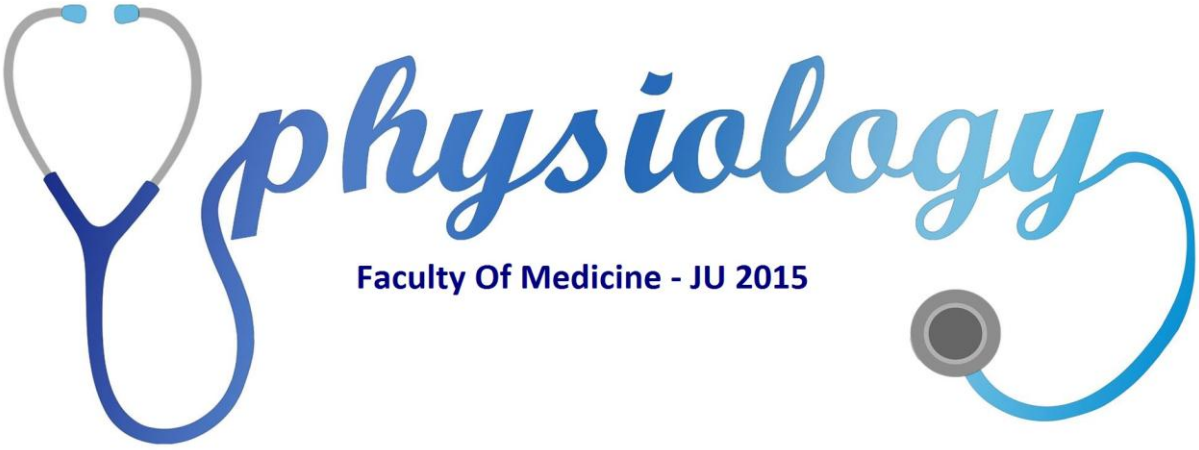

Signal Transduction - Lecture 14

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Two main factors should be present for a response to be elicited:

1. Ligand
2. Receptors

Receptors are not present in constant amounts in the cell:

- They are dynamic:
  - Either synthesized or broken down.
    - This is determined primarily and mainly by signaling.
- 2 ways of regulation:
  - Up regulation/Sensitization
    - Increase in receptors number by increase in synthesis or by increase in insertion into the plasma membrabe to be exposed to the ligands.Usually occurs to increase response of hormone to the stimulus(bigger effect)
    - Also called "priming"
    - Cells become more sensitive to stimulus

*RULE: More stimuli→ stimulates negative feedback in receptor production.
Thus, there will be something called Down Regulation:

- Down Regulation/Desensitization
  - Decrease in number of receptors
  - Could occur through
    - **Inactivation** of some receptor molecules/intracellular molecules
    - **Temporary engulfing** of the receptor molecules, away from the receptor hormone interaction are
    - **Destruction** of the receptors
    - **Decreased production** of receptors
  - Cells become less sensitive to hormone as a result of→
    -
    - Long term or continuous stimuli
  - One way to reduce Down Regulation
    - Having a pulse-like-stimulus- ON and OFF
      - this will make sure anytime there is a stimulus the number of receptors will not decrease
  - Clinical Correlation: Diabetes II- Insulin Insensitivity
    - Normally, Insulin stimulates sugar uptake in cells after each meal. In patients with Insulin Insensitivity, Insulin is highly stimulated due to high sugar intake, which will induce downregulation of receptors. Consequently, the cells become desensitized to Insulin and no response to it occurs. Thus, sugar stays in the blood in very high amounts which can be deleterious.
    - This is prevented by a pulsatile stimulus.
  - Diabetes I → insulin is not enough.

- Hormonal Half Life: the time that is required to reduce the normal physiological amount of a hormone by half(50% of it)
    - Represents the time it takes for a hormone to clear out from the blood.
        - somehormones have short half livesand some have long half life time.
        - It depends on the metabolism of the free hormones and the amount of (free) hormones level (unbound to proteins) in the blood
    - Ex- if the half life of a hormone is 4 hours, then after 8 hours you wouldn't find it in the blood.

- Affinity Constant/Binding Constant:
    - Affinity describes how strong the hormone binds to its receptors.
    - The constant is called K_d or Ka: dissociation or association constant
        - K_d is an equilibrium constant that is equal to :

$$K_d = \frac{[R][L]}{[R \cdot L]}$$

Where: [R]- unbound receptor
[L]- unbound ligand
[R—L] is the receptor-ligand complex(bound)

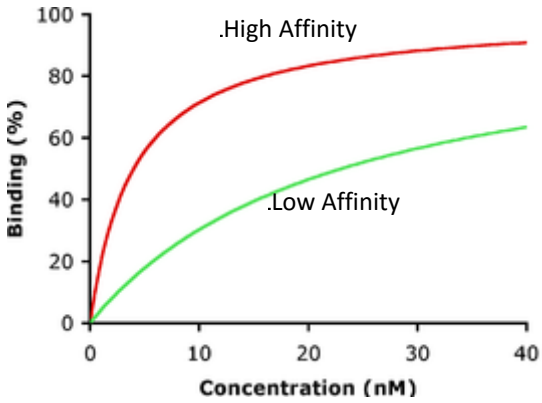
*** It is the amount of free ligand(dissociated) divided by the amount of ligand that is bound to receptors(associated).

Thus, it describes **association** and **disassociation** of the hormone with its receptor at **equilibrium.**

- One importance of K_d is that it tell us how much we need of a hormone in order to have good binding at equilibrium.
    - If we have a hormone with a **low affinity** for receptor:
        - The ligands will not bind to the receptors easily and many ligands will be dissociated(unbound). As a result K_d will be high and not many receptors will be bound. In order to elicit a response we need more ligands bound to receptors. Therefore, we need more hormone in order to have more ligands and shift equilibrium to have more bound receptors.
    - If we have a hormone with high affinity for receptors:
        - The hormone will be bound to most of the receptors, K_d is low and we won't need large amounts of the hormone or ligand to elicit the response

    - Summary:
        - Low affinity- High K_d- less bound ligands→ we need more concentration of the hormone to elicit response

- High affinity- Low K_d- more bound ligands→ we don't need large concentration of hormone to elicit response



In this picture the high affinity hormone had 80% binding at 10 nM. The low affinity hormone had only 30% binding at 10 nM. So we need a lot more of the low affinity hormone to cause a response.

Sometimes high amounts of hormones cause a totally different response than the lower amounts of hormones.

The most important thing to know is that the hormone should be in a physiological reference range that is compatible with its affinity.

In the human body, affinity is very high for endogenous(internal) ligands. Affinity for some drugs might be lower.

- Specifity
  - Each receptor may bind to different ligands specifically.
  - Each different ligand has a different affinity for the same receptor. Depending on the chemical structure of the ligand.
  - Types of ligands:
  - 1. Agonist: binds to receptor and induces a stimulus(down stream signaling)
    The most common in our body
    - Might be inhibitory/excitatory- it does not matter as long as it induces the receptors to function
  - 2. Antagonist:
    - Synthesized to block the function of agonists.
      They bind to receptor and prevent the response to happen. Saturates and blocks/prevents responses elicited by endogenous ligands.

- Types of receptors:
  - Ligand-gated ion channel:
    - Acetylcholine is the ligand
    - The channel is on the post-synaptic membrane
    - When Ach binds to the channels, it induces depolarization
      - Na+ flows in and the action potential is transmitted
  - G-Protein coupled receptor
    - Largest family of receptors
    - 7 transmembranehelix spanning domains

- They are bound to G-proteins
- The G-protein is:
    - Heterotrimeric with alpha, beta, and gamma subunits
- Mechanism of action :

1. Ligand binds to receptors
2. Conformational change causes receptors ors bind to the G-protein
3. Normally, the alpha subunit has a GDP attached to it. In this step, GTP will switch with GDP and GTP will be bound to the alpha subunit. – JEF activity causes GTP-GDP exchange. JEF is an enzyme that adds GTP.
4. The GTP bound alpha(active) binds to different enzymes in order to induce changes.
    a. Could stimulate adenylate cyclase(for example) to produce cAMP(2^{nd} messenger)
    b. The Gs subunit is activiated- G-stimulatory(the active alphs form)
        i. Gs is very important for cAMP synthesis
5. cAMP binds to protein kinase A. PkA is compsed to 2 parts:
    a. 2 units are regulatory
    b. 2 units are catalytic

    ** When these two units are bound to each other, PKA is inactive.

    I. When cAMP binds to the regulatory part of PKA, these 2 parts separate. PKA is now active and is works to phosphorylate.
        - Kinases either phosphorylate the amino acid serine or threonine, or both of them together.
            - Why serine and threonine specifically? They have hydroxyl groups and these are the parts that are phosphorylated.

    II. After being phosphorylated, proteins either become active or inactive.

**Just as we have Kinase, we have phosphatase action.

- Now that the signal caused a response, we have to find a way to turn off the signal because everything in the body needs to be balanced.
    - We cannot depend on the hormonal life spane, so we need to act on every step along the pathway:

1) Inactivate G-alpha(which is active when bound to GTP).
    - We can have an exchange of GDP for GTP(releasing GTP). This is done by the GAP enzyme. This enzyme has a GTP-ase activity.
    - This GTP-ase activity exists intrinsically in the G-protein.
    - Now there is no free Gs or G-alpha(they bind back to inhibitory beta and gamma), and adenylate cyclase becomes inactive. cAMP production ceases.

2) Phosphodiesterase stimulation:
    - An enzyme that turns cAMP to AMP and thus there is no longer a second messenger.
    - cAMP is what stimulates phosphodiesterase., so, cAMP is what stimulates its degradation.
    - PKA can also phoshorylate Phosphodiesterase and stimulate its activity.

- Thus, the more cAMP we have, the higher the rate at which it is degraded, and this is needed to turn off the signal or the message.

3) Desensitization/Down regulation
    - Decreasing sensitivity
    - PKA can phosphorylate the receptor.
      The receptor now has a tag.
      The tag is identified by a regulatory protein called Beta-arrestin.
      B-arrestin binds to receptors and this induces invagination/calathrin coated endocytosis of the whole associated area.
    - Beta-arrestin activation also stimulates the B-arresin to bind to phosphodiesterase. Phosphodiesterase becomes in close proximity with cAMP and stimulates its breakdown.

4) Phosphatase can also remove the phosphate group added by PKA to cellular proteins.

- There several types of G-alpha:

1. G-alpha stimulatory (Gsalpha)
    - It is called stimulatory because it increases the production of cAMP
2. G-alpha inhibitory(Gialpha)
    - Inhibitory because it reduces/inhibits cAMP production
    - This is GTP bound but it doesn't activate adenylate cyclate, rather it inhibits it.

☒ Beta-Gamma complex:
    - When bound to the alpha subunit, it deactivates it(inhibitory)
    - They can bind to certain isoforms of adenylate cyclase and deactivate it.

- one example of GPCR ; is a receptor for norepinephrine/ epinephrine,called the B-adrenergic receptor
    a. They are stimulatory and activate the Gs subunit- thus increases the concentration of cAMP.
- Another example is Rhodopsin in the retina(a GPCR) does not need a ligand. It is stimulated by light.
- Some proteins can modulate and modify the localization and affinity of GPCRS. They might increase their diversity, causing them to dimerize or form oligomeric complexes. Thus the GPCR might be present in different forms..

- ✓ Clinical Correlations:
  - Cholera Toxin:
    - Causes ADP ribosylation, a chemical reaction that adds ADP to a molecule covalently
    - This acts on Galpha(activated form)
    - Gs no longer can exchange GDP for GTP (cant release GTP) and it stays permanently activated
    - Because it's a covalent bond, it's very hard to break and is irreversable
    - Affected receptors are G-protein linked receptors in the Gastro-Intestinal tract
      - In the GI tract, there are lots of electrolytes in the lumen. When these receptors are active, Cl and many other electrolytes flow out of the GI accompanied by water and dehydration occurs as diarrhea.
  - Pertussis Toxin(whooping cough disease):
    - Causes Gia(inhibitory) unable to release GDP. GDP remains bound to it, so it won't be ab;le to be activated.
    - Thus we have an inactivation of the inhibitory G-protein, so excitation results from absence of the inhibition.
    - This produces the same results as the cholera toxin.