Chapter 8- Receptors and Messengers

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Introduction

- Receptor – specific areas of proteins
  - embedded in the cell membrane
  - nucleus

ligand (endogenous/exogenous) and binding domain, biological response

secondary messengers, possibility of intervention
Introduction

- secondary messengers, possibility of intervention

agonists and antagonists (several groups of xenobiotics, not just drugs)

Cyclic adenosine monophosphate (cAMP)

Propranolol (the S isomer is 100 times more active than the R isomer)
The Chemistry of the Ligand-Receptor Binding

- Full spectrum of chemical bonding

ligand to receptor – diffusion or transport proteins

Key:

= sections of the structure of the receptor.

Dipole–dipole bonding

Hydrophobic bonding

Hydrogen bonding

Charge transfer complex

Ion–dipole bonding

Ionic bonding
The Chemistry of the Ligand-Receptor Binding

- Full spectrum of chemical bonding
  - charge-transfer complexes

- hydrophobic bonding and London dispersion forces
Structure and Classification of Receptors

- Family 1
  - endogenous ligand: fast neurotransmitters
  - nAChR, GABA$_A$ or glutamate receptors
  - general structure

Four/five subunits with total of 16-20 membrane-spanning domains.
Structure and Classification of Receptors

- Family 2
  - endogenous ligand: hormones and slow transmitters
  - mAChR and noradrenergic receptors (it is coupled to the effector system by G-protein)
  - general structure
Structure and Classification of Receptors

- **Family 3**
  - endogenous ligand: insulin and growth factors
  - insulin receptors (it is linked to tyrosine kinase)
  - general structure

![Diagram of receptor structure](image)
Structure and Classification of Receptors

- **Family 4**
  - endogenous ligand: steroid hormones, thyroid hormones, vitamins (D), retinoic acid
  - antidiuretic hormone (ADH) or vasopressing receptors
  - general structure

![Diagram of receptor structure](image-url)
Structure and Classification of Receptors

- Further classification
  - e.g. mAChR or nAChR, even further $m_1$AChR – $m_5$AChR
  - $\alpha$ or $\beta$ adrenoreceptors

Adrenaline (epinephrine)  Acetylcholine (ACh)  Noradrenaline (norepinephrine)

Nicotine  Muscarine
Ligands activate or deactivate (inhibit) – primary messengers:
- primary messengers: hormones, neurotransmitters, other endogenous substances, or xenobiotics (drugs, bacteria, virus)

Hormones:

- **Steroidal hormones**
  - Testosterone (androgen)
  - Estradiol (estrogen)

- **Peptide hormone**
  - Oxytocin (uterine stimulant)
  - L-Thyroxine (thyroid hormone)

- **Prostaglandins**
  - PGE₁ (gastric juice suppressant)
  - PGF₂α (stimulates contractions of uterine smooth muscle)
  - autocoids
General Mode of Operation

- Ligands activate or deactivate (inhibit) – primary messengers
  - primary messengers: hormones, neurotransmitters other endogenous substances, or xenobiotics (drugs, bacteria, virus)

**Neurotransmitters:**

- **Glycine**
  \[ \text{H}_3\text{NCH}_2\text{COO}^- \]

- **Met-enkephalin**
  \[ \text{H-Try-Gly-Gly-Phe-Me}(\text{OH}) \]

- **Leu-enkephalin**
  \[ \text{H-Try-Gly-Gly-Phe-Leu}(\text{OH}) \]

- **GABA** (\(\gamma\)-Aminobutyric acid)
  \[ \text{H}_3\text{NCH}_2\text{CH}_2\text{CH}_2\text{COO}^- \]

- **Angiotensin II**
  \[ \text{H-Phe-Pro-His-Ile-Tyr-Val-Arg-Asp}(\text{OH}) \]

- **Glutamic acid**
  \[ \text{H}_3\text{NCH}_2\text{CH}_2\text{COOH} \]

- **Substance P**
  \[ \text{H-Met-Leu-Gly-Phe-Phe-Gln-Gln-Pro-Lys-Pro-Arg}(\text{OH}) \]

- **Dopamine**
  \[ \text{HO-CH}_2\text{CH}_2\text{NH}_2 \]

- **Noradrenaline**
  \[ \text{HO-CHCH}_2\text{NH}_2 \]

- **Acetylcholine**
  \[ \text{(CH}_3)_3\text{NCH}_2\text{CH}_2\text{OCOCH}_3 \]

- **Serotonin (5-HT)**
  \[ \text{HO-CH}_2\text{CH}_2\text{NH}_2 \]
General Mode of Operation

- Mode of action: Superfamily 1/2

The weak ionic bonding is converted into stronger ionic bonding by a change in the receptor’s conformation. This change in conformation opens the ion channel.

The very weak hydrogen bonding is converted into stronger hydrogen bonding by a change in the receptor’s conformation. Change in conformation attracts a G-protein.
General Mode of Operation

- Superfamily 1 – ion channel control
  
e.g. nAChR
General Mode of Operation

- Superfamily 2 – most receptors have one polypeptide chain
• Superfamily 2 – role of G proteins
General Mode of Operation

- Superfamily 2 – role of G proteins

Several steps

Membrane

Activated AC

ATP

AMP

Phosphodiesterase

Protein kinase (inactive)

Protein kinase (active)

Activates various enzyme systems that transmit the original message from the primary messenger

ATP

AMP

cAMP
General Mode of Operation

- Superfamily 2 – role of G proteins

![Chemical Diagram]

- Membrane
- Active phospholipase
- Activates PKC
- PIP_2 → DAG
- Various intracellular processes
- Intracellular Ca^{2+} ions released
- ATP, ADP
- DAG-1-phosphate
- PIP_2, PIP
- DAG
- IP_3
General Mode of Operation

- Superfamily 3

Proteins with an SH2 domain are attracted to the phosphorylated tyrosine–kinase domain, activating enzyme systems and transcription factors leading to a cellular response.
General Mode of Operation

- **Superfamily 4**

![Diagram showing the general mode of operation of a protein with binding domain, zinc fingers, C-Terminal, DNA binding domain, and N-Terminal.]

-Molecular structures of:
  - **Trans-Retinoic Acid**
  - **Hydrocortisone (cortisol)**
  - **Vitamin D₂ (cholecalciferol)**
  - **Triiodothyronine (T₃)**
Ligand-Response Relationships

• L – R binding  loss of energy (affinity)

\[ K_D = \frac{[L][R]}{[L \rightarrow R]} \]

\[ pD_2 = - \log K_D = - \log EC_{50} \]

\[ K_a = \frac{[L \rightarrow R]}{[L][R]} \]
Ligand-Response Relationships

- Experimental Determination of L-R curves

Diagram:
- Amplifier
- Recorder
- Isometric transducer
- Air line
- Drug in
- Physiological solution used to surround and clean the tissue
- Tissue sample
- Heating coil to warm drug to 37°C
- Constant temperature bath (37°C)
Ligand-Response Relationships

- **Experimental Determination of L-R curves**

  **An example of an experimental trace**

  - **Concentration of agonist**
  - **Response**
  - **EC$_{50}$**

  **Plots of the dose–response results**
Ligand-Response Relationships

• Agonist Concentration-Response Relationships

% of the maximum response of the control A

- EC\textsubscript{50} of drug A
- EC\textsubscript{50} of drug B
- EC\textsubscript{50} of drug C
- Log [agonist]

\[ \text{Response (\% max)} \]

- \text{ACh} \quad EC_{50} 3 \times 10^{-7} \text{M}; \alpha, 1.00
- \text{pilocarpine} \quad EC_{50} 3 \times 10^{-6} \text{M}; \alpha, 0.77
- \text{BuCh} \quad EC_{50} 3 \times 10^{-6} \text{M}; \alpha, 0.2

\[
\begin{align*}
\text{CH}_3\text{COOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3^+ \\
\text{Acetylcholine (ACh)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3^+ \\
\text{Butylcholine (BuCh)}
\end{align*}
\]

\[
\begin{align*}
\text{Pilocarpine}
\end{align*}
\]
Ligand-Response Relationships

• Antagonist Concentration-Response Relationships

![Diagram showing agonist's normal response and antagonist's ideal case.](Image)

- EC₅₀ of drug A
- EC₅₀ of drug A in the presence of an antagonist concentration X₁
- EC₅₀ of drug A in the presence of an antagonist concentration X₂
- EC₅₀ of drug A in the presence of an antagonist concentration X₃
Ligand-Response Relationships

- Antagonist Concentration-Response Relationships

(a) $\text{Receptor + Agonist} \iff \text{Receptor–Agonist} \rightarrow \text{Normal response}$

(b) $\text{Antagonist + Receptor + Agonist} \iff \text{Agonist + Receptor–Antagonist} \iff \text{Receptor–Agonist + Antagonist}$

\[ K_1 \]

$\downarrow$

$\text{Reduced response}$
Ligand-Response Relationships

- Antagonist Concentration-Response Relationships

(a) Graph showing the concentration response relationships for various agonists (X₁, X₂, X₃) with increasing concentration of X.

(b) Schematic diagram illustrating the interaction between an agonist and an antagonist at an allosteric site on the receptor.

Graph showing the percentage of endogenous ligand bound to the receptor as a function of log [Displacing agent], with IC₅₀ indicated.
Ligand-Response Relationships

- Partial Agonists – act both ways
  - multiple pharmacophores that act differently
  - reasonable but not perfect fits
Ligand-Response Relationships

- Desensitization

\[\text{Response} \rightarrow \% \text{ Response} \]

(a)

(b)
Ligand-Receptor Theories

- Clark’s occupancy theory

\[
\frac{E}{E_{\text{max}}} = \frac{[\text{DR}]}{[\text{RT}]} = \frac{[D]}{K_D + [D]}
\]

\[K_D = EC_{50}\]
Ligand-Receptor Theories

- Clark’s occupancy theory

  new developments:
  - many D-R complex formations are not reversible
  - the R sites are not always independent
  - not every D-R formation is bimolecular
  - max response maybe obtained before every R is occupied
  - the response is not linear to the proportions of receptors occupied

- Ariens and Stephenson (1950s) – intrinsic activity/efficacy

\[ \alpha = \frac{E_{\text{max}} \text{ of a drug}}{E_{\text{max}} \text{ of the most active agonist in the same structural series}} \]

\[ \frac{E}{E_{\text{max}}} = \frac{[DR]}{[R_T]} = \frac{\alpha [D]}{K_D + [D]} \]
Ligand-Receptor Theories

• The Rate Theory (Paton, 1961)
  - stimulation only when the ligand first occupies the receptor
  - second conformational change – more stable complex
  - when ligand leaves further stimulus can occur
  - type of activity is independent of the number of receptors, it depends on the rate of binding/release

  correlation : poor

• The Two-State Model
  - receptors exist in an active/inactive state (relaxed/R, tensed/T)
  - equilibrium between the two states
• Agonists

Receptor: Histamine

Endogenous ligand:

Agonists:

2-Methylhistamine

4-Methylhistamine

2-(2-Pyridyl)ethylamine

β-Adrenergic

Isoprenaline

Terbutaline

Pirbuterol
Drug Action and Design

• Agonists

<table>
<thead>
<tr>
<th>Structure</th>
<th>Torsion</th>
<th>angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$COOCH$_2$CH$_2$\textsuperscript{+}N(CH$_3$)$_3$ Cl$^-$</td>
<td></td>
<td>+ 85</td>
</tr>
<tr>
<td>Acetylcholine chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$COOCH(CH$_3$)CH$_2$\textsuperscript{+}N(CH$_3$)$_3$ I$^-$</td>
<td></td>
<td>+ 85</td>
</tr>
<tr>
<td>(+) Acetyl-2$S$-methylcholine iodide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$COOCH$_2$CH(CH$_3$)\textsuperscript{+}N(CH$_3$)$_3$ I$^-$</td>
<td></td>
<td>+ 89</td>
</tr>
<tr>
<td>(−) Acetyl-2$R$-methylcholine iodide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$COOCH(CH$_3$)CH(CH$_3$)\textsuperscript{+}N(CH$_3$)$_3$ I$^-$</td>
<td></td>
<td>+ 76</td>
</tr>
<tr>
<td>Acetyl-1$R$,2$S$-dimethylcholine iodide</td>
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</tbody>
</table>

24X activity

(-) Acetyl-2$S$-methylcholine chloride

400X activity

(+) Acetyl-2$R$-methylcholine chloride

2$S$,3$R$,5$S$-muscarine iodide

2$R$,3$S$,5$R$-muscarine iodide
• Antagonists

**Receptor:**

**Endogenous ligand:**

**Antagonist:**

- **α-Adrenergic**
  - Tolazoline
  - Dibenamine
  - Prazosin

- **H<sub>1</sub>-Histamine**
  - Diphenhydramine hydrochloride
  - Trippamamine hydrochloride
  - Promethazine hydrochloride
• Case study 1 – CNS drugs
citalopram – antagonist antidepressant
Drug Action and Design

- Case study 1 – CNS drugs
  - citalopram – antagonist antidepressant

![Molecular structures of drugs](image)

- Talopram
- Citalopram
- Fluoxetine (Prozac)
- Fluvoxamine
- Paroxetine
- Sertraline

S-Citalopram (Escitalopram)  R-Citalopram
• Case study 1 – CNS drugs
citalopram
Drug Action and Design

• Case study 2 – β blockers (β adrenoreceptor antagonists in the heart)

Key: * = chiral centre

Isoprenaline

Dichloroisoprenaline

Pronethalol

Propranolol

Oxprenolol

Alprenolol

Timolol

Nadolol

(a)

(b)

\[
\begin{align*}
\text{Ar} + \text{ClCH}_2\text{CHO} & \xrightarrow{\text{NaOH/H}_2\text{O}} \text{ArOCH}_2\text{CH}_2\text{O} \\
\text{ArOCH}_2\text{CH}_2\text{O} & \xrightarrow{\text{R-NH}_2} \text{ArOCH}_2\text{CH}_2\text{NH-R}
\end{align*}
\]
**Case study 2 – β blockers (β adrenoreceptor antagonists in the heart)**

<table>
<thead>
<tr>
<th></th>
<th>α₁</th>
<th>β₁</th>
<th>β₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>vasodilation</td>
<td>decreases blood glucose level</td>
<td>decreases heart rate</td>
<td>bronchoconstriction decreases blood glucose</td>
</tr>
<tr>
<td>pupil constriction</td>
<td>increase in gut secretions and motility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>causes impotence</td>
<td></td>
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</tbody>
</table>

![Molecular structures of Epanolol, Primidolol, Xamoterol, Labetalol, and Celiprolol]
• Case study 2 – selective β blockers (β adrenoreceptor antagonists in the heart)

- **Sotalol**
- **Practolol**
- **Atenolol**
- **Betaxolol**
- **Bisoprolol**
- **Esmolol**
- **Metoprolol**