Neuromuscular Junction - Pharmacology

1. Some pharmacological principles, illustrated by ACh at the NMJ

2. Targets for drug action at the NMJ

3. Drugs affecting ACh Receptors (activation)
   - competitive and depolarising block

4. Drugs affecting ACh Esterase (inactivation)

5. Drugs affecting synthesis, storage and release of ACh

Reading
Rang et al. Pharmacology
Most drugs are effective because they bind to proteins:

- Enzymes
- Carriers
- Ion channels
- Receptors

General Principle of Pharmacology: 1
General Principle of Pharmacology: 2

Drugs are useful as:

- Experimental tools
- Clinical treatments
General Principle of Pharmacology: 3

The concept of the receptor, and most kinds of receptors, have been identified by the selective effects of specific drugs.
General Principle of Pharmacology: 4

Drug assays are based on:

- Ligand binding (→ affinity, $K_D$)
- Biological response (→ efficacy, EC50)
General Principle of Pharmacology: 5

When “doing drugs” a good pharmacologist needs to know:

• Target specificity/Ligand specificity
• Agonist/Antagonist
• Dose/Response
• Affinity ($K_D$)/Efficacy ($EC50/IC50$)
• Competitive/Non-competitive
• Clinical/Non-clinical uses
• Side-effects
The biological responses normally measured in skeletal muscle are:

- muscle contraction (twitch/tetanus);
- transmitter release (EPCs/EPPs);
- receptor/ion channel response (nAChR channels)
...add $\mu$-conotoxin
...add d-tubocurarine
Acetylcholine

CH₃–C–O–CH₂–CH₂–N⁺–CH₃
  ^
  |  choline
  |
CH₃

acetate

Quaternary nitrogen
The “nicotinic” ACh Receptor
Testing drugs on ACh receptors

Iontophoresis

Bath application

10 µM ACh

2 mV

60 s

Patch clamp

Application of neurotransmitter to membrane patch

Channels closed

Channels open

20 msec
Ligand Binding

α-bungarotoxin
General scheme for Agonist-Receptor Interaction
(illustrated by ACh binding to its Receptors)

\[
ACh + R \rightleftharpoons ACh-R \rightleftharpoons ACh-R^* \\
\text{bound} \quad \text{activated} \\
\downarrow \quad \uparrow \\
ACh-R^0 \\
\text{“desensitized”}
\]

\[
K_D = \frac{[ACh].[R]}{[ACh-R]} = \sim 80 \mu M
\]
Ligand-gated (e.g. AChR)  Voltage-gated (e.g. NaV)
Ligand-gated (e.g. AChR)  Voltage-gated (e.g. NaV)

\[ \text{Na}^+ \quad \text{K}^+ \]
**nACh Receptor Pharmacology - Neuromuscular Junction**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist/blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>nicotine</td>
<td>d-tubocurarine (curare)</td>
</tr>
<tr>
<td>acetylcholine</td>
<td>α-bungarotoxin</td>
</tr>
<tr>
<td>carbachol</td>
<td>atracurium</td>
</tr>
<tr>
<td>decamethonium</td>
<td>pancuronium</td>
</tr>
<tr>
<td>suxamethonium (I)</td>
<td>suxamethonium (II)</td>
</tr>
</tbody>
</table>
## V-gated Ion-channel Pharmacology

<table>
<thead>
<tr>
<th>Channel</th>
<th>Antagonist/blocker</th>
</tr>
</thead>
</table>
| Na channels | tetrodotoxin (TTX)  
saxitoxin (STX)  
μ-conotoxin  
lignocaine |
| K channels | Cs⁺  
tetraethylammonium (TEA)  
4-aminopyridine (4-AP) |
| Ca channels | Cd²⁺, Mg²⁺  
N-type  
ω-conotoxin  
P-type  
ω-agatoxin  
L-type  
dihydropyridines (DHP) |
Drugs that stimulate ACh Receptors (agonists)

Acetylcholine

Nicotine

Carbachol

Decamethonium

Succinylcholine
Drugs that block ACh Receptors (antagonists)

Acetylcholine

Tubocurarine
Drugs that block ACh Receptors (antagonists)

Example: d-tubocurarine

Example: α-bungarotoxin
Drugs that block ACh Receptors (antagonists)

α-bungarotoxin

Control

10µm α-BTX
**ACh antagonists that are used clinically**

![Acetylcholine](Image)

![Pancuronium](Image)

![Atracurium](Image)
- Not broken down by AChE (nmj)
- Broken down by BuChE (blood plasma)
- Short lasting
- Not counteracted by cholinesterase inhibitor
- Used clinically for brief muscle relaxation
- “Depolarising blocker”
Sux

Na⁺

K⁺

Na⁺
Sux

Na$^+$

K$^+$

Na$^+$
Other targets in neuromuscular pharmacology:

- Synthesis
- Storage
- Release
- Action
- Inactivation
Drugs that block ACh esterase

Acetylcholine

Edrophonium

Neostigmine

Sarin

Antidote: Pralidoxime iodide
Drugs that inhibit transmitter synthesis/storage

Acetyl CoA + Choline $\xrightarrow{\text{Choline acetyltransferase (ChAT)}}$ ACh

**Choline uptake**

Hemicholinium-3 (HC-3)

**Vesicle filling**

AH5183 (Vesamicol)
Drugs that inhibit transmitter release

**Ca$^{2+}$ channel blockers**

**Botulinum toxins = ‘SNARE’ blockers**
EPPs in μ-conotoxin/d-tubocurarine

EPP in low Ca/high Mg

\[ \text{Mg}^{2+} \uparrow \]

\[ \text{Ca}^{2+} \downarrow \]
0 CaDirect+Ca  +4AP  +Mg  +4AP  DirectTTX
K channel blockers and some toxins enhance transmitter release

α-latrotoxin

BEFORE

During

AFTER
Targets of drug action at the neuromuscular junction

- \(\alpha\)-bungarotoxin
- d-tubocurarine
- atracurium
- suxamethonium
- hemicholinium
- vesamicol
- botulinium (A-D)
- edrophonium
- neostigmine
- sarin
Summary
1. Drugs with effects on neuromuscular synapses may be tested in many ways: bioassay of muscle contractions, iontophoresis during intracellular recording; or patch-clamp recording from single receptors and channels.
2. Every step in the cycle of ACh synthesis, storage, release, activation and inactivation is a potential target for drug action at the NMJ.
3. Drugs affecting storage and release:
   - 4-AP, TEA, latrotoxin, botulinum toxin, agatoxin
   - hemicholinium, vesamicol
4. Drugs that block ACh Esterase:
   - edrophonium, prostigmine, neostigmine, sarin
5. Drugs affecting ACh Receptors:
   - carbachol, decamethonium, suxamethonium
   - tubocurarine, a-bungarotoxin, atracurium
6. Clinical uses of drugs acting at the NMJ:
   - muscle relaxants as adjunct to anaesthesia in surgery
   - treatment of neuromuscular disease