Principles of Neuropharmacology
Understanding the brain
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Contents

I. Basics
   Pharmacodynamics (PD), receptor binding
   Pharmacokinetics (PK), blood brain barrier

II. Neuropharmacology trends
   Drug development
   FDA approvals
Neuropharmacology goal

„Apply information about **drugs and their mechanisms of action** to develop safer, more effective treatments and eventually curative and preventive measures for a host of **nervous system abnormalities**“

*Nestler E.J., McGraw Hill*

Neuropsychopharmacology:
how drugs that influence nervous system functioning

Psychopharmacology:
the effect of drugs on psychologic parameters, emotions and cognition

Behavioral Neuropharmacology:
how drugs affect human behavior, including drug dependence and addiction

Molecular Neuropharmacology:
study of neurons and chemical interactions to develop drugs for neurological function
Pharmacodynamics (PD)

Pharmacokinetics (PK)

How drugs act on a living body, and which are the biochemical and physiological effects, dependent on drug concentration
Pharmacodynamics

Drug targets

A. RECEPTORS
- Agonist/inverse agonist
- Transduction mechanisms: Ion channel opening/closing, Enzyme activation/inhibition, Ion channel modulation, DNA transcription
- Antagonist: No effect, Endogenous mediators blocked

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

C. ENZYMES
- Inhibitor: Normal reaction inhibited
- False substrate: Abnormal metabolite produced
- Prodrug: Active drug produced

D. TRANSPORTERS
- Normal transport: Transport blocked
- Inhibitor or false substrate: Abnormal compound accumulated

Rang & Dales Pharmacology
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Pharmacodynamics

Neuropharmacologic agents
  Peripheral nervous system drugs
  Central nervous system (CNS) drugs

Mechanism
  Axonal conduction (non-selective drugs)
  Synaptic transmission (highly selective)

Effect on synaptic transmission
  Transmitter synthesis ②  (increase, decrease or enhance)
  Transmitter release ⑦
  Receptor binding ⑨  (activation, enhancement or inhibition of activation)
  Re-uptake ⑪
Receptor binding

Specific, saturable and stereoselective reaction between a (Ligand) and Receptor

\[
L + R \overset{k_{on}}{\rightleftharpoons} L-R \quad \text{Equilibrium}
\]

\[
K_D = \frac{k_{off}}{k_{on}} = \frac{[L][R]}{[L-R]}
\]

B (fractional occupancy) =
Bound receptors/total receptors =
\[
\frac{[L-R]}{[R]+[L-R]} = \frac{[L]}{[L]+K_D}
\]

When \([L] = K_D\) then \(B\) is 0.5

\[K_D \text{ (1/affinity) = ability of a drug to bind a receptor site)}\]
concentration of drug required to occupy 50% of sites at equilibrium
\(B_{max}\) total amount of binding (extrapolation)

Binding studies do not give information on Receptor function

GraphPadSoftware
Receptor binding

![Graphs showing receptor binding](image)

The graphs illustrate the binding of agonists and antagonists to receptors, with specific focus on the Langmuir model. The diagrams depict the concentration-response relationships, highlighting the binding of agonists and antagonists at different concentrations. The graphs also show the calculation of EC₅₀ and IC₅₀ values, indicating the concentrations at which a response is half-maximal for agonists and antagonists, respectively.

*Note: Diagrams and specific values are not transcribed here.*
Receptor binding

Potency:
strength of binding drug-target
(comparison of drugs A...D effects)

Selectivity:
if a drug binds only one receptor (therapeutic window)

Specificity:
able to alter a disease process while leaving other processes unaffected

Efficacy:
maximal biological effect of an agonist

Ex: Therapeutic window of Yohimbine

Affinity

Physiological action

Binding

Activation

Response

Taylor & Francis 1997; Lippincott-Raven, 1997; Churchill Livingstone, 1990
Receptor binding

*In vitro:*

Radioactive:
Cell harvester for radioligand binding assays

*In vivo:*
Positron Emission Tomography (PET)

*Non-radioactive:*
- Surface Plasmon Resonance (SPR, Biacore) binding in real time
- Fluorescence resonance energy transfer (FRET)
- Fluorescence polarization (FP)....
Receptor binding ligands

**Full agonist:** binds to receptor and initiates or alters a cellular activity
  
  Gamma-aminobutyric acid (GABA) to GABA-receptors

**Superagonist:** higher efficacy than endogenous agonist
  
  Synthetic superagonists of Vit D nuclear receptor

**Partial agonist:** binds to receptor site but induces less cellular activity than agonist
  
  Gaboxadol (hypnotic), GABA_A-receptor

**Antagonist:** affinity for the receptor but does not stimulate it
  
  **Competitive:** competes for agonist binding site. **Reversible** or **Irreversible**.
  
  **Non competitive:** binds reversibly to another site which reduces the binding of the agonist (**allosteric binding**)
Receptor binding Antagonism

**A**
Reversible competitive antagonism

Fractional agonist occupancy

![Graph showing reversible competitive antagonism with curves for different antagonist concentrations](image)

**B**
Irreversible competitive antagonism

Fractional agonist occupancy

![Graph showing irreversible competitive antagonism with curves for different antagonist concentrations](image)
Inverse agonist: opposite effect to agonist, negative efficacy (there has to be some constitutive receptor activation)

Convulsant benzodiazepines (RY023), GABA\textsubscript{A} receptor, no clinical use

Allosteric modulators: bind to sites in the receptor other than the agonist site, modify agonist activity

Benzodiazepines potentiate GABA effect
Receptor binding
Allosteric modulation

Agonist

Affinity modulation

Allosteric drug

Efficacy modulation

Orthosteric Agonism

Allosteric agonism

Response
Receptor binding
Complexity

$\text{GABA}_A$ receptor
Ligand-gated ion channel, postsynaptic, pentameric

$\text{GABA}$ (gamma-aminobutyric acid) endogenous agonist (reduces excitability)
  - **Muscimol** agonist from mushroom (sedative-hypnotic)
  - **Bicuculline** antagonist (convulsant, epilepsy)
  - **Benzodiazepines** positive allosteric (anticonvulsant, sedatives)
  - **Flumazenil** benzodiazepine antagonist (overdose)
  - **β-carboline** inverse agonist intensifies anxiety
  - **Picrotoxin** channel blocking (convulsant)

Others: ETOH, barbiturates, anaesthetic agents, neurosteroids, potentiate GABA effect
Pharmacodynamics (PD)

Pharmacokinetics (PK)

How a living body acts on drugs

“measurement of changes of drug concentrations in blood plasma along time in relation to dose as a result of absorption, metabolism, distribution and excretion”
Pharmacokinetics (PK)

**ADME**

- **Absorption** (delivery route)
- **Distribution** (where drug goes)
- **Metabolism** (transformation)
- **Excretion** (elimination)

**Bioavailability**: How much of the administered drug reaches its target
Pharmacokinetics (PK)

**Bioavailability:**
- Route of administration
- Dose
- Onset of action
- Peak action time and duration
- Frequency of dosing...

Clinical studies:
Therapeutic effect/adverse effects

- Lipid: water partition coefficient
- Plasma protein binding
- Stability once absorbed...
- **Blood Brain Barrier (BBB)**
Blood Brain Barrier

“Highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the CNS”
Physiological delivery

Modify drug that it may use endogenous nutrient transporters
L-DOPA transported by the Large-neutral amino acid carrier

Receptor-mediated transcytosis
ligand (or antibody against the receptor) conjugated to the drug
Transferrin receptor (e.g. transferrin coated nanoparticles)
Insulin receptor
LDL receptor

Pharmacological delivery

Drug modification
- decreasing drug size
- increasing lipophilicity
- linking it to a lipophilic carrier (pro-drug)
- Increasing stability and solubility: micelles, polymers

Blocking active efflux of P-glycoproteins: with certain L-type Ca\(^{2+}\) channel blockers

Disrupt BBB temporarily: Mannitol (change osmotic properties e.g. chemotherapeutics; Oregon Univ. OHSU)
Neuropharmacology Trends
Drug development
FDA approvals

„In 2009, from 10.974 clinical trials, 250 targeted depression and more than 5.500 oncology indications “...

Griffin, R., National Academies Press, 2010

....6.7% people affected by depression in the US
... 50 million americans affected by neurological disorders
... 600 aprox. neurological disorders
... 8% global health burden

Medicines in Development, 2015 Report
Drug development

Drug development typically takes 3-6 years for clinical candidates and another 6-7 years for the drug to become available, resulting in a total of 9-13 years. The financial investment is significant, ranging from $800 million to $1.4 billion.

- **3-6 YEARS**
  - **5,000-10,000 compounds**
  - 250
- **6-7 YEARS**
  - 5
  - 1
  - **0.5-2 Y**

**Clinical Candidates**
- **Drugs**
- **20-100 volunteers**
- **100-500 volunteers**
- **1000-5000 volunteers**

**Preclinical Development**
- **Phase 1**
  - PK tolerability
- **Phase 2**
  - Efficacy
- **Phase 3**
  - Safety & Efficacy
- **Launch (Phase 4)**
  - Indication
  - Discovery & expansion

**Med. Chem & SAR**
- **250 volunteers**

**Discovery**
- **Optimisation**
- **Medicinal Chemistry**
- **Structure-based drug design**
- **Selectivity screens**
- **ADMET screens**
- **Cellular/Animal disease models**
- **Pharmacokinetics**
- **Toxicology**
- **In vivo safety pharmacology**
- **Formulation**
- **Dose prediction**

**Development**
- **Lead Optimisation**
- **High-throughput Screening (HTS)**
- **Fragment-based screening**
- **Focused libraries**
- **Screening collection**

**Lead Discovery**
- **High-throughput Screening (HTS)**
- **Assay development**
- **Biochemistry**
- **Clinical/Animal disease models**

**Target Discovery**
- **Target identification**
- **Microarray profiling**
- **Target validation**
- **Assay development**
- **Biochemistry**
- **Clinical/Animal disease models**

Modified from J. Overington
„Learning from historical drug discovery data“ EMBL presentation

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Drug development
Discovery phase

Target discovery and validation:
- Discovery of a biomolecule of interest and evaluate its potential as target
- Design a bioassay to measure biological activity (drug potency),
- Perform high-throughput screening to find hits
- Hit to lead (Medicinal Chemistry)

EC<sub>50</sub> Concentration of agonist where 50% of its maximal effect is observed
IC<sub>50</sub> Concentration of an antagonist where 50% of its maximal antagonistic effect is observed
Drug development

Pre-clinical Phase

Non human studies necessary before getting EMEA/FDA permission for human studies

- **Pharmacological testing (safety pharmacology):** no cardiac dysrhythmias, blood pressure changes, ataxia

- **Preliminary toxicological testing:** maximum non toxic dose (for 28 days in two species)
  
  *Post mortem* examination: histological and biochemical signs of damage

- **PK testing:** To link pharmacological and toxicological effects to plasma concentration and drug exposure

- **Chemical and pharmaceutical development:** to assess feasibility of large-scale synthesis and purification, stability, formulation for clinical studies

*EMEA*: European Agency for the Evaluation of Medicinal Products

*FDA*: Food and Drug Administration
Drug development
Pre-clinical Phase

Parameters

**LD$_{10}$**  *Lethal* dose 10%: dose that causes death in 10% of animals tested
(3R animal ethical use: Replacement, Reduction and Refinement)

**TD$_{50}$**  Median *toxic* dose: dose at which (or above) 50% of animals tested had toxic effects

**ED$_{50}$**  Median *effective* dose: Minimum dose to show desired activity in half the members of a population after a specific duration of time

**Therapeutic index**  It is a safety parameter

\[ \text{Therapeutic index} = \frac{\text{LD}_{10}}{\text{ED}_{50}} \text{ in animals} = \frac{\text{TD}_{50}}{\text{ED}_{50}} \text{ in humans} \]

Large difference: *medication is safe*
Drug development

Clinical Phase

Compounds are tested for efficacy, side effects and potential dangers in volunteers

<table>
<thead>
<tr>
<th>Stage of Development</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-100 healthy/p</td>
<td>100-500 patients</td>
<td>$10^3$ patients</td>
<td>long term</td>
</tr>
<tr>
<td>End Point</td>
<td>Safety</td>
<td>Efficacy</td>
<td>Efficacy</td>
<td>Efficacy</td>
</tr>
<tr>
<td>Specific End Point</td>
<td>Safety Profile Tolerability</td>
<td>Cardiac Output</td>
<td>Reduction in Mortality Rate</td>
<td>Reduction in Mortality Rate</td>
</tr>
<tr>
<td>Types of Studies</td>
<td>Different Indications; Single or Multiple Dose</td>
<td>Placebo Controlled; Dose Escalation</td>
<td>Placebo Controlled; Long Term Follow Up</td>
<td>Comparative; New Indications</td>
</tr>
</tbody>
</table>

Modified from M. Silverman
BioStrategies Consulting Ltd
## General classification of drugs acting on the CNS

<table>
<thead>
<tr>
<th>Class</th>
<th>Definition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>General anaesthetic agents</td>
<td>Drugs used to produce surgical anaesthesia</td>
<td>Isoflurane, desflurane, <strong>propofol</strong>, etomidate</td>
</tr>
<tr>
<td>Analgesic drugs</td>
<td>Drugs used clinically for controlling pain</td>
<td><strong>Opiates</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neuropathic pain – carbamazepine, gabapentin,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>amitriptyline, duloxetine</td>
</tr>
<tr>
<td>Anxiolytics and sedatives</td>
<td>Drugs that reduce anxiety and cause sleep</td>
<td><strong>Benzodiazepines</strong> (e.g. diazepam -Valium-,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chlordiazepoxide, flurazepam, clonazepam)</td>
</tr>
<tr>
<td>Antiepileptic drugs</td>
<td>Drugs used to reduce seizures</td>
<td><strong>Carbamazepine</strong>, <strong>valproate</strong>, lamotrigine</td>
</tr>
<tr>
<td>Synonym: anticonvulsants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotic drugs</td>
<td>Drugs used to relieve the symptoms of schizophrenic illness</td>
<td><strong>Selective serotonin reuptake inhibitors</strong>,</td>
</tr>
<tr>
<td>(antischizophrenic drugs)</td>
<td></td>
<td><strong>tricyclic antidepressants</strong>, monoamine oxidase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inhibitors</td>
</tr>
<tr>
<td>Antidepressant drugs</td>
<td>Drugs that alleviate the symptoms of depressive illness</td>
<td><strong>Selective serotonin reuptake inhibitors</strong>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>tricyclic antidepressants</strong>, monoamine oxidase</td>
</tr>
<tr>
<td>Psychomotor stimulants (psychostimulants)</td>
<td>Drugs that cause wakefulness and euphoria</td>
<td><strong>Amphetamine</strong>, <strong>cocaine</strong>, methylphenidate,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>caffeine</strong></td>
</tr>
<tr>
<td>Psychotomimetic drugs</td>
<td>Drugs that cause disturbance of perception (particularly visual hallucinations) and of behaviour in ways that cannot be simply characterised as sedative or stimulant effects</td>
<td>Lysergic acid diethylamide (<strong>LSD</strong>), mescaline, MDMA (<strong>ecstasy</strong>, 3,4-methylenedioxymethamphetamime)</td>
</tr>
<tr>
<td>(hallucinogens)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognition enhancers</td>
<td>Drugs that improve memory and cognitive performance</td>
<td><strong>Acetylcholinesterase inhibitors</strong>: donepezil,</td>
</tr>
<tr>
<td>(nootropic drugs)</td>
<td></td>
<td>galantamine, <strong>rivastigmine</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>NMDA receptor antagonists</strong>: <strong>memantine</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others: <strong>piracetam</strong>, <strong>modafinil</strong></td>
</tr>
</tbody>
</table>

*Modified from Rang & Dales Pharmacology*
FDA approvals since 1950s

**Psychiatric disorders:** 78 NME  
**Neurological disorders:** 79 NME

**Prevalent indications**

**Targets**

Kinich MS, Drug Discovery Today, 2015

*NME*: New molecular entities
FDA approvals in psychiatric disorders

**Shortcomings:**
- Small-molecule libraries composition biased (e.g. GPCRs, ion channels)
- More inhibitors and antagonists than agonists
- 7 of 15 agonist impact GABA-A receptor
- 13 of 20 reuptake inhibitors target the Serotonin transporter
- Narrow subset of existing animal models....

*Modified from Kinch MS, Drug Discovery Today, 2015*
Neurodegenerative Diseases

„Conditions characterised by progressive cell loss with abnormal production, accumulation or misfolding of proteins“

- **Disease-modifying drugs**
- **Symptomatic antidementia agents**
  - cholinesterase inhibitors (galantamine)
  - NMDA receptor antagonist (memantine)
- **Psychotropic drugs**

(Cummings Nat. Drug Discov. 2006)
Difficulties in drug discovery for CNS diseases

Complex diseases, single targets?
CNS disorders are polygenic with environmental and epigenetic components, animal models to validate drug discovery are difficult, tissue availability...

...drugs that modulate more than one target (non-selective) are better?

Selective serotonin reuptake inhibitors:
Increase the extracellular level of serotonin by limiting reabsorption into the presynaptic cell

Table 2: Antidepressants with complex modes of action are superior to single-action antidepressants

<table>
<thead>
<tr>
<th>Prototypical drug</th>
<th>Class</th>
<th>Mode*</th>
<th>Molecular target(s)</th>
<th>Phase of testing</th>
<th>Efficacy vs SSRI</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electro-convulsive therapy</td>
<td>Somatic therapy</td>
<td>C</td>
<td>Undefined</td>
<td>In use for decades</td>
<td>Greater efficacy$^{58}$</td>
<td>None</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Tricyclic antidepressant</td>
<td>C</td>
<td>NET, SERT, 5-HT$<em>{2A}$, 5-HT$</em>{3C}$, 5-HT$_{6}$, $\alpha_1$-adrenergic, muscarinic</td>
<td>In use for decades</td>
<td>Slight advantage$^{41}$</td>
<td>Generic</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Serotonin-selective reuptake inhibitor</td>
<td>S</td>
<td>SERT</td>
<td>In use for &gt;10 years</td>
<td>N/A</td>
<td>Eli Lilly</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>Dual serotonin/norepinephrine reuptake inhibitor</td>
<td>C</td>
<td>NET; SERT</td>
<td>In use 10 years</td>
<td>Slight advantage$^{41,42,53}$</td>
<td>Wyeth</td>
</tr>
<tr>
<td>Pindolol</td>
<td>5-HT$_{1A}$ partial agonists/SSRI combination</td>
<td>C</td>
<td>5-HT$_{1A}$</td>
<td>Several double-blind, placebo controlled clinical trials completed; both drugs approved for use</td>
<td>Combination &gt; than SSRI alone in uncomplicated depression$^{59}$ but not in refractory or chronic depression$^{51}$</td>
<td>Generic</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>Dual serotonin/norepinephrine reuptake inhibitor</td>
<td>C</td>
<td>NET; SERT</td>
<td>NDA submitted</td>
<td>Unknown; predicted to be &gt; than SSRIs</td>
<td>Eli Lilly</td>
</tr>
</tbody>
</table>

How to develop best „non-selective“ drugs?

1- High-throughput screening: single molecular targets

2- Behaviour-based screening: Drug-responses in entire organisms (2 antidepressants with no affinity for known targets: YKP10A, INN00835)

3- Genomic approaches: Ability to modify the coordinated expression of gene families

4- Structure-based drug design: many molecular targets high degree of structural similarity

Multitarget drug discovery

Example: Ladostigil for Alzheimer’s disease in Phase II Clinical Trials

- Protective against oxidative stress-induced neuronal apoptosis
- Increase brain-derived nerve factor expression
- Neutroprotective

Modified from Bolognesi, Current Op in Chem Biol, 2009
Conclusions:

- Drug discovery: multidisciplinary enterprise (*in silico, in vitro, in vivo*)
- New targets in neuropharmacology
- New chemical libraries
- New animal models
- New approaches which integrate behavioural, genomic, and medicinal chemistry studies..... **INNOVATION**

420 Medicines in pipeline for neurological disorders

Monoclonal antibodies

Thank you