Veterinary Bioscience: Cells to Systems

Lecture 12 – Receptors and signalling pathways 1:
Signal transduction and second messengers

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Intended Learning Outcomes

1. Describe the main signal transduction pathways involved in cell signalling: i) ligand- gated ion channels, ii) G-protein coupled receptors, iii) receptor enzymes and iv) nuclear receptors (class I and class II); in order to understand how drugs and hormones stimulating these pathways cause their actions.
2. Describe through the use of examples how G protein signal transduction pathways are regulated; in order to understand how hormone and drug actions are modulated and switched off.
3. Describe how bacterial toxins such as cholera toxin and pertussis toxin are able interfere with heterotrimeric G protein signalling; in order to understand how bacterial toxins may cause disease via these signalling mechnisms.

Keywords
Cell signalling, receptors, signal transduction, ion channels, G Protein-coupled receptors, G proteins.

SIGNAL TRANSDUCTION PATHWAYS

The ability of cells or tissues to respond to a particular hormone or signalling molecule (**ligand**) is governed exclusively by the presence of specific **receptor** molecules either upon plasma membranes or within responsive cells. Each cell has only a limited set of receptors (out of the thousands possible) so each cell is therefore able to restrict the range of signals that it responds to.

Ligand binding to its receptor induces profound changes within target cells. There are three fundamental mechanisms by which such changes occur:

1. Altered ion channel permeability.
2. Activation of enzymes and other dynamic molecules: Most enzymes shuttle between conformational states that are catalytically active versus inactive, on versus off. Many hormones affect their target cells by inducing such transitions, usually causing an activation of one of more enzymes. Because enzymes are catalytic and often serve to activate additional enzymes, a seemingly small change induced by hormone-receptor binding can lead to widespread consequences within the cell.
3. Modulation of gene expression: Stimulating transcription of a group of genes clearly can alter a cell's phenotype by leading to a burst of synthesis of new proteins. Similarly, if transcription of a group of previously active genes is shut off, the corresponding proteins will soon disappear from the cell.

The binding of the specific receptor by the ligand signals the cell by the process of **signal transduction** whereby the message is converted from one form to another.

HORMONE RECEPTORS AND SIGNAL TRANSDUCTION

Signal transduction is any process by which a cell converts one kind of signal or stimulus into another. Processes referred to as signal transduction often involve a sequence of biochemical reactions inside the cell, which are carried out by enzymes and linked through second messengers. Such processes take place in as little time as a millisecond or as long as a few seconds. Slower processes are rarely referred to as signal transduction.
In many transduction processes, an increasing number of enzymes and other molecules become engaged in the events that proceed from the initial stimulus. In such cases the chain of steps is referred to as a "signaling cascade" or a "second messenger pathway" and often results in a small stimulus eliciting a large response.

1. The ligand (external signal) is the primary messenger.
2. Receptor binding produces second messengers (intracellular signalling molecules) within the target cell.
3. These relay and often amplify in a cascade the cellular response.

All hormone receptors exhibit **specificity** and **saturability.** For example adrenaline (adrenergic) receptors have the capacity to bind adrenalin, but not insulin or testosterone or other unrelated hormones. They can, however, bind molecules structurally related to adrenaline which may alternatively compete with **(antagonists)** or replace **(agonists)** adrenaline.

Considerable information about a how a hormone acts can be gained by knowing the type of receptor it uses. Despite the molecular diversity of hormones, all hormone receptors can be categorized into one of two types, based on their location within the cell:

1. Cell surface receptors.
Hydrophilic hormones such as insulin and adrenaline cannot diffuse across the plasma membrane and indeed are not required to enter cells to mediate their effects. Instead they bind to specific receptors located in the plasma membrane of the responsive cell and this engagement results in the **transduction** of a signal across the plasma membrane.

2. Nuclear or cytoplasmic receptors.
Hydrophobic hormones (e.g. steroids and thyroid hormones) are able to traverse the plasma membrane even without a specific transport system. Hormones of this type often have specific binding molecules (receptors) located either within the cytoplasm or the nucleus of the responsive cell.

CELL SURFACE RECEPTORS

Serum hormone concentrations are extremely low (10^{-9}M to 10^{-11}M) and therefore these receptors must have high affinity for the hormone. When a membrane impermeable hormone (or ligand) such as adrenalin or insulin binds to its receptor, it activates the receptor. This binding to its receptor occurs through a number of specific weak non-covalent bonds by fitting into a specific binding site. High receptor affinity occurs when low concentrations of a ligand will result in binding of most of the cognate receptors whereas low receptor affinity occurs when a high concentration of the ligand is required for most receptors to be occupied. The activation of the receptor by its ligand typically involves a conformational change in the receptor protein (e.g., it undergoes changes in the folding of its primary amino acid chain in one or more regions)

This often results in the generation of short term messages (eg 2nd messengers) that modify the activity of pre-existing proteins within cells. Different types of signals may be generated depending upon the receptor and ligand. For example, it may allow the receptor/ligand to bind to other proteins (e.g., enzymes) forming an activated receptor complex. The activated receptor complex then activates downstream effectors (enzymes) that in turn lead to changes in the physiology, behaviour or shape of the cell. Or it may directly lead to the activation of transcription factors that will enter the nucleus to regulate gene activity.

Plasma membrane receptors for different hormones generally are not closely related in structure although all have three common requirements.
(i) An extracellular domain or segment for specific ligand (hormone) recognition
(ii) A means of attaching to the plasma membrane.
(iii) A means of transmitting the signal that hormone is present on the outside to the cytoplasm (signal transduction).

SIGNAL TRANSDUCTION

Signal transduction is defined as the ability of a cell to change behaviour in response to a receptor-ligand (hormone) interaction.
For this to occur there must be:

1. **Recognition**. Receptor must be able to detect the signal (i.e.specifically bind and recognize hormone). The hormone is the primary messenger.
2. **Transduction** of the extracellular message into an intracellular signal. Ligand binding causes a conformational chane in the receptor and triggers catalytic activities to the receptor or causes the receptor to interact with cytoplasmic or membrane enzymes.
3. **Transmission** of second messenger signal which often results in activation of a catalytic cascade and activation of an appropriate effector. Effectors include ion channels, enzymes and transcription factors.
4. **Modulation of an effector**. Results in activation of protein kinases (adding phosphate groups onto other proteins) and phosphatases (removing phosphate groups), thereby altering enzyme activity.
5. An **appropriate response** of the cell to the initial stimulus. Summation and integration of multiple signaling pathways.
6. **Termination of the response**. Inbuilt controls and feedback pathways.

Among the most important second messengers are cAMP, cGMP, diacyglycerol (DAG), inositol 1,4,5-triphosphate (IP_3) and Ca^{2+}. Changes in these second messengers triggers a rapid alteration in one or more enzymes and non-enzymic proteins.

Many intracellular signaling proteins behave as molecular switches. The reception of a signal activates them and causes them to pass the signal through the cell, after which they can be switched off until another signal is received.
Molecular switches fall into two main classes: the first is a very large class of proteins that are activated by phosphorylation (i.e. the addition of a phosphate to the protein). Often many of the steps in a signal transduction pathway is the phosphorylation/dephosphorylation of particular effector proteins. **Protein kinases** add phosphates (phosphorylate) protein whereas **protein phosphatase** catalyses the removal of phosphates from proteins. The second class of intracellular signalling proteins is G proteins that are activated by the binding of a guanine nucleotide.

MAIN TYPES OF SIGNAL TRANSDUCTION PATHWAYS

Cell surface receptors can be grouped into 3 main pathways that following ligand binding function using different intracellular signal pathways.

1. **Ligand gated ion channel receptors**. The ligand (external signal) is the primary messenger. Integrated membrane receptor is part of an ion channel, and ligand binding results in opening or closing of the ion channel.
2. **G-protein coupled receptors.** These integral plasma membrane receptors have seven membrane spanning helices and work via a G protein (heterotrimeric guanosine triphosphate GTP binding complex.
3. **Enzyme linked receptors** (catalytic receptors). Receptors are integral plasma membrane proteins that are enzymes themselves (or part of an enzyme complex)

1) LIGAND GATED ION CHANNEL

Integrated membrane proteins where the receptor is part of an ion channel (ionotropic receptor). Gated ion channels open or close following binding of the ligand. The binding of a neurotransmitter such as ACh results in transient opening of the channel, thus altering the ion permeability of the cell giving an electrical signal. ACh opens Na^+ channels thus changing in the membrane potential. Other ion channels such as K^+, Ca^{2+} or Cl^- are regulated by ligand gated ion channels. Other examples include receptors for γ-aminobutyric acid (GABA), glutamate and glycine.

2) G-PROTEIN COUPLED RECEPEPTORS (GPCRs)

Key points:

1. G-proteins are guanine nucleotide-binding proteins that exist as heterotrimers in combinations of different α, β, and γ subunits
2. G proteins (**GTP-binding proteins)** bind to GPCRs and act as a molecular switch that permit binding of GTP when activated (on position) and off when GTP hydrolyses to GDP
3. Heterotrimeric G-proteins are receptor linked, transduce signals and when active bind to enzymes and other proteins modifying their activity (switching on or off).

G proteins are a large family built from at least 23 different α subunits, 6 β subunits and 12 γ subunits. These different subunits allows for the formation of different varieties of G-proteins (eg Gs, Gi, Gq); each being specific for a particular set of receptors and a particular set of downstream target proteins thus providing diversity for signal transduction events.

Key point: Act as a molecular switch that can be turned on and then quickly be turned off again based on their binding to a single guanine nucleotide (GDP or GTP).

Main G protein functions:

1. Activate kinases that can phosphorylate enzymes (on or off).
2. Bind potassium or calcium ion channels in neurotransmission.
3. Release or stimulate the production of second messengers such as cyclic AMP (cAMP), diacylglycerol (DAG), inositol phospholipids IP_3 and calcium ions.

Examples:

A) G proteins acting via adenylate cyclase (membrane bound enzyme)

(i) Ligand binding changes the receptor conformation allowing Gs protein to bind.
(ii) Gs protein (Gα subunit) is bound to a single GDP molecule and it is switched off and inactive.
(iii) Gα now releases its bound GDP and takes up GTP which in turn causes the heterotrimeric G-protein to dissociate to its Gα-GTP and G$\gamma\beta$ components. Either Gα or G$\gamma\beta$ can activate other cellular components (target protein(s)).
(iv) Gα will remain an activating messenger until the GTP is hydrolyzed (GTP Ú GDP +Pi). The inactive Gα-GDP then reassociate with the G$\gamma\beta$ complex (inactive state switch is off).
(v) GTPase activity allows G proteins to convert GTP to GDP. Now inactive state.

Adrenalin, G proteins and cAMP

Binding of adrenalin to the β-adrenergic receptor activates G-proteins. GαGTP initiates a biochemical cascade that activates adenylate cyclase (adenyl cyclase) which in turn hydrolyses ATP to 3', 5' cyclic AMP (cAMP).

cAMP is an example of a secondary messenger.

Protein kinase A (PKA) is a cAMP-dependent kinase. Binding of cAMP to PKA releases regulatory

subunits bound to PKA. PKA once activated phosphorylates a number of proteins by transferring a phosphate from the ATP to a serine or threonine on a target enzyme. For example phosphorylase kinase (glycogen breakdown turned **on** in liver and muscle). In this later case PKA phosphorylates the regulatory subunits of inactive glycogen phosphorylase kinase which activates it, to in turn activate glycogen phosphorylase. The end result is the activation of several enzymes involved in glycogen breakdown and release of glucose

Important regulatory control points

A series of events now occur to reverse the metabolic stimulus mediated by adrenalin. These events prevent uncontrolled depletion of metabolic energy reserves (glycogen and triglycerides) and ensure that continual adrenergic stimulation remains essential for the continuation of the metabolic response.

1. The adrenalin concentration in blood diminishes and the adrenalin-receptor conjugate on the plasma membrane dissociates. Many receptor hormone conjugates are internalized and 'new' receptors expressed.
2. The GTP bound to Gα hydrolysis to GDP and the "ground state" G protein reforms resulting in the deactivation of adenylate cyclase. Reactivation via the G protein system occurs only while adrenalin-receptor conjugates persist on the plasma membrane of the responding cell.
3. Degradation of cAMP is catalysed by specific a phosphodiesterase
4. Intracellular protein phosphatases remove the phosphate groups from the key enzymes affected by their addition in the first place. The balance between kinases (adding phosphates) and phosphatases (removing) activity is important in promoting signalling events.

All of the reactions of the adenylate cyclase activation cascade occur rapidly and the system, once activated, decays rapidly. Adrenergic receptor and the activation of the second messenger system results in a large amplification of the response as several Gβ. However, the occupation of single α-GTP molecules are generated each of which can activate an adenylate cyclase molecule. These in turn activate multiple enzyme products. These factors together account for the potency of adrenalin and for the reversibility of its effect.

Several hormones use the same second messenger:

A great many messengers rely on just one class of molecules the G proteins to direct the flow of signals from the receptor to the rest of the cell. Some hormone pairs act additively. For example glucagon and adrenalin on liver cells. Glucagon upon binding its receptor also activates adenylate cyclase via Gs and elevates cAMP levels in the same liver cells that adrenalin acts upon.

Others act antagonistically (adrenalin Gs and prostaglandin Gi). Prostaglandins also acts upon adenylate cyclase, but to **inhibit** not to activate it. The prostaglandin receptor, when engaged, promotes the dissociation of a **different** G protein which releases an **inhibitory** Giα-GTP subunit. The α_2 adrenergic receptor also mediates it affects via Giα proteins. This inhibitory subunit binds to quiescent adenylate cylcase and **prevents its activation** by adrenalin or glucagon. Thus the precise metabolic status of a tissue at any time is controlled by the levels of the hormones for which it has receptors.

Cholera toxin and G proteins

The bacterium vibrio cholera secretes a toxin which is an ADP-ribosylating enzyme. It penetrates mammalian cell and splits NAD^+ into ADP-ribose and nicotinamide. ADP -ribose binds to the dissociated Gα.

NAD^+ + Gα --> ADP-Gα + nicotinamide

The covalently modified Gα derivative behaves like a normal Gα-GTP activator of adenylate cyclase, but it decays very slowly; thus the adrenalin activation cascade, once triggered, cannot be turned off. Although the toxin can operate on many cell types its main victims are the gut epithelial cells at the site of a v. Cholera colonisation. The adrenergic activation system in these cells results in the activation of a molecular pump which actively secretes water and Cl^- into the lumen of the gut. Serious diarrhoea results with dehydration.

It was recently shown that an adrenalin antagonist was therapeutically useful in controlling equine bacterial diarrhoea and the probable basis for the action of this drug (phenoxybenzamine) is its ability to prevent the generation of dissociated Gα proteins for bacterial toxins allow ADP-ribosylate binding in gut cells.

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