Topic 10 Drugs of the Nervous System

Ch 19,20 Patrick
Part VI- Nervous system -Corey
Part 1: Cholinergics & anticholinesterases

1. Nerve Transmission
2. Neurotransmitter
3. Transmission
4. Cholinergic receptors
   4.1. Nicotinic receptor
   4.2. Muscarinic receptor - G Protein coupled receptor
5. Cholinergic agonists
   5.1. Acetylcholine as an agonist
   5.2. Nicotine and muscarine as cholinergic agonists
   5.3. Requirements for cholinergic agonists
6. SAR for acetylcholine
7. Binding site (muscarinic)
8. Active conformation of acetylcholine
9. Instability of acetylcholine
10. Design of cholinergic agonists
11. Uses of cholinergic agonists
CHOLINERGIC NERVOUS SYSTEM
1. Nerve Transmission

Peripheral nervous system
1. Nerve Transmission

Peripheral nervous system

CNS (Somatic)

CNS (Autonomic)
- Sympathetic
- Parasympathetic

Synapse
- Ach (N)
- NA

Adrenal medulla
- Adrenaline

Smooth muscle
- Cardiac muscle

Skeletal muscle
- Ach (N)
1. Nerve Transmission

Synapses

- Nerve impulse
- Vesicles containing neurotransmitters
- Release of neurotransmitters
- Receptor binding and new signal

Dimensions: 100-500 Å
2. Neurotransmitter

Acetylcholine (Ach)
3. Transmission process

Signal in nerve 1

- Acetylcholine
- Vesicle
- Acetylcholinesterase enzyme
- Cholinergic receptor
3. Transmission process

Vesicles fuse with membrane and release Ach
3. Transmission process
3. Transmission process

- Receptor binds Ach
- Induced fit triggers 2° message
- Triggers firing of nerve 2
- Ach undergoes no reaction
3. Transmission process

- Ach departs receptor
- Receptor reverts to resting state
- Ach binds to acetylcholinesterase
3. Transmission process

Ach hydrolysed by acetylcholinesterase

Acetylcholine $\xrightarrow{\text{Acetylcholinesterase}}$ Acetic acid + Choline
3. Transmission process

Choline binds to carrier protein

Carrier protein for choline
3. Transmission process

Choline transported into nerve
3. Transmission process

Ach resynthesised

E 1 = Choline acetyltransferase

\[
\text{Choline} + \text{SCoA} \rightarrow \text{Acetylcholine}
\]
3. Transmission process

Ach repackaged in vesicles
4. Cholinergic receptors

Receptor types

- Not all cholinergic receptors are identical
- Two types of cholinergic receptor - nicotinic and muscarinic
- Named after natural products showing receptor selectivity

Acetylcholine is natural messenger for both receptor types

Nicotine

L-(+)-Muscarine

Activates cholinergic receptors at nerve synapses and on skeletal muscle
Activates cholinergic receptors on smooth muscle and cardiac muscle
Peripheral nervous system

CNS (Somatic)

CNS (Autonomic)
- Sympathetic
- Parasympathetic

Synapse

Ach (N)

Adrenal medulla

Adrenaline

Ach (N)

NA

Smooth Muscle
Cardiac Muscle
4.1 Nicotinic receptor

Control of Cationic Ion Channel:

- Receptor
- Binding site
- Messenger

Five glycoprotein subunits traversing cell membrane

Induced fit ‘Gating’ (ion channel opens)
4.1 Nicotinic receptor

The binding sites

Cell membrane

Binding sites

2xα, β, γ, δ subunits

Ion channel

Two ligand binding sites mainly on α-subunits
4.2 Muscarinic receptor - G Protein coupled receptor

Activation of a signal protein

- Receptor binds messenger leading to an induced fit
- Opens a binding site for a signal protein (G-protein)
4.2 Muscarinic receptor - G Protein coupled receptor

Activation of membrane bound enzyme

- G-Protein is split and subunit activates a membrane bound enzyme
- Subunit binds to an allosteric binding site on enzyme
- Induced fit results in opening of an active site
- Intracellular reaction is catalysed
5. Cholinergic agonists

5.1 Acetylcholine as an agonist

**Advantages**
- Natural messenger
- Easily synthesized

**Disadvantages**
- Easily hydrolysed in stomach (acid catalysed hydrolysis)
- Easily hydrolysed in blood (esterases)
- No selectivity between receptor types
- No selectivity between different target organs
5. Cholinergic agonists

5.2 Nicotine and muscarine as cholinergic agonists

Advantages

- More stable than Ach
- Selective for main cholinergic receptor types
- Selective for different organs

Disadvantages

- Activate receptors for other chemical messengers
- Side effects
5. Cholinergic agonists

5.3 Requirements for cholinergic agonists

- Stability to stomach acids and esterases
- Selectivity for cholinergic receptors
- Selectivity between muscarinic and nicotinic receptors
- Knowledge of binding site
- SAR for acetylcholine
6. SAR for acetylcholine

Quaternary nitrogen is essential

Bad for activity
6. SAR for acetylcholine

- Distance from quaternary nitrogen to ester is important
- Ethylene bridge must be retained

Bad for activity
6. SAR for acetylcholine

Ester is important

Bad for activity
6. SAR for acetylcholine

Minimum of two methyl groups on quaternary nitrogen

Bad for activity

Active
6. SAR for acetylcholine

Methyl group of acetoxy group cannot be extended

Bad for activity
6. SAR for acetylcholine

Conclusions:
- Tight fit between Ach and binding site
- Methyl groups fit into small hydrophobic pockets
- Ester interacting by H-bonding
- Quaternary nitrogen interacting by ionic bonding
7. Binding site (muscarinic)
7. Binding site (muscarinic)
7. Binding site (muscarinic)

- Possible induced dipole dipole interaction between quaternary nitrogen and hydrophobic aromatic rings in binding site
- N\(^+\) induces dipole in aromatic rings
8. Active conformation of acetylcholine

- Several freely rotatable single bonds
- Large number of possible conformations
- Active conformation does not necessarily equal the most stable conformation
8. Active conformation of acetylcholine

Rigid Analogues of acetylcholine

- Rotatable bonds ‘locked’ within ring
- Restricts number of possible conformations
- Defines separation of ester and N

Muscarinic receptor

Nicotinic receptor
9. Instability of acetylcholine

- Neighbouring group participation
- Increases electrophilicity of carbonyl group
- Increases sensitivity to nucleophiles
10. Design of cholinergic agonists

Requirements

- Correct size
- Correct pharmacophore - ester and quaternary nitrogen
- Increased stability to acid and esterases
- Increased selectivity
10. Design of cholinergic agonists

Use of steric shields

Rationale

- Shields protect ester from nucleophiles and enzymes
- Shield size is important
- Must be large enough to hinder hydrolysis
- Must be small enough to fit binding site
10. Design of cholinergic agonists

Methacholine

Properties

- Three times more stable than acetylcholine
- Increasing the shield size increases stability but decreases activity
- Selective for muscarinic receptors over nicotinic receptors
- S-enantiomer is more active than the R-enantiomer
- Stereochemistry matches muscarine
- Not used clinically

![Chemical structures](image-url)
10. Design of cholinergic agonists

Use of electronic factors

- Replace ester with urethane
- Stabilises the carbonyl group
10. Design of cholinergic agonists

Properties

- Resistant to hydrolysis
- Long lasting
- NH$_2$ and CH$_3$ are equal sizes. Both fit the hydrophobic pocket
- NH$_2$ = bio-isostere
- Muscarinic activity = nicotinic activity
- Used topically for glaucoma
10. Design of cholinergic agonists

Steric + Electronic factors

Properties

- Very stable
- Orally active
- Selective for the muscarinic receptor
- Used to stimulate GI tract and urinary bladder after surgery

\[
\text{Bethanechol}
\]

\[
\text{H}_2\text{N-C(O)O-Me}^+ \quad \text{NMe}_3
\]
10. Design of cholinergic agonists

Nicotinic selective agonist

\[ \text{Me} \quad \text{C} \quad \text{O} \quad \text{O} \quad * \quad \text{asymmetric centre} \]

\[ \text{NMe}_3 \quad \text{Me} \]

* asymmetric centre
11. Uses of cholinergic agonists

Nicotinic selective agonists

Treatment of myasthenia gravis

- lack of acetylcholine at skeletal muscle causing weakness

Muscarinic selective agonists

• Treatment of glaucoma
• Switching on GIT and urinary tract after surgery
• Treatment of certain heart defects. Decreases heart muscle activity and decreases heart rate
Peripheral nervous system

CNS (Somatic)

Somatic Muscle

CNS (Autonomic)

Sympathetic

Parasympathetic

Synapse

Ach (N)

NA

Skeletal Muscle

AUTONOMIC

Synapse

Adrenal medulla

Adrenaline

Smooth Muscle
Cardiac Muscle
Contents

Part 1: Cholinergics & anticholinesterases

1. Nerve Transmission
2. Neurotransmitter
3. Transmission process
4. Cholinergic receptors
    4.1. Nicotinic receptor
    4.2. Muscarinic receptor - G Protein coupled receptor
5. Cholinergic agonists
    5.1. Acetylcholine as an agonist
    5.2. Nicotine and muscarine as cholinergic agonists
    5.3. Requirements for cholinergic agonists
6. SAR for acetylcholine
7. Binding site (muscarinic)
8. Active conformation of acetylcholine
9. Instability of acetylcholine
10. Design of cholinergic agonists
11. Uses of cholinergic agonists
CHOLINERGIC NERVOUS SYSTEM
1. Nerve Transmission

Peripheral nervous system
1. Nerve Transmission

Peripheral nervous system
1. Nerve Transmission

Synapses

Nerve impulse

100-500Å

Receptors

Vesicles containing neurotransmitters

Release of neurotransmitters

Receptor binding and new signal

New signal
Part 2: Cholinergics & anticholinesterases

12. Cholinergic Antagonists (Muscarinic receptor)
   12.1. Atropine
   12.2. Hyoscine (scopolamine)
   12.3. Comparison of atropine with acetylcholine
   12.4. Analogues of atropine
   12.5. Simplified Analogues
   12.6. SAR for Antagonists
   12.7. Binding Site for Antagonists

13. Cholinergic Antagonists (Nicotinic receptor)
   13.1. Curare
   13.2. Binding
   13.3. Analogues of tubocurarine
12. Cholinergic Antagonists (Muscarinic receptor)

- Drugs which bind to cholinergic receptor but do not activate it
- Prevent acetylcholine from binding
- Opposite clinical effect to agonists - lower activity of acetylcholine
12. Cholinergic Antagonists (Muscarinic receptor)

Clinical Effects

• Decrease of saliva and gastric secretions
• Relaxation of smooth muscle
• Decrease in motility of GIT and urinary tract
• Dilation of pupils

Uses

• Shutting down digestion for surgery
• Ophthalmic examinations
• Relief of peptic ulcers
• Treatment of Parkinson’s Disease
• Anticholinesterase poisoning
• Motion sickness
12.1 Atropine

- Racemic form of hyoscyamine
- Source - roots of belladonna (1831) (deadly nightshade)
- Used as a poison
- Used as a medicine
  - decreases GIT motility
  - antidote for anticholinesterase poisoning
  - dilation of eye pupils
- CNS side effects - hallucinations
12. Cholinergic Antagonists (Muscarinic receptor)

12.2 Hyoscine (scopolamine)

- Source - thorn apple- *Datura*-jimsonweed
- Medical use - treatment of motion sickness
- CNS effects, hallucinations
12. Cholinergic Antagonists (Muscarinic receptor)

12.3 Comparison of atropine with acetylcholine

- Relative positions of ester and nitrogen similar in both molecules
- Nitrogen in atropine is ionised
- Amine and ester are important binding groups (ionic + H-bonds)
- Aromatic ring of atropine is an extra binding group (vdW)
- Atropine binds with a different induced fit - no activation
- Atropine binds more strongly than acetylcholine
12. Cholinergic Antagonists (Muscarinic receptor)

12.4 Analogues of atropine

- Analogues are fully ionized
- Analogues unable to cross the blood brain barrier
- No CNS side effects

Ipratropium  
(bronchodilator & anti-asthmatic)

Atropine methonitrate  
(lowers GIT motility)
12. Cholinergic Antagonists (Muscarinic receptor)

12.5 Simplified Analogues

Pharmacophore = ester + basic amine + aromatic ring

Amprotropine

Tridihexethyl bromide

Propantheline chloride
12. Cholinergic Antagonists (Muscarinic receptor)

12.5 Simplified Analogues

Tropicamide (ophthalmics)

Cyclopentolate (ophthalmics)

Benztropine (Parkinson’s disease)

Benzhexol (Parkinson’s disease)

Pirenzepine (anti-ulcer)
12. Cholinergic Antagonists (Muscarinic receptor)

12.6 SAR for Antagonists

```
R₂N
CH₂
CH₂
O
C
O
CH
R'
```

R' = Aromatic or Heteroaromatic

**Important features**

- Tertiary amine (ionised) or a quaternary nitrogen
- Aromatic ring
- Ester
- \( N\)-Alkyl groups (R) can be larger than methyl (unlike agonists)
- Large branched acyl group
- R’ = aromatic or heteroaromatic ring
- Branching of aromatic/heteroaromatic rings is important
12. Cholinergic Antagonists (Muscarinic receptor)

12.6 SAR for Antagonists

[Chemical structures of active and inactive molecules are shown here]
12. Cholinergic Antagonists (Muscarinic receptor)

12.6 SAR for Antagonists vs. Agonists

<table>
<thead>
<tr>
<th>SAR for Antagonists</th>
<th>SAR for Agonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiary amine (ionized) or quaternary nitrogen</td>
<td>Quaternary nitrogen</td>
</tr>
<tr>
<td>Aromatic ring</td>
<td></td>
</tr>
<tr>
<td>Ester</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td>(N)-Alkyl groups ((R)) can be larger than methyl</td>
<td>Ester</td>
</tr>
<tr>
<td>(R' = \text{aromatic or heteroaromatic} )</td>
<td>(N)-Alkyl groups = methyl</td>
</tr>
<tr>
<td>Branching of Ar rings important</td>
<td>(R' = H )</td>
</tr>
</tbody>
</table>

12. Cholinergic Antagonists (Muscarinic receptor)
12. Cholinergic Antagonists (Muscarinic receptor)

12.7 Binding Site for Antagonists

- Acetylcholine binding site
- van der Waals binding regions for antagonists
12. Cholinergic Antagonists (Muscarinic receptor)

12.7 Binding Site for Antagonists
13. Cholinergic Antagonists (Nicotinic receptor)

13.1 Curare

- Extract from curare plant: *Strychnos toxifera*
- Used for poison arrows
- Causes paralysis (blocks acetylcholine signals to muscles)
- Active principle = tubocurarine
13. Cholinergic Antagonists (Nicotinic receptor)

**Pharmacophore**
- Two quaternary centres at specific separation (1.15nm)
- Different mechanism of action from atropine based antagonists
- Different binding interactions

**Clinical uses**
- Neuromuscular blocker for surgical operations
- Permits lower and safer levels of general anaesthetic
- Tubocurarine used (previously) as neuromuscular blocker but side effects
13. Cholinergic Antagonists (Nicotinic receptor)

13.2 Binding

a) Receptor dimer

protein complex
(5 subunits)
diameter=8nm

8nm

9-10nm

b) Interaction with tubocurarine

Probably not bridging Ach sites

Tubocurarine
Acetylcholine binding site
13. Cholinergic Antagonists (Nicotinic receptor)

13.3 Analogues of tubocurarine

- Decamethonium
  - Long lasting
  - Long recovery times
  - Side effects on heart

- Suxamethonium
  - Esters incorporated
  - Shorter lifetime (5 min)
  - Fast onset and short duration
  - Side effects at autonomic ganglia
13. Cholinergic Antagonists (Nicotinic receptor)

13.3 Analogues of tubocurarine

- Steroid acts as a spacer for the quaternary centres (1.09nm)
- Acyl groups are added to introduce the Ach skeleton
- Faster onset than tubocurarine but slower than suxamethonium
- Longer duration of action than suxamethonium (45 min)
- No effect on blood pressure and fewer side effects
13.3 Analogues of tubocurarine

- Design based on tubocurarine and suxamethonium
- Lacks cardiac side effects
- Rapidly broken down in blood both chemically and metabolically
- Avoids patient variation in metabolic enzymes
- Lifetime is 30 minutes
- Administered as an i.v. drip
- Self destruct system limits lifetime

**Atracurium**
13. Cholinergic Antagonists (Nicotinic receptor)

13.3 Analogues of tubocurarine

Atracurium stable at acid pH
Hofmann elimination at blood pH (7.4)
13. Cholinergic Antagonists (Nicotinic receptor)

13.3 Analogues of tubocurarine

Mivacurium

- Faster onset (2 min)
- Shorter duration (15 min)
Contents

Part 3: Cholinergics & anticholinesterases

14. Acetylcholinesterase
   14.1. Role
   14.2. Hydrolysis reaction catalysed
   14.3. Effect of inhibition
   14.4. Structure of enzyme complex
   14.5. Active site - binding interactions
   14.6. Active site - Mechanism of catalysis

15. Anticholinesterases
   15.1. Physostigmine
   15.2. Mechanism of action
   15.3. Physostigmine analogues
   15.4. Organophosphates
   15.5. Anticholinesterases as ‘Smart Drugs’
14. Acetylcholinesterase

14.1 Role

- Hydrolysis and deactivation of acetylcholine
- Prevents acetylcholine reactivating receptor
14. Acetylcholinesterase

14.2 Hydrolysis reaction catalysed

Acetylcholine $\rightarrow$ Acetic acid + Choline

active $\rightarrow$ inactive
14. Acetylcholinesterase

14.3 Effect of inhibition

- Inhibitor blocks acetylcholinesterase
- Ach is unable to bind
- Ach returns to receptor and reactivates it
- Enzyme inhibitor has the same effect as a cholinergic agonist
14. Acetylcholinesterase

14.4 Structure of enzyme complex

collagen
14. Acetylcholinesterase

14.5 Active site - binding interactions

- Anionic binding region similar to cholinergic receptor site
- Binding and induced fit strains Ach and weakens bonds
- Molecule positioned for reaction with His and Ser
14. Acetylcholinesterase

14.6 Active site - Mechanism of catalysis

Serine (Nucleophile)  Histidine (Base)

Histidine (Base catalyst)

Histidine Acid catalyst
14. Acetylcholinesterase

14.6 Active site - Mechanism of catalysis

Histidine

Basic catalyst
14. Acetylcholinesterase

14.6 Active site - Mechanism of catalysis

Histidine (Acid catalyst)

Histidine Basic catalyst

Histidine
14. Acetylcholinesterase

- Serine and water are poor nucleophiles
- Mechanism is aided by histidine acting as a basic catalyst
- Choline and serine are poor leaving groups
- Leaving groups are aided by histidine acting as an acid catalyst
- Very efficient - $100 \times 10^6$ faster than uncatalysed hydrolysis
- Acetylcholine hydrolysed within 100 µsecs of reaching active site
- An aspartate residue is also involved in the mechanism
14. Acetylcholinesterase

The catalytic triad

- An aspartate residue interacts with the imidazole ring of histidine to orient and activate it
15. Anticholinesterases

- Inhibitors of acetylcholinesterase enzyme
- Block hydrolysis of acetylcholine
- Acetylcholine is able to reactivate cholinergic receptor
- Same effect as a cholinergic agonist
15. Anticholinesterases

15.1 Physostigmine

- Natural product from the African calabar (ordeal) bean- *Physostigma*
- Carbamate is essential (equivalent to ester of Ach)
- Aromatic ring is important
- Pyrrolidine N is important (ionized at blood pH)
- Pyrrolidine N is equivalent to the quaternary nitrogen of Ach

![Chemical structure of Physostigmine](http://www.floradelaterre.com/index.php?id=19)
15.2 Mechanism of action

Physostigmine

Mechanism of action steps:
1. Initial interaction
2. Intermediate state
3. Final state
15.2 Mechanism of action

Rate of hydrolysis slower by $40 \times 10^6$
15.2 Mechanism of action

Stable carbamoyl intermediate

Carbonyl group 'deactivated'
### 15.3 Physostigmine analogues

#### Miotine
- Simplified analogue
- Susceptible to hydrolysis
- Crosses BBB as free base
- CNS side effects

#### Neostigmine
- Fully ionized
- Cannot cross BBB
- No CNS side effects
- More stable to hydrolysis
- Extra $N$-methyl group increases stability

**Chemical Structures**

Miotine: \[
\text{Me-N}_2\text{CH-NMe}_2\text{O}n\text{C-CH}_3\text{NMe}_2
\]

Neostigmine: \[
\text{Me-N}_3\text{O}n\text{C-CH}_3\text{NMe}_3\]

(ionised at blood pH)
15.4 Organophosphates

a) Nerve gases

Dyflos
(Diisopropyl fluorophosphonate)

- Agents developed in World War 2
- Agents irreversibly inhibit acetylcholinesterase
- Permanent activation of cholinergic receptors by Ach
- Results in death
b) Mechanism of action

- Irreversible phosphorylation
- P-O bond very stable
15.4 Organophosphates

c) Medicinal organophosphate

- Used to treat glaucoma
- Topical application
- Quaternary N is added to improve binding interactions
- Results in better selectivity and lower, safer doses
15.4 Organophosphates

d) Organophosphates as insecticides

- Relatively harmless to mammals
- Agents act as prodrugs in insects
- Metabolised by insects to produce a toxic metabolite
15.4 Organophosphates

d) Organophosphates as insecticides

PARATHION (Inactive Prodrug)

MAMMALS

Phosphorylates enzyme

INSECTS

Oxidative desulphurisation

Active drug

Phosphorylates enzyme

DEATH

Mammalian Metabolism

Inactive & excreted
15.4 Organophosphates

e) Design of Organophosphate Antidotes

**Strategy**

- Strong nucleophile required to cleave strong P-O bond
- Find suitable nucleophile capable of cleaving phosphate esters
- Water is too weak as a nucleophile
- Hydroxylamine is a stronger nucleophile

\[
\text{NH}_2\text{OH} + \text{RO-PO}_2\text{OR} \rightarrow \text{H}_2\text{N-PO}_2\text{OR} + \text{ROH}
\]

- Hydroxylamine is too toxic for clinical use
- Increase selectivity by increasing binding interactions with active site
15.4 Organophosphates

e) Design of Organophosphate Antidotes

- Quaternary N is added to bind to the anionic region.
- Side chain is designed to place the hydroxylamine moiety in the correct position relative to phosphorylated serine.
- Pralidoxime 1 million times more effective than hydroxylamine.
- Cannot act in CNS due to charge - cannot cross bbb.
15.4 Organophosphates

e) Design of Organophosphate Antidotes

Active Site (Blocked)

Active Site (Free)
15.4 Organophosphates

e) Design of Organophosphate Antidotes

- **Prodrug for pralidoxime**
- **Passes through BBB as free base**
- **Oxidised in CNS to pralidoxime**
15.5 Anticholinesterases as ‘Smart Drugs’

- Act in CNS
- Must cross blood brain barrier
- Used to treat memory loss in Alzheimer’s disease
- Alzheimer’s causes deterioration of cholinergic receptors in brain
- Smart drugs inhibit Ach hydrolysis to increase activity at remaining receptors
15.5 Anticholinesterases as ‘Smart Drugs’

Tacrine (Cognex)
Toxic side effects

Donepezil

Metrifonate
(organophosphate)

Galanthamine
(daffodil and snowdrop bulbs)

Rivastigmine (Exelon)
(analogue of physostigmine)

Anabaseine
(ants and marine worms)

Xanomeline