Lecture 2.3: Pharmacodynamics, Quantifying Antagonism, Allosteric Modulation, and Assay Systems

1. Agonism Spectrum and Receptor Occupancy vs. Activation

- **Occupancy vs. Activation:**
  - Receptor occupancy (binding) is governed by **affinity** and follows the law of mass action.
  - Receptor activation (producing a stimulus and downstream response) requires **efficacy**.
  - A concentration-occupancy curve does not automatically match a concentration-response curve because receptor-effector coupling is non-linear and subject to signal amplification.
- **Full Agonists:**
  - Possess high efficacy and efficiently couple receptor occupancy to downstream activation.
  - Can elicit a maximum operational tissue response ($E_{\max}$) without needing to occupy all available receptors in the tissue, demonstrating the phenomenon of **receptor reserve** (spare receptors).
- **Partial Agonists:**
  - Possess intermediate or lower efficacy.
  - Must occupy a significantly higher fraction (or all) of the available receptor pool to achieve their submaximal peak response.
  - Their maximal attainable response is lower than that produced by a full agonist in the same tissue system.
  - *Therapeutic Utility:* Despite lower efficacy, partial agonists serve as valuable clinical therapeutics (e.g., stabilizing receptor tone without overactivation or acting as competitive functional blockers in the presence of high endogenous agonist tone).
- **Antagonists & Inverse Agonists:**
  - Classical neutral antagonists possess affinity but zero intrinsic efficacy under basal, non-constitutive conditions.
  - In settings with constitutive receptor activation (e.g., specific activating mutations or receptor overexpression), certain antagonists reduce basal receptor signaling below baseline, displaying **negative intrinsic efficacy** (inverse agonism).

Practice Multiple-Choice Questions

Question 1

What structural characteristic defines the transmembrane topology of all canonical G protein-coupled receptors?

A) An intracellular amino terminus with four hydrophobic domains

B) A single polypeptide chain crossing the membrane bilayer seven times

C) Multiple alpha-helical subunits organized around a central aqueous ion pore

D) Two identical polypeptide dimers that span the plasma membrane twice

Question 2

What biochemical event triggers the dissociation of the G-alpha subunit from the G-beta-gamma heterodimer?

A) Hydrolysis of bound cyclic adenosine monophosphate by phosphodiesterases

B) Proteolytic cleavage of the amino-terminal tail by cell-surface metalloproteinases

C) Phosphorylation of specific serine residues on intracellular loop three by GRK2

D) Displacement of bound GDP followed by the rapid binding of GTP

Question 3

How does the small GTPase RhoA become activated following the stimulation of a G-12/13-coupled receptor?

A) The liberated G-alpha subunit activates Rho-GEFs that swap GDP for GTP on RhoA.

B) G-12/13 directly phosphorylates intracellular calcium-binding proteins in the cytoplasm.

C) Active G-alpha subunits hydrolyze membrane phospholipids to release arachidonic acid.

D) The associated G-beta-gamma dimer opens inwardly rectifying potassium channels.

Question 7

What physiological consequence distinguishes *extrasynaptic* GABA receptor activation from *synaptic* GABA receptor activation?

A) Extrasynaptic activation produces brief, millisecond-scale phasic inhibition.

B) Extrasynaptic activation mediates prolonged, long-term tonic inhibition because neurotransmitters linger longer outside the synaptic cleft.

C) Extrasynaptic receptors are permeable exclusively to sodium cations.

D) Extrasynaptic receptors open only in the complete absence of GABA.

Question 8

What primary neurochemical alteration characterizes standard traditional pharmacological treatments for most forms of epilepsy?

A) Enhancing or prolonging inhibitory GABAergic neurotransmission to counteract excess brain excitation.

B) Permanently destroying all postsynaptic glutamate receptors.

C) Accelerating the degradation of intracellular adenosine triphosphate.

D) Blocking all potassium leak channels across the entire cortex.

Question 9

Why do patients harboring specific *gain-of-function* mutations in GABA receptor genes often experience worsening clinical symptoms when administered traditional GABA-enhancing anti-seizure medications?

A) Gain-of-function mutations cause the blood-brain barrier to become completely impermeable to drugs.

B) Enhanced GABA activity in certain gain-of-function variants paradoxical triggers network instability and severe hyperexcitability.

C) Traditional drugs chemically convert GABA molecules into toxic heavy metals.

D) Gain-of-function receptors destroy all surrounding microglial cells.

Answer Key & Explanations

1. **A** — While ~35–40% of marketed drugs target GPCRs, they act on only a small subset of the ~800 human GPCRs, leaving roughly 85% currently unexploited in modern pharmacopeia.
2. **C** — Potency reflects the concentration required to generate a defined response level (such as an EC_{50}) and depends on system parameters like receptor expression and pathway amplification.
3. **D** — An inverse agonist selectively stabilizes the inactive receptor state, reducing baseline constitutive activity that occurs in the absence of an agonist.
4. **B** — The orthosteric pocket recognizes the same endogenous molecule across an entire receptor family, resulting in conserved amino acid sequences that make subtype-selective orthosteric drug design challenging.
5. **D** — Doxazosin blocks α_1-adrenergic receptors in the target prostate tissue, but because these receptors are also present throughout the vascular smooth muscle, it produces on-target, off-tissue vasodilation and hypotension.
6. **C** — Pure PAMs have no intrinsic signaling ability on their own; their action relies entirely on modulating the receptor when an endogenous orthosteric agonist binds.
7. **A** — Allosteric effects are saturable because they depend on allosteric site occupancy and a finite cooperativity factor, preventing runaway signaling even at high concentrations.
8. **B** — Cinacalcet binds allosterically to the calcium-sensing receptor (CaSR) on parathyroid chief cells to enhance sensitivity to extracellular calcium, treating secondary hyperparathyroidism.
9. **C** — In allosteric ternary complex models, alpha (α) represents the affinity cooperativity factor governing how allosteric binding changes orthosteric ligand affinity.
10. **A** — NAMs with negative affinity cooperativity ($\alpha < 1$) destabilize the orthosteric complex, accelerating the dissociation rate (k_{off}) of the bound agonist.
11. **B** — Biased agonism occurs because ligands selectively stabilize distinct active receptor conformations that favor coupling to specific transducers (e.g., G-proteins vs. β-arrestins).
12. **C** — Oliceridine was developed to retain analgesic Gα_i signaling while avoiding β-arrestin 2 recruitment, which is linked to respiratory depression and constipation.
13. **A** — Observational or system bias can occur when different assay formats have differing degrees of downstream amplification or sensitivity, creating an illusion of ligand-directed pathway selectivity.
14. **B** — Structurally distinct ligands can induce different receptor conformations that alter GRK phosphorylation patterns ("barcoding"), producing distinct downstream β-arrestin signaling outcomes.
15. **C** — Neutral allosteric ligands occupy the allosteric binding pocket without altering orthosteric signaling parameters ($\alpha = 1, \beta = 1$), serving as competitive antagonists against PAMs and NAMs.

2. Quantifying Competitive Antagonism and the Schild Analysis

- **Characteristics of Orthosteric Competitive Antagonism:**
  - The antagonist binds reversibly to the same binding site (orthosteric site) as the endogenous agonist.
  - The interaction is **surmountable**: increasing the concentration of the agonist displaces the antagonist and fully restores the maximal response ($E_{\max}$).
  - On a semi-logarithmic plot, adding fixed concentrations of a competitive antagonist produces **parallel, rightward shifts** of the agonist concentration-response curve without depressing $E_{\max}$.
- **Experimental Paradigms:**

  1. *Parallel Curve Shift:* Generating full agonist concentration-response curves in the absence and presence of increasing concentrations of the antagonist under equilibrium conditions.
  2. *Displacement/Reversal:* Fully activating the receptor system with an agonist and titrating increasing concentrations of the antagonist to observe dose-dependent relaxation or suppression back to baseline.

- **Terminology of Dissociation Constants:**
  - K_D: Conventionally denotes the equilibrium dissociation constant of an *agonist*.
  - K_B: Denotes the equilibrium dissociation constant of an *antagonist* (reflecting its binding affinity at equilibrium).
- **The Dose Ratio (r) and the Schild Equation:**
  - The dose ratio (r) represents the fold-increase in agonist concentration required to produce the same biological response in the presence of a given concentration of antagonist $[B]$:

$$r = \frac{EC_{50}\ \text{(in presence of antagonist)}}{EC_{50}\ \text{(control)}}$$

  - Derived from the Gaddum-Schild relationship under equilibrium conditions:

$$r - 1 = \frac{[B]}{K_B}$$

  - Taking the logarithm yields the linear **Schild equation**:

$$\log_{10}(r-1) = \log_{10}[B] - \log_{10} K_B$$

- **The Schild Plot:**
  - Plots $\log_{10}(r-1)$ on the y-axis against $\log_{10}[B]$ on the x-axis across multiple antagonist concentrations.

Lecture 5.2 – Drug Activity at GPCRs

1. GPCRs in Physiology and Drug Discovery

A. Physiological Significance

- G-protein coupled receptors (GPCRs), also termed 7-transmembrane (7TM) receptors, are integral membrane proteins embedded within the plasma membrane.
- They detect a diverse array of extracellular stimuli, including photons (light), monoamines, peptide hormones, lipid mediators, ions, and neurotransmitters.
- GPCR activation triggers intracellular signaling cascades that mediate vital physiological processes such as sensory perception (vision, taste, olfaction), nociception (pain), cardiovascular regulation, and endocrine homeostasis.
- Aberrant GPCR function is implicated in widespread pathophysiological conditions, including heart failure, chronic obstructive pulmonary disease, endocrine syndromes, neurodegenerative disorders, and diverse malignancies.

B. Current Therapeutic Landscape and Untapped Potential

- Approximately 35% to 40% of all prescription drugs approved by the US FDA target GPCRs.
- Despite their clinical importance, approved drugs target only a small subset of the roughly 800 distinct GPCRs encoded in the human genome.
- Over 85% of human GPCRs remain unexploited or "undrugged," representing a substantial reservoir for novel drug discovery.
- Historically, identifying selective small molecules for GPCRs has been challenging due to structural complexities and membrane-bound instability; however, recent advances in structural biology (particularly cryo-electron microscopy) and computational screening are opening previously inaccessible targets.

C. Complexity of Downstream GPCR Signaling

- **Heterotrimeric G-Protein Coupling:** GPCRs transduce signals through distinct G-protein alpha families ($G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, $G\alpha_{12/13}$), each regulating specific second-messenger systems (such as cyclic AMP generation, intracellular calcium mobilization, and Rho kinase cascades).
- **Desensitization and Scaffolding:** Activated receptors are phosphorylated by G-protein coupled receptor kinases (GRKs), which promotes the recruitment of arrestin scaffolding proteins (β-arrestin 1 and 2).
- **Trafficking and Compartmentalized Signaling:** β-arrestin recruitment uncouples G-proteins, induces clathrin-mediated endocytosis, and directs receptors toward endosomal recycling or lysosomal degradation. GPCRs can also generate sustained, non-canonical signals from intracellular compartments such as endosomes.

2. Fundamental Pharmacological Parameters

A. Affinity

- **Definition:** The thermodynamic strength of reversible physical binding between a ligand and its receptor.
- **Molecular Basis:** Driven by non-covalent interactions (hydrogen bonds, electrostatic salt bridges, van der Waals forces, hydrophobic contacts) within the binding pocket.
- **Quantification:** Experimentally measured via equilibrium dissociation constants (K_d or K_i). A smaller dissociation constant reflects higher binding affinity.

B. Efficacy

- **Definition:** The relative capacity of a bound ligand to stabilize active receptor conformations and generate a functional biological response.
- **Measurement:** Evaluated in comparison to a reference standard (typically the full endogenous agonist) and represented as the maximal achievable response ($E_{\max}$) or intrinsic efficacy.
- **Functional Classes:**
    - *Full Agonist:* Produces the maximal biological response attainable in a given test system.
    - *Partial Agonist:* Possesses intermediate intrinsic efficacy, producing a sub-maximal response even at full receptor occupancy.
    - *Neutral Antagonist:* Binds the receptor without altering baseline conformational equilibrium, occupying the pocket and blocking agonist binding.
    - *Inverse Agonist:* Preferentially binds and stabilizes the inactive conformation, suppressing constitutive (basal) signaling in constitutively active GPCR systems.

C. Potency

- **Definition:** The concentration or dose of a drug required to elicit a defined magnitude of biological response (typically quantified as EC_{50} or IC_{50}).
- **Operational Nature:** Potency is not an isolated molecular property; it is a composite parameter governed by affinity, intrinsic efficacy, receptor expression density, and downstream signal amplification in a given tissue.
- **Graphical Shifts:** In concentration-response plots, higher potency corresponds to a leftward shift along the concentration axis, whereas lower potency corresponds to a rightward shift.

Lecture 8 – Stems Cells as Therapy and in Drug Development

1. Stem Cell Fundamentals and Classification

Definition of a Stem Cell

- An unspecialized cell defined by two essential functional capabilities:
  - **Self-Renewal:** The ability to undergo mitotic division to generate an identical, undifferentiated daughter cell.
  - **Differentiation:** The capacity to commit to a lineage pathway and produce mature, functional specialized progeny.

Potency and Classification

- **Tissue (Adult / Somatic) Stem Cells:**
  - **Multipotent:** Restricted to producing specialized cell types typical of their tissue of origin.
  - **Finite Lifespan In Vitro:** When isolated and cultured in vitro, tissue stem cells undergo replicative senescence and eventually arrest division.
  - **Physiological Role:** Facilitate physiological tissue homeostasis, everyday turnover, and targeted regeneration after injury or pathology.
- **Pluripotent Stem Cells:**
  - **Pluripotency:** Capable of differentiating into specialized derivatives of all three primary embryonic germ layers: **ectoderm**, **mesoderm**, and **endoderm**.
  - **Indefinite Self-Renewal:** Exhibit prolonged proliferative capacity in vitro without senescing, functioning as immortal cell populations under appropriate culture conditions.
  - **Subtypes:**
    - *Embryonic Stem Cells (ESCs):* Derived from the inner cell mass of pre-implantation blastocysts. Historically faced ethical, legal, and financial restrictions regarding embryo destruction.
    - *Induced Pluripotent Stem Cells (iPSCs):* Groundbreaking discovery published in 2006 by Kazutoshi Takahashi and Shinya Yamanaka (2012 Nobel Prize). Somatic cells (such as dermal fibroblasts) are genetically reprogrammed back to an unspecialized, pluripotent state using four core transcription factors: **Oct3/4, Sox2, Klf4, and c-Myc** (OSKM factors).
    - *Reprogramming Evolution:* Early methods utilized integrating retroviruses. Modern clinical-grade translational protocols employ non-integrating strategies—such as synthetic modified messenger RNA (mRNA), recombinant proteins, episomal plasmids, or Sendai virus vectors—to avoid insertional mutagenesis.

2. Physiological Tissue Stem Cell Reservoirs

- **Broad Organ Distribution:** Tissue-resident stem cell pools inhabit the brain, eye, skin, lungs, gastrointestinal tract, liver, pancreas, and kidneys.
- **Continuously Renewing Tissue Systems:**
  - **Hematopoietic System (Bone Marrow):**
    - Driven by Hematopoietic Stem Cells (HSCs) that replenish circulating blood and immune cells.
    - High daily cellular output: A single individual generates approximately 5,400 kg of granulocytes over a 70-year lifespan.
    - Divergent cellular half-lives: Erythrocytes circulate for ~120 days, whereas granulocytes turnover rapidly (often within hours to a single day) to maintain immune surveillance.
  - **Intestinal Epithelium:**
    - Governed by $Lgr5^+$ (leucine-rich repeat-containing G-protein coupled receptor 5-positive) crypt base columnar stem cells.
    - Responsible for continuous renewal of the functional intestinal monolayer (enterocytes, goblet cells, Paneth cells, enteroendocrine cells).
- **Hematopoietic Lineage Hierarchy:**
  - The multipotent HSC sits at the apex, maintaining quiescence and self-renewal.
  - Differentiation proceeds through committed progenitor compartments (transit-amplifying pools).
  - This hierarchy amplifies cell numbers without forcing the primary HSC pool to undergo continuous division, shielding genomic integrity from replication-induced mutations.

3. Clinical Cell Therapies

Hematopoietic Stem Cell Transplantation (HSCT)

- The most established and routinely practiced clinical stem cell therapy.
- **Preclinical Proof of Concept:** Lethally irradiated mice suffer hematopoietic failure; intravenous infusion of donor HSCs reconstitutes the entire myeloid, erythroid, and lymphoid blood compartments.
- **Transplantation Approaches:**
  - **Autologous HSCT (Approx. 60% of cases):**
    - Uses the patient's own stem cells.
    - Cells are mobilized from the bone marrow niche into peripheral circulation using granulocyte colony-stimulating factor (**G-CSF** analogues) or the CXCR4 antagonist **plerixafor**.
    - Mobilized $CD34^+$ cells are harvested via apheresis and cryopreserved.
    - The patient undergoes high-dose conditioning chemotherapy/radiotherapy to ablate the underlying malignancy (e.g., lymphoma, myeloma).
    - Cryopreserved HSCs are re-infused to rescue and reconstitute hematopoiesis.

2. Components of a Comprehensive Non-Clinical Dossier

A complete non-clinical data package for a New Chemical Entity (NCE) comprises three foundational pillars:

A. Pharmacology Studies

- **Primary Pharmacodynamics:** Assesses the on-target mechanism of action directly related to the therapeutic indication, confirming efficacy and target engagement.
- **Secondary Pharmacodynamics:** Evaluates off-target activity at secondary receptors, ion channels, enzymes, and transporters to identify potential adverse effects outside the intended pathway.
- **Safety Pharmacology:** Focuses on the acute functional effects of a drug on vital physiological systems essential for life (the core battery):
    - Central Nervous System (CNS): Motor activity, coordination, behavior, reflexes, and body temperature regulation.
    - Respiratory System: Respiratory rate, tidal volume, and oxygen saturation.
    - Cardiovascular System: Blood pressure, heart rate, electrocardiographic parameters (QTc interval), and specific ion channel assays (such as hERG potassium channel inhibition).

B. Pharmacokinetic (ADME) Studies

- Evaluates absorption, tissue distribution, biotransformation pathways, and routes of excretion in the same animal species used in pivotal toxicology studies.
- Compares animal and human metabolic profiles to confirm that human metabolites are formed in animal test species, ensuring that metabolite-driven toxicities are identified prior to clinical trials.

C. Toxicology Studies

- **Single-Dose (Acute) Studies:** Evaluates immediate adverse reactions across a range of high doses.
- **Repeated-Dose Studies:** Evaluates sub-acute (typically 14 days), sub-chronic (28 to 90 days), and chronic (6 months in rodents, 9 months in non-rodents) toxicities to identify cumulative organ damage and reversibility following treatment cessation.
- **Genotoxicity:** Assesses mutagenic and clastogenic potential through in vitro bacterial and mammalian assays alongside in vivo micronucleus testing.
- **Carcinogenicity:** Identifies tumorigenic potential through long-term exposure models.
- **Developmental and Reproductive Toxicity (DART):** Evaluates impacts on male and female fertility, embryonic and fetal development, and postnatal development.

3. Regulatory Data Integration, Exposure Comparisons, and Risk Assessment

Weight-of-Evidence (WoE) Assessment

Regulatory reviewers synthesize diverse experimental findings using a structured weight-of-evidence approach:

- **Dose Dependency:** Confirming whether the incidence and severity of an observed effect increase systematically with escalating doses.
- **Biomarker and Pathological Correlation:** Linking biochemical alterations to physical organ damage (e.g., verifying whether elevated serum transaminases correlate with macroscopic liver enlargement or microscopic hepatocellular necrosis).
- **Cross-Study Consistency:** Determining whether pathological trends reproduce across study durations and different animal models.
- **Metabolite Attribution:** Determining whether adverse findings stem from the parent drug or specific circulating metabolites.

Toxicokinetic Exposure Comparisons and Safety Margins

- Systemic drug exposure provides a more reliable metric than administered nominal dose (mg/kg) when comparing animal findings to human risk.
- **Exposure Metrics:**
  - $C_{\max}$**:** Peak systemic plasma concentration (critical for assessing acute, peak-driven toxicities).
  - AUC **(Area Under the Curve):** Total systemic exposure over a dosing interval (critical for evaluating cumulative or chronic toxicities).
- **Relative Exposure Ratio:**

$$\text{Exposure Ratio} = \frac{\text{Animal Exposure Metric at Steady State } (AUC \text{ or } C_{\max})}{\text{Human Exposure Metric at Recommended Dose } (AUC \text{ or } C_{\max})}$$

- **Threshold Doses:**
  - **NOEL (No Observed Effect Level):** The highest tested dose that produces no measurable biological, physiological, or pathological alteration.
  - **NOAEL (No Observed Adverse Effect Level):** The highest tested dose that does not produce a statistically or biologically significant adverse reaction. Minor adaptive changes (such as transient biomarker shifts) may be present, provided animal health is not compromised.
  - **LOAEL (Lowest Observed Adverse Effect Level):** The lowest tested dose that produces a statistically or biologically significant adverse effect, defining the operational threshold of toxicity.
- **Clinical Safety Confidence:** A higher exposure margin between the animal NOAEL and human clinical exposure provides greater confidence that clinical use will not elicit the toxicities observed at supratherapeutic levels in animals.