase activity, for a period of time, activating many enzymes

Hormones release control

Feedback inhibition:

♣Ultrashort loop ♣ Short loop ♣ Long loop

Hormones classes

- Source-target: endocrine (secreted in the blood; distance; stability; concentration); paracrine and autocrine.

- Chemical composition:

  Polypeptides (the majority): such as Pituitary hormones; Hypothalamic releasing hormones; Insulin, Growth factors, and others.

  All hypothalamic, pituitary, digestive hormones
  All pituitary hormones are made from single polypeptide chains EXCEPT: TSH; FSH; LH (homodimers) – glycoproteins (≈ 25 kDa)

Hormone	Structure
GHRH	44
TRH	3
GnRH	10
CRH	41
ADH	9
Vasopressin	9
Angiotensin I	10
Angiotensin II	8
Insulin	51
Glucagon	29

- Insulin is a large hormone
- TRH has only 3 amino acids, which is the shortest hormone ever known.
- Angiotensin II is synthesized from Angiotensin I by angiotensin converting enzyme (therapy target), by removing two amino acids (10 to 8).
- Oxytocin and Vasopressin differ in 2 amino acids only, although having the same number of amino acids.

Amino acid derivatives: Modified amino acids. Such as Adrenalin, Thyroid hormones.

Amines - derived from tyrosine or tryptophan TH, dopamine, epinephrine, melatonin

Amino acid	hormone
Tryptophan	Melatonin; Serotonin
Phenylalanine -- Tyrosine	Epinephrine; Norepinephrine; T3 and T4; Dopamine

- **Steroids**: cholesterol-related molecules. have four *steran* rings.

A. Sex hormones - are divided into 3 groups
1. Male sex hormones or Androgens
2. Female sex hormones or Estrogens
3. Pregnancy hormones or Progestines

B. Hormones of Adrenal Cortex
1. Mineralocorticoids: aldosterone. ...
2. Glucocorticoids: cortisol. ...
3. Adrenal androgens: male sex hormones mainly dehydroepiandrosterone (DHEA) and testosterone

- Mechanism of action:

Hormones that bind to intracellular receptors (lipid-soluble hormones):
- Steroids
- Thyroid hormones
- Calcitriol, retinoic acid

Hormones that bind to cell surface receptors (According to second messenger):
- cAMP (β adrenergic factor, glucagon, ACTH)
- cGMP (atrial natriuretic factor, Nitric oxide)
- Calcium or phosphatidyl inositol (oxytocin, TRH)
- Kinase or phosphatase cascade (insulin, GH)

General Features of Hormone Classes

	Class I	Class II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Actions	Slow	Fast
Solubility	Hydrophobic	hydrophilic
Needs protein carrier?	yes	No
Plasma half life	Long	short
Receptor	Intracellular (cytoplasmic OR nuclear)	transmembrane
mediator	Receptorhormone complex	Second messengers: cAMP, cGMP, Ca2+ , kinase cascades, metabolites of

		phosphoinositols

Peptide Hormones Synthesis

There are three ways of peptide hormones synthesis:

4- From precursor polypeptides
   - One gene may code more than one hormone (POMC gene is an example; *P*oly*O*pio*M*elano*C*ortine)
   - The cleavage depends on specific enzymes
5- From precursor polypeptides:
   Vasopressin and Oxytocin are an example. Synthesis occurs in separate cell bodies of hypothalamic neurons.
6- From Pre-pro-hormones
   An example is Insulin. Preproinsulin is turned into proinsulin by cutting the signal peptide. Insulin is produced from the proinsulin by cutting the C peptide. Insulin has 2 disulfide bridges in its active structure.

Signal transduction

Transduction: conversion of one form of a signal to another so as cells can produce many kinds of responses in different ways. Amplification is a MUST. Signal (polar, large) should bind receptors which are intrinsic and trans-membrane, and have Intra- & extracellular domains.

Second messengers have the ability to diffuse to other cellular compartments. Amplification of the signal happens at this point of signal transduction. The function of second messengers is enzyme activation or membrane channels. Some second messengers are common in multiple signaling pathways (≈ 30 hormones use cAMP!!!). This permits fine tuning but can pose problems.

Types of second messengers include:

♣ Small molecules: cAMP, cGMP, Ca+2

♣ Phosphorylation through kinases

Signal Termination

Is it important?

Signal termination keeps cells responsive to new signals and prevents energy wasting. Failure of termination may cause problem (GH & cancer).

How it is achieved?

♣ Degradation of the second messenger

♣Dephosphorylation by hydrolysis

Membrane associated receptors

7-Trans-membrane Helix Receptors (7TM)

7TM is a receptor that has 7 α-helices. It has many Serine and Threonine residues, which enables its phosphorylation, and so its conformational change.

But how are the 7 helices stabilized in the membrane?

- helices are rich with hydrophobic amino acids.
- all the H-bonds are hidden from the lipophilic environment.

How does it work?

Binding of the ligand induces the receptor conformational change. That results with Serine and Threonine phosphorylation.

The receptor functions

- Smell, Taste, Vision
- Neurotransmission
- Hormone Secretion
- Chemotaxis
- Exocytosis
- Cell Growth and development
- Viral Infection
  All these receptors share the same basic structure; however, they differ in their specificity and effects.

  Refer to the slides for visual illustrations and chemical structures.

END

*G protein is attached to the membrane because it is covalently bound to fatty acids (one on alpha, one on gamma subunit).

*Hormone binding→conformational change in receptor→conf. change in G protein→ alpha subunit loses GDP and takes GTP to get activated→ detach from βɣ dimer, and go for several targets: phospholipase C, ion channels (open or close them), or more commonly: Adenylatecyclase.

*Aden. Cyclase is a membrane enzyme, consists of 12 membrane spanning α helices, 2 intracellular domains. It converts ATP→cAMP.

* Each receptor is bound to at least 1 G protein, but it may be bound to more (like 100 G proteins with 1 receptor).So 1 hormone binds→100 G protein activated→100 aden. Cyclase activated→ much more cAMP produced. (**SIGNAL AMPLIFICATION**).

*Sometimes, Aden. Cyclase is inhibited by Gα. This depends on the nature of the receptor (some receptors are inhibitory) and the nature of the Gα subunit itself.

*cAMP targets protein kinase A (PKA). It is an enzyme, consists of 2 catalytic subunits and 2 regulatory subunits, which bind 4 cAMP molecules to detach from the catalytic, thus activating the kinase activity.

*PKA phosphorylates target proteins. This can either lead to activation or inhibition (famous example: Glycogen synthase gets inactivated if phosphorylated).

*Termination of the signal occurs by : 1-Hormone dissociating from the receptor (because they are bound non-covalently).
2-Slow GTPase activity in α subunit (GTP→GDP, so Gα is inactivated).
3-Phosphodiesterase breaking cAMP.
4-Phosphorylation of the receptor, making it attract/bind a protein called β-Arrestin, so it masks the receptor. So even if the hormone is bound, the receptor cannot activate G protein because it's masked (hormone bound + no response…this is desensitization).

*Cholera toxin: toxin that binds GPCR in intestine→ HUGE production of cAMP→release of Cl, pumping of Na, water out by osmosis→excessive and life-threatening diarrhoea.
****

*Active Gα targeting Phospholipase C (PLase C) : a membrane protein, consists of a Catalytic domain, a Membrane binding domain(PH and C2), and a G protein binding domain (to get activated).

*This enzyme has isozymes (multiple forms in different tissues). Only PLase C β has the G protein binding domain, and it is the one involved in this pathway.

*PLase C breaks phosphatidyl inositol 4,5-bisphosphate (PIP2) to inositol 1,4,5 trisphosphate (IP3-the main 2nd messenger) and diacylglycerol (DAG). IP3 is h.philic, it leaves the membrane and binds calcium channels on sarcoplasmic reticulum to cause Ca release. Four IP3s are required for full opening of the channel, but 3 can do the work (considerable opening). Released Ca (positively charged) targets negatively charged Calcium binding proteins, and Protein kinase C (PKC; membrane enzyme), that gets fully activated by binding of both Ca and DAG to it.

*Binding of IP3 to the channel is cooperative; meaning the binding of the first IP3 makes the binding of the 2nd easier, which makes the 3rd easier, which makes the 4th easier.

*PKC domains: 1-Membrane binding domain 2-Catalytic domain
3-DAG binding domain
4-Calcium binding domain
5-Pseudosubstrate domain: it fits in the active site of PKC (like the substrate), but it contains Ala instead of Ser/Thr, so it's not phosphorylated. It keeps the enzyme inactive (by blocking/closing the active site, by working as a competitive inhibitor). When Ca and DAG bind, this domain is displaced, exposing the active site and activating the enzyme.

*Termination is by removing IP3 phosphates or by addition of new phosphate producing IP4 (faster), both ways make IP3 inactive. And when the cell has the time, it removes IP4 phosphates, but the last added phosphate must not be removed first, because this will produce active IP3 and keep the signal running.

*Clinical hint: Lithium based drugs prevent IP3 recycling in the CNS→ IP3 remains active → treat depression.

*Characteristics of Ca:
1- Positively charged, so it binds Ca binding proteins (contain negative charges).
2-Huge difference between sER and cytosol (10000 times), the release has huge impact
3- It can make 6-8 bonds (ligations) with oxygen of amino acids. So it's tightly bound.
4-It is bulky, so when bound to protein, it causes the desired conformational change.

*Ca binding proteins:
1-Parvalbumin: the 1st one discovered, has 6 α helices (A-E), Ca binds in a loop between helices E&F. This Ca binding structure (helix-loop-helix) is called an EF hand, and is present in other proteins.
Note: helix-loop-helix: bind Ca, while helix-turn-helix: smaller, bind DNA.
2-Calmodulin: 2 globular regions, each has 2 EF hands, so it can bind 4 Ca ions. When Ca binds→conf. change→active→activate other proteins like Calmodulin dependent protein kinase and **Ca ATPase pump→to pump Ca back and terminate the signal.**

*Ca pump: 80% of proteins on sER surface, energy expensive (2Ca pumped: 1ATP), directly –fast- activated after Ca channels opening, 10 helices,↓ATP→tetany&R.mortis.

Receptor Tyrosine Kinases Cascade

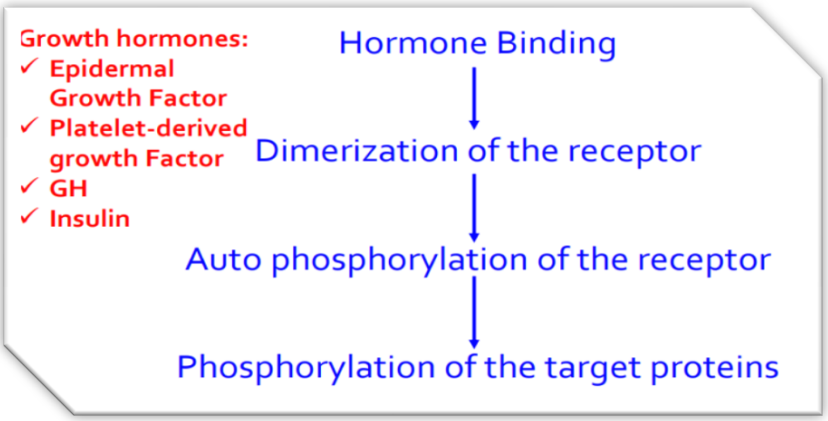
This receptor is a kinase enzyme, and the pathway involves Tyrosine amino acid phosphorylation. This pathway is used by most growth-related hormones (Insulin, GH, growth factors…). There are two classes of this receptor:

- Monomer, which dimerizes after ligand binding. All receptors of this family are monomers except for insulin receptor.
- Dimer, and the subunits are bound by disulfide bridges; such as insulin receptor.

Receptor Domains

Span the membrane, several subclasses (class II, Insulin R), hormone receptor & tyrosine kinase portion

The Pathway

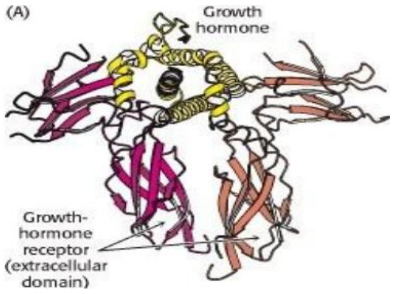


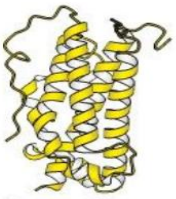
- Binding of the ligand leads to conformational change, which results with monomers dimerization. Dimerization is a hallmark of this pathway. Binding has two forms:
  3- One ligand binds to a monomer and another binds to another monomer. Conformational changes of the monomers leads to their dimerization. So 2 ligands bind here.
  4- One ligand binds to a dimer receptor (ex. Insulin receptor).
- Dimerization induces a conformational change that leads to auto- and cross-phosphorylation of the Tyrosine residues in the coupling domain, and thus fully activating the receptor. Notice that the monomers phosphorylate themselves and each other.
- This receptor has its second receptor (tyrosine kinase) within itself. So, it does not need a second messenger system.
- Target activities may be alterations in membrane transport of ions & amino acids & the transcription of certain genes; Phospholipase C is one of the targets. Insulin-sensitive protein kinase: activates protein phosphatase 1.
  activation of an originally dimerized receptor (ex. Insulin receptor) is similar to the activation of the monomer receptor, and involves:

  Binding – conformational change – activation – tyrosine residues phosphorylation – kinase activity

Growth Hormone & GH receptor

- GH:
  - Monomeric Protein
  - 217 Amino Acids
  - Compact Four-helix Bundle
- GH receptor (cooperative binding)
  - 638 A.Acid
  - Extracellular Domain (≈250 A.A) & Intracellular Domain (≈350 A.A)
  - Single Membrane-Spanning Helix
  - Monomeric (free) vs. Dimeric (bound)

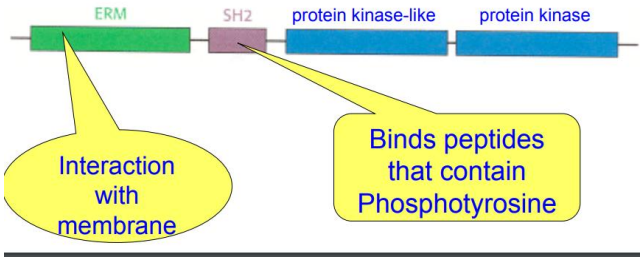



Human growth hormone

Janus Kinase

JAK is bound to the monomers that get dimerized. JAK also has Tyrosine residues.

JAK kinase domains



JAK kinase domains include:

- Membrane binding domain (to be close to the receptor)
- Kinase domain
- SH2 domain (discussed below)

Dimerization of the receptor monomers allows auto- and cross-phosphorylation occurs between the two JAK kinases, resulting with their activation. Activated JAK kinases phosphorylate target molecules, and STAT, or *SignalTransducer&Activators ofTranscription*, is the most common one. STAT leads to transcription activation, having the DNA as its final target.

STAT is phosphorylated by JAK kinase on a Tyrosine residue nearthe carboxyl terminus. Phosphorylated Tyrosine binds to SH2 domain of anotherSTAT 5 molecule. SH2 domain is a phosphorylated Tyrosine binding domain. (SH2 domain is present in JAK kinase as well as STAT molecules.)

After that, the resultant STAT dimer heads towards its final target, the DNA, to activate transcription.

Note that JAK/STAT pathway is an example of the pathways tgat follow the binding of a ligand to a receptor tyrosine kinase.

Note: If JAK2 remains active it will produce Cancer

Examples on Receptor Tyrosine Kinases

Epidermal Growth Factor Receptor :♣Monomeric (inactive) ♣ EGF binding

⎩Dimerization ⎩Cross Phosphorylation ⎩Activation

Insulin Receptor:♣ Tetramer (2 α ; 2 β), dimer (2 αβ pairs) ♣ Disulfide bridges

Insulin Binding →Activation of the Kinase

RAS Protein

RAS protein is a monomeric G protein. It works in a similar manner to α-subunit of G proteins. So, its activation includes:

Ligand binding leads to receptor activation – RAS conformational change – GDP for GTP replacement – activation – activates another effector protein

RAS protein also has a slow GTPase activity that leads to GTP for GDP replacement and signal termination.

RAS includes several groups or subfamilies.

RAS has a major role in growth, differentiation, cellular transport, motility etc…

Mammalian cells contain 3 Ras proteins.

Mutation of RAS GTPase domain: Loss of ability to hydrolyze GTP. Ras is locked in "ON" position. Continuous stimulation of growth.

***Eicosanoids** act locally (autocrine and paracrine), all members have 20 carbons, they have short half life and are very potent. They include Prostaglandins(PGs), Thromboxanes(TXs), and Leukotrienes (LTs).

*All eicosanoids are made from Arachidonic acid, which is attached to carbon 2 of glycerol in membrane lipids. Phospholipase A2 breaks that bond and release AA. This is the rate limiting step in eicosanoid synthesis. Then AA is modified by cyclooxygenases to give PGs/TXs, or lipoxygenase to give LTs.

*Differences in structure: AA: no rings, 4 double bonds
PGs: 5 membered ring……..TXs: 6 membered ring
LTs: no rings, at least 3 conjugated double bonds (double-single-double-single-double-single)

*Eicosanoids have diverse and sometimes opposing actions. Some promote v.dilation, some v.constriction.

***Steroids** are made of cholesterol (27 carbons). All have 4 steran rings (17 carbons), which cannot be cleaved by the body; it's conjugated and released with bile (small amount with urine).

*What differs between steroids is what is attached to the rings. There are 18/19/21 carbon steroids.

21 carbon: Pregnenolone, progesterone, Aldosterone, cortisol.
19 carbon: testosterone
18 carbon: Estrogen (testosterone is converted to estrogen by Aromatase)

*Nitric Oxide (NO): local action, made by nitric oxide synthase NOS (Arginine +O2→citrulline +NO).

*NOS has many isoforms in different tissues, such as neurons, macrophages and smooth muscles of blood vessels, where it produces NO (local vasodilator).

*Treatment of Angina Pectoris (less blood to the heart) is by nitrates (due to v.dilatory action).
*Septic shock is when bacterial toxins cause huge NO synthesis→v.dilation→severe hypotension.

*thyroid hormones: [tyrosine with a phenol (benzene ring and OH)]+ 3 iodines (T3) **or** 4 iodines (T4).

*Catecholamines are synthesized as follows:
1-Phenylalanine is hydroxylated to tyrosine. 2-Tyrosine is hydroxylated to DOPA
3-DOPA is decarboxylated to dopamine. 4- dopamine is hydroxylated to norepinephrine
5-Norepinephrine is methylated to epinephrine

Note: If step 1 is deficient→phenylketonuria. If step 2 is deficient→Albinism.

*they are degraded by monoamine oxidases (MAO, remove amine group from backbone) or Catechol-o-methyl transferase (COMT, add methyl to catechol ring). If we inhibit MAO→Active catecholamines→treat depression.

*Protein hormones: synthesis is by
1-One long protein, cleaved to many hormones (POMC→MSH,ACTH,endorphins)
2-Immature protein, cleaved to mature one (preproinsulin→proinsulin→insulin)
3-parent gene, surrounded by codons that make it express one hormone in one place and another hormone in another place (Neurophysin→Oxytocin or vasopressin).

*Protein hormones can be excreted in urine or degraded in plasma, but mainly they are degraded by endocytosis of the hormone, then the vesicle fuses with a lysosome (energy independent process).

*Intracellular proteins (not for protein hormones), they are degraded by the Ubiquitin-proteasomal pathway (energy dependent).