B. 7 Neuromuscular blocking agents

a. Explain the physiology of neuromuscular transmission and how this may be interfered with to produce muscle relaxation.

Acetylcholine is the neurotransmitter of the NMJ. Choline is synthesized in the liver and actively taken-up by nerve cells. It is condensed with acetyl-CoA derived from the TCAC which is present in the cell cytoplasm. This reaction is catalyzed by choline acetyltransferase present in the nerve terminal which is inhibited by acetylcholine and acetylcholinesterase. ACh is stored in vesicles in the nerve terminal.

Vesicles are grouped in the nerve terminal away from the junctional surface and move to sites adjacent to the junction where they are bound to synapsin. The mobilization and release of vesicles is promoted by stimulation of the prejunctional nicotinic receptor and inhibited by stimulation of the prejunctional muscarinic receptor.

In response to the rise in intracellular Ca$^{2+}$, protein kinase II dephosphorylates synapsin and allows vesicles to bind to synaptophysin which results in release of their contents. There is continuous slow release of the contents of vesicles from the nerve terminal, resulting in miniature end-plate potentials (MEPPs). ACh is hydrolyzed to choline and acetate by cholinesterase in the synaptic cleft. These are then reabsorbed by the nerve terminal.

The neuromuscular junction consists of a nerve terminal (of a motor neurone) adjacent to an end plate on skeletal muscle. The nerve terminal contains around 300,000 vesicles containing acetylcholine. When an action potential arrives at the nerve terminal, an influx of Ca$^{2+}$ triggers the release of the contents of around 125 vesicles of acetylcholine into the synaptic cleft. The acetylcholine diffuses rapidly to its receptors on the end plate where it opens ion channels in the muscle cell membrane, allowing a rapid influx of Na$^+$, generating an EPSP and triggering an action potential (by opening voltage-dependent channels) which propagates through the T-tubule system and causes a release of Ca$^{2+}$ from the sarcoplasmic reticulum. This intracellular Ca$^{2+}$ binds to troponin C, allowing binding of actin to tropomyosin and contraction of muscle fibrils with the hydrolysis of ATP.

The influx of Ca$^{2+}$ lasts around 50 ms in skeletal muscle. The action potential lasts only 1-5 ms and the acetylcholine is broken down within a few milliseconds by acetylcholinesterase in the subneural clefts. The low intracellular concentration of Ca$^{2+}$ is rapidly restored by active transport of the Ca$^{2+}$ back into the sarcoplasmic reticulum. A single action potential generates only a brief contraction of skeletal muscle. A sustained (tetanic) contraction requires summation of a rapid series of action potentials.

The pharmacological methods by which neuromuscular transmission is blocked include competitive blockade of the acetylcholine receptor with an antagonist (non-depolarizing block) or non-competitive blockade with an agonist which is not broken down by acetylcholinesterase (depolarizing). Non-competitive blockade works to prevent muscle contraction initially by maintaining the end plate in a depolarized state and blocking the ion channel at the receptor, thereby blocking the repeated action potentials required to produce sustained contraction (phase I block). Prolonged exposure to depolarizing blockers causes a desensitization of the end plate resulting in a phase II block similar to that caused by the non-depolarizing blockers.

Transmission can also be interfered with at other stages. Latrotoxin (Red-back spider venom) increases vesicle release, depleting ACh. Botulinum toxin inhibits vesicle release from the nerve terminal.

After blockade, transmission can be enhanced ("reversed") by cholinesterase inhibiting drugs which increase the life of ACh in the synaptic cleft. Higher doses of these drugs inhibit transmission by causing a depolarizing block. 4–aminopyridine and tetraethylammonium block K$^+$ channels in the nerve terminal, delaying repolarization and increasing the amount of ACh released.

Open channel block is produced by depolarizing muscle relaxants but can also be produced by NDBs if a depolarizing agent is given while a high concentration of NDB is present. The NDB binds in the open channel of the ACh receptor, producing a prolonged...
block. This is a clinical problem if a reversal agent is given while the patient is deeply paralyzed.

There is substantial redundancy in transmission at the NMJ. The quantity of ACh released and the number of nicotinic receptors are both in large excess. Normal VC and TOFC 0 is seen despite block of 75% of receptors by an NDB. Normal inspiratory force is observed with 50% blocked and sustained head lift with 33% blocked.

b. Describe the post-junctional receptor.

Nicotinic post-junctional receptors are grouped at the “shoulders” of the subneural clefts. The receptor is a cone-shaped protein consisting of five subunits which binds with two molecules of acetylcholine. The ion channel when opened admits Na\(^+\), K\(^+\) and Ca\(^{2+}\), but the main effect at the time of opening is influx of Na\(^+\).

The receptor is a pentamer composed of four different units (\(\alpha_2\beta\varepsilon\delta\)) all of which span the cell membrane. The ACh receptor sites are on the \(\alpha\) subunits. There is a central ion channel which opens due to conformational changes with both receptor sites are occupied by agonists.

The nicotinic receptors on nerve tissue are composed of a different combination of units (\(\alpha_2\beta_3\)) as are fetal receptors (\(\alpha_2\beta_\gamma\delta\)), which are found on the muscle cell surface away from the neuromuscular junction and are also expressed in denervated muscle.

c. Outline the properties of an ideal neuromuscular blocking agent.

- Non-depolarizing action
- Rapid onset (within one circulation time)
- Short duration, suitable for infusion
- Rapid metabolism to inactive products
- Antagonized by cholinesterase inhibitors
- Actions confined to the NMJ
- Not transferred across the placenta or blood-brain barrier
- No local or systemic side-effects
- Compatibility with other drugs and solutions
- Long shelf life without refrigeration
- Cheap
- Made by chemical synthesis
- Sterilizable

d. Apprise different methods of monitoring the neuromuscular junction.

A transcutaneous nerve stimulator placed over the ulnar nerve, producing contraction of adductor pollicis is commonly used to assess the degree of neuromuscular junction block. Other sites used include the phrenic, facial, posterior tibial and lateral popliteal nerves. Other skeletal muscles have different sensitivities to neuromuscular blocking agents from the diaphragm. The ulnar nerve is the best validated.

Skin is prepared by removal of excess hair, abrasion to remove some of the stratum corneum and cleaning with alcohol. Pregelled electrodes are placed over the ulnar nerve, distally at the lateral border of FCU 1-2 cm proximal to the proximal skin crease at the wrist and proximally as close as practical to the distal electrode in the line of the nerve. The positive electrode is proximal.

An initial threshold for stimulation is established with 1 Hz stimuli: the minimum current required to produce a twitch in the thumb. This current is tripled (minimum 20 mA) to determine a supramaximal stimulus which should stimulate all fibres in the ulnar nerve.

The most common method of assessment is the “train-of-four”. A train of four impulses at 0.5 s intervals is applied to the ulnar nerve and the contraction of adductor pollicis is palpated or measured with a force transducer. The result is quantified as a count.
(TOFC) or a T4/T1 ratio (TOFR). Without a junction blocker, the four twitches are of equal strength, at TOFR ≥ 0.7, spontaneous ventilation is safe. With a count of 1 or 0, intubation should be possible. Reversal should be given with a TOFC of 3 or 4, a TOFC of 1 or 2 increases the risk of inadequate reversal in recovery.

In recovery or when TOFC is 4 with little fade, double-burst stimulation of two brief 50 Hz stimuli 0.75 s apart gives a more readily palpable fade for manual monitoring of residual paralysis. Fade is due to blockade of prejunctional nicotinic receptors causing a failure of the normal increase in ACh mobilization following a stimulus.

With deep paralysis all four twitches become absent, and a post-tetanic count allows monitoring of paralysis: a 5 s 50 Hz stimulus is followed by a 3 second pause and then stimuli every second, the number before complete fade being counted. Repetitive stimuli increase synthesis and release of ACh, producing post-tetanic facilitation. Some facilitation will be present for five minutes following a PTC. To guarantee no coughing or diaphragmatic movement on intubation, a PTC of 1 or 0 is required. A PTC of 9 is equivalent to a TOFC of 1. The time from PTC 1 to TOFC 1 is documented for most relaxants (pancuronium 40 min, atracurium 9 min)

Clinical assessment of the degree of relaxation is also made by the surgeon, particularly in intraabdominal surgery and in recovery with assessment of head lift for 5 s.

e. Give a detailed account of the pharmacology of suxamethonium including its undesirable properties.

Class
depolarizing neuromuscular blocking agent, used to achieve rapid, brief paralysis of skeletal muscle.

History
This class of agent was discovered in the late 1940s by Paton & Zaimis, who initially investigated decamethonium, a similar compound without ester linkages.

Physical
The structure of suxamethonium is two ACh molecules linked through the acetate methyl groups: \((\text{CH}_3)_3\text{N}^+-\text{(CH}_2)_2\text{O-OC-CH}_2\text{-CH}_2\text{-CO-0-(CH}_2)_2\text{-N}^+(\text{CH}_3)_3\).

Chemical
Presentation is as a 2 ml vial containing 100 mg in solution. Storage is at 4°C, with 5% loss of potency over 3 months at 20°C. It is a small polar molecule, readily soluble in water.

Pharmacokinetics
administered as IV injection
rapid distribution to ECF
rapid hydrolysis by plasma cholinesterase. 90% hydrolyzed before distribution to the NMJ. \(\rightarrow\) succinylmonocholine (+ choline) \(\rightarrow\) succinic acid + choline. 14% of succinylmonocholine is renally cleared. Spontaneously hydrolyzes slowly at physiological pH.
inactive genetic variants of plasma cholinesterase produce a greatly prolonged half life: \(t\frac{1}{2}\beta\) 1 h.

Pharmacodynamics
binds to post-synaptic nicotinic ACh receptors at the NMJ, resulting in the fixing open of the associated Na⁺ channel. This produces a brief depolarization, seen as fasciculation, particularly in the face, and then blocks transmission of normal impulses to the muscle until the suxamethonium is hydrolyzed. Fasciculation may also result from antidromic conduction. The blockade is not competitive. This is known as phase I block.
minimal affinity for preganglionic nicotinic receptors.
repeated or prolonged administration produces a state of phase II block: a more prolonged block with features similar to non-depolarizing blockade: fade on train-of-four, recovery with neostigmine administration.

Clinical use

Relaxants 2.B.7.3 James Mitchell (3 December 2009)
dosage: 1 to 2 mg/kg IV, with a higher dose required if a NDB has been given first. Higher dose in neonates and children.

onset of action: one circulation time
duration: 3-5 minutes (except in suxamethonium apnoea)

advantages
- rapid, complete relaxation with a standard dose
- clinical signs of onset: fasciculations
- brief duration
- cheap

disadvantages
- common
  - post operative muscle pains, common in younger patients. bradycardia from vagotonic effect
  - increased intraabdominal pressure
  - increased intraocular pressure
  - increased intracranial pressure (all prevented with NDB)
- release of K⁺
  - rise of 0.5-0.7 mmol/l in normal patients
due to depolarization and trauma with fasciculation
- extrajunctional ACh receptors
  - seen in burns, denervation, prolonged immobility
  - rise up to 9 mmol/l described
  - in muscle trauma rise of 3-4 mmol/l described
  - period of danger is quoted variously
    - burns 1-12 weeks or 1 day to 6 months
    - prevention attempted with NDB (ineffective)
    - salbutamol has some effect

uncommon
- abnormal plasma cholinesterase results in very prolonged action:
  - suxamethonium apnoea
  - may trigger malignant hyperpyrexia, masseter spasm
  - development of phase II block with repeated use

Plasma cholinesterase
coded for by autosomal gene

variants
- N normal
- D dibucaine (cinchocaine) resistant
- F fluoride resistant
- S silent
- C₅ increased activity (coded at a different locus)
- D, F and S types have reduced (or no) activity in hydrolysing suxamethonium
- 95% of people are NN

assays
- direct assay of PIChE activity using benzoylcholine as the substrate
dibucaine number
  - % inhibition by 10⁻⁵ mol/l cinchocaine in vitro (low in D)
fluoride number
  - % inhibition by 5x10⁻⁵ mol/l fluoride in vitro (low in F and D)
scoline number
  - activity assay (?method) high with normal hydrolysis

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Relaxants 2.B.7.4 James Mitchell (3 December 2009)
f. Describe the structure-activity relationships of the non-depolarizing muscle relaxants including the newer steroidal and benzyl-isoquinolinium muscle relaxants.

Most of the muscle relaxants bear a resemblance to acetyl choline in part of their structure. This is most obvious in suxamethonium which consists of two ACh molecules joined by their acetate methyl groups.

Chain length determines specificity for neuromuscular rather than ganglionic synapses for the -methonium series of agents consisting of a carbon chain with quaternary nitrogens at each end. Though suxamethonium would be about the right length to bind both binding sites on the nicotinic receptor if it were a straight molecule, it in fact requires two molecules to activate a nicotinic receptor as it exists in a bent conformation. The distance from the quaternary nitrogen to the van de Waals radius of an oxygen atom is 4.4 Å for optimal muscarinic action and 5.9 Å for nicotinic action.

Most of the non-depolarizing agents can be grouped into steroids and isoquinolinium derivatives. The steroid agents include pancuronium, vecuronium, rocuronium. They each have two ACh-like regions at each end of the molecule. The region on the D-ring is most important for binding at the nicotinic ACh receptor. The A-ring moiety confers more muscarinic blocking action.

This is pancuronium. Removal of the 2β-methyl group adjacent to the A-ring yields vecuronium, which produces less tachycardia as it lacks anti-muscarinic activity. Other substitutions of the end rings give rocuronium and pipecuronium.

The isoquinolinium muscle relaxants consist of large rigid functional groups at either end of a chain resembling suxamethonium. Nicotinic potency and specificity are associated with a distance between quaternary nitrogen atoms of 20-21 Å.
This is atracurium, in which the order of the ester oxygen and carbonyl groups in the chain are reversed compared to ACh. Its chiral centres are circled.

Doxacurium and mivacurium have different lengths in the central chain and extra functional groups. Not all neuromuscular blocking agents have a quaternary nitrogen atom nor a region resembling ACh e.g. β-erythroidine.

Novel blocking agents under development include bistropine diesters and asymmetrical chlorofumarates. Both these classes of compounds are somewhat similar in arrangement to the benzylisoquinolinium compounds with a flexible chain including carbonyl or ester linkages joining large rigid groups containing a quaternary nitrogen.

g. Describe the pharmacokinetics of non-depolarizing muscle relaxants and the role of prejunctional receptors as well as the effects of renal and hepatic disease.

<table>
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<tr>
<th>Relaxant</th>
<th>V_d</th>
<th>t'₁/₂α</th>
<th>t'₁/₂β</th>
<th>clearance</th>
<th>elimination in urine</th>
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</table>

The non-depolarizing muscle relaxants are administered by intravenous injection or infusion. They are polar compounds and so have a limited V_d. Blood levels fall rapidly initially with redistribution.

The older agents tubocurarine, metocurine and gallamine are not metabolized, but excreted unchanged, gallamine by renal clearance and the others 50-60% by renal clearance.

The steroidal agents are hydroxylated in the 3 and 17 positions in the liver with some reduction in potency. The long-acting agents, pancuronium, doxacurium and pipercuronium are predominantly renally excreted (60-90%) while the shorter-acting agents vecuronium and rocuronium are predominantly excreted in bile (75-90%) either unchanged or as 3-hydroxy derivatives.

The isoquinolinium agents are hydrolyzed either spontaneously (atracurium) or by plasma cholinesterase (mivacurium) to inactive metabolites. The action of these agents is still affected by renal failure (which lowers plasma cholinesterase activity) and hepatic failure (due to hepatic metabolism of metabolites of atracurium).

Effective durations of action range from around 15 minutes for mivacurium, 20-35 minutes for vecuronium, rocuronium and atracurium to more than 35 minutes for pancuronium and tubocurarine.
Hepatic impairment increases the $V_d$ and $t_{1/2,\beta}$ (of agents undergoing hepatic metabolism or excretion) and reduces their clearance. This increases the initial dose requirement but prolongs the duration of action.

Renal impairment greatly reduces the clearance of renally cleared agents (e.g. pancuronium) and so increases the duration of action. It is also associated with a reduction in plasma cholinesterase activity and so slows metabolism of mivacurium and suxamethonium and reduces their dose requirements.

Non-depolarizing agents exert their action predominantly by acting as competitive antagonists to ACh at the nicotinic receptor site. At high doses they may also cause direct blockade of the ion channel. On the prejunctional cell membrane they block sodium channels, inhibiting the depolarization of the membrane and the release of ACh. They also block prejunctional nicotinic receptors, inhibiting the mobilization of ACh vesicles.

**h. Describe the factors that may modify responses to muscle relaxants.**

The factors which affect the response to suxamethonium are given above: primarily factors affecting plasma cholinesterase activity and the degree of K⁺ release with administration of suxamethonium.

**Pharmacokinetic**
- adequate circulation required for distribution: determines rate of onset.
- reduced $V_d$ in elderly
- rate of metabolism dependent on:
  - plasma cholinesterase for mivacurium, suxamethonium
  - ↓ in pregnancy, burns
  - hepatic function for vecuronium and rocuronium
  - ↑ metabolism with enzyme induction
- elimination dependent on:
  - renal function for many agents, especially tubocurarine and gallamine
  - temperature
  - perfusion
  - blood loss may be a significant contributor in some cases

**Pharmacodynamic**
- receptors:
  - deficiency in myasthenia gravis
  - up-regulation in UMN lesions
- drugs:
  - open channel block
    - aminoglycosides, Ca²⁺ channel blockers, local anaesthetics, Li⁺
  - closed channel block
    - quinidine, tricyclics, naloxone
    - Mg²⁺ decreases ACh release
  - block antagonized by cholinesterase inhibitors
  - also increased block in:
    - hypothermia, acidosis, hypokalaemia, hypercalcaemia

**Other**
- reduced motor neurone activity due to volatile anaesthetics (isoflurane >> halothane). Isoflurane also acts at the NMJ.
- other drugs:
  - diuretics, ganglion blockers

**i. Describe the systemic side effects of muscle relaxants.**
The adverse effects of suxamethonium are given above.
Administration of non-depolarizing muscle relaxants causes weakness and then paralysis of skeletal muscle, acting most rapidly on small, fast-twitch muscles and last on the postural muscles and diaphragm.

Cardiovascular
- Isoquinolinium agents other than doxacurium cause histamine release and thus vasodilation and hypotension.
- Gallamine and pancuronium antagonize cardiac muscarinic receptors, causing an increase in heart rate.
- In high doses, tubocurarine causes blockade of autonomic ganglia, leading to hypotension and reduced intestinal motility.

Histamine release
- The obsolete agents dTC and metocurine showed strong histamine release.
- Mivacurium causes marked histamine release.
- Suxamethonium and atracurium cause some histamine release.
- Causes flushing, hypotension and bronchoconstriction.

**The pharmacology of sugammadex.**

**Class**
- Sugammadex is a specific binding agent for rocuronium and to a lesser extent vecuronium.

**History**
- Sugammadex was brought to market in 2008-9.

**Physical**
- Presented as 2 ml and 5 ml ampoules containing 100 mg/ml.

**Chemical**
- Sugammadex is a modified gamma cyclodextrin. It consists of eight modified dextrose moieties with 1,4α linkages in a ring.

**Pharmacokinetics**
- $V_d = 15 l$, $t_{1/2}^α = 2.9$ min, $t_{1/2}^β = 2.2$ h, clearance 91 ml/min with linear kinetics.
- No significant protein binding.
- Minimal hydroxyapatite binding.
- No significant metabolism.
- Clearance decreases proportional to creatinine clearance.

**Pharmacodynamics**
- Binding steroidal relaxants in circulation produces offset of relaxation due to the high concentration gradient from the site of effect to the plasma compartment.
- No direct physiological effect.
- Can bind other steroids and reduce the free fraction (e.g. OCP).
- May increase APTT and INR after high dose, bleeding effect not known.

**Clinical Use**
- **Routine reversal**
  - PTC 1-2: 4 mg/kg gives TOFR 0.9 in 3 minutes.
  - TOFC 2: 2 mg/kg gives TOFR 0.9 in 2 minutes.
- **Immediate reversal**
  - 3 minutes after 1.2 mg/kg rocuronium: 16 mg/kg gives TOFR 0.9 in 90 s.
- **Redosing relaxant**
  - Use of 0.6 mg/kg rocuronium is not recommended for 6 h after 2 mg/kg or 12 h after 16 mg/kg.
  - Non-steroidal relaxants (e.g. cisatracurium) can be used at any time.