# Veterinary Bioscience: Cells to Systems

## Lecture 13 – Receptors and signalling pathways 2:

## G-protein receptors, enzyme receptors and intracellular receptors

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**Intended Learning Outcomes**

1. Describe how different types of G proteins (Gs, Gi ,Gq, Gt signalling) can be linked to different intracellular signalling pathways, in order to understand how drugs and hormones stimulating G Protein-coupled receptors cause their actions.
2. Describe how endothelial cells can be stimulated to produce nitric oxide; in order to compare this mechanism of vasodilation with the actions of drugs directly targetting vascular smooth muscle.
3. Describe how enzyme-linked receptors activate downstream effector proteins; in order to understand how hormones such as growth factors and insulin cause their action.
4. Understand how some hormones act through intra-cellular receptors, leading to changes in gene transcription via hormone response elements; in order to appreciate the longer time course over which these hormones cause their effects.

**Keywords**
Cell signalling, receptors, signal transduction, G Protein-coupled receptors, G proteins, enzyme linked receptors, intracellular receptors, hormone response elements.

**G protein families**

| G protein | Receptor | Signalling pathway |
|---|---|---|
| Gs | $\beta$-adrenergic receptors, glucagon, histamine, serotonin | Stimulatory, increase cAMP |
| Gi | $\alpha$2-adrenergic receptors | Inhibitory, decrease cAMP |
| Gq | $\alpha$1-adrenergic receptors, some muscarinic receptors; histamine | IP3, DAG<br>Increase cytoplasmic $Ca^{++}$ |
| Gt | Light receptors in eye | Transducin, Increase cGMP phosphodiesterase (catalytic) Decrease cGMP |

**Gq proteins acting via a phospholipase (in cell membrane).**
Activation of a Gq protein activates phospholipase C which activates IP3 and DAG. These in turn activate **protein kinase C** and **Calmodulin** dependent protein kinases. $\alpha$1 adrenergic receptor when occupied by adrenalin promotes an intracellular amplification cascade which does not result in the elevation of cAMP levels. Instead, it uses the Gq protein and activates the enzyme PLC (phospholipase C) which mediates the breakdown of a lipid molecule, phosphatidyl-inostol diphosphate (PIP2), into inositol triphosphate (IP3) and diacylglycerol (DAG).

IP3 mediates the release of intracellular $Ca^{2+}$ from the endoplasmic reticulum to the cytosol and $Ca^{2+}$ ions, along with DAG, the other product of PIP2 hydrolysis, bind to and thereby activate **protein kinase C (PKC)**, an enzyme distinct in specificity to the cAMP-dependent protein kinase. PKC, when active, can

phosphorylate other proteins and either activate or inhibit their enzymatic activities. The end result of the cascade initiated by PKC activation depends upon the tissue or cell type which is triggered by hormone but PKC mediated cascades often result in cellular activation.

## $Ca^{2+}$ & Calmodulin

$Ca^{2+}$ ions are important second messengers for many responses. $Ca^{2+}$ is removed from the cytoplasm by $Ca^{2+}$pumps or Na/$Ca^{2+}$ exchangers so levels in cytoplasm are normally low. $Ca^{2+}$ is stored in the endoplasmic reticulum (ER) lumen or in mitochondria. IP3 releases $Ca^{2+}$ from the ER.
One of the subunits of phosphorylase kinase is the protein calmodulin: it is a calcium binding protein which undergoes a pronounced conformational shift upon $Ca^{2+}$ binding. This shift is transmitted allosterically to the active site of the enzyme which increases catalytic efficiency; In the case of adrenalin the cascade is accelerated. Calmodulin occurs in many cell types and acts as an accelerating protein cofactor or enzyme modifier for a number of enzymes only if $Ca^{2+}$ levels are high. It accounts for the ability of $Ca^{2+}$ to act as a second messenger. Example given in lectures, Acetylcholine binding M3 Gq receptor on endothelial cells leading to release of calcium and calmodulin activation of NO synthase.

## $G_t$ proteins regulating ion channels

Some G proteins regulate $Na^+$ channels via a phosphodiesterase. Used in photo transduction. A photon interacts with a receptor (rhodopsin) in the retina of the eye and activates $G_{t\alpha}$ protein–transducin. The subunit activates phosphodiesterase which hydrolyzes and lowers cGMP levels. This closes cGMP activated $Na^+$ channels which are kept open in the dark.

## G βγ subunits can also interact with downstream effectors.

Para sympathethic nerves in the heart release acetylcholine which binds to its muscarinic $M_2$ receptor and activates a coupled $G_i$ protein. $G\gamma\beta$ complex (not $G\alpha$) is the signalling component and binds to and opens a $K^+$ channel that reduces heart rate. The $K^+$ channel recloses when the $G\alpha$ subunit inactivates itself by hydrolysing its bound GTP and reassociates with the $\gamma\beta$ complex to form an inactive G protein.

## NITRIC OXIDE SIGNALLING

Nitric oxide (NO) is a very small signalling molecule that plays an important role in controlling blood flow and blood pressure. The binding of acetylcholine, bradykinin, shear stress or adenine nucleotides causes the release of NO in vascular endothelial cells that causes the smooth muscle cells surrounding the blood vessels to relax causing vasodilation. In response to stimuli such as acetylcholine, NO synthase is activated within vascular endothelial cells

1. Binding of acetylcholine to Gq protein linked receptors on endothelial cells causes $IP_3$ production.
2. $IP_3$ releases calcium ions from endoplasmic reticulum.
3. $Ca^{2+}$ ions and calmodulin form a complex which activates NO synthase which converts arginine to citrulline and NO.
4. NO rapidly diffuses from endothelial cell into adjacent smooth muscle cells.
5. Acts only locally - quickly converted to nitrates and nitrites (half-life 5-10 sec).
6. In smooth muscle cell, NO activates a soluble guanylyl cyclase (in the cytoplasm) to make cyclic GMP (cGMP) from the nucleotide GTP.

cGMP activates protein kinase G relaxes muscle proteins by phosphorylating muscle proteins which result in vasodilation of the blood vessels and increase blood flow. Sildenafil (Viagra) dilates pulmonary blood vessels (and enhances penile erection) by blocking the degradation of cGMP prolonging the NO signal. Whereas nitroglycerine, which gets converted to NO in the circulation, is used to treat heart failure by reducing systemic vascular resistance and dilating coronary blood vessels.

**3) CATALYTIC ENZYME-LINKED RECEPTORS**

Ligand activates a receptor that is also an enzyme. Activation of receptor causes receptor dimer formation which activates their kinase function and allows them to phosphorylate one another. This process in turn activates a complex of intracellular signalling proteins.

Examples include receptors for insulin , insulin like growth factor, epidermal growth factor (EGF), nerve growth factor (NGF) and platelet derived growth factor (PDGF).

These receptors contain both $\alpha$ and $\beta$ subunits. The $\alpha$ subunit is on the exterior membraneside and binds the hormone and the $\beta$ subunit which traverses the membrane contains the enzyme tyrosine kinase.

1) The insulin receptor phosphorylates tyrosine residues (autophosphorylation) and insulin- receptor substrates (IRS). The IRS proteins are docking proteins to which various downstream effector proteins bind and become activated. Phosphatidylinositol 4,5-bisphosphate (PIP2) becomes phosphorylated to form PIP3, and it leads to major changes in glucose and protein metabolism eg activation glycogen synthetase and recruitment of glut 4 transporters

**Key aspects of hormone signalling via cell surface receptors**

1) **Specificity:**
   a) A high receptor ligand specificity ensures that only the required target cell is influenced by the first messenger.
   b) The binding site and ligand share complementary structures
   c) Non-covalent interactions similar to enzyme-substrate and antigen - antibody
   d) Ligand binding to the receptor relies on the law of mass action
2) **Amplification**
   a) First messengers are often short-lived and in low concentrations
   b) Induce key intracellular signalling proteins to behave as a molecular switch.
   c) Amplification proceeds via enzyme cascades
3) **Integration**
   a) Cells frequently receive multiple signals
   b) There are many reciprocal pathways within cells (e.g. glycolysis & gluconeogenesis)
   c) Pathways and systems controlling them must be coordinated to provide an integrated cellular response
4) **Rapid decay**
   a) Since this category of hormones is designed primarily for short-term, reversible messages the system must **decay** relatively rapidly.
5) **Desensitization**
   a) The aim is to produce a rapid and major cellular response to a transient signal
   b) But occasionally the signal persists and the threshold for eliciting the transduction pathway is increased – i.e. sensitivity to 1st messenger. Cells develop a decreased responsiveness following repeated agonist binding.
   c) The desensitisation is often achieved by a feedback loop
   d) The target may be the receptor – affinity, activity or expression

Often desensitization of the cell to a ligand depends upon receptor down-regulation which occurs by removal of the receptor from the cell surface by endocytosis or alterations to the receptor that reduces its capacity to bind its ligand.

Other mechanisms include receptor uncoupling via molecules such as $\beta$-arrestin that bind to phosphorylated G proteins phosphorylated by G protein coupled receptor kinases. $\beta$-arrestin uncouple the G$\alpha$ subunit of heterotrimeric G proteins.

The response of a cell to a particular signalling molecule varies not only as to whether specific receptors exist but also depends on the function of the cell and therefore what proteins and molecules are present within that cell. For example, the neurotransmitter acetylcholine exerts different effects on specialized cells. Heart muscle cells respond to acetylcholine by reducing the force and rate of contractions whereas salivary cells respond to the same signal (acetylcholine) by secreting saliva.

## HORMONES THAT BIND TO INTRACELLULAR RECEPTORS

Nuclear receptors (NR) are important in cell signalling as they act as transcription factors. A number of important hydrophobic signalling molecules such as steroid hormones, thyroid hormones, Vitamin A & D and retinoic acid produce their effects by binding to **intracellular receptors** located either in the cytoplasm or nucleus. The ligand bound intracellular receptors are transcription factors that regulate the expression of target genes by binding to specific DNA sequences termed **hormone-responsive elements** (HRE) or nuclear receptors.

There are two main classes of nuclear receptors. Class I receptors consist of steroid hormone receptors for androgen estrogen and glucocorticoid hormones. Here the NR in absence of the ligand is located in the cytosol. Cortisol diffuses across cell membrane into cytoplasm. Hormone binding to NR in cytoplasm results in dissociation of heat shock proteins, dimerization of receptors and translocation via nuclear pores to the nucleus where they bind to glucocorticoid response elements leading to transcription. The various nuclear receptors display specific cell and tissue distributions thus the genes affected depend upon the complement of receptors in the cell and the affinity of these receptor-ligand complexes for a particular HRE.

Non-steroidal hormone receptors form class II and include retinoid acid receptor (RAR) and thyroid hormone receptor (TR) that are located in the nucleus. Thyroid hormone is transported across cell membrane and enter the nucleus. The TR heterodimerized to retinoic receptor is bound to a corepressor protein and thyroid (ligand binding) results in dissociation of the corepressor and recruitment of a co-activating protein. Activates a RNA polymerase that leads to transcription of a selected set of genes.

The intracellular localization of different unoccupied receptors varies. The glucocorticoid and mineralocorticoid receptors are mainly cytoplasmic, the estrogen and progesterone receptors are primarily nuclear and the thyroid and retinoic acid receptors are bound to DNA. Cytoplasmic receptors are complexed to heat shock proteins (HSPs). Hormonal binding causes a conformational change in these receptors and stimulates dissociation of inhibitory HSPs from the receptors. HSPs stabilize the receptors and prevent receptor binding to DNA. This dissociation permits the receptors to dimerise. The complexes then translocate to the nucleus where they bind to DNA and activate or inhibit certain genes. The HRE is located in the promoter, which is ‘upstream’ from the hormone-responsive genes. The homone receptor complex is then thought to act as a transcription factor, modulating the transcription rate for hormone-responsive genes and in some cases altering post-transcriptional steps, thereby changing the levels of specific messenger RNAs (mRNAs). The various nuclear receptors display specific cell and tissue distributions thus the genes affected depend upon the complement of receptors in the cell and the affinity of these receptor-ligand complexes for a particular HRE.

## BIBLIOGRAPHY:


1. Boron WF and Boulpaep EL: Medical Physiology, 3rd Ed. Elsevier, 2017.
2. Costanzo, LS: Physiology, 6th Ed. Elsevier, 2018.
3. Hall JE: Guyton and Hall Textbook of Medical Physiology, 13th Ed. Elsevier, 2016.
4. Koeppen BM and Stanton BA: Berne & Levy Physiology, 7th Ed. Elsevier, 2018.
5. Voet, D et al. Fundamentals of Biochemistry.5$^{th}$ Edition 2016