Mechanism of action-1

- receptors: mediators of hormone action, membrane associated vs. intracellular
- receptors: measurements of receptor - ligand interaction, regulation mechanism
- surface-receptors: kinases, phosphatases, G activities, ligand-gated ion channels
- intracellular receptors: steroid, thyroid, retinoid and arylhydrocarbon receptors
- mechanism of thyroid, gonad and adrenal hormone action: gene regulation by T3, P4,T, E2, ALD, CORT and their receptors
- in addition, neurosteroids’ mechanism of action: genomic actions, actions at cell surface through their membrane receptors
- genetic control of hormone biosynthesis
- permissive action, steroid / thyroid hormone
- endocrine pathologies, action mechanism
- a review using expression of a polypeptide hormone controlled by a liposoluble hormone

Today’s lecture

- sensor
- negative feedback “story line”
- integrator center
- a “reflex arc” a base for a control model
- diagram for a control system as that present in a refrigerator
- effector
- efferent “story line”

... if story lines are linked through an integrator, then you have “control”...
Today’s lecture

For each hormone, the student should know
1. Its cell of origin
2. Its chemical nature, including
   a. Distinctive features of its chemical composition
   b. Biosynthesis
   c. Whether it circulates free or bound to plasma proteins
   d. How it is degraded and removed from the body
3. Its physiological actions
   a. At the whole body level
   b. At the tissue level
   c. At the cellular level
   d. At the molecular level
   e. Consequences of inadequate or excess secretion
4. What signals or perturbations in the internal or external environment evoke or suppress its secretion
   a. How those signals are transmitted
   b. How that secretion is controlled
   c. What factors modulate the secretory response
   d. How rapidly the hormone acts
   e. How long it acts
   f. What factors modulate its action

I suggest you put this information into a table YOU design!!!

Membrane

- What does a cell membrane look like?
- What does a receptor look like?
- What does a receptor in a membrane look like?
- What does an enzyme look like?
- What does an enzyme in a membrane look like?
- What does an ion channel look like?
- What does an ion channel in a membrane look like?
All Receptors

• How do hormones and receptors interact?
• What is affinity and what is specificity?
• What is a conformation change?
• What is the relation between binding and biological effect?
• What are spare receptors?
• What is the life cycle of a hormone receptor?

\[
\begin{align*}
H + R &\rightleftharpoons HR \\
\text{bound hormone (HR)} &
\end{align*}
\]

\[
\begin{align*}
\frac{H \cdot R}{HR} &= \frac{k_1}{k_2} = k_d \\
= &\text{bound hormone (HR)}
\end{align*}
\]

Scatchard plot

single binding
slope = -1/kd
capacity

double binding

half saturation
affinity = kd

bound hormone (HR)

free hormone (H)

binding capacity

high affinity / low capacity
low affinity / high capacity

All Receptors

Specific hormone binding

% reduction in the number of receptors

number of receptors occupied for maximal biological response (100%)

number of occupied receptors per cell x 1000

biological response as % of maximum

hormone concentration (M)

0 5 10 15 20

0 5 10 15 20

0 50 100

10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7}

0 50 100 150 200

0 5 10 15 20

0 5 10 15 20

0 10 20

0 100 200 300 400

0 10 20 30 40

0 50 100 150

10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7}
Membrane Receptors

Transducer, comparator, amplifier, crosstalk

Membrane Receptors

Surface - receptors: kinases, phosphatases and GC activities, ligand-gated ion channels, transport

Golgi

RER

Lysosomes

mRNA

nucleus

Next lecture

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Nuclear Receptors

- gene, exons, introns
- cis - acting, trans - acting
- transcription, translation
- splicing
- RNA cap
- polyA tail

- Hormonal action can be regulated at the level of transcription, translation, RNA turnover, protein turnover, and post-translational modification.

Nuclear Receptor Structure

<table>
<thead>
<tr>
<th>Protein</th>
<th>DNA Binding Region</th>
<th>Hormone Binding Region</th>
<th>COOH</th>
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<tbody>
<tr>
<td>E2</td>
<td>1-563</td>
<td>1-946</td>
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<tr>
<td>P4</td>
<td>1-946</td>
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<td>CORT</td>
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<td>1-917</td>
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<tr>
<td>Vit D</td>
<td>1-427</td>
<td></td>
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</tr>
</tbody>
</table>
Zinc Fingers Structure

Structure of nuclear receptors

General modular structure

- Modulator domain
- AF-1 function
- Serine/threonine phosphorylation targets
- DNA binding
- Zinc fingers
- Hinge
- Ligand binding

Structure of zinc finger

Zinc finger module 1

NH₂
P box

Zinc finger module 2

D box

COOH

Transcription Factors Structure

Structure of transcription factors

Leucine zipper

DNA binding domain

Helix-loop-helix

Leucine residues
DNA methylation is a phenomenon occurring on the DNA known to consist of four bases. One of them, cytosine, exists in a "normal" and a methylated version, i.e., with a methyl group attached, but only when directly followed by the base guanine. The consequences of methylation lie in the regulation of gene expression: methylated cytosines in the promoter region of a gene lead to inactivation, thus acting as an "on" and "off" switch for genes. This is a naturally occurring mechanism to prevent all genes in a tissue/cell to be expressed at a time. As all cytosines in a CG-context (i.e., in front of a guanine) are known, it is possible to analyze the patterns of methylated and unmethylated cytosines in the genome and to identify the pattern that is typical for a specific tissue and type of disease. Once differentially methylated cytosines for a certain disease are known, their detection enables an exact diagnosis at a very early stage, molecular classification and the likely reaction of a patient to treatment. Epigenomics can obtain this information based on its robust proprietary technology.
Intracellular receptors for steroids are transcription factors. Their Zn fingers are binding regions which attach to the promoter segment of DNA.

Nuclear Receptor for Steroids

Inactive transcription

Active transcription

Nuclear Receptor for T3 Hormone

Inactive transcription

Active transcription
Nuclear Receptor for T3 Hormone

• In its “free” state T3R binds to its HRE as homo-dimer, or as a hetero-dimer with retinoid-X (e.g. Vit A). The carboxy-terminus of T3R interacts with TFIIIB preventing the formation of a stable pre-initiation complex and, together with a co-repressor, silences transcription.

• Upon T3 binding, its receptor undergoes a conformational change (“magic”), dissociation of the co-repressor, a decreased interaction of the T3R with the carboxy-terminus TFIIIB and an increase interaction of the T3R amino-terminus with TFIIIB.

• These changes facilitate TFIIIB binding an assembly of a stable pre-initiation complex, the binding of RNA polymerase II and the activation of transcription initiation.

Efficiency, permissiveness

cAMP ----> PKA ----> channel / enzyme

Protein synthesis

Steroid S + R ----> SR

DNA

additional transcription factor

mRNA

Cellular response

Efficiency, permissiveness

DNA
Permissive Action

- Action on specific mRNA synthesis could cause an increase in the number of membrane receptors, which might increase the production of cyclic nucleotides, thus leading to an increase cellular response to hormones acting on the plasmalemma.

- Thyroid or steroid hormones could increase or decrease the amount of cyclic nucleotide - dependent protein kinases PK or amount of substrate available for phosphorylation by cAMP or cGMP - dependent PK.

- Thyroid and steroid hormones could enhance the synthesis of a protein that could act as an inhibitor of another protein (e.g., phosphoprotein phosphatase) whose action is antagonistic to cyclic nucleotide action.

Pathologies

- Theoretically, genetic pathologies can be associated with each step of the biosynthetic pathway leading to the production of a particular enzyme or protein.

- Congenital adrenal hyperplasia due to gene deletion or to point mutation of the 21 - hydroxylase enzyme (“beard lady”).

- Testicular feminization due to point mutations scattered throughout the androgen receptor gene, cause decrease amounts of functional androgen receptors, altered sexual differentiation &feed-back regulation (“beware of single bars”).

- Vit D - dependent rickets due to a single point mutation in tip of one of theDNA - Zn fingers binding domain of Vit D receptor, thus making it unable to interact and transcriptionally regulate Vit D - responsive genes (“link to rickets and osteoporosis”).
For example, let us consider the effect of a steroid on the genomic regulation of a peptide hormone. (the mechanism of action of a peptide hormone will be covered next lecture)
“Consensus” gene encoding a prototypical peptide hormone

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Endocrine Physiology

levels of organization

structure - function

homeostatic regulation

Potential control points for regulation of gene expression in hormone production
Models for activation of gene expression

“Cis” model

“Trans” model

Consensus gene

Mechanisms of transcriptional repression

Competition
Sequestration
Quenching / tethering
Active

(“or fat Albert and the buck - buck game”)
Activation of specific transcription factors by steroids

Cell-surface receptor coupled signal transduction pathways involved in activation of nuclear transcription factors
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