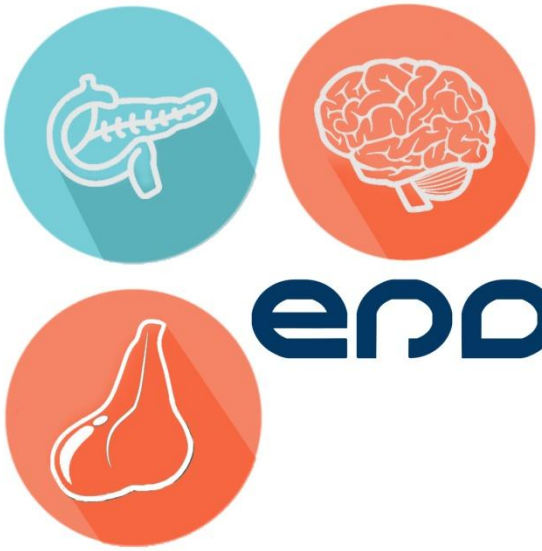

endocrine system

Biochemistry

Sheet Slide

number	1
Done by	Abdullah Al Zibda
Correction	AbdelMuez siyam
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In this sheet, we will discuss some focal points of endocrine biochemistry, biochemistry of hormones and receptors and we will introduce the topic of signal transduction. This sheet, as well as other biochemistry course sheets, **is summarized in a special section at the end of the sheet**. Make sure to follow up with the slides while studying the sheet.

What are hormones?

Hormones are body-synthesized chemicals which are secreted in *low amounts*. They control body systems physiology and functions. Chemically, they can belong to more than one family of molecules (proteins (majority), steroids or amino acid derivatives) as discussed later. Hormones can be classified according to many characteristics (source-target relation; structure; mechanism of action), as discussed later.

Functions of Endocrine System and Hormones

The endocrine system and the nervous system act individually and together to regulate the human body. They control body homeostasis all the time at all levels. They also affect each other; some hormones also work as neurotransmitters.

Hormones function in:

- They help maintain homeostasis

- Mediate responses to external stimuli

- Play roles in growth and development

Regulatory loops

Any system that regulates many functions must be highly regulated in the first place. Endocrine system can be controlled at the level of:

- The secreting gland (ultra-short loop)
- A gland controls other glands (short loop)
- The control of the last gland (long loop)

Slide 4

Focal biochemical insights in endocrinology

Some functional notes in endocrinology are chemically unjustified, so let us illustrate examples of these complex points, following them with biochemical discussion.

1- Hormones are secreted in low amounts. Moreover, when secreted in the blood, they get diluted. But still they perform their function that requires receptor saturation!

2- A hormone with a specific chemical structure performs different functions in the different body tissues while binding to the same receptors (present in different tissues).
3- Less than 50 hormones have been identified. Such small number of hormones controls all the cells in the body, which are 75 trillion in number and belong to more than 200 types.
4- 30 of the hormones produce the same second messenger; *cAMP*.
5- There are many other molecules in the body that have similar chemical features as hormones, but they cannot propagate messages like hormones; the receptors recognize the hormones and not the other similar molecules.

How is that?!

- Affinity: the receptors of the hormones a have high affinity to bind hormones in low concentrations and not the other molecules (complementary structures). *"Points 1& 5."* This concept is similar to the affinity of the enzymes to the substrates, which allows them to catalyze the reactions.

  Affinity is measured by K_d, or the association constant, which is equal to the division of the rate of dissociation on the rate of association. Its unit is mol/L. the lower the K_{ad} value, the more the affinity (more association; less dissociation), and vice versa.K_d values of the most hormone-receptor reactions run between 10^{-9} and 10^{-11}; very low values and so very high affinity, and so high specificity.

  K_d is interpreted as the concentration needed to cause effective binding. But effective binding is not enough to cause the response, but rather **saturation** is. For saturation to occur, a concentration of 20X of K_d value of the hormone is needed.

- Differences in gene expression between cells: although all cells share the same genetic material, they differ in gene expression. So, each type of cells has a distinctive set of proteins. cAMP, then, which is a second messenger helps in signal transduction as discussed later, will affect different proteins in different cell types; activating different pathways. *"Points 2;3."*

- Potency: different hormones have different potencies. So, if two hormones share the same second messenger and affect the same cell, saturation of one hormone's receptors will result with different rate of second messenger production than the saturation of another hormone's receptors.*"Point 4."*

Conclusion:

One hormone → several cell types

One cell type → several hormones

One hormone → several effects

Factors that 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

$Ka = [H\text{-}R] / \{[H] \times [R]\}$

$Kd = \{[H] \times [R]\} / [H\text{-}R]$

Receptors Biochemistry

Receptors are the first point of effect of hormones. Some present on the cell surface, and others present in the cell cytoplasm or nucleus (of lipid soluble hormones). The hormones whose receptors are on the cell surface will need a second messenger system to transduce the signal to the interior of the cell. However, lipid soluble hormones can directly cross the cell membrane, and so **do not** need that; in this case the functional unit is the hormone-receptor complex.

Receptors are proteins

Why are enzymes and receptors always *proteins* and not lipids, sugars or nucleic acids?

What is really striking about proteins is their ability to undergo structural conformational change after binding to specific molecules.

So, for example, a ligand may bind a receptor, which will induce a conformational change in the receptor, enabling it to induce a conformational change in bound proteins, like G protein as discussed later. Such conformational change is followed by the replacement of GDP to GTP at the α subunit. This will result in having another conformational change in the α subunit, which will dissociate from the G protein complex and bind to the following protein in the pathway, which may be an adenylyl cyclase, which will lead to a conformational change in the protein, activating it, and so forth.

Receptor domains

The domain is a part of the protein that is responsible for a special function. If a domain was cut and unfolded, it will refold back to its original form, and will be able to perform its function again. Knowing the domains of the different proteins helps us identify the function of the whole protein.

All receptors have at least two functional domains, which are:

 Recognition domain (binding domain): binds the ligand (in the cytoplasm, the nucleus or on the external leaflet of the plasma membrane).

 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, as dicussed in the following section.

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Signal Amplification

Amplification of signal allows the secreted hormone to exert its function even while being secreted in low concentrations. But if amplification did not exist, the needed hormonal concentration would become high, and that does not happen in nature, because:

1- Hormonal synthesis would then spend plenty of the body energy.
2- The secretion of hormones in low amounts allows their regulation to be easy. Such regulation would be harder if hormones were secreted in high amounts.
3- When high concentration is needed to perform a function, then such settings mean that the binding affinity is diminished, which means that the binding is not specific anymore, which is a disastrous fact that leads to no regulation of the physiological functions and metabolism in the best way.

Signal amplification can occur by:

- 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 classes

Hormones can be classified in many ways; according to the chemical composition; the solubility; location of receptors or the nature of the signal used to mediate hormonal action. Some of such classes are listed below:

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

- Chemical composition:

Polypeptides: such as Pituitary hormones; Hypothalamic releasing hormones; Insulin, Growth factors, and others. To this group belong the majority of the hormones in our body.

Amino acid derivatives: such as Adrenalin, Thyroid hormones. Such hormones are derived from amino acids, which get modified to form the hormone.

Steroids: cholesterol-related molecules.

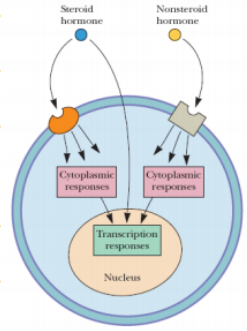
- Mechanism of action:

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

	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Action	Slow	Fast
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma $t_{1/2}$	Long (hrs - days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, kinase cascades, metabolites of phosphoinositols



Group I:

- These hormones have a carrier protein to carry them from the source to the target through the blood, since they are hydrophobic and cannot stand the blood environment. This slows their action; because

they need to bind and then must be released and then bind to the receptors, and each reaction is governed by the affinities between the compounds.

- Another thing that contributes to their slow effect is that they usually target the DNA, activating gene transcription and translation, and all these processes need time to produce the final function.
- This is also related to their long half-lives.
- No second messenger cascade is required in these hormones pathways.

Group II:

- These hormones are water-soluble, and so do not need carrier proteins. And so, they are fast and bind extracellular trans-membrane receptors. Their half-lives are short.
- A second messenger system is required to transduce the extracellular messege to the interior. In such pathways, signal amplification occurs.

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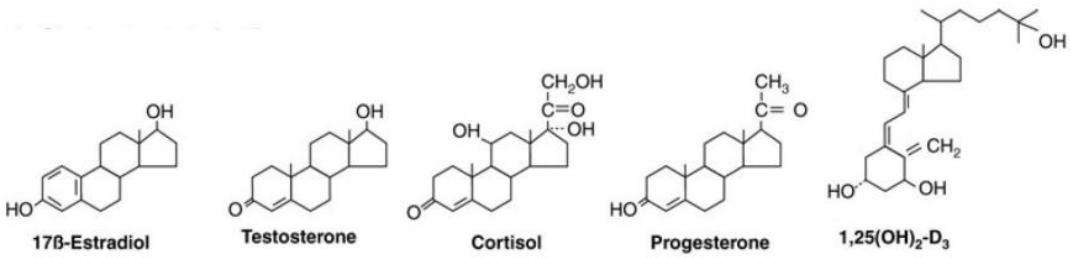
Examples on Hormones Families

Steroids

All steroid hormones are derived from cholesterol, and modifications on its structure give rise to the other hormones. They have four *steran* rings.

Structural note: one modification transforms Estrogen hormone to Testosterone.



A. Sex hormones - are divided into 3 groups

- Male sex hormones or Androgens

- Female sex hormones or Estrogens

- Pregnancy hormones or Progestines

B. Hormones of Adrenal Cortex

- Mineralocorticoids: aldosterone... .

-Glucocorticoids: cortisol... .

-Adrenal androgens: male sex hormones mainly dehydroepiandrosterone (DHEA) and testosterone

Non-steroid hormones

A. Peptide and protein hormones

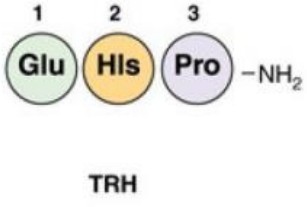
- All hypothalamic, pituitary, digestive hormones

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

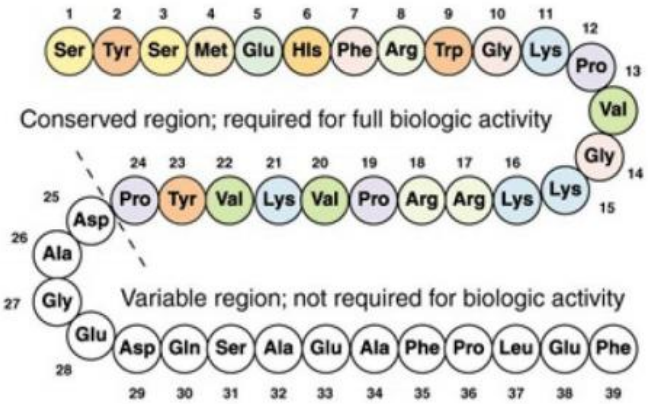
Hormone	Structure
GHRH	44
TRH	3
GnRH	10
CRH	41
ADH	9
Vasopressin	9
Angiotensin I	10
Angiotensin II	8
Insulin	51
Glucagon	29

These hormones vary in size. Numbers in the table are not for memorization, but you must know the distinctive ones, and which hormones are large or small. For example, Insulin is a large hormone, whereas TRH has only 3 amino acids, which is the shortest hormone ever known.Note that Angiotensin II is synthesized from Angiotensin I by angiotensin converting enzyme (therapy target), by removing two amino acids (10 to 8).

Note that Oxytocin and Vasopressin differ in 2 amino acids only, athough having the same number of amino acids (nine).

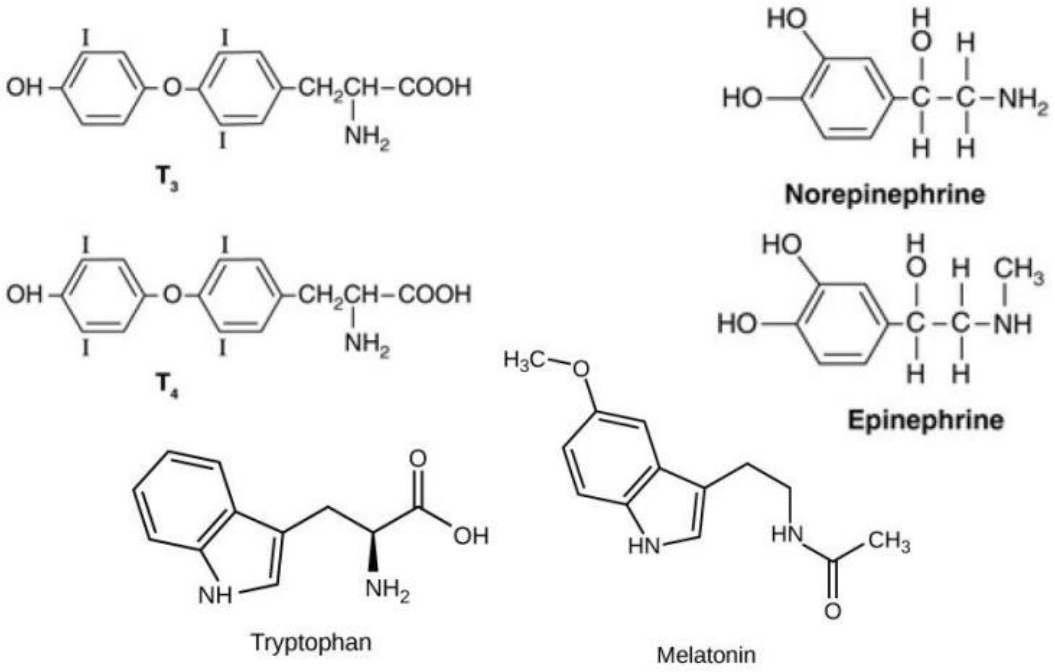


TRH



ACTH

B. Amino acid derivatives



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

Tyrosine has a phenol group. Addition of another phenol group gives thyroid hormones T_3 or T_4 (according to the number of iodine ions). Hydroxylation of Tyrosine results with DOPA. DOPA is decarboxylated to produce dopamine, which is converted to Norepinephrine. Epinephrine results from the methylation of Norepinephrine. Phenylalanine hydroxylation results with Tyrosine.

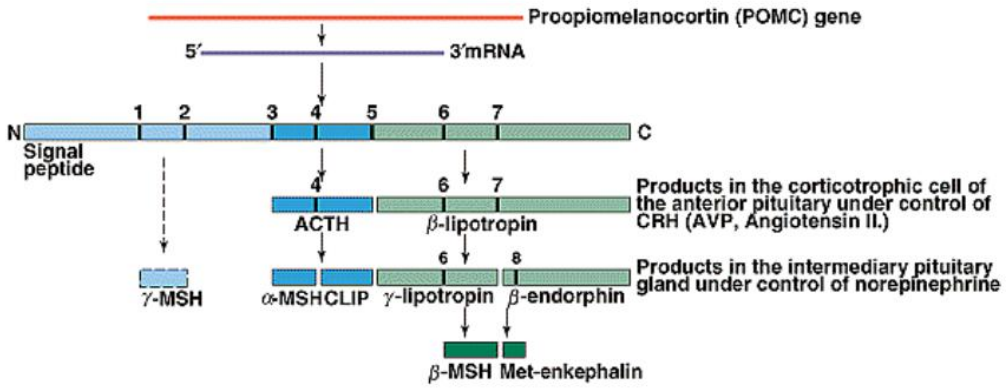
Tryptophan gives Serotonin after its decarboxylation, and gives Melatonin after its backbone methylation.

Peptide Hormones Synthesis

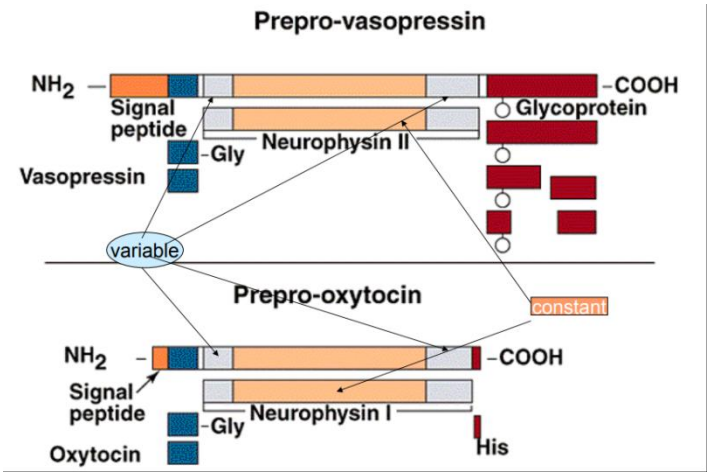
There are three ways of peptide hormones synthesis:

1- From precursor polypeptides: parent protein is fractionated into smaller single hormones
   - 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

Poly: not mature
opio: analgesics
Melano: Melanine-related
Cortine: Cortisol-related



2- From precursor polypeptides: protein coding sequence preceded by other sequences. Vasopressin and Oxytocin are an example. Synthesis occurs in separate cell bodies of hypothalamic neurons.



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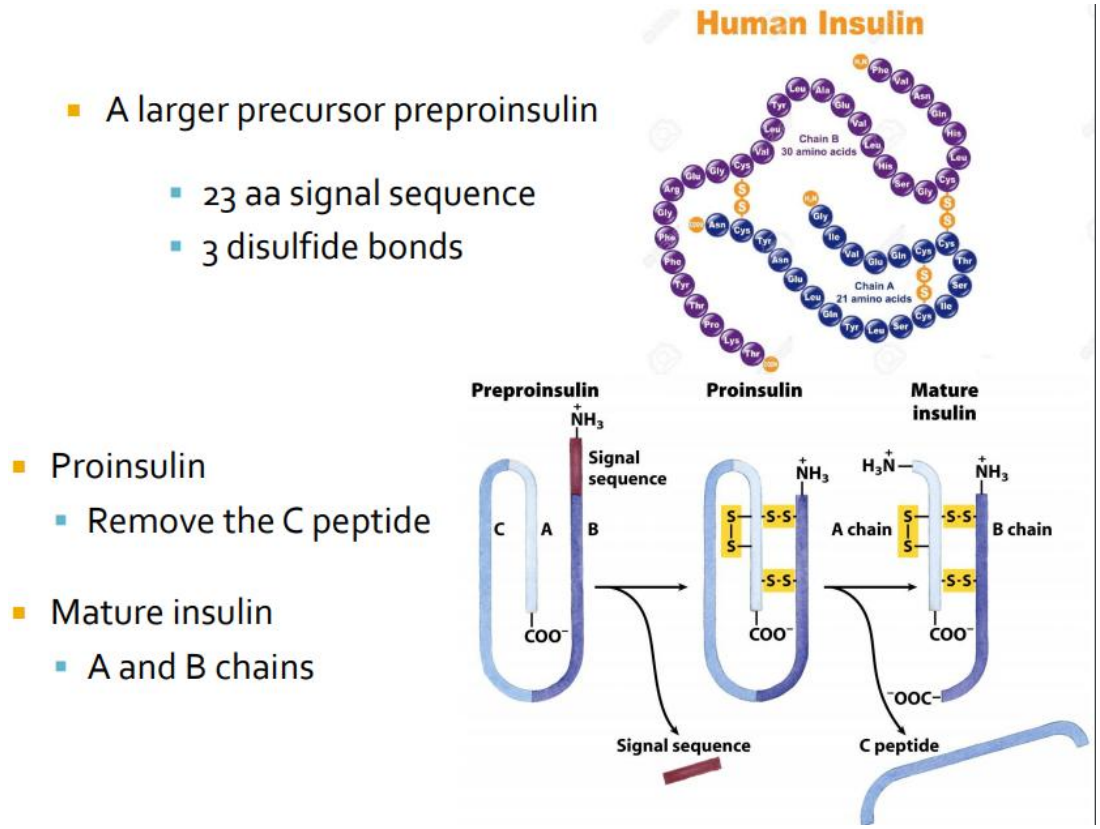
3- From Pre-pro-hormones: immature big protein is cut and modified to produce the active structure. An example is Insulin. Preproinsulin is turned into proinsulin by cutting the signal peptide. Insulin is produced from the proinsulin by cutting the C peptide. Insulin has 2 chains in its active structure: A and B.



Hormones Interactions on the Target Cell

As a cell may be affected by more than one hormone, they may have separate actions, or they be cooperate with each other. In the latter case, they may have many forms of interactions, which include:

1- Permissive effects: one hormone enhances the effect of a later hormone. Here, order is important; the first hormone prepares the cell to be affected by the second hormone. If preceded the first, the second cannot affect the cell.
   - Estrogen up-regulates progesterone receptors in uterus
   - Thyroid hormone increases the effect of epinephrine on breakdown of triglycerides in adipocytes
2- Integrative effects: hormones produce complementary effects on different tissues.
   PTH and Calcitriol increase ECF calcium.
3- Synergistic effects: boost the functions of each other. Both FSH and estrogen are necessary for normal oocyte development. FSH and testosterone together increase spermatogenesis.
4- Antagonistic effect: oppose each other; such as Insulin and Glucagon.

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.

Is that enough?

The need for second messenger:because of having few number of hormone molecules, in addition to the restriction in the movement of the trans-membrane receptors, which are stuck in the membrane.

Second messengers

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

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Membrane associated receptors

7-Trans-membrane Helix Receptors (7TM)

7TM is a receptor that has 7 α-helices which are swung in the cell membrane. 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?

Although the membrane interior is lipophilic, the H-bond rich helices stand stable inside. That is possible either by a little increase in the hydrophobic content of the helix **OR**. By maximizing all the H-bonds between the amino acids, and are hidden from the lipophilic environment, so the helix is hydrophilic in nature, but it cannot make any H-bonding with anything exterior.

How does it work?

Binding of the ligand induces the receptor conformational change, this activates G protein. In some part of its job, serine and Threonine residues get phosphorylated. (to be discussed).

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.

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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
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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

Signal amplification can occur by:

- 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
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Angiotensin I	10
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- 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):
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- 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.

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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.

  <u>Refer to the slides for visual illustrations and chemical structures.</u>

<u>END</u>