• receptors: mediators of hormone action, membrane associated vs. intracellular
  – receptors: measurements of receptor - ligand interactions, regulation mechanisms
  – surface - receptors: kinases, phosphatases G couple receptor (GCPR) activities, ligand-gated ion channels
• intracellular receptors: steroid, thyroid, retinoid and arylhydrocarbon receptors
  – genetic control of hormone formation
  – permissive actions, steroids / thyroid hormones
  – endocrine pathologies, action mechanism
  – review using expression of a polypeptide hormone as example

Mechanism of action-1

This lecture main topics

• mechanism of steroid hormone action: genomic actions, actions at the cell surface, neurosteroids
• mechanism of thyroid hormone action: gene regulation by T3 and the thyroid hormone receptor

Today’s lecture

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

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

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 principal 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

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 ?
• 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?

**Scatchard plot**

- bound / free
- H R / H
- single binding slope = -1/k_d
- double binding
- capacity
- bound hormone (HR)

**Receptor-1**

- k_1
- k_2
- H R
- H R
- = k_1
- k_2
- = k_d
- bound hormone (H R)
- half saturation
- affinity = k_d
- free hormone (H)
- high affinity / low capacity
- low affinity / high capacity

**Receptor-2**

- number of occupied receptors per cell x 1000
- biological response as % of maximum
- % reduction in the conc. of receptors
- number of receptors occupied for maximal biological response (100%)
- Specific hormone binding
- Biological response
- hormone concentration (M)
Receptor-3

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

Golgi

RER

mRNA

Lysosomes

Golgi

RER

mRNA

Lysosomes

Receptor-3

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

Transducer, comparator, amplifier, crosstalk

Gs

GI

AC -> cAMP -> PKA -> intermediaries

AC -> cAMP -> PKA -> intermediaries

AC -> cAMP -> PKA -> intermediaries
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.
Zinc Fingers Structure

Transcription Factors Structure
DNA methylation is a phenomenon occurring on the DNA known to consist of four bases. One of them, cytosine, exists in a "normal" and in 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.
Zn fingers are binding regions of transcription factor proteins which attach to the promoter segment of DNA.
Nuclear Receptor for Thyroid H.-2

- In its “free” state T3R binds to its HRE as homodimer, or as a heterodimer with retinoid-X. The carboxy-terminus of T3R interacts with TFIIB preventing the formation of a stable preinitiation complex and, together with a co-repressor, silences transcription.
- Upon T3 binding, its receptor undergoes a conformational change, dissociation of the co-repressor, a decreased interaction of the T3R with the carboxy-terminus TFIIB and an increase interaction of the T3R amino-terminus with TFIIB.
- These changes facilitate TFIIB binding an assembly of a stable preinitiation complex, the binding of RNA polymerase II and the activation of transcription initiation.

Efficiency, permissiveness

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

Cellular response

Protein synthesis

mRNA

Steroid S + R ----> SR

DNA

additional transcription factor
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

• Testicular feminization due to point mutations scattered throughout the androgen receptor gene, cause decrease amounts of functional androgen receptors, altered sexual differentiation and feed-back regulation

• Vit D-dependent rickets due to a single point mutation in the tip of one of the Zn fingers of the DNA binding domain of the Vit D receptor, thus making it unable to interact and transcriptionally regulate Vit D-responsive genes
“Review” of the mechanism of action of intracellular receptors

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)

General scheme of steroid hormone action. Steroid hormones penetrate the plasma membrane and bind to intracellular receptors in the nucleus or cytoplasm. Hormone binding activates the receptor, which forms complexes with other proteins and binds to specific acceptor sites (hormone response elements, HRE) on DNA to initiate transcription and formation of the proteins that express the hormonal response. The steroid hormone then is cleared from the cell.

“Consensus” gene encoding a prototypical peptide hormone

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)

Activation of steroid hormone receptors. Inactive receptors associated with other proteins react with hormone, shed their associated proteins, and change their conformation. They can then form dimers that bind DNA and a variety of nuclear peptide regulators of gene transcription. 59 kDa = a protein with a mass of 59 kilodaltons; Hsp90 = 90 kDa heat shock protein; Hsp70 = 70 kDa heat shock protein.
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

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)

Translation. A molecule of transfer RNA (tRNA) charged with its specific amino acid, phenylalanine, and already linked to the growing peptide chain, is positioned on the mRNA by complementary pairing of triplet of nucleotides with its codon of three nucleotides in the mRNA. A second molecule of tRNA charged with its specific amino acid, tryptophan, has docked at the adjacent triplet of nucleotides and awaits the action of ribosomal enzymes to form the peptide bond with phenylalanine. Linking the amino acid to the peptide chain releases it from its tRNA and allows the empty tRNA to dissociate from the ribosome. A third molecule of tRNA, which brought the preceding molecule of leucine, is departing from the left, while a fourth molecule of tRNA, carrying its cargo of glutamine, arrives from the right and waits to form the complementary bonds with the next codons in the mRNA that will bring the glutamine in position to be joined to tryptophan at the carboxyl terminus of the peptide chain. The ribosome moves down the mRNA adding one amino acid at a time until it reaches a stop codon. (Adapted from Alberts et al. (1994) Molecular Biology of the Cell. New York: Garland Publishing.)
"Consensus" gene encoding a prototypical peptide hormone

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)

Post-translational processing. The leader sequence or signal peptide of proteins destined for secretion enters the cisternae of the endoplasmic reticulum even as peptide elongation continues. In the endoplasmic reticulum (1) the leader sequence is removed, (2) the protein is folded with the assistance of protein chaperons, (3) sulfhydryl bridges may form, and (4) carbohydrate may be added (glycosylation). The partially processed protein (5) is then entrapped in vesicles that bud off the endoplasmic reticulum and (6) fuse with the Golgi apparatus, where glycosylation is completed, and (7) the protein is packaged for export in secretory vesicles in which the final stages of processing take place.
“Consensus” gene encoding a prototypical peptide hormone

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)

Exocytosis. 1. Immature secretory vesicles bud off the trans-Golgi stacks. 2. Maturation of the vesicle includes emission of some proteins and water, acidification of vesicle contents, and condensation of enclosed proteins to form dense core granules. 3. Upon receipt of a signal for exocytosis (i.e., the presence of the peptide hormone), the vesicle is recruited to the plasma membrane. 4. In preparation for exocytosis, the vesicle is tethered to the membrane (docking). 5. An energy-dependent interaction forms a loose association of special proteins (SNARE proteins) in the membranes of the vesicle and the plasma membrane, "priming" the vesicle to respond to a secretory stimulus. 6. Secretion is triggered by an increase in cytoplasmic calcium that produces conformational changes in the SNARE proteins that brings the membranes into close apposition so that fusion occurs and a secretory pore is formed. 7. Expansion of the pore as the vesicle membrane is incorporated into the plasma membrane releases vesicular contents into the extracellular fluid.

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, or leap frog, game")

Activation of specific transcription factors by steroids
Cell-surface receptor coupled signal transduction pathways involved in activation of nuclear transcription factors