Drug Targets

Four major drug targets:

- **RECEPTORS**
  - Agonist: Direct
  - Transduction mechanisms
  - Antagonist: No effect
  - Endogenous mediators blocked

- **ION CHANNELS**
  - Blockers: Permeation blocked
  - Modulators: Increased or decreased opening probability

- **ENZYMES**
  - Inhibitor: Normal reaction inhibited
  - False substrate: Abnormal metabolite produced
  - Pro-drug: Active drug produced

- **TRANSPORTERS**
  - Normal transport
  - Inhibitor: or
  - Transport blocked
  - False substrate: Abnormal compound accumulated

Exceptions: Colchicin (acts on tubulin), Cyclosporin (acts via immunophillins), etc.
## Drug Targets

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Agonists</th>
<th>Antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinic ACh receptor</td>
<td>Acetylcholine</td>
<td>Tubocurarine</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>α-Bungarotoxin</td>
</tr>
<tr>
<td>Beta-adrenoceptor</td>
<td>Noradrenaline (norepinephrine)</td>
<td>Propranolol</td>
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<tr>
<td>Histamine (H₁-receptor)</td>
<td>Isoprenaline</td>
<td>Mepyramine</td>
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<tr>
<td>Histamine (H₂-receptor)</td>
<td>Histamine</td>
<td>Impromidine</td>
</tr>
<tr>
<td>Insulin receptor</td>
<td>Insulin</td>
<td>Ralitinidine</td>
</tr>
<tr>
<td>Opiate (μ-receptor)</td>
<td>Morphine</td>
<td>Naloxone</td>
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<tr>
<td>5-HT₂-receptor</td>
<td>5-Hydroxytryptamine</td>
<td>Ketanserin</td>
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<tr>
<td>Dopamine (D₂ receptor)</td>
<td>Dopamine</td>
<td>Chlorpromazine</td>
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<tr>
<td>Insulin receptor</td>
<td>Insulin</td>
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<tr>
<td>Oestrogen receptor</td>
<td>Ethinylestradiol</td>
<td>Tamoxifen</td>
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<tr>
<td>Progesterone receptor</td>
<td>Norethisterone</td>
<td>Danazol</td>
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</table>

### Ion channels

<table>
<thead>
<tr>
<th>Ion channels</th>
<th>Blockers</th>
<th>Modulators</th>
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</thead>
<tbody>
<tr>
<td>Voltage-gated sodium channels</td>
<td>Local anaesthetics</td>
<td>Veratridine</td>
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<tr>
<td>Renal tubule sodium channels</td>
<td>Tetrodotoxin</td>
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<tr>
<td>Voltage-gated calcium channels</td>
<td>Amiloride</td>
<td>Aldosterone</td>
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<tr>
<td>Voltage-gated potassium channels</td>
<td>Divalent cations (e.g. Ca²⁺)</td>
<td>Dihydropyridines</td>
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<tr>
<td>ATP-sensitive potassium channels</td>
<td>4-Aminopyridine</td>
<td>Many neuromodulators</td>
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<tr>
<td>GABA-gated chloride channels</td>
<td>Picrotoxin</td>
<td>Cromokalim</td>
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<tr>
<td>Glutamate-gated (NMDA) cation channels</td>
<td>Dizocilpine, Mg²⁺</td>
<td>Sulphonylureas</td>
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<tr>
<td></td>
<td>Ketamine</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Benzodiazepines</td>
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<td></td>
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<td>Glycine</td>
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# Drug Targets

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Inhibitors</th>
<th>False substrates</th>
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</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Neostigmine</td>
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<tr>
<td></td>
<td>Organophosphate</td>
<td></td>
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<tr>
<td>Choline acetyltransferase</td>
<td>Aspirin</td>
<td>Hemicholinium</td>
</tr>
<tr>
<td>Cyclooxygenase</td>
<td>Allopurin</td>
<td></td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Captopril</td>
<td></td>
</tr>
<tr>
<td>Angiotensin-converting enzyme</td>
<td>Acelozolamide</td>
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<tr>
<td>Carbonic anhydrase</td>
<td>Simvastatin</td>
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<tr>
<td>HMG-CoA reductase</td>
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<tr>
<td>Dopa decarboxylase</td>
<td>Carbidopa</td>
<td>Methyldopa</td>
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<tr>
<td>Monoamine oxidase-A</td>
<td>Iproniazid</td>
<td></td>
</tr>
<tr>
<td>Monoamine oxidase-B</td>
<td>Selegiline</td>
<td>MPTP</td>
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<tr>
<td>Dihydrofolate reductase</td>
<td>Trimeprinn</td>
<td></td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>Methotrexate</td>
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<tr>
<td>Enzymes involved in DNA synthesis</td>
<td>Cytarabine</td>
<td>Cytarabine</td>
</tr>
<tr>
<td>Enzymes of blood clotting cascade</td>
<td>Azathioprine</td>
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<tr>
<td>Plasminogena</td>
<td>Aciclovir</td>
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<tr>
<td>Thymidine kinase</td>
<td>Sequenvir</td>
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<tr>
<td>HIV protease</td>
<td></td>
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<tr>
<td>Reverse transcriptase</td>
<td>Didanosine, zidovudine</td>
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<tr>
<td>Carriers</td>
<td>Inhibitors</td>
<td>False substrates</td>
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<tr>
<td>Choline carrier (nerve terminal)</td>
<td>Hemicholinum</td>
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<td>Noradrenaline uptake 1</td>
<td>Tricyclic antidepressants</td>
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<tr>
<td></td>
<td>Cocaine</td>
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<tr>
<td>Noradrenaline uptake (vesicular)</td>
<td>Reserpine</td>
<td>Amphetamine</td>
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<tr>
<td>Weak acid carrier (renal tubule)</td>
<td>Probenecid</td>
<td>Methyldopa</td>
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<tr>
<td>Na⁺/K⁺/Cl⁻ co-transporter (loop of Henle)</td>
<td>Loop diuretics</td>
<td></td>
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<tr>
<td>Na⁺/K⁺ pump</td>
<td>Cardiac glycosides</td>
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<tr>
<td>Proton pump (gastric mucosa)</td>
<td>Omeprazole</td>
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<tr>
<td>Others</td>
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<td></td>
</tr>
<tr>
<td>Immunophilins</td>
<td>Ciclosporin, tacrolimus</td>
<td></td>
</tr>
<tr>
<td>Tubulin</td>
<td>Colchicine</td>
<td></td>
</tr>
</tbody>
</table>
Drug Targets: Receptors

Responses to the extracellular environment involve cell membrane or intracellular receptors whose engagement modulates cellular components that generate, amplify, coordinate and terminate postreceptor signaling via (cytoplasmic) second messengers.

Transmembrane signaling is accomplished by only a few mechanisms:

- **Transmembrane ion channels**: open or close upon binding of a ligand or upon membrane depolarization
- **G-protein-coupled receptors**: Transmembrane receptor protein that stimulates a GTP-binding signal transducer protein (G-protein) which in turn generates an intracellular second messenger
- **Nuclear receptors**: Lipid soluble ligand that crosses the cell membrane and acts on an intracellular receptor
- **Kinase-linked receptors**: Transmembrane receptor proteins with intrinsic or associated kinase activity which is allosterically regulated by a ligand that binds to the receptor’s extracellular domain
Drug Targets: Receptors

1. Ligand-gated ion channels (ionotropic receptors)
   - Hyperpolarisation or depolarisation
   - Cellular effects
   - Time scale: Milliseconds
   - Examples: Nicotinic ACh receptor

2. G-protein-coupled receptors (metabotropic)
   - Change in excitability
   - Second messengers
   - Ca\(^{2+}\) release
   - Protein phosphorylation
   - Cellular effects
   - Time scale: Seconds
   - Examples: Muscarinic ACh receptor

3. Kinase-linked receptors
   - Protein phosphorylation
   - Gene transcription
   - Protein synthesis
   - Cellular effects
   - Time scale: Hours
   - Examples: Cytokine receptors

4. Nuclear receptors
   - Gene transcription
   - Protein synthesis
   - Cellular effects
   - Time scale: Hours
   - Examples: Oestrogen receptor
Receptors

- Rapidly acting (milliseconds) transmembrane ion channels: Multi-unit complexes with central aqueous channel. Upon binding of a ligand, channel opening allows a specific ion travel down its concentration gradient $\Rightarrow$
  
  **Transient cell membrane depolarization** (post synaptic potential)
  
  - Example: Nicotinic acetylcholine receptor: Pentameric structure (two alpha chains, one each beta, gamma, and delta chain). Activation occurs by binding of two molecules ACh to the alpha subunits, triggering the opening of the channel for Na$^+$ and K$^+$ ions.

  **Myasthenia gravis**: Autoimmune disease caused by inactivating antibodies against NAcHR
Many other types of transmembrane ion channels ==> Ion channels are common drug targets!

• **Voltage-gated channels:**
  • Gating: controlled by membrane polarization/depolarization
  • Selectivity: Na\(^+\), K\(^+\) or Ca\(^+\) ions

• **Intracellular ligand-gated channels:**
  • Ca\(^+\) controlled K\(^+\) channel
  • ATP-sensitive K\(^+\) channel
  • IP\(_3\)-operated Ca\(^+\) channel (in the ER membrane)
Calcium as Second Messenger

\[ \text{Ca}^{++} \rightarrow \text{very important in regulating cellular and physiological responses} \]

- Extracellular concentrations are 2 mM (EM, blood), and levels in cytoplasmic vesicles and the ER can reach up to 10 mM.
- Baseline cytosolic \( \text{Ca}^{2+} \) concentration is around 100 nM in resting cells.

- High gradient makes this a very fast and sensitive signaling system: only slight changes in membrane permeability will result in dramatic changes in the concentration of \([\text{Ca}^{2+}]_i\).
- Low level of \([\text{Ca}^{2+}]_i\) is also necessary to facilitate a phosphate oriented cellular metabolism (high calcium and high phosphate concentrations are incompatible!!)
  
  \[ \rightarrow \text{Evolutionary challenge: Maintain calcium gradient} \] !!!

- Evolvement of proteins that bind \( \text{Ca}^{2+} \) with high affinity, but reject magnesium!

- **Two classes of Ca-binding proteins:**
  - membrane-integrated (unlimited capacity --> transporter systems: Ca-channels, calcium pumps)
  - non-membranous (limited capacity --> not only buffering, but processing of signal through conformational changes that enable interaction with target proteins: Calmodulin, Troponin C ...)

<table>
<thead>
<tr>
<th></th>
<th>ECF</th>
<th>ICF</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K^+ )</td>
<td>4.5</td>
<td>160</td>
</tr>
<tr>
<td>( Na^+ )</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>( Cl^- )</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>( \text{Ca}^{++} )</td>
<td>2.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Calcium as Second Messenger

Sources of Ca\(^{++}\):  
- **Extracellular compartment**: (predominantly in nerve, cardiac and smooth muscle cells)  
  Three types of plasma-membrane localized calcium channels:  
  - **Voltage operated calcium channels**:  
    Action potential depolarizes plasma membrane, which results in the opening of “voltage” dependent calcium channels (channels can be opened by increase in extracellular K\(^{+}\)).

  Each channel protein has four homologous domains, each containing six membrane spanning \(\alpha\)-helices (the fourth one functions as the “voltage” sensor.)
Calcium as Second Messenger

Three types:

<table>
<thead>
<tr>
<th>Type</th>
<th>Properties</th>
<th>Location/Function</th>
<th>Blockers</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>High activation threshold; slow inactivation</td>
<td>Plasma membrane of many cells; main Ca^{++} source for contraction in smooth and cardiac muscle</td>
<td>Dihydropyridines; verapamil; diltiazem</td>
</tr>
<tr>
<td>N</td>
<td>Low activation threshold; slow inactivation</td>
<td>Main Ca^{++} source for transmitter release by nerve terminals</td>
<td>ω-Conotoxin (snail venom)</td>
</tr>
<tr>
<td>T</td>
<td>Low activation threshold; fast inactivation</td>
<td>Widely distributed; important in cardiac pacemaker and Purkinje cells</td>
<td>Mibefradil; (verapamil; diltiazem)</td>
</tr>
</tbody>
</table>

- **Ligand gated calcium channels:**
  Calcium channels opened after ligand binding to the receptor (e.g. glutamate/NMDA receptor; ATP receptor; nicotinic ACh receptors (muscarinic ACh receptors signal through G-Proteins --> slower), prostaglandin receptors

- **Store operated calcium channels:**
  Activated by emptying of intracellular stores, exact mechanism unknown
Calcium as Second Messenger

**Intracellular compartment:** (predominantly in muscle cells)

- Calcium stored in mM concentrations in endo/sarcoplasmatic reticulum bound to *Calsequestrin*. (Previously mitochondria were thought to play an important role as Ca^{++}-stores, but the uptake rate is 10x lower than that of the ER/SR -> not useful)

Calcium release from the ER/SR is regulated by two receptors in the ER/SR membrane:

- **Ryanodine receptors (RyR):**
  - Named after sensitivity to Ryanodine: plant alkaloid, irreversible inhibitor
  - Very important in skeletal muscle: direct coupling of RyRs with the dihydropyridine receptors of the T-tubules (dihydropyridine receptors are closely related to the L-type Ca^{++} channels) (see Ca^{++} effects)
  - Activity of RyRs in non-muscle cell lacking T-tubules regulated by cyclic ADP ribose
  - Caffeine: reversible activator of RYRs

- **IP_3^-Receptors (IP_3R):**
  - Inositol-1,4,5-triphosphate is produced through the activity of receptor activated phospholipases C --> diffuses through cytoplasm and binds IP_3R
Calcium as Second Messenger

Removal of Ca$$^{2+}$$:

- **Ca$$^{2+}$$ - pumps:**
  
  Activity of these pumps is induced by increases in cytosolic calcium.

  - **Plasma membrane Na$$^+$$/Ca$$^{2+}$$ exchanger** (mainly in excitable cells, e.g. cardiac cells)
    
    three Na$$^+$$- ions are exchanged for one Ca$$^{2+}$$-ion
    
    *Digitalis alkaloids:* Na$$^+$$/ K$$^+$$-ATPase inhibitors => intracellular Na$$^+$$ raises => Na$$^+$$/Ca$$^{2+}$$ exchange less efficient => Ca$$^{2+}$$ intracellular increases => stronger contractions

  - **Plasma membrane Ca$$^{2+}$$-ATPase (PMCA)**
    
    two Ca$$^{2+}$$- ions are transported per ATP molecule hydrolyzed; regulated by CaM, PKA or PKC

  - **SR/ER Ca$$^{2+}$$-ATPase (SERCA):**
    
    80% (!) of integral membrane protein of SR
    
    target of *thapsigargin* ( => Ca$$^{2+}$$-release from intracellular stores)

- **Ca$$^{2+}$$ - buffers:**
  
  Low affinity (!) but high capacity (50-100 Ca$$^{2+}$$-ions/molecule)
  
  - Calsequestrin (very acidic, 37% of amino acids are aspartic and glutamic acid),
  
  - Calreticulin, Parvalbumin
Calcium as Second Messenger

Ca\textsuperscript{++} Sensors:

• Annexins:
  
  Family of proteins w/ common feature that they interact w/ membranes in a Ca\textsuperscript{++}- dependent manner. 
  
  Low affinity for Ca\textsuperscript{++}-ions restricts action to membrane proximity (high local Ca\textsuperscript{++} conc.); 
  
  implicated in the regulation of PLA2, cytoskeletal (re)organization and vesicle movement

• EF-hand proteins:
  
  named after the shape created by the E and F \(\alpha\)-helices of the Ca\textsuperscript{++}-binding domain; high affinity
Calcium as Second Messenger

- **Calmodulin:**
  - ubiquitous expression; binds 4 Ca\(^{++}\)-ions; acts through stimulation of either protein kinases (CaMKs)
  - or protein phosphatases (Calcineurin); also activates cAMP phosphodiesterase

- **Troponin C:**
  - restricted expression, regulates contraction of skeletal and heart muscle
Calcium as Second Messenger
Calcium as Second Messenger

Function of cytoplasmic free Ca$^{++}$:

- **Muscle contraction:**
  - **Skeletal and cardiac muscle:**
    - Contraction (=actin-myosin interaction) controlled by proteins on actin filaments (tropomyosin w/ troponin)
    - **Troponin I** inhibits formation of *cross-bridges* between actin and myosin => muscle relaxed.
    - **Troponin C** combines with Ca$^{2+}$-ions and blocks the action of Troponin I => muscle contracted
Calcium as Second Messenger

- **Smooth muscle:**
  Contraction controlled by proteins acting either on actin…
  - NO Troponin=>regulation occurs through the CaM binding *Caldesmon*:
    Low [Ca$^{++}$]: Caldesmon forms complex with actin and tropomyosin => access of myosin to actin restricted
    =>muscle relaxed.

  … or on *myosin*
  - Myosin light chains inhibit actin-myosin interaction:
    phosphorylation of *myosin light chain* (MLC) by *MLC kinase* (MLCK) relieves this inhibition =>
    phosphorylated myosin is able to interact w/ actin => contraction
Calcium as Second Messenger

- Neuronal excitibility and secretion:
  - Increase of [Ca$^{++}$] induces fusion of the synaptic vesicles with the plasma membrane => this causes exocytosis of neuro-transmitters into the synaptic cleft.
Calcium as Second Messenger

- **Immune response:**
  - TCR stimulation $\Rightarrow$ [Ca$^{++}$] increases $\Rightarrow$ activates *Calcineurin* $\Rightarrow$ dephosphorylates NFATc on ser/thr $\Rightarrow$ NFATc translocates to nucleus where it combines with NFATn and induces transcription of IL-2 gene $\Rightarrow$ T cell proliferation

  Calcineurin: target of immunesuppressive drugs FK506 and ciclosporin, which form a complex with immunophilins and compete with Ca$^{++}$/CaM for binding to calcineurin $\Rightarrow$ no NFATc activation
G-Protein-coupled Receptors

**GPCR:** Large family of receptors with a probable common evolutionary precursor. Transmembrane protein that is serpentine in shape, crossing the lipid bilayer seven times.

**G-Proteins:**

- Guanine nucleotide binding proteins: participate in reversible, GTP-mediated interactions.

- **Common features:**
  - bind GDP and GTP with high affinity, but adopt different structure depending on the bound nucleotide.
  - GTP-bound complex has high affinity for other proteins ("acceptor"), affecting their enzymatic activity
  - possess intrinsic GTPase activity that is usually activated by interaction with regulatory proteins (e.g. GAPs)
  - covalent attachment of various lipids (myristoylation, palmitoylation,...) is responsible for membrane targeting

- **Additional control exerted through:**
  - GTPase Activating Proteins (GAPs): function on small GTP binding proteins
  - Guanine-nucleotide Exchange Factors (GEFs): promote GDP release
  - Regulators of G-protein Signaling (RGSs): similar to GAPs, but act on heterotrimeric G-Proteins
G-Protein-coupled Receptors

G-Proteins:

Two major groups:

- "Small GTP binding proteins" (act downstream of receptor: ras, rac etc.) => see growth factor receptor signaling
- "Heterotrimeric G-proteins" (directly coupled to receptor and enzyme):
  - Coupled to 7 transmembrane spanning receptors: (β-adrenergic R, PG-R)
  - All members are heterotrimeric, consisting of α, β and γ subunits
G-Protein-coupled Receptors

α, β and γ subunits:

- α-subunit (23 isoforms): contains the GTP/GDP binding site, is responsible for identity
- β (5 isoforms) and γ (12 isoforms) subunits: are identical or very similar; interchangeable in vitro;
  - most of them are ubiquitously expressed; membrane anchored through prenylation of Gβ

<table>
<thead>
<tr>
<th>Gα Subclass</th>
<th>Effect</th>
<th>Associated Effector Protein</th>
<th>2nd Messenger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gs</td>
<td>↑</td>
<td>Adenylate cyclase</td>
<td>cAMP</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>Ca²⁺ channel</td>
<td>Ca²⁺</td>
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<tr>
<td></td>
<td>↓</td>
<td>Na⁺ channel</td>
<td>Change in membrane potential</td>
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<tr>
<td>Gs</td>
<td>↓</td>
<td>Adenylate cyclase</td>
<td>cAMP</td>
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<td>Ca²⁺ channel</td>
<td>Ca²⁺</td>
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<tr>
<td>Gq</td>
<td>↑</td>
<td>Phospholipase C</td>
<td>IP₃, DAG</td>
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<tr>
<td>Go</td>
<td>↑</td>
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<tr>
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<td>Ca²⁺ channel</td>
<td>Ca²⁺</td>
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<tr>
<td>Gt</td>
<td>↑</td>
<td>cGMP phosphodiesterase</td>
<td>cGMP</td>
</tr>
</tbody>
</table>

- Gq and
g-olf (expressed only in olfactory cells) are coupled to PLCβ
- Gt (=Transducin) is coupled to a cGMP phosphodiesterase and is expressed only in the rod cells of the retina (these cells are INactivated by light!). Photons hit Rhodopsin => activated opsin is generated => facilitates GTP loading of Gt => activates cGMP phosphodiesterase => cGMP (keeps Na⁺ and Ca²⁺ channels open to cause membrane depolarization => neuro-transmitter release) converted to 5’GMP (inactive => channels closed) => membrane polarization => NO neurotransmitter release)
G-Protein-coupled Receptors

Main targets:

**Phospholipase Cβ:**

see Lipid and Inositol Signaling

**Adenylate cyclase:**

- Two repeats of six transmembrane α-helices and two catalytic domains that convert ATP into cAMP
- Activated or inhibited by G-proteins (a brain specific isoform is also activated through activated CaM):
  GTP-bound G_{αs} activates AC, GTP-bound G_{αi} inhibits activity (Forskolin: direct activator of AC => cAMP↑↑)
G-Protein-coupled Receptors

Cholera-toxin: causes ADP-ribosylation of $G_{\text{sst}}$ \(\Rightarrow\) release of GTP inhibited \(\Rightarrow\) $G_{\text{sst}}$ trapped in active form. cAMP regulates secretion of fluid into the intestinum \(\Rightarrow\) enormous loss of liquid and electrolytes \(\Rightarrow\) death!

Pertussis-toxin: causes ADP-ribosylation of $G_{\text{ikt}}$, release of GDP inhibited \(\Rightarrow\) $G_{\text{ikt}}$ locked in its inactive form \(\Rightarrow\) can not inhibit AC!
Cyclic Nucleotides

Targets of cAMP:

Protein Kinase A (PKA):

- Consists of four subunits: two regulatory and two catalytic subunits => after cAMP binding to the regulatory subunits the catalytic subunits dissociate and translocate to the target substrates.
- First identified process regulated by PKA was glycogenolysis (PKA phosphorylates glycogen phosphorylase kinase which in turn activates glycogen phosphorylase. PKA also phosphorylates and inactivates glycogen synthase => release of glucose)
Cyclic Nucleotides

- PKA also phosphorylates transcription factors: CREB
  - CRE (cAMP response element) in the promoter of cAMP responsive genes
  - CREB becomes phosphorylated by PKA that translocated to the nucleus
Cyclic Nucleotides

- **Guanylate Cyclase:**
  - Yields cyclic GMP
  - Second messenger in only a few cell types (intestinal mucosa, vascular smooth muscle)
  - cGMP stimulates cGMP-dependent protein kinases
  - Action terminated by hydrolysis of cGMP and dephosphorylation of protein kinase substrates
  - Nitric Oxide activates cytoplasmic guanylyl cyclase:
Cyclic Nucleotides

Phosphodiesterases:
- Attenuate G-protein/AC coupled-receptor derived signals by converting cAMP to 5’AMP, or cGMP to 5’GMP
- several families; activation creates feed-back loops

- Phosphodiesterase inhibitors:
  Methylxanthines:
  Caffeine, theophylline => enhance and prolong the signals originating from adrenergic receptors

Sildenafil (Viagra®):
Specific PDE V inhibitor

<table>
<thead>
<tr>
<th>ISOENZYME FAMILY</th>
<th>REGULATORY CHARACTERISTICS</th>
<th>KNOWN FUNCTIONAL EFFECTS OF ISOENZYME INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ca²⁺, calmodulin-regulated with different $K_m$ values for cGMP and cAMP hydrolysis</td>
<td>CNS modulation; vasorelaxation</td>
</tr>
<tr>
<td>II</td>
<td>cGMP-stimulated cAMP hydrolysis with high $K_m$ for cAMP</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>cGMP-inhibited cAMP hydrolysis; low $K_m$ for cAMP and cGMP</td>
<td>Positive inotropism; vascular and airway dilation; inhibition of platelet aggregation; stimulation of lipolysis; inhibition of cytokine production*</td>
</tr>
<tr>
<td>IV</td>
<td>Low $K_m$ for cAMP hydrolysis</td>
<td>Airway smooth muscle relaxation; inhibition of inflammatory mediator release; CNS modulation; gastric acid secretion</td>
</tr>
<tr>
<td>V</td>
<td>High and low $K_m$ isoforms for cGMP specific hydrolysis</td>
<td>Platelet aggregation inhibition</td>
</tr>
<tr>
<td>VI</td>
<td>Activity regulated by interaction with transducin</td>
<td>Photoreceptor phosphodiesterase</td>
</tr>
<tr>
<td>VII</td>
<td>Low $K_m$ for cAMP hydrolysis</td>
<td>Abundant in skeletal muscle; present in heart and kidney</td>
</tr>
</tbody>
</table>
Regulation of Receptors

- Receptors not only initiate regulation of physiological and biochemical function but are themselves subject to many regulatory and homeostatic controls.
- Controls include regulation of synthesis and degradation of the receptor by multiple mechanisms; covalent modification, association with other regulatory proteins, and/or relocalization within the cell.
- Modulating inputs may come from other receptors, directly or indirectly.
- Receptors are always subject to feedback regulation by their own signaling outputs.
Regulation of Receptors

• Heterologous desensitization:
  – Four residues in the cytosolic domain of the β-adrenergic receptor can be phosphorylated by PKA
  – Activity of all Gs protein – coupled receptors, not just the β-adrenergic receptor, is reduced

• Homologous desensitization:
  – Other residues in the cytosolic domain of the β-adrenergic receptor are phosphorylated by the receptor-specific β-adrenergic receptor kinase (BARK)
  – BARK only phosphorylates the β-adrenergic receptor which facilitates β-arrestin binding to the phosphorylated receptor
Receptor Desensitization
Receptor Down-Regulation

- Slower onset (hours to days), more prolonged effect
- Decreased synthesis of receptor proteins
- Increase in receptor internalization and degradation
- Internalization involves endocytosis of receptor: the endocytic vesicle may ultimately return the receptor to the cell surface, or alternatively may deliver the receptor to a lysosome for destruction.
- Endocytic vesicles are associated with phosphatases which can clear phosphate from a receptor and ready it for reuse before returning it to the plasma membrane.
Nuclear Receptors

- Lipid soluble ligands that penetrate cell membrane (corticosteroids, mineralocorticoids, sex steroids, Vitamin D, thyroid hormone)
- Receptors contain DNA-binding domains and act as ligand-regulated transcriptional activators or suppressors (characteristic lag period of 30 minutes to several hours):

  Ligand binding of the receptors triggers the formation of a dimeric complex that can interact with specific DNA sequences ("Response Elements") to induce transcription. The resulting protein products possess half-lifes that are significantly longer than those of other signaling intermediates ⇒ Effects of nuclear receptor agonists can persist for hours or days after plasma concentration is zero.

Nuclear Receptor Subfamilies

- Group I
  - Cytoplasm
  - Nucleus
  - Binding site
  - Receptors: Steroid hormones
  - (3)

- Group II
  - Cytoplasm
  - Nucleus
  - Binding site
  - Receptors: Retinoids, Thyroid hormone, Vitamin D, Ecdysone
  - (0-5)

- Group III
  - Cytoplasm
  - Nucleus
  - Binding site
  - Receptors: NGFI-B, SF-1
  - (0)

- Group IV
  - Cytoplasm
  - Nucleus
  - Binding site
  - Receptors: HNF4
  - (1)
Nuclear Receptors

• **Examples:**
  – Glucocorticoids: Inhibit transcription of COX-2; induce transcription of Lipocortin
  – Mineralcorticoids: Regulate expression of proteins involved in renal function
  – Retinoids (Vit A derivatives): Control embryonic development of limbs and organs; affect epidermal differentiation => dermatological use (Acne)
  – PPARs (Peroxisome Proliferation-Activated Receptors): control metabolic processes:
    • **PPARα:** Target of **Fibrates** (cholesterol lowering drugs: stimulate β-oxidation of fatty acids)
    • **PPARγ:** Target of **Glitazones** (anti-diabetic drugs: induce expression of proteins involved in insulin signaling => improved glucose uptake)