DRUG-RECEPTOR INTERACTION

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- Sites of Drug action
- Various Protein Target for Drug action
- Drug - Receptor Binding
- Agonist & Antagonist and Dose-Response Curve
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- Conclusion
INTRODUCTION

- What is Drug?
- Effects of Drug
  - Type of Response ~ Effect
  - How & where it is produced ~ Action.
  
  So, Effect is measured while action is identified.

- How drug acts,
- Where it acts,
- How it produces the pharmacological response.
SITES OF DRUG ACTION

- **Extra Cellular Sites**
  - **Antacids** - Neutralize Gastric Acidity
  - **Chelating Agents** - Complexes with Heavy Metals.
  - **Osmotic Purgatives** - Retaining Fluid inside Intestine.

- **Intracellular Sites**
  - **Sulpha Drugs** - Interfering Synthesis of Folic acid
  - **5-Flourouracil** - Replacing Uracil into RNA

- **Cellular Sites**
  - **Acetyl Choline** - Motor end plate of skeletal muscles
  - **Ranitidine** - $H_2$-receptor of Parietal cell
TARGETS FOR DRUG ACTION

- Protein targets for drug action on mammalian cells -
  1. Receptor
  2. Ion Channels
  3. Enzymes
  4. Carrier Molecules
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<th>Type of target</th>
<th>Effectors</th>
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<td>Divalent cations (e.g. Cd²⁺)</td>
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Macromolecular component of organism that binds drug and initiates its effect.

Specific Macromolecular Protein.

Membrane bound or Intracellular.

Capable to bind with specific functional groups of drugs.

3-Dimensional Configuration.
Most drugs act (bind) on receptors

- In or on cells
- Chemical Bond: Ionic, Hydrogen, Hydrophobic, Vander Waals & Covalent.
- Exact requirements (Size, Shape, Stereo specificity)
- Saturable
- Agonists (Salbutamol), or Antagonists (Propranolol)

Receptors have signal transduction mechanism
**AFFINITY** - Attractiveness B/W Drug & Receptor.
* Covalent bonds stable & essentially irreversible.
* Other bonds strong or weak, but usually reversible.

**Efficacy** - Ability of a bound drug to change receptor in a way that produces effect.

Some drugs possess affinity but NOT efficacy.
Cell Membrane

Bound Endogenous Activator (Agonist) of Receptor

Active Cell Surface Receptor

Cell Membrane

Extracellular Compartment

Intracellular Compartment

Cellular Response
Cell Membrane

Extracellular Compartment

Displaced Endogenous Activator (Agonist) of Receptor

Bound Antagonist of Receptor (Drug)

Cell Membrane

Inactive Cell Surface Receptor

Intracellular Compartment
Drugs-receptor interaction

\[
\text{Drug + Free Receptor} \quad \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} \quad \text{Drug-receptor Complex}
\]

Where: 
- \( D \) = drug concentration
- \( \text{DR} \) = concentration of drug-receptor complex
- \( 100 - \text{DR} \) = free receptor concentration

- Drug binding obey the law of mass action.

At equilibrium,

\[
[D] \times [R] \times k_1 = [\text{DR}] \times k_{-1}
\]

so that:

\[
[D] [R] = \frac{k_1}{k_{-1}} [\text{DR}]
\]

\[
k_1/k_{-1} = \text{dissociation constant (kd)}
\]

\text{Lower \( kd \) = more potent drug}
- Rate of change rapid at first and becomes progressively smaller as dose increased.
- Eventually, increments in dose produce no further change in effect i.e., maximal effect for that drug is obtained.
- Difficult to analyze mathematically.
Log Dose Scale

- Transforms hyperbolic curve to sigmoid (almost straight line)
- Compresses dose scale
- Proportionate doses occur at equal intervals
- Straightens line
- Easier to analyze mathematically
EFFECTIVENESS, TOXICITY, LETHALITY

ED50 (Median Effective Dose 50) -
* 50% Population manifests a given effect.

TD50 (Median Toxic Dose 50) -
* 50 percent Population manifests a given toxic effect.

LD50 (Median Lethal Dose 50) -
* Dose which kills 50 percent of the subjects.

Therapeutic Index (TI) = \( \frac{TD50 \text{ or } LD50}{ED50} \)

- Provides a very crude measure of safety of drug.
- Higher the TI = Safer the drug.
- TI vary from: 1.0 (some cancer drugs) to >1000 (penicillin).
Receptor Families: Signal Transduction Mechanism

- Ion-channel linked receptor
- G-protein coupled receptor
- Enzyme linked receptor
- Nuclear receptor
Ion-channel linked receptor

- Located on cell membrane.
- Directly coupled with Effectors (channels).
- Takes millisecond to produce action.
- Mainly involved in fast Synaptic transmission.
- Examples are—
  - $\eta$-Ach receptor
  - $GABA_A$ receptor
  - Glutamine receptor
  - Glycine receptor
  - $5-HT_3$ receptor
**Structure:**

* Made of Oligo protein containing four subunits which enclosing a cylindrical Ion channel.

* Each subunit have 4-5 Transmembrane Segments. Which crosses the lipid bi layer 4-times.
Ion channel receptors

1. Signal molecule binds as a ligand at a specific site on the receptor.
2. Conformational changes open the channel allowing ions to flow into the cell.
3. The change in ion concentration within the cell triggers cellular responses.
4. Ligand binds; channel opens; ions flow through.
5. Change in ion concentration triggers cellular responses.
6. Ligand dissociates; channel closes.
G-protein coupled receptor

- Also called metabotropic receptor.
- Located on cell membrane.
- Coupled through G-protein with Effectors.
- Effector may be channels or enzymes.
- Take seconds to produce action.
- Mainly involved in Hormones & slow transmission.
- Examples -
  - m-Ach receptor
  - Dopamine receptor
  - Adrenergic receptor
  - Opiate receptor
**G protein-linked receptors**

Structure:

- Single polypeptide chain that crosses the lipid bilayer 7 times, resulting in 7 transmembrane helices.
- There’s a G protein attached to the cytoplasmic side of the membrane (functions as a switch).
Chemical signal binds as a ligand to a G protein-linked receptor

G protein linked receptor changes shape and interacts with a G protein

Interaction causes GDP to be displaced and GTP to be bound to the G protein

The active G protein binds to another protein, usually an enzyme

The enzyme is activated

G protein hydrolyzes GTP back to GDP

G protein releases from the enzyme and the reaction stops

(a) G-protein system in inactive form

(b) G-protein system in action

(c) Return to inactive form

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C-AMP Activation
“G” refers that protein binds Guanine nucleotides (GDP, GTP)

G proteins integral membrane protein, i.e. heterotrimers ($\alpha\beta\gamma$);

G proteins have similar $\beta$ and $\gamma$ subunits, but differ in type of $\alpha$-subunits;

When G-protein activated, $\alpha$ subunit dissociates to interact with an enzymes that generate second messengers (e.g. cAMP).
The Nobel Prize in Physiology and Medicine 1994

"for their discovery of G-proteins and the role of these proteins in signal transduction in cells"

Alfred G. Gilman
USA
1941-

Martin Rodbell
USA
1925-1998
Enzyme linked receptor

- Located on Cell membrane.
- Also known as kinase linked receptor.
- Coupled with intracellular Tyrosine kinase.
- Take minutes to produce Action.
- Mainly involved in Growth factor and certain hormones.

- Examples -
  - Insulin receptor
  - Cytokinase receptor
Exist as individual polypeptides

Very large binding domain, present in extra cellular and

large effector domain present in intracellular.

Directly linked tyrosine kinase which binds to specific Protein.
Ligands bind to both receptors

The two receptor polypeptides aggregate forming a dimer

Each phosphorylates (using ATP) the tyrosines on the tail of the other polypeptide

Activates the tyrosine-kinase parts of the dimer

Relay proteins bind to the phosphorylated tyrosines (may activate 10 or more different transduction pathways)

Receptor proteins are now recognized by relay proteins inside the cell

(a) Inactive tyrosine-kinase receptor system

(b) Activated system
Nuclear receptors

- Also known as Gene-Transcription receptor.
- Present in intracellular either Cytoplasm or Nucleus.
- Intracellular protein so agonist must first inter cells.
- Coupled via DNA.
- Take hours to produce action.
- Examples -
  * Steroid receptor
  * Thyroid receptor
  * Vit-D receptor
Heat Sensitive Protein (HSP-90), DNA binding domain (zinc finger), Transcriptional control domain and Ligand binding domain.

- Agonist binding release HSP-90, then exposes DNA binding domain.
- Exposed DNA binding domain associate with DNA of specific gene; stimulate RNA polymerase activity; Specified m-RNA synthesized.
- m-RNA regulate production of specific proteins that gives specific physiological functions.
1. Agonist

   Na

   Activation of conductance

2. Agonist

   Na

   G-Protein Activation

   Generation of Second Messenger

   Activation of Cell Signaling

3. Agonist

   Phosphorylation of Tyrosines on Key Signaling Molecules

   Activation of Cell Signaling

4. Agonist

   Transport to the Nucleus

   Activation of transcription and translation
Drug Discovery and Development

**Preclinical studies**
- Research team formed and objectives set
- Novel chemicals synthesized
- Chemicals tested for efficacy and safety in test tubes and animals. Results used to choose drug candidate.
- Formulation, stability scale-up synthesis, chronic safety in animals
- Company files Investigational New Drug (IND) application with FDA

**Clinical studies**
- Drug is approved for marketing
- FDA reviews NDA
- Company files New Drug Application (NDA)
- Phase III: large clinical trials in many patients
- Phase III: studies in patients (efficacy)
- Phase I: studies in healthy humans (toleration)

*Nature Reviews | Drug Discovery*
Checkpoints

- Early Development:
  - Pre-clinical
  - Phase I
  - Phase Ila
  - Phase IIb

- Late Development:
  - Phase IIIa
  - Phase IIIb
  - Phase IV

- Post Registration:
  - MAA/NDA
  - Launch decision
  - Post registration

Key Events:
- Human trials begin
- Healthy human safety
- Patient efficacy / safety
- Selection of dose and regimen
- MAA/NDA go / no go
- Launch decision
- Post registration
Safety & toxicity in animals

- Acute toxicity profile
- Chronic toxicity profile
  -- 14 day toxicity test in one rodent and one non-rodent species before use in man.
  -- 3 month study read out at 28 days
  -- longer studies (12 & 24 month)

Three dose levels (below, about, well above human dose).

It is insufficient to use doses which are not toxic; the doses producing toxic effects and the nature of these effects MUST be established.
Clinical Trials

- Research studies involving people
- Try to answer scientific questions and find better ways to prevent, diagnose, or treat disease
- Clinical trials translate results of basic scientific research into better ways to prevent, diagnose, or treat disease
- The more people take part, the faster we can:
  - Answer critical research questions
  - Find better treatments and ways to prevent disease
Clinical testing

- Phase 0 (non-clinical)
- Phase 1 (volunteers)
- Phase 2 (patients)
- Phase 3 (large scale multi-centre)
- Phase 4 (post registration monitoring)

Phases can also be defined by the information you are trying to get out of the testing.
Clinical trials
(The Way We Make Progress Against Disease)

Drug action depends on:

- Pharmacodynamics
- Pharmacokinetics and dose regimen
- Drug interactions
- Receptor sensitivity of patient
- Mood/personality of patient & doctor
- Patients expectations and past experience
- Social environment of patient
- Clinical state of patient

Clinical trial controls these variables and examines action of drug in defined set of circumstances
Most drugs act through receptors.

Interaction b/w drug & receptor can be described mathematically and graphically.

Higher therapeutic index, Safer the drug.

There are 4 common signal transduction mechanism.

Second messengers carry signal inside cell; often use protein phosphorylation as a signaling device.
Thank you
Various types of $G$-protein families $\alpha$-subunits

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<th>$G$ protein</th>
<th>Signal</th>
<th>Effected enzyme</th>
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<td>epinephrine, glucagon</td>
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