Neuroscience with Pharmacology  

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The Neuromuscular Junction: Pharmacology

Several important general principles of pharmacology are illustrated by drugs that act at neuromuscular junctions (NMJ’s) and indeed some of those principles were developed by pharmacological study of the actions of acetylcholine at the NMJ. For example, the concepts of dose-dependence, efficacy, affinity, competitive and non-competitive antagonism, reversible and irreversible binding, and desensitization, are all very well illustrated by drugs acting at the NMJ. The effects of these drugs on neuromuscular transmission can be measured in several ways: including muscle tension measurements, electrophysiological recordings, and chemical or radioactive ligand-binding assays.

Drugs acting at the NMJ are used: i) as research tools; ii) therapeutically, as treatments for various neuromuscular disorders; and iii) cosmetically, e.g. ‘botox’ for ironing-out wrinkling of facial skin. Clinical uses of neuromuscular blocking drugs (functional antagonists) include surgery to increase muscle relaxation under anaesthesia. On the other hand, drugs that enhance neuromuscular function (functional agonists) are used clinically to treat diseases like myasthenia gravis or Lambert-Eaton Myasthenic Syndrome (LEMS).

An important principle is that specific drugs interfere selectively with the different cellular mechanisms involved in neuromuscular transmission (synthesis, storage, release, action and inactivation of transmitter). Interestingly, a common chemical feature of many of these drugs is presence of a quaternary nitrogen (N\(^+\) group, which is also a key feature of the acetylcholine (ACh) molecule itself.

1. Drugs affecting Synthesis:
Ethylcholine mustard aziridinium ion (AF64A, MEChMAz) has been reported to be a specific irreversible inhibitor of choline acetyltransferase (ChAT), preventing synthesis of acetylcholine and thereby reducing transmitter release. Some styrylpyridine compounds also inhibit ChAT. Hemicholinium-3 inhibits synthesis of ACh indirectly, by blocking the membrane transporter responsible for uptake of choline, a limiting substrate for ChAT, from the extracellular fluid.

2. Drugs affecting Storage:
The acetylcholine transporter in the membranes of synaptic vesicles is blocked by vesamicol, preventing filling of the vesicles with acetylcholine, thereby inhibiting synaptic transmission

3. Drugs affecting Release:
   a. Inhibitors. Transmitter release by exocytosis is strongly dependent on Ca\(^{2+}\) ion concentration. Mg\(^{2+}\) ions compete with Ca\(^{2+}\), depressing exocytosis - and hence reducing the quantal content of EPPs - in direct proportion to [Mg\(^{2+}\)]. Nerve terminals contain voltage-gated Ca-channels that regulate the amount of Ca\(^{2+}\) entry. These channels are of the “P/Q” sub-type, blocked by \(\omega\)-conotoxin or \(\omega\)-agatoxin (from marine snails), which therefore block transmitter release. Transmitter release is also blocked irreversibly by botulinum toxins (from Clostridium botulinum bacteria), which have different specificities for SNARE proteins involved in transmitter release. For example, the light-chain component of botulinum toxin Type A (BoNTx-A) has enzymic activity that cleaves SNAP-25, a t-SNARE protein essential for fusion of synaptic vesicles with the nerve terminal plasma membrane. Botulinum toxins are potentially lethal because blocking neuromuscular transmission in respiratory muscles paralyses breathing. However, at low doses, “Botox” has both clinical uses (such as reducing spasticity in patients with cerebral palsy) and cosmetic uses (facial wrinkles; excessive sweating).

   b. Enhancers: Drugs that increase transmitter release (functional agonists) include 4-aminopyridine, which works by blocking presynaptic potassium channels, prolonging the duration of the presynaptic action potential. The prolonged depolarization allows more Ca\(^{2+}\) ions to enter the terminal through voltage-gated Ca-channels, increasing vesicular fusion rate and hence EPP quantal content. The active component of black widow spider venom, \(\alpha\)-latrotoxin produces massive, uncontrolled discharge of all synaptic vesicles in motor nerve terminals, until the terminals are completely emptied, first producing seizures then paralysis. The mechanism is
complex but thought to involve binding to endogenous receptors of ‘latrophilins’, whose natural function is presently unknown.

4. Drugs affecting Action:
   a. Inhibitors (antagonists) This is the main area of neuromuscular synaptic pharmacology. The binding of ACh to its receptors at NMJ epitomises the general equation: A+R⇔AR⇔AR*, with the ‘starred’ state of the agonist-receptor (AR) complex corresponding to the open-pore state of a ligand-gated ion channel. In fact, two antagonists were extremely important in the identification of the nicotinic acetylcholine receptor at NMJ: d-tubocurarine (a plant alkaloid in curare, a tree bark-extract used by Amazonian natives in manufacturer of poisoned hunting darts) and α-bungarotoxin, a component of the venom of the Taiwanese banded krait, a poisonous snake. Tubocurarine is a reversible competitive antagonist that vies with acetylcholine for binding to the α-subunits of ACh receptors, thus reducing the response to ACh released by motor nerve terminals. Binding of tubocurarine is reversible: that is, it can be out-competed by elevating the ACh concentration. By contrast, α-bungarotoxin binds to AChR with much higher affinity and is effectively an irreversible competitive antagonist. This property made the toxin a vital tool in the isolation and characterization of the ACh receptor. The different sub-classes of muscarinic and nicotinic ACh receptors were initially defined by their differential sensitivities to neuromuscular blocking drugs, confirmed subsequently using molecular biological techniques. Nowadays, derivatives of tubocurarine are used clinically as muscle relaxants and as adjuncts to anaesthesia. These derivatives include pancuronium and atracurium. In addition, allosteric (conformational) changes in ACh receptors, or steric hindrance to ACh binding, reduce the affinity of receptors for ACh and therefore its effects. These non-competitive antagonists include phencyclidine (PCP, “angel-dust”) and methyl violet-10B (crystal violet).

   b. Enhancers (agonists). Cholinergic agonists that act at NMJ’s include carbachol and decamethonium. They maintain the open state of the ligand-gated channels that are integral to the ACh receptor. Acetylcholine itself is also, of course, an agonist of the ACh receptor but molecules like ACh and decamethonium are sometimes referred to as partial agonists, because they have complex or bimodal effects, producing first excitation then inhibition of muscle depolarization and contraction. This is partly due to desensitization that occurs with a sustained concentration of agonist. Another drug with complex agonist-antagonist behaviour is suxamethonium, a drug used surgically to produce transient muscle relaxation. This drug is a “depolarizing blocker”. It first produces sustained activation of ACh receptors, then the maintained depolarization leads to inactivation of voltage-gated Na-channels, blocking action potentials and thus producing muscle relaxation.

5. Drugs affecting Inactivation:
ACh is inactivated in the synaptic cleft by the action of the enzyme acetylcholinesterase (AChE). This enzyme is voracious! It is capable of rapidly catalysing hydrolysis of more than 10 times the amount of ACh (into acetate and choline) actually released from nerve endings, within 1 millisecond. This makes it a very effective terminator of ACh’s action. Inhibitors of AChE included edrophonium, neostigmine and prostigmine. These are used clinically in the diagnosis and treatment of myasthenic disorders, for example to distinguish myasthenia gravis (MG) from Lambert-Eaton Myasthenic Syndrome (the “tension test” is positive for relieving symptoms and signs of MG, a disease of ACh receptors; but negative for LEMS, a disease of presynaptic Ca-channels). Other, highly toxic antagonists of AChE included sarin, a “nerve gas” that kills by phosphorylating AChE, causing the seizures that prolonged exposure of NMJ’s and other cholinergic synapses to these agents produces. Antidotes include pralidoxime, usually combined with atropine (the latter to reduce the effects of ACh on muscarinic receptors). Common pesticides, still in widespread use in the Far East, also contain organophosphorus compounds, such as paraoxon or dimethoate, that poison AChE, with complex pathophysiological effects when ingested.

Further Reading