How do drugs work? Drug Targets

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Dept Pharmacology & Clinical Pharmacology

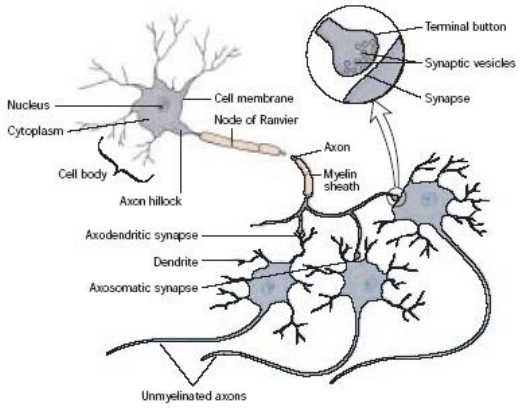
Learning Objectives

- Describe the potential drug targets within a human body.
- Describe the role of receptors, enzymes, ion channels and transporters in drug action.
- Understand how drugs bind to receptors, and define the principles of affinity, efficacy and potency and be aware of the influence of the tissue on these properties.
- Understand the concentration response curve and what information can be gained from it.
- Differentiate between inverse agonism, agonism and antagonism and explain them using the two state model of receptor activity.
- Differentiate between different types of antagonism and understand their impact on the concentration response curve.

Mechanism of Drug Action

- Most drugs produce their effects by **binding** to protein molecules. Drug binding often leads to a conformational change in the protein. Four primary drug targets are:
  - Ion channels.
  - Enzymes.
  - Carrier molecules.
  - Receptors.
- The only important exception to proteins as target sites is DNA on which a number of antitumour and antimicrobial drugs act.

Cell-to-cell communication

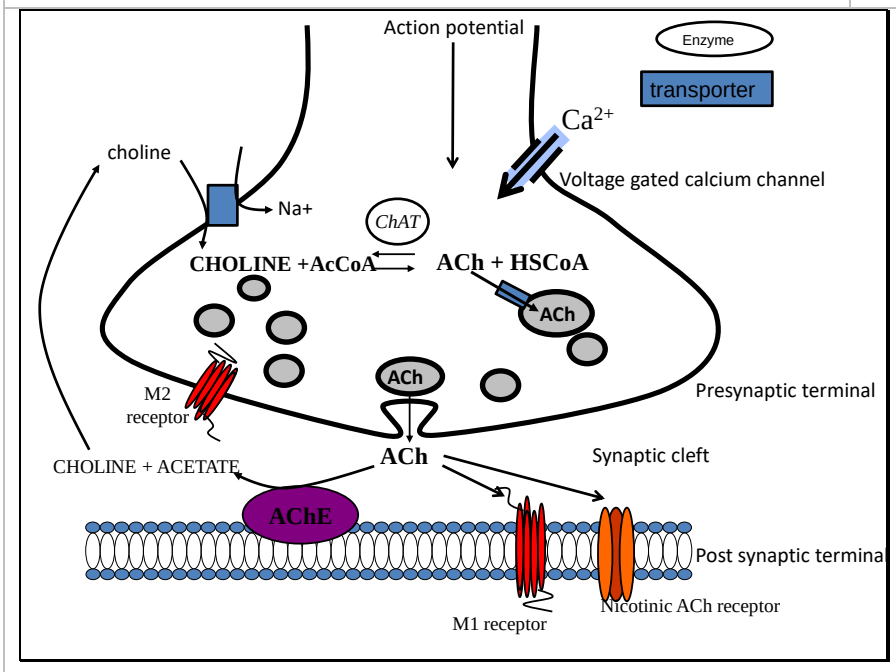


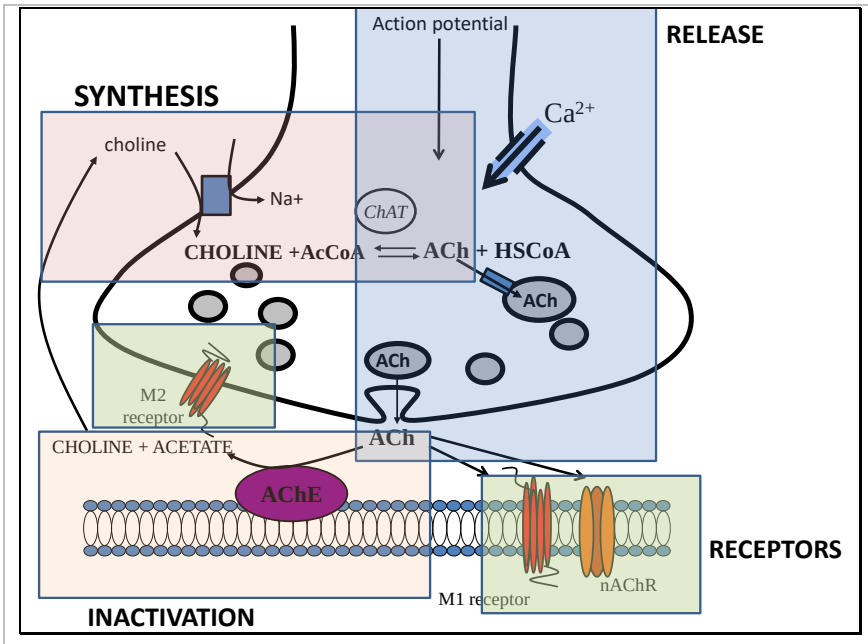
Neuroscience of psychoactive substance use and dependence. I. World Health Organization. ISBN 92 4 156235 8

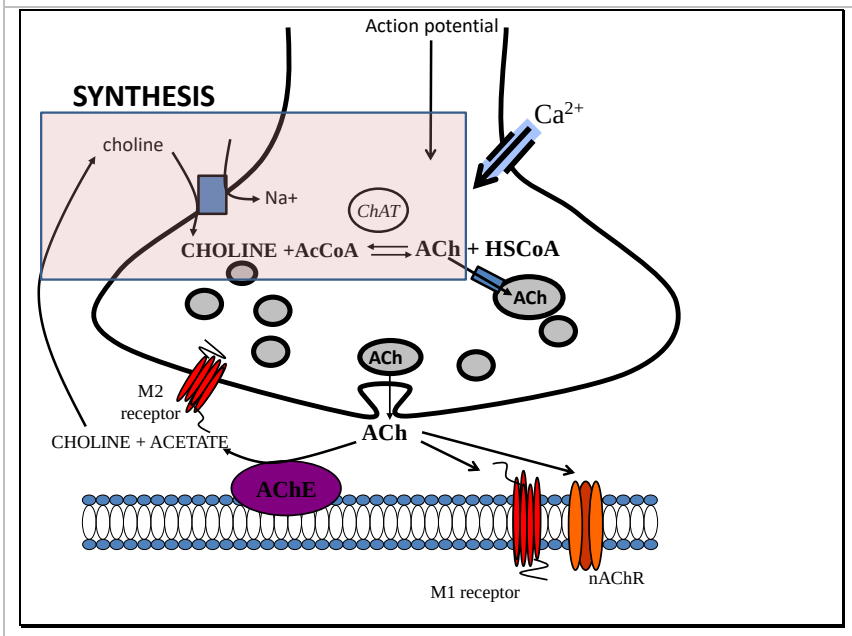
Neurotransmission is a four step process…..

- Neurotransmitter synthesis
- Neurotransmitter release
- Action on receptors
- Inactivation

- Drugs can act on all of these processes – we will look at these in more detail using the cholinergic system….




Action potential
RELEASE
SYNTHESIS
Ca2+
choline
Na+
ChAT
CHOLINE +AcCoA
ACh + HSCoA
ACh
ACh
M2
receptor
CHOLINE + ACETATE
ACh
AChE
RECEPTORS
nAChR
M1 receptor
INACTIVATION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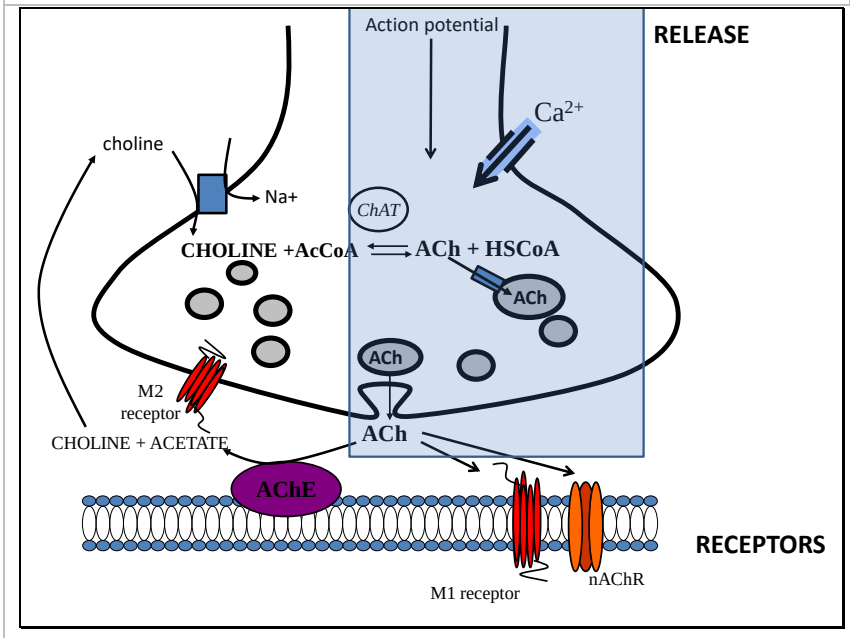
Synthesis

- Two key sites of action
  - Choline transporter – critical to uptake of choline, rate limiting step in ACh synthesis.
  - Choline Acetyl Transferase (ChAT) - enzyme involved in synthesis of choline

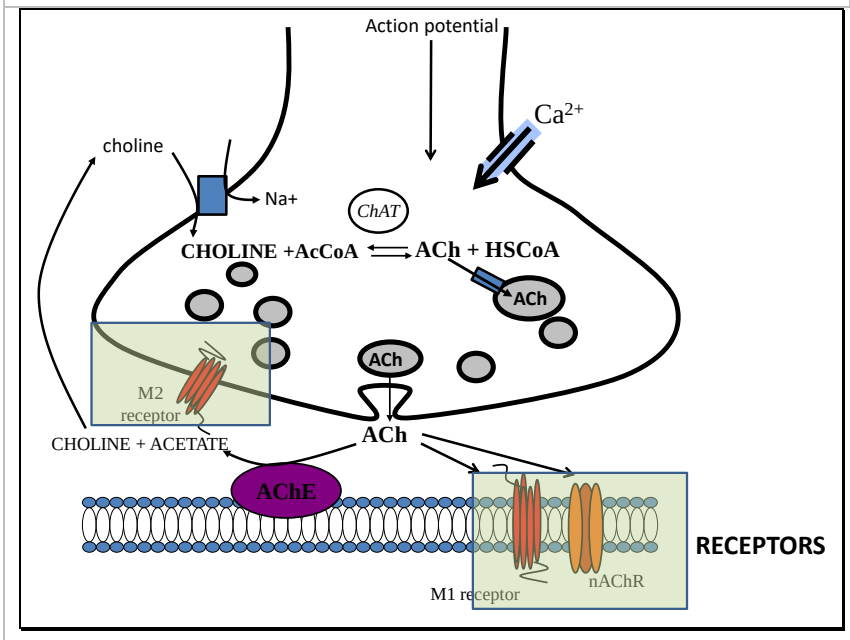
➢ What effect would drugs that inhibited these proteins have on neurotransmission?



Release

- ACh is packaged in synaptic vesicles by the vesicular ACh transporter.
- Vesicles are held in the cytoskeleton by Ca2+ sensitive vesicle membrane proteins (VAMPs).
- When an action potential reaches the terminal, voltage dependent calcium channels open and Ca2+ rushes in triggering vesicular fusion with the cell membrane and release of ACh into the synapse

➢ Sites of Drug Action? – what effect?



Receptors

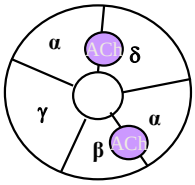
- Proteins which specifically recognise a particular neurotransmitter/hormone and upon binding undergo a conformation change leading to activation/inhibition of cell signalling.
- Four main families of receptor:
  - Ligand Gated Ion Channels (Ionotropic receptors)
  - G-protein coupled receptors
  - Tyrosine kinase/cytokine receptors
  - Nuclear/Steroid Hormone Receptors

	Ligand Gated Ion Channels	GPCR	Kinase-linked receptor	Nuclear Receptor
Location	Membrane	Membrane	Membrane	Intracellular
Effector	Ion channel	Channel or enzyme	Enzyme	Gene Transcription
Coupling	Direct	G-protein	Direct	Via DNA
Examples	Nicotinic, GABAa	Dopamine, cannabinoid, adenosine, muscarinic GABAB	Insulin, growth factor, cytokine	Steroid, thyroid hormone receptors
Structure	Oligomeric assembly of subunits surrounding pore	Monomeric structure of 7 transmembrane domains	Single transmembrane helix linking extracellular receptor to intracellular kinase domain	Monomeric structure with separate receptor and DNA binding domains.

Ligand Gated Ion Channels

- Mediate fast signal transmission at synapses (action occurs in a fraction of a millisecond, and is equivalent to about 10^7 ions per second)
- All are multi-subunit complexes
- All have three important properties:
  - They are activated in response to specific ligands
  - They conduct ions through the otherwise impermeable cell membrane
  - They select among different ions

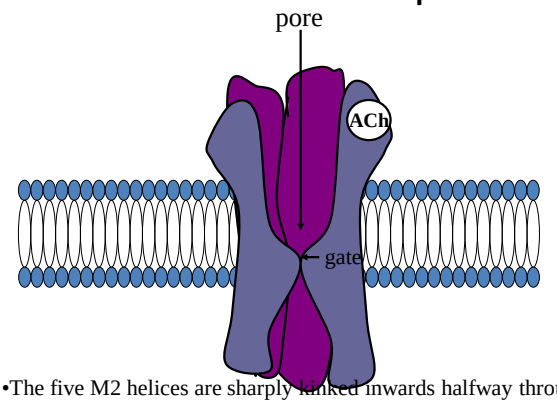
Nicotinic Acetylcholine Receptors



Neuronal
nAChR

•Subunits clustered around central receptor channel
•Each subunit has 4 transmembrane domains (20 TM domains in total)
•ACh binding site is a dimer formed by 3 or more peptide loops on a subunit and 2 loops on adjacent subunit (complementary component).
•Each ACh binding site sites at the interface between one of the two alpha-subunits and its neighbour.
•Binding to both sites is needed for channel opening.

The Receptor Pore



•The five M2 helices are sharply kinked inwards halfway through the membrane forming a constriction.
•The M2 helices are believed to snap to attention when Ach binds, opening the channel

The receptor pore

- Ion conductivity is highly selective, and determined by amino acids in TM2.
- Most excitatory neurotransmitters (ACh, Glutamate) cause an increase in Na+ and K+ permeability.
- The inside of the cell therefore becomes more positive (depolarised) and has an increased probability for an action potential.
- For nACh, mutation of a critical residue in TM2 changes the ion channel from being cation selective to being anion selective.

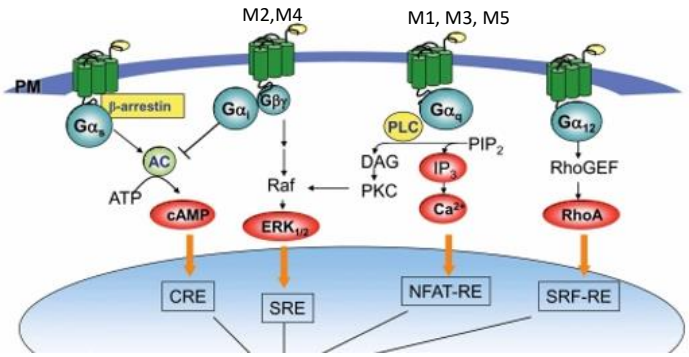
Ionotropic Receptors As Drug Targets

- **$GABA_A$** – benzodiazepines and barbiturates (sedation and anxiolytic effects). Muscimol (hallucinogenic mushroom). Flumazinal.
- **Glutamate** – ketamine (aneasthetic). Major target for neuroprotection and anti-convulsants, but to date all compounds have shown major adverse effects (predominantly hallucinations)
- **Nicotinic**– nicotine, pancuronium (antagonist) used as muscle relaxants during anaesthesia.

Families Of Receptors

- Ligand Gated Ion Channels (Ionotropic receptors)
- G-protein coupled receptors
- Tyrosine kinase/cytokine receptors
- Nuclear/Steroid Hormone Receptors

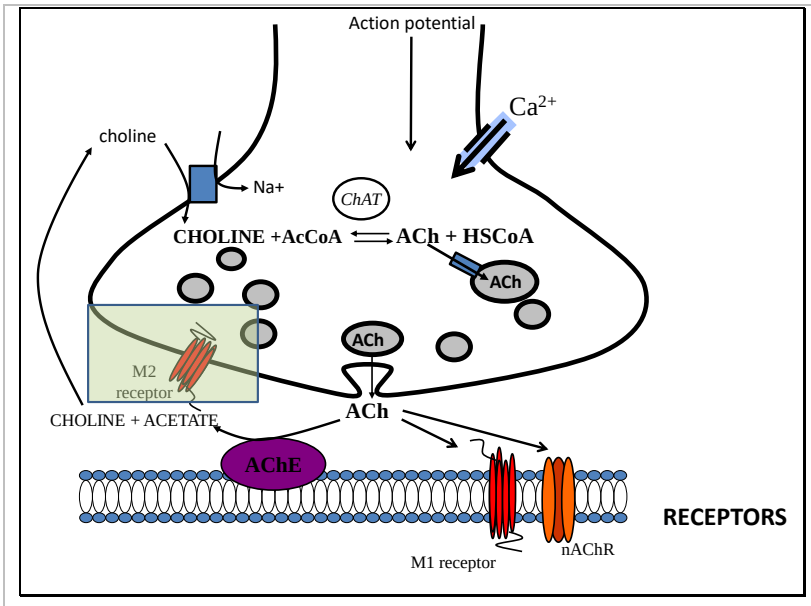
Key GPCR signalling pathways



Clinical condition	Drug	References‡
Alzheimer's disease Cognitive impairment	M_1, M_2 or mixed M_1/M_2 agonist; M_2 antagonist§	51,52,54–56, 58,69
Sjögren's syndrome	M_1, M_3 or mixed M_1/M_3 agonist	15,25,114
Schizophrenia	M_1, M_4 or mixed M_1/M_4 agonist	18,74,75
Parkinson's disease	M_1, M_4 or mixed M_1/M_4 antagonist	14,18,22,72
Type 2 diabetes	M_3 agonist (peripherally acting)	29
Obesity	M_3 antagonist (centrally acting)	16,129
Peptic ulcer disease‖	M_3 or mixed M_3/M_5 antagonist	116,117
OAB, COPD	M_3 antagonist	27,94,95,101
Irritable bowel syndrome Gastrointestinal spasms	M_3 or mixed M_2/M_3 antagonist	15,28,105–110
Antinociception	M_4 agonist	26
Wound healing	M_4 agonist, M_3 antagonist	121
Cerebrovascular insufficiency	M_5 agonist	19,69
Drug addiction and withdrawal	M_5 antagonist	83–85

*The proposed therapeutic uses of the indicated agents are based on gene targeting and/or pharmacological data (see text for details). ‡Note that only studies dealing with muscarinic acetylcholine receptor (*mAChR*) mutant mice have been included in this table. §Although several pharmacological studies suggest that M_2 receptor-preferring antagonists can enhance cognition in experimental animals[67,68], studies with *M2R*$^{-/-}$ mice have shown that the complete lack of M_2 receptors is associated with cognitive deficits (see text for details)[59]. ‖Note that pirenzepine and telenzepine, two M_1 receptor-preferring antagonists, are used for the treatment of peptic ulcer disease in Europe, Japan and Canada. COPD, chronic obstructive pulmonary disease; OAB, overactive bladder.

Potential therapeutic uses of mAChR subtype-selective compounds*
From: :
***Nature Reviews Drug Discovery* 6, 721-733 (2007)**
doi:10.1038/nrd2379



Pre-Synaptic Receptors

- Presynaptic receptors are usually Gi linked.
- Activation of them leads to inhibition of voltage sensitive Ca^{2+} channels
- This results in decreased neurotransmitter release (feedback loop)
- Because the pre-synaptic receptors are pharmacologically distinct from the post-synaptic receptors, specific drugs can be designed to target these receptors.
- Drugs which block presynaptic receptors can result in a 10 fold increase in neurotransmitter release.

Other drugs acting through GPCRs

- β-adrenoceptor – propranolol, isoprenaline
- Adenosine receptors – caffeine, theophylline
- Dopamine receptors – L-dopa, haloperidol, bromocryptine
- Opioid receptors – morphine, codeine
- Serotonin receptors – buspirone, ondansetron, LSD
- Muscarinic receptors – atropine
- Cannabinoid receptors – cannabis, rimonabant, Sativex

Families Of Receptors

- Ligand Gated Ion Channels (Ionotropic receptors)
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Tyrosine Kinase Receptors

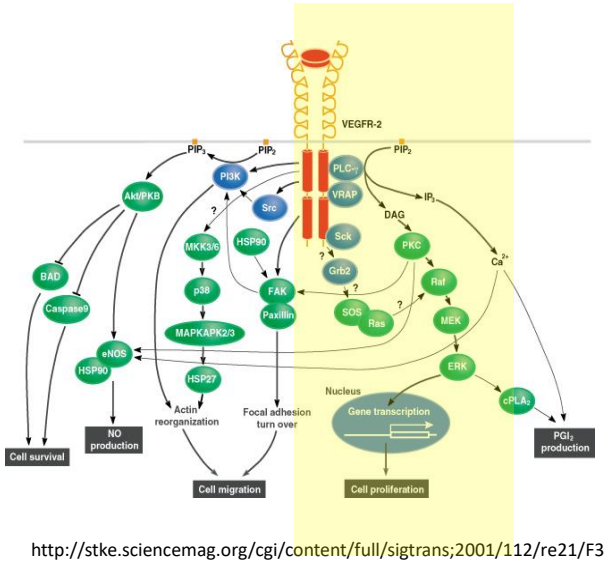
- Receptor functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins.
- Tyrosine kinase receptors mediate the actions of growth factors, cytokines and certain hormones (eg insulin).

Vascular Endothelial Growth Factor Receptors

- Essential for angiogenesis during development, pregnancy, wound healing
- Also in pathophysiological conditions eg cancer, rheumatoid arthritis, cardiovascular disease.
- Multiple receptors/multiple ligands, we will look briefly at VEGFR2

VEGFR2

- Ligand stimulated receptor dimerisation
- Autophosphorylation of tyrosine residues in cytoplasmic domain
- Associates with SH2 domain proteins
- Activation regulates a multitude of biological functions
  - Endothelial cell survival
  - Endothelial cell proliferation
  - Endothelial cell migration
  - NO and PGI2 production
  - Increase vascular permeability



http://stke.sciencemag.org/cgi/content/full/sigtrans;2001/112/re21/F3

Proliferation pathways

- Receptor activation leads to activation of PLCγ by phosphorylation.
- PLC γ-hydrolyses PIP2 to DAG + IP3
- DAG activates PKC
- PKC activation leads to activation of ERK via Raf and MEK
- ERK activation leads to increased gene transcription

What therapies might target these pathways?

- Angiogenesis inhibitors?
- Angiogenesis stimulators?