Neuroscience with Pharmacology 2

Neuromuscular Junction 2: Pharmacology

1. Principles and methods for studying ACh pharmacology at the NMJ
2. Targets for drug action at the NMJ (synthesis, storage, release, action, inactivation)
3. Drugs affecting postsynaptic (nicotinic) ACh Receptors

Targets in neuromuscular pharmacology:

Acetylcholine

"Quaternary" nitrogen

Drugs acting at the NMJ are useful as:
- Experimental tools
- Clinical treatments
When "doing drugs" a good pharmacologist needs to know:

- Target specificity/Ligand specificity
- Agonist/Antagonist
- Dose/Response
- Efficacy (EC50/IC50)/Affinity (K_D)
- Competitive/Non-competitive
- Clinical/Non-clinical uses
- Side-effects

Drug assays at the NMJ are based on:

- Biological response (-->efficacy, EC50)
- Ligand binding (-->affinity, K_D)

Several techniques can be used to assay the effects of drugs at NMJ’s

1. Muscle contraction
2. Intracellular EPP recording
3. Iontophoresis/patch clamp
4. Ligand binding

Measuring muscle contractions in response to release of neurotransmitter by nerve stimulation...

Measuring EPP’s...

Action potential
...add µ-conotoxin
...add d-tubocurarine
Testing drugs on ACh receptors

Iontophoresis

Bath application

Patch clamp

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Ligand binding visualised...

α-bungarotoxin

10µm α-BTX

Ligand binding visualised...

α-bungarotoxin

But normally measured chemically (e.g. radioactively-labelled ligand)...
Targets of drug action at the neuromuscular junction

- α-bungarotoxin
- d-tubocurarine
- atracurium
- suxamethonium
- hemicholinium
- vesamicol
- botulinum (A-D)

Drugs that inhibit transmitter release

- P/Q Ca²⁺ channel blockers
- Botulinum toxins + "SNARE" blockers

K channel blockers and some toxins enhance transmitter release

- α-latrotoxin

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

\[ \text{ACh} \ast \text{R} \Rightarrow \text{ACh-R} \Rightarrow \text{ACh-R}^* \]

- "bound"
- "activated"

- ACh-R⁰
- "desensitized"

\[ K_D = \frac{[\text{ACh}][\text{R}]}{[\text{ACh-R}]} \approx 80 \, \mu\text{M} \]

3. Drugs affecting postsynaptic (nicotinic) ACh Receptors
The nicotinic ACh Receptor at NMJ

nACh Receptor Pharmacology - Neuromuscular Junction

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist/blocker</th>
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<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>
- 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"
Summary

1. Every step in the cycle of ACh synthesis, storage, release, activation and inactivation is a potential target for drug action at the NMJ.

2. Drugs affecting release
   - 4-AP, α-latrotoxin, botulinum toxin, agatoxin

3. Drugs that block ACh Esterase:
   - edrophonium, prostigmine, neostigmine, sarin
   - TEA, 4-AP, α-latrotoxin, botulinum toxin, agatoxin

4. Drugs affecting storage and synthesis:
   - hemicholinium, vesamicol
   - AF64A

5. Drugs affecting ACh Receptors:
   - carbachol, decamethonium, suxamethonium
   - tubocurarine, α-bungarotoxin, atracurium

6. Clinical uses of drugs acting at the NMJ:
   - muscle relaxants as adjunct to anaesthesia in surgery
   - treatment of neuromuscular disease