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Neuromuscular Blocking Agents

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Neuromuscular blocking agents used in clinical medicine act by interfering with the action of the endogenous neurotransmitter acetylcholine (ACh) on the nicotinic cholinergic receptor at the neuromuscular junction (NMJ), thereby inhibiting receptor-coupled transmembrane ion movements necessary to initiate muscle contraction (Bouzat et al., 2004; Unwin, 2005). The end result of this action is skeletal muscle paralysis and muscular relaxation (Bowman, 2006). Neuromuscular blocking (NMB) agents are used in veterinary medicine as adjuncts to general anesthesia. The most common indications for a NMB agent in veterinary medicine are to aid in the placement of an endotracheal tube, to be used concurrently with sedative/hypnotic agents to facilitate mechanical ventilation, to enhance muscle relaxation for a variety of surgical conditions (e.g. ophthalmological procedures), or as part of a balanced anesthetic technique to reduce the amount of inhalation anesthetic required (Keegan, 2015). The sporadic use of NMB agents in veterinary medicine is in stark contrast to the widespread use of NMB agents in human anesthesia and is largely a result of interspecies differences. For example, most species seen in veterinary medicine can be easily intubated with an endotracheal tube after administration of an intravenous anesthetic induction agent without the additional need of a NMB agent, while intubation in man is often more difficult and may necessitate deeper muscle relaxation (Bozeman et al., 2006).

Development

Development of NMB drugs represents a colorful history in the field of pharmacology. Interesting reviews of the course of these events have been presented by Betcher (1977), Bisset (1992), and Lee (2005). Neuromuscular

blocking drugs originated with the discovery of curare, a tarlike mixture of plant material used as a poison by South American Indians. The actual ingredients of the poison for arrows, blowgun darts, and spears were known only to a local “pharmacist,” who was often the tribal medicine man of a region. Thus the botanical preparations obtained by explorers could not be identified as to content; they were simply classified according to the containers in which they were packaged. Tubo-, para-, or bamboo-curare was contained in cutoff bamboo tubes; this mixture was usually obtained from southern Amazon tribes. The plant origin of tube-curare preparations was primarily Menispermaceae (*Chondrodendron tomentosum*). Calabash-curare was packaged in hollow gourds or calabashes; it was the most active preparation. Pot-curare came in small earthenware pottery from the central part of the Amazon basin; this concoction often contained plants other than Menispermaceae (McIntyre, 1972). The most important constituent isolated from curare was *d*-tubocurarine (Wintersteiner and Dutcher, 1943).

Original studies in the 19th century by Claude Bernard (1856) (Bowman, 2006) demonstrated that curare prevented the muscle contraction elicited by stimulation of the motor nerve. It did not, however, affect the central nervous system (CNS), prevent response to direct stimulation of the muscle, or depress axonal conductance. It was proposed that curare acted at the nerve–muscle junction. Reports since then have substantiated, clarified, and extended observations concerning the neuromuscular blocking properties of curare alkaloids. Early results stimulated active research into the chemical structural requirements of curare-like compounds, leading to the discovery of other types of NMB agents.

Neuromuscular blocking agents possess chemical structural groups that allow interaction of these agents with the nicotinic cholinergic receptor (Brejc et al., 2001). However, these drugs cause distinctly different effects from the endogenous mediator ACh. According

to the drug's mechanism of action at the nicotinic postjunctional receptor, NMB drugs are classified as either competitive nondepolarizing agents or as depolarizing agents. Competitive nondepolarizing NMB agents occupy the receptor so that ACh cannot access its binding site and upon binding these compounds fail to trigger transmembrane ion movement, resulting in muscle paralysis for the duration of their effect. Depolarizing agents act in a more complicated manner and initially cause membrane depolarization, often characterized by muscle fasciculation, before blockade and muscle paralysis occur (Hibbs and Zambon, 2011).

Impulse Transmission at the Somatic Neuromuscular Junction

Prior to discussing individual NMB agents, impulse transmission at the somatic NMJ will be reviewed in relation to sites of action of different drugs. General concepts of cholinergic transmission are discussed in detail in Chapters 6 and 8.

Physiological and Anatomic Considerations

A representation of a somatic NMJ synapse and proposed sites of drug actions are shown in Figure 10.1. Terminal

branches of a motor axon lose their myelin sheath and embed within invaginations of the cell membrane of the skeletal muscle cell; these invaginations are termed synaptic gutters. A synaptic gutter, in turn, has many microinvaginations or infoldings, called junctional folds. The space within the synaptic gutter between the nerve ending and the muscle cell is called the synaptic cleft. *Presynaptic* refers to nerve axon elements, whereas *postsynaptic* refers to constituents of the muscle cell.

Vesicular structures localized within cholinergic nerve terminals represent storage sites for ACh (see Chapter 6). As an axonal action potential arrives at the nerve terminal, it increases the release of ACh from the storage vesicles into the synaptic cleft. This step (excitation–secretion coupling) is dependent upon an action potential activating voltage-gated Ca^{++} channels that shift extracellular Ca^{++} into the neuron and/or release Ca^{++} bound to superficial membranes of the nerve terminal. The rise in intracellular Ca^{++} triggers fusion of the storage vesicles with the plasma membrane, and neurotransmitter release. The ACh released into the synaptic cleft binds to specialized receptor sites on the postsynaptic membrane and causes depolarization of the muscle cell. The exclusively nicotinic cholinergic receptors located on the plasma membrane surface of the muscle cell are clustered in high density in the

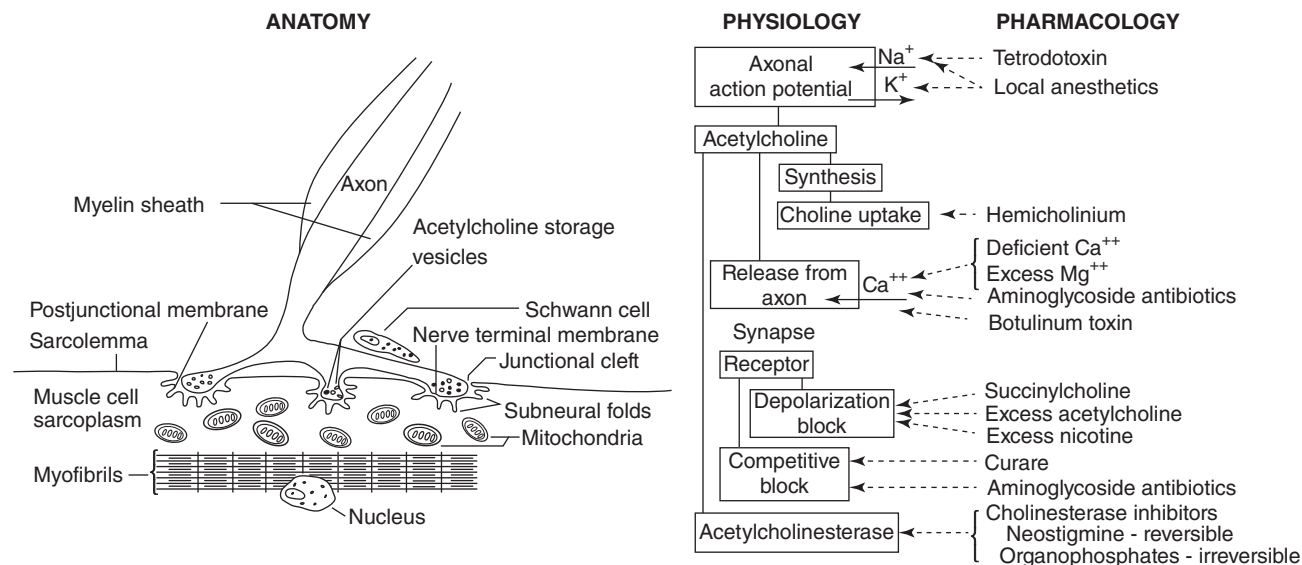


Figure 10.1 Schematic representation of a somatic neuromuscular junction (synapse), related physiological pathways, and proposed sites of action of various pharmacological agents. An axonal action potential (AP) is characterized by an influx of Na^+ and an efflux of K^+ . Tetrodotoxin and saxitoxin inactivate Na^+ pathways. Local anesthetics block Na^+ and K^+ pathways. Choline uptake into the neuron is blocked by hemicholinium; synthesis of ACh is prevented. As the AP arrives at the nerve terminal, it instigates inward movement of Ca^{++} ; this triggers discharge of ACh into the junctional cleft. A lack of Ca^{++} or an excess of Mg^{++} decreases release of ACh. Aminoglycoside antibiotics also interfere with Ca^{++} -dependent release of ACh. Botulinum toxin inhibits ACh release. Succinylcholine (depolarizing neuromuscular blocking agents) cause persistent depolarization block of the motor end-plate region, as does excess ACh and nicotine. Curare and atracurium (competitive neuromuscular blocking agents) compete with ACh for postsynaptic receptors but do not cause depolarization. Aminoglycoside antibiotics decrease sensitivity of the postsynaptic membrane to ACh. Catabolism of ACh by acetylcholinesterase is inhibited by reversible and irreversible anticholinesterase agents; ACh accumulates. Source: Modified from Hibbs and Zambon, 2011; Couteaux, 1972.

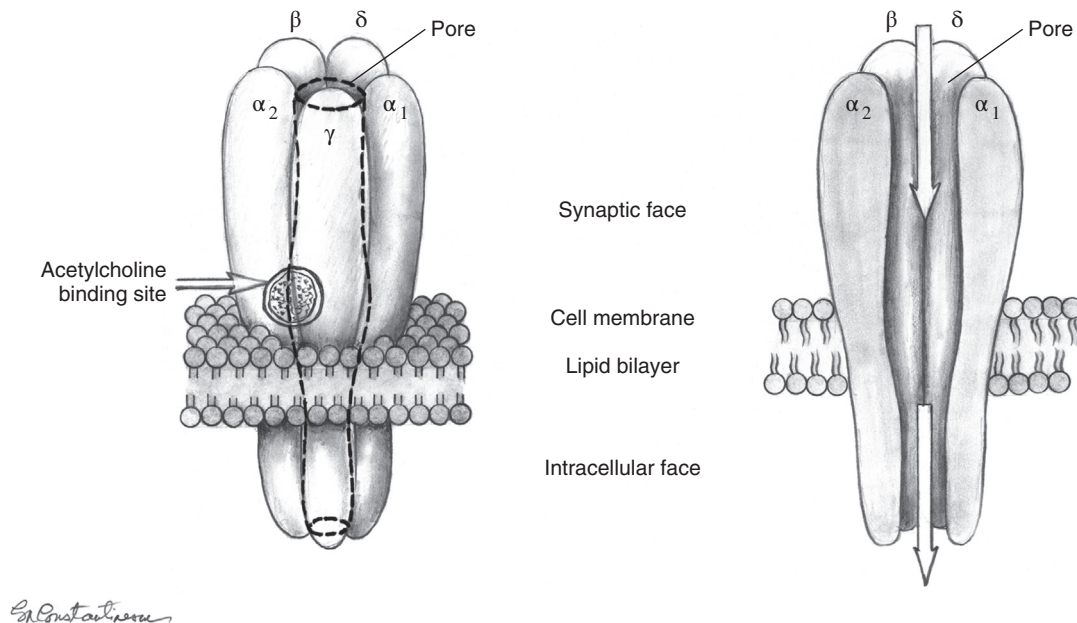


Figure 10.2 Schematic representation of the nicotinic cholinergic receptor. The receptor is embedded across the cell membrane lipid bilayer, presenting a synaptic face to the neuroeffector junction between the neuron and innervated cell, and an intracellular face within the cytoplasm. The receptor comprises a pentameric configuration of four separate subunits with a stoichiometric ratio of $\alpha_2\beta\gamma\delta$; in adult muscle $\alpha_2\beta\epsilon\delta$. The α subunits contain the primary ligand binding sites for recognition of acetylcholine and related agents. Subunit arrangement forms an internal pore that allows passage of select ions upon receptor activation and resulting membrane depolarization (see text). Source: Redrawn from Unwin et al. (1988) by Dr. Gheorghe M. Constantinescu, University of Missouri.

junctional folds of the postsynaptic membrane (Huh and Fuhrer, 2002).

It requires the activation of a large number of nicotinic cholinergic receptors to excite a single muscle fiber and stimulate muscle contraction. At the same time the process requires a very rapid termination of response. A single synaptic vesicle contains roughly 7000–12,000 molecules of ACh and a single motor axon action potential may trigger the fusion of 40–300 vesicles depending on the species or type of NMJ studied (Steinbach and Wu, 2004). After release into the synaptic cleft the ACh reaches high concentration rapidly, where it can bind to cholinergic receptors. However, ACh released into the synaptic cleft also can bind to the enzyme acetylcholinesterase (AChE), which hydrolyzes ACh to choline and acetate thus inactivating it. All unbound, extraneuronal ACh is rapidly metabolized by the AChE enzyme, which is localized in the motor end-plate region. Although AChE may be bound in part to presynaptic elements, it is concentrated at the postsynaptic membrane (Inestrosa and Perelman, 1990; Hucho et al., 1991). The relative ratio of binding sites for ACh on the nicotinic cholinergic receptor to AChE binding sites at the NMJ is approximately 10 : 1 (Steinbach and Wu, 2004). Due to the rapid rate of hydrolysis of ACh by AChE and the comparatively slow release of ACh from relatively large numbers of nicotinic receptors at the motor end-plate, the concentration of free ACh within

the synaptic cleft is rapidly reduced after a single action potential.

The Nicotinic Receptor and Structure–Activity Relationships

The nicotinic cholinergic receptor is a pentameric molecule of about 290 kilodaltons that spans the bilayer of the postsynaptic membrane at the NMJ (Figure 10.2). The receptor comprises five individual subunits in a stoichiometric ratio of $\alpha_2\beta\gamma\delta$; the γ -subunit is replaced by an ϵ -subunit in muscle from adult animals. Each subunit presents an extracellular and intracellular surface and also contains sequences of hydrophobic amino acids that are the likely regions embedded within the membrane bilayer (Hibbs and Zambon, 2011). The five subunits of each individual receptor complex are elongated perpendicular to the postsynaptic membrane and are arranged circumferentially to form a rosette around a central lumen (Figure 10.2). This central transmembrane channel of the receptor complex represents the membrane pore for ion fluxes instigated by agonist activation of the receptor (Unwin, 2005). Agonist and antagonist binding sites are restricted to the α -subunits (Kistler et al., 1982). Whereas ACh evokes receptor activation upon binding to the α -subunits, occupation of these same sites by antagonists prevents effective receptor activation

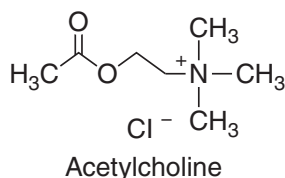
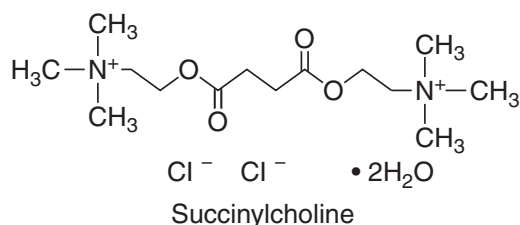
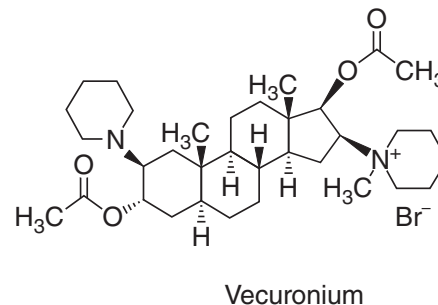
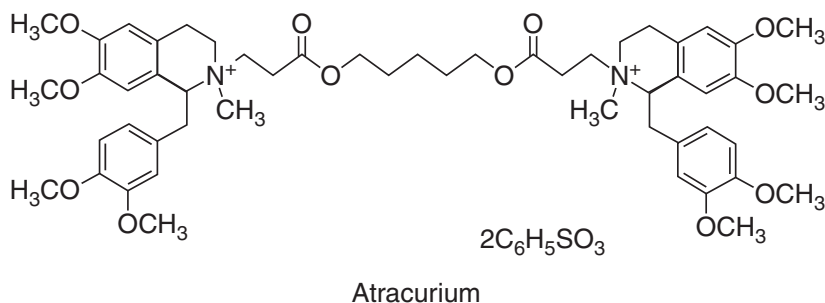
NICOTINIC CHOLINERGIC NEUROTRANSMITTER**DEPOLARIZING AGENT****COMPETITIVE NONDEPOLARIZING AGENTS**

Figure 10.3 Chemical structures of agents interacting with the nicotinic cholinergic receptor at the neuromuscular junction. The cholinergic neurotransmitter, acetylcholine, contains a cationic nitrogen moiety that forms a bond with receptor binding site. Two molecules of acetylcholine must bind to a nicotinic receptor to activate the ion channel. The depolarizing neuromuscular blocking agent, succinylcholine, is depicted. The chemical structure of succinylcholine is equivalent to two acetylcholine molecules fused in sequence. A single molecule of succinylcholine can bind to both of the nicotinic binding sites on the cholinergic receptor inactivating the receptor. Competitive nondepolarizing NMB agents from the benzyisoquinolinium family (atracurium) and the aminosteroid group (vecuronium) are represented. These compounds are complex bulky molecules but contain the important dual cationic nitrogen moieties in a rigid configuration that allows occupation of both of the nicotinic binding sites on the cholinergic receptor and effectively inactivating the receptor.

(Karlin, 2002). The muscle becomes paralyzed, whether in response to a competitive nondepolarizing blocking agent or to transient activation by a depolarizing blocking agent. Chemical structures of several commonly used NMB agents are shown in Figure 10.3 to demonstrate structural differences of the competitive, nondepolarizing and the depolarizing types of NMB agents.

Based on general chemical structural characteristics, Bovet (1951) placed neuromuscular blocking agents into two basic categories. One group is characterized by large, bulky, rigid molecules; members of this group include *d*-tubocurarine, vecuronium, atracurium, and pancuronium, all of which are the competitive, nondepolarizing neuromuscular blockers. The other group is characterized by long, slender, flexible molecules that allow free bond rotation. Decamethonium and succinylcholine are in this group; these agents represent member of the depolarizing NMB agents. The dichotomy in basic structural arrangement of competitive, nondepolarizing and depolarizing agents has been offered as a partial explanation for dissimilar effects evoked by interaction of these agents with the nicotinic cholinergic receptor.

Among other requirements, receptors contain two anionic (negatively charged) binding sites separated by

set distances. These sites are essential for electrostatic bonding of the cationic (positively charged) nitrogen moiety of ACh (and exogenous chemicals) to the receptors (Brejc et al., 2001). All NMB agents, are quaternary ammonium compounds, containing the cationic nitrogen regions necessary to interact at the ACh binding site. Despite the variety of chemical structures represented among the modern nondepolarizing NMB agents, a maximum bond distance between the two quaternary groups present in any of these molecules is typically fixed at 1.0 ± 0.1 nm. Depolarizing NMB agents that vary in length and have free bond rotation may have a distance between quaternary ammonium groups up to a maximum bond distance of 1.45 nm (Hibbs and Zambon, 2011).

Occupation of negatively charged binding sites on the receptor by the neurotransmitter, ACh activates influx of Na^+ and flux of K^+ along their respective concentration gradients, resulting in membrane excitation. Occupation of these sites by the molecularly rigid competitive agents stabilizes the receptor so that the membrane channel is not activated. Depolarizing agents initially act similarly to ACh. Because of their flexible structure, they allow initial channel activation and ion flow but for some reason

result in a persistent interruption in ion flow through the receptor so that additional changes in electrical potential are not achieved.

Pharmacological Considerations

The NMJ is quite susceptible to alteration by selective pharmacological agents. Various drugs, toxins, electrolytes, and other agents alter in different manners the synthesis, storage, release, receptor interactions, and catabolism of ACh. Several important factors affecting cholinergic transmission are outlined in Figure 10.1.

Hemicholinium is a choline-transport inhibitor that interferes with choline reuptake into cholinergic neurons; although this agent has no current clinical application, it is used widely in research application (Inazu et al., 2013). Acetylcholine synthesis is prevented by lack of choline, classified as an essential nutrient in the B vitamin complex family (Ferguson and Blakely, 2004). Existing vesicular stores of ACh are exhausted upon nerve stimulation, and a gradual weakening and eventual paralysis will result without reuptake and synthesis.

Nerve conduction is affected by only a few substances. Local anesthetics in immediate contact with the motor nerve axon act to stabilize the nerve by inactivating both Na^+ and K^+ channels so that axonal action potential propagation is halted. The puffer fish poison tetrodotoxin and the shellfish poison saxitoxin decrease the permeability of excitable membranes to Na^+ (but not K^+); thus axonal action potentials are not generated and paralysis results. These toxins do not cause an initial depolarization of nerves; they act noncompetitively, are approximately 100,000 times more potent than cocaine or procaine, and are frequently used in research. Clinical cases of fatal food poisoning have also been attributed to ingestion of these substances from their natural source (Cusick and Saylor, 2013).

Botulinum toxin is a potent substance (lethal dose for a mouse is 4×10^7 molecules) produced by *Clostridium botulinum*. It is ingested rarely by humans, more often in cattle, horses, poultry and waterfowl and often is fatal (Johnson et al., 2010). It acts within the motor axon to prevent ACh release into the NMJ by interfering with fusion of cholinergic synaptic vesicles with the plasma membrane (Dressler et al., 2005).

Magnesium ions (Mg^{++}) interfere with release of ACh from the nerve terminal by competing for the transport mechanisms responsible for mobilization of Ca^{++} into the nerve. Magnesium uncouples the excitation–secretion coupling process. An insufficient concentration of Ca^{++} produces similar effects. Magnesium also acts postsynaptically to decrease the effectiveness of ACh to activate receptors.

Aminoglycoside antibiotics (i.e., neomycin–streptomycin group) inhibit release of ACh from

motor nerves by decreasing availability of Ca^{++} at superficial membrane binding sites of the axonal terminal, thereby inhibiting the excitation–secretion coupling process. These antibiotics also reduce sensitivity of the postsynaptic membrane to ACh (Adams, 1984).

Cholinesterase inhibitors (see Chapter 8) decrease the hydrolytic activity of AChE and pseudocholinesterase (Taylor, 1991; Hibbs and Zambon, 2011). ACh rapidly accumulates at receptor sites. Muscle fasciculations, spasms, convulsions, and eventually apnea occur after overdosage with cholinesterase inhibitors.

Postjunctional Mechanisms of Neuromuscular Blockade

The pharmacological effects of clinically useful NMB drugs are a result of their direct alteration of the ability of ACh to activate postsynaptic nicotinic cholinergic receptors (Hibbs and Zambon, 2011).

Competitive Nondepolarizing Agents

Nondepolarizing NMB agents compete with ACh for available nicotinic cholinergic receptors at the neuromuscular postsynaptic membrane and, once occupying these receptors, prevent the ability of ACh to produce a motor response. The historical prototype of this group of drugs is *d*-tubocurarine (tubocurarine chloride, USP, Tubarine), a naturally occurring monoquaternary alkaloid obtained from the bark of the South American plant *Chondrodendron tomentosum*. Today *d*-tubocurarine is not used clinically and has long been replaced with similarly acting agents, including atracurium, cisatracurium, pancuronium, vecuronium, rocuronium, mivacurium, doxacurium, and, most recently, gantacurium. Pharmacological characteristics of representative NMB agents in modern use are summarized in Table 10.1.

Ultra refined experimental techniques helped verify the primary site of action of competitive blocking agents (Bowen, 1972; Hubbard and Quastel, 1973). Although *d*-tubocurarine binds to cholinergic receptors in the same region as ACh, and appears to have the same or similar affinity as ACh for cholinergic receptors, *d*-tubocurarine does not exhibit receptor-activating properties, it has no depolarizing activity, and therefore does not cause a motor end-plate potential. Moreover, the *d*-tubocurarine–receptor interaction renders affected receptors unavailable for interaction with ACh. ACh-induced end-plate potentials are reduced to subthreshold levels or abolished in curarized muscles. In the absence of induced end-plate potentials and subsequent muscle action potentials, the muscle relaxes and is, in fact, paralyzed.

Based on competitive interaction between nondepolarizing agents and ACh, cholinesterase inhibitors were

Table 10.1 Characteristics of neuromuscular blocking agents

Drug	Onset	Duration	Elimination	Dosing	Constant rate infusion
<i>Depolarizing agents</i>					
Succinylcholine	1–2 min	6–10 min	Hydrolysis by plasma cholinesterase	0.3–0.4 mg/kg (C) 0.1–0.2 mg/kg (F) 0.12–0.15 mg/kg (E)	
<i>Competitive nondepolarizing agents</i>					
Atracurium	5 min (C)	30–40 min (C)	Ester hydrolysis, Hofmann elimination	0.1–0.2 mg/kg (C) 0.1–0.25 mg/kg (F) 0.07–0.15 mg/kg (E)	0.18–0.36 mg/kg/h (C) 0.17 mg/kg/h (E)
Cisatracurium	5 min (C)	30–40 min (C)	Hofmann elimination	0.075–0.3 mg/kg (C) 0.05–0.3 mg/kg (F)	
Doxacurium	40 min (C)	100–120 min (C)	Renal and biliary elimination of unchanged drug	3.5 µg/kg (C)	
Vecuronium	5 min (C)	30–40 min (C)	Hepatic metabolism biliary and renal excretion	0.1 mg/kg (C) 0.025–0.1 mg/kg (F) 0.1 mg/kg (E)	
Rocuronium	1–2 min (C)	20–30 min (C)	Hepatic uptake and Biliary excretion	0.1–0.6 mg/kg (C) 0.1–0.6 mg/kg (F) 0.3–0.6 mg/kg (E)	0.2 mg/kg/h (C)
Pancuronium	5 min (C)	30–60 min (C)	Hepatic metabolism and renal elimination of active metabolites	0.07–0.1 mg/kg (C) 0.06–0.1 mg/kg (F) 0.12 mg/kg (E)	
Gantacurium	1–2 min (C)	5 min (C)	Ester hydrolysis, cysteine adduction	0.06 mg/kg (C) 0.06 mg/kg (F)	

Species indicated are canine (C), feline (F) and equine (E).

found to be effective in antagonizing the effects of these blocking agents (Taylor, 2011). Cholinesterase inhibitors prevent the enzymatic catabolism of ACh. More ACh is available for interaction with cholinergic receptors and thereby decreases effectiveness of competitive blocking agents. This relationship has been exploited clinically in successful efforts to terminate the effects of nondepolarizing agents. However, cholinesterase inhibitors do not antagonize effects of the other class of neuromuscular blockers, the depolarizing drugs.

Nondepolarizing NMB agents are classified as pachycurares, or bulky molecules having their amine functions incorporated into rigid ring structures. Two groups of synthetic pachycurares contain the drugs in common use in medicine today: the aminosteroids and the benzyliisoquinoliniums. The aminosteroids maintain their interonium distance by an androstane skeleton while the benzyliisoquinoliniums maintain this atomic distance by linear diester-containing chains (Lee, 2001).

Benzyliisoquinolinium Compounds

Atracurium is a *bis*-benzyltetrahydroisoquinolinium with isoquinolinium nitrogens connected by a diester-containing hydrocarbon chain. Its action is intermediate with a dose-dependent onset of action of approximately 5 minutes and duration of approximately 30 minutes in dogs (Jones, 1983). Repeated doses are generally not cumulative, so longer-term maintenance can be achieved via a constant-rate infusion (Playfor et al., 2000). Atracurium undergoes ester hydrolysis, Hofmann

elimination reaction, and likely other nonhepatic routes for biotransformation, making the drug appropriate for use in hepatic or patients with renal insufficiency. In a Hofmann elimination reaction, a quaternary ammonium group is converted to a tertiary amine by cleavage of a carbon–nitrogen bond. This process does not require enzymatic activity. Hofmann elimination is both a pH and temperature-sensitive reaction in which higher pH and temperatures favor drug breakdown. Clinical consequences of this are important in that patient acidemia and hypothermia may hinder drug breakdown (Playfor et al., 2000). Additionally, the drug should be kept refrigerated and is supplied at a pH of 3.25–3.65 to slow degradation.

The marketed atracurium product has ten isomers. These isomers are separated into three geometric isomer groups that have been designated *cis*–*cis*, *cis*–*trans*, and *trans*–*trans* according to their configuration about the tetrahydroisoquinoline ring system (Wastila et al., 1996). Cisatracurium is the 1*R* *cis*–1'*R* *cis* isomer of atracurium and represents approximately 15% of the marketed atracurium product's weight yet produces roughly 50% of atracurium's neuromuscular blocking activity. Cisatracurium (Nimbex) is available as a stand-alone product separate from atracurium. Cisatracurium is metabolized by Hofmann elimination entirely. The presence of portosystemic shunt and hepatic insufficiency did not affect the rate of onset or duration of action of cisatracurium in dogs receiving 0.1 mg/kg IV followed by repeat doses of 0.03 mg/kg IV (Adams et al.,

2006). In general, cisatracurium has a similar duration and clinical effects to atracurium.

Following intravenous injection, atracurium spontaneously decomposes into laudanosine and a quaternary monoacrylate. Laudanosine in high quantities is a CNS stimulant and may result in seizures, hypotension, and tachycardia (Chapple et al., 1987). Laudanosine, unlike atracurium, is dependent upon hepatic clearance, so theoretically laudanosine plasma concentrations may become elevated in patients with hepatic dysfunction. Practically, laudanosine-induced toxicity is unlikely in clinical patients unless atracurium is used in large doses and/or for prolonged periods (Chapple et al., 1987). Nonetheless, another aminosteroid may be selected to avoid this remote issue entirely.

d-Tubocurarine, the prototypical benzoisoquinolone NMB agent, is associated with histamine release and resulting hypotension. Although atracurium has the potential to result in histamine release, this requires several times the ED₉₅ dose (effective dose producing a 95% reduction in twitch height) before appreciable amounts of histamine are released, making problems such as hypotension and tachycardia rarely observed in clinical cases (Scott et al., 1986; Hackett et al., 1989). In isoflurane-anesthetized dogs, 0.2 mg/kg IV atracurium did not result in significant changes to intraocular pressure, mean arterial pressure, heart rate, or central venous pressure (McMurphy et al., 2004). A notable exception to atracurium and cisatracurium's minimal cardiovascular profile may be in dogs with X-linked muscular dystrophy. Affected golden retrievers in one retrospective study that received 0.1 mg/kg cisatracurium had marked increases in heart rate ($115 \pm 64\%$) and blood pressure ($33.5 \pm 31\%$) that lasted 10 and 30 minutes, respectively (Staffieri et al., 2011).

Atracurium has been widely used with positive clinical success across a variety of species including, but not limited to, dogs, cats, and horses. In horses and dogs, the use of atracurium in conjunction with aminoglycoside antibiotics, specifically gentamycin, has been shown to augment neuromuscular blockade; however, this effect appears minimal and has not been reported to impact recovery quality (Hildebrand and Hill, 1994; Martinez et al., 1996). Atracurium has also been investigated for use in a urethral flushing solution in cats and dogs to help facilitate manual bladder expression in patients with spinal cord injuries and in obstructive urethral plugs (Galluzzi et al., 2012; Galluzzi et al., 2015).

Two additional benzyliisoquinolinium compounds, mivacurium and doxacurium, are reported in the veterinary literature but are infrequently used in clinical practice today. Mivacurium (Mivacron) is a short-acting NMB agent in humans (15–20 minutes' duration); however, this duration of effect appears markedly prolonged in dogs. Although mivacurium resulted in

minimal hemodynamic changes, in dogs administered 0.05 mg/kg IV it appeared to have a long half-life and slow clearance under halothane anesthesia with duration of effect up to 151.0 ± 38.50 minutes (Smith et al., 1999a, 1999b). Clinical observations in cats suggest that mivacurium has a shorter duration of action compared to that in dogs. Mivacurium is unique amongst the benzyliisoquinolinium compounds in that it is metabolized by plasma cholinesterases to a monoester and this attribute, as a result of interspecies differences in plasma cholinesterases, may explain its variable duration of action between species. At the time of writing, mivacurium is not currently available within the United States.

Doxacurium (Nuromax) has a slow onset of action and long duration (>50 minute) of effect in man. Doxacurium may result in an increased propensity for histamine release and cardiovascular side effects when compared to other benzyliisoquinolinium compounds. Work in dogs would suggest that 2.1 µg/kg IV approximates an ED₅₀ while 3.5 µg/kg IV approximates an ED₉₅ with duration of action at 108 ± 31 minutes (Martinez et al., 1996). Due to increased cost, minimal reported advantage, and lack of availability and clinical experience, atracurium is typically selected over other available benzyliisoquinolinium compound for veterinary patients.

Aminosteroid Neuromuscular Blocking Agents

Pancuronium (Pavulon) was the first aminosteroid to be introduced as a NMB agent. The drug has a dose-dependent onset of approximately 5 minutes and long duration of action, up to 60 minutes in the dog (Gleed and Jones, 1982). Because repeated doses have a cumulative effect, constant-rate infusions are avoided. A large proportion of pancuronium is excreted by the kidney. The remainder is metabolized via the liver, making the duration of action increased in patients with renal and hepatic dysfunction. In addition to exerting its effect on post-synaptic nicotinic receptors at the NMJ, pancuronium also has a vagolytic effect by blocking cardiac muscarinic action, which may result in an increase in heart rate. Decreases to systemic vascular resistance, pulmonary vascular resistance, and coronary and renal artery dilation via prostaglandin I₂ release have also been noted in the dog (Hackett et al., 1989; Sai, 1998). In dogs, cats, pigs, and horses doses between 0.06 and 0.12 mg/kg IV have been reported (Gleed and Jones, 1982; Hildebrand et al., 1989; Miller et al., 1978; Veres-Nyékí et al., 2012). Pancuronium is stable at room temperature for 6 months.

Removal of a single positively charged methyl group from pancuronium creates vecuronium, an aminosteroid NMB agent that is essentially devoid of cardiovascular effects (Morris et al., 1983; Jones, 1985b). This molecular modification has several other important clinical implications, including molecular instability in solution which

necessitates vecuronium be kept as a lyophilized powder and only reconstituted immediately prior to administration with stability of only 24 hours once reconstituted. Additionally, vecuronium has a shorter duration of action than pancuronium and an increase in lipid solubility, which results in greater biliary elimination than with pancuronium (Hill et al., 1994). Vecuronium has a dose-dependent onset of action of approximately 5 minutes and generally an intermediate duration of action of approximately 30 minutes, making its clinical utility similar to atracurium.

Vecuronium undergoes both hepatic metabolism, biliary clearance from the liver as parent compound, and about 25% undergoes renal elimination. Patients with renal or hepatic insufficiency may experience prolonged recovery if increased doses of vecuronium are administered. Dogs with diabetes mellitus have been shown to have a shorter duration of effect from vecuronium based upon both tactile train-of-four (TOF) and electromyography (Clark et al., 2012). The potency and duration of vecuronium does not, however, appear to be altered in dogs with autosomal recessive centronuclear myopathy (Martin-Flores et al., 2015b). In horses, it appears that a dose greater than 0.1 mg/kg IV would be needed for complete neuromuscular blockade and that this effect would have a uniquely long duration in this species, lasting over 120 minutes (Martin-Flores et al., 2012a, 2012b). Additionally, one case report documents that a dosage of 0.5 mg/kg IV edrophonium failed to completely reverse prolonged vecuronium-induced neuromuscular blockade in anesthetized dog as judged by peripheral nerve stimulation (Martin-Flores et al., 2011).

Rocuronium bromide (Zemuron), another member of the aminosteroid NMB agent family, lacks the acetyl ester that is found in the steroid nucleus of pancuronium and vecuronium and is less potent but similar in molecular weight to vecuronium. Rocuronium has a more rapid onset compared with atracurium and vecuronium, but similar duration of action (Carson et al., 1990; Gyermek et al., 2002). Additionally, rocuronium appears to be largely devoid of cardiovascular side effects, including no significant histamine release (Hudson et al., 1998). The drug is eliminated primarily via hepatic clearance while a small fraction is eliminated via the kidney. Detectable levels of rocuronium metabolites are not found. Rocuronium is stable for 60 days at room temperature. Rocuronium at clinically relevant concentrations may also bias the accuracy of lithium dilution cardiac output monitoring by interacting with the LiDCO sensor (Ambrisko et al., 2013).

In cats, a dosage of 0.6 mg/kg IV rocuronium had an onset time of 46 ± 11 seconds, produced no change to subject heart rate, and took 20.7 ± 5.4 minutes for TOF ratios to return to 0.9, a level consistent with full recovery from neuromuscular blockade (Auer and Mosing, 2006;

McGrath and Hunter, 2006). Sixty seconds after rocuronium administration in cats, paralysis of the internal laryngeal muscles and conditions suitable for endotracheal intubation occurred that were comparable to topical lidocaine (Moreno-Sala et al., 2013). In dogs, rocuronium at 0.3 mg/kg and 0.6 mg/kg IV resulted in an onset of neuromuscular blockade in 2 ± 0.9 minutes and 1.1 ± 0.6 minutes with total recovery occurring at 23.8 ± 6.6 minutes and 31.9 ± 6.5 minutes respectively (Auer, 2007). Following a loading dose of 0.5 mg/kg IV, rocuronium was found to be suitable for constant-rate infusion at 0.2 mg/kg/h for up to 146 minutes (Alderson et al., 2007). Doses of rocuronium as low as 0.03–0.075 mg/kg IV in isoflurane-anesthetized dogs resulted in a centralized globe position and acceptable conditions for ophthalmic procedures (Briganti et al., 2015). In horses undergoing ophthalmic surgery, a dose of 0.3 mg/kg IV rocuronium produced effective neuromuscular blockade in 2.3 ± 2 minutes with a central globe position in 31 ± 2.8 seconds and a clinical duration of 32 ± 18.6 minutes in all 20 horses studied (Auer and Moens, 2011).

Asymmetric Mixed-onium Chlorofumarates

Gantacurium represents a new class of nondepolarizing NMB agents called asymmetric mixed-onium chlorofumarates, which are structurally distinct from the traditional aminosteroids and benzyliisoquinolinium compounds. Gantacurium has an ultrashort duration of action and is degraded in plasma by pH sensitive chemical hydrolysis and inactivation by cysteine adduction. It does not undergo Hofmann elimination. In dogs anesthetized with thiopental, nitrous oxide, and isoflurane, the ED_{95} was 0.06 mg/kg IV with an onset time of 107 seconds and duration of action of 5.2 minutes (Heerd et al., 2004). In cats following dexmedetomidine and propofol, IV gantacurium at 0.5 mg/kg abolished laryngospasm in 100% of cats and induced apnea for 3 ± 1.5 minutes (Martin-Flores et al., 2015a). At clinical doses, cats and dogs do not appear to have appreciable cardiovascular effects; however, in humans, transient cardiovascular side effects were observed at doses beginning at three times the ED_{95} and were consistent with histamine release (Belmont et al., 2004). Recovery is accelerated with AChE inhibitors such as edrophonium. In humans, exogenous administration of cysteine can also accelerate the antagonism of gantacurium-induced neuromuscular blockade. Gantacurium is not stable in aqueous solution and, like vecuronium, is provided as a lyophilized powder that is reconstituted prior to administration.

Trisquaternary Ether Neuromuscular Blocking Agents

Two drugs are represented in this class of compounds that currently have no clinic significance for use in North America, primarily due either to significant side effects or lack of a currently available marketed product. Gallamine

(Flaxedil) is a NMB agent first developed in 1947 (Raghavendra, 2002). Its desirable qualities were rapid onset of action (3 minutes) and intermediate to long duration of action in the range of 40 minutes. Gallamine has significant parasympatholytic activity and is associated with tachycardia and hypertension (Lee, 2001; Clark and Mitchelson, 1976). Gallamine can also cause histamine release. Approximately 1 mg/kg gallamine causes complete muscle paralysis in both dogs and cats within 1–2 minutes after IV injection and lasts 15–20 minutes. A hypotensive response may be induced in cats with gallamine but is infrequently observed in dogs.

Alcuronium (Alloferin) is another trisquaternary ether NMB agent whose profile is distinguished by a long duration of effect, rendering it generally less desirable than the short to intermediate acting agents (Diefenbach et al., 1995). The mean duration of effect for alcuronium reported in dogs is 70 minutes (Jones et al., 1978). This agent relies entirely on renal elimination for its clearance, undergoing no biodegradation, and relevant concentrations of alcuronium can still be detected in the plasma up to 12 hours postinitial administration of neuromuscular blockade (Diefenbach et al., 1995). This agent is not marketed for use within the United States.

Depolarizing Agents

Succinylcholine chloride, USP (Quelicin, Anectine, Sucostrin, Suxamethonium), and decamethonium bromide, USP (Syncurine, C-10), are members of the depolarizing group of NMB agents. These drugs exert their paralytic effects by interfering with ACh-mediated depolarization of the postsynaptic membrane. In contrast to the well-defined mechanism of the competitive agents, certain aspects of the mechanism(s) of depolarizing neuromuscular blockers are continually debated.

Only succinylcholine is currently available for use within veterinary medicine as a depolarizing NMB agent. However, its utility has dwindled and even this agent has largely been replaced by newer nondepolarizing NMB agents because of succinylcholine's side-effect profile and potential patient monitoring challenges. Succinylcholine is composed of two molecules of ACh linked back to back through acetate methyl groups. Succinylcholine, like ACh, stimulates cholinergic receptors at the NMJ and at nicotinic ganglionic and muscarinic autonomic sites, ultimately opening the ion channel at the nicotinic cholinergic receptor. Succinylcholine elicits a prolonged depolarization of the neuromuscular end-plate that does not allow the postsynaptic membrane to completely repolarize and therefore renders the neuromuscular motor end-plate unresponsive to the normal action of ACh. Because of the initial stimulatory depolarizing action, transient contraction of muscle cells occurs

after administration of succinylcholine. Clinically, this is characterized in the intact animal by asynchronous muscular contractions of the head, body trunk, and limbs. Fasciculations are not always apparent in anesthetized animals. Because of the persistent depolarization of the postsynaptic nicotinic membrane, continued impulse transmissions are blocked and a flaccid paralysis ultimately ensues. The mechanism of depolarizing NMB agent action has historically been described as biphasic.

Phase I block: Initially a phase I block results from depolarization of the neuromuscular motor end-plate region by succinylcholine resulting in an increased and persistent permeability of the postsynaptic membrane to sodium and potassium ions, specifically an influx of sodium ions into the cell and an efflux of potassium ions out of the cell. The ion channel pores subsequently close and become inactivated. As a result, ACh cannot act as a neurotransmitter, and impulse transmission to evoke a muscle response fails. The membrane potential must be reset in order for the channel to be reactivated again. When ACh causes depolarization it is immediately hydrolyzed by AChE within the synaptic cleft and its effect is very short lived. Succinylcholine causes a persistent alteration in membrane potential because it is not hydrolyzed but must slowly diffuse away from the NMJ and be metabolized by plasma cholinesterases. It should be pointed out that ACh, when in excess, also causes persistent depolarization block of cholinergic synaptic junctions (Appiah-Ankam and Hunter, 2004).

Phase II block: Phase II block occurs in some instances after prolonged exposure or large doses of a depolarizing agent and motor end-plate sensitization and is characterized by a change from the depolarizing block to one that more closely resembles the effect of nondepolarizing NMB agents. The actual mechanisms involved are poorly understood and opinion is contradictory as to this transition. Zaimis (1959) believes, for example, that confusion has occurred because in some species some blocking agents have a “dual mechanism”; that is they cause some effects that resemble depolarization block and cause other effects that resemble competitive blockade (Galindo and Kenney, 1974).

After exposure of isolated nerve-muscle preparations to succinylcholine, the initial peak level of depolarization subsides. Subsequently, the end-plate becomes transiently sensitive to depolarizing agents. Gradually, a competitive-like blockade results and seems to be at least partially susceptible to reversal by cholinesterase inhibitors. Though the overall importance of phase II block has not been clearly defined for depolarizing NMB agents, its potential for occurrence and recognition is important.

As a group, the depolarizing NMB agents cause depolarization of receptor areas of muscle fibers sometime during their course of action. In general, unlike non-depolarizing NMB agents, administration of an AChE inhibitor (e.g., edrophonium, neostigmine) will not reverse the effects of depolarizing NMB agents such as succinylcholine and may in fact delay recovery.

Succinylcholine has a rapid onset of effect and an ultrashort duration of action, which has made the agent historically ideal to assist in rapid human endotracheal intubation. The short duration of action (approximately 6–10 minutes) of succinylcholine is due to its rapid hydrolysis by plasma cholinesterase (pseudo-cholinesterase, butyrylcholinesterase) to succinylmonocholine and choline. Because there is limited plasma cholinesterase activity at the NMJ, the neuromuscular blockade that occurs from succinylcholine is terminated by the drug's diffusion away from the NMJ back into the circulation. This has important clinical consequences in that the drug is not reversible by AChE inhibitors and factors that decrease plasma cholinesterase activity may prolong recovery. Common factors seen in veterinary patients that may decrease plasma cholinesterase activity include hepatic disease, advanced age, malnutrition, neoplasia, pregnancy, burns, monoamine oxidase inhibitors, terbutaline, esmolol, and metoclopramide (Birch et al., 1956; Lepage et al., 1985; Kao et al., 1990; Barabas et al., 1986).

Hansson (1956) reported that the IV ED₅₀ (dose that reduced muscle twitch by 50%) of succinylcholine in the sciatic nerve-gastrocnemius muscle preparation of anesthetized dogs was 0.045–0.060 mg/kg. This dose did not effectively paralyze the respiratory muscles, however, and 0.085 mg/kg was required to induce transient apnea, whereas 0.11 mg/kg and 0.22 mg/kg were needed to cause apnea for 18–21 minutes and 23–27 minutes, respectively. In unanesthetized dogs, IM administration of 0.12 mg/kg succinylcholine caused ataxia in 5 minutes and forced abdominal respiration in 7 minutes; recovery was apparently complete in 30 minutes. In clinical situations, 0.3 mg/kg succinylcholine administered intravenously will usually afford good muscle relaxation in dogs, whereas in the cat, 1 mg/kg may be required. In dogs, Hansson (1956) reported that 0.15 mg/kg succinylcholine was effective in paralyzing the diaphragm during thoracotomy procedures. However, Eyster and Evans (1974) suggested the use of 0.5 mg/kg succinylcholine for muscle relaxation in dogs during thoracotomy for open-heart surgery. This dose was also reported to control muscle twitches evoked by inadvertent stimulation of nerves during use of electrocautery. Duration of paralysis varies and should be closely monitored.

In rhesus monkeys, 1–2 mg/kg succinylcholine administered intravenously has been used for restraint for tuberculosis testing and endotracheal intubation

(Lindquist and Lau, 1973). In pigs, approximately 2 mg/kg succinylcholine is effective. Much smaller amounts (0.01–0.02 mg/kg) are required in cattle and sheep. Hansson (1956) reported that 0.13–0.18 mg/kg succinylcholine is required to immobilize unanesthetized horses. However, the generally accepted dose of succinylcholine in horses, when used alone, is 0.088 mg/kg (Lumb and Jones, 1973).

Succinylcholine historically has been used without anesthesia in horses for casting and restraint during brief surgical procedures such as castration. This practice is not condoned, because no anesthesia is afforded for painful procedures, severe fright is evoked, and pronounced cardiovascular disturbances and even myocardial damage may result. Succinylcholine should not be used as a sole restraining agent during surgical procedures but only in conjunction with a general or local anesthetic.

Frequent side effects from succinylcholine have resulted in the drug largely being replaced in veterinary medicine by newer agents. Succinylcholine stimulates all cholinergic autonomic receptors, including nicotinic receptors on both sympathetic and parasympathetic ganglia and muscarinic receptors in the sinus node of the heart (Galindo and Davis, 1962). As a prominent clinical manifestation of generalized autonomic stimulation, cardiac dysrhythmias, including sinus bradycardia, junctional ectopy, and ventricular dysrhythmia, may occur. At lower doses negative chronotropic effects predominate and at higher doses tachycardia may occur.

In humans, the administration of succinylcholine to an otherwise healthy patient raises plasma potassium levels by approximately 0.5 mEq/l. This phenomenon results because with channel activation by acetylcholine, sodium moves into the cells, and potassium exits to the extracellular space. This mild increase in potassium is generally well tolerated in healthy patients and does not result in arrhythmias. However, in patients with preexisting hyperkalemia the additional rise in plasma potassium levels could be of clinical concern. The combination of severe metabolic acidemia and hypovolemia has, in both rabbits and man resulted, in severe and life threatening hyperkalemia (Antognini and Gronert, 1993; Schwartz et al., 1992).

Succinylcholine may cause a rise in intraocular pressure (IOP). The mechanism by which the drug increases IOP is not known but may involve contraction of tonic myofibrils or choroidal blood vessel dilation or both. In man the increased IOP occurs within 1 minute after injection, peaks between 2 and 4 minutes, and subsides by 6 minutes (Pandey et al., 1972). Succinylcholine is also known to increase intragastric pressure, presumed to be due to fasciculation of abdominal skeletal muscles. Succinylcholine can be a potential trigger for malignant hyperthermia in susceptible patients and

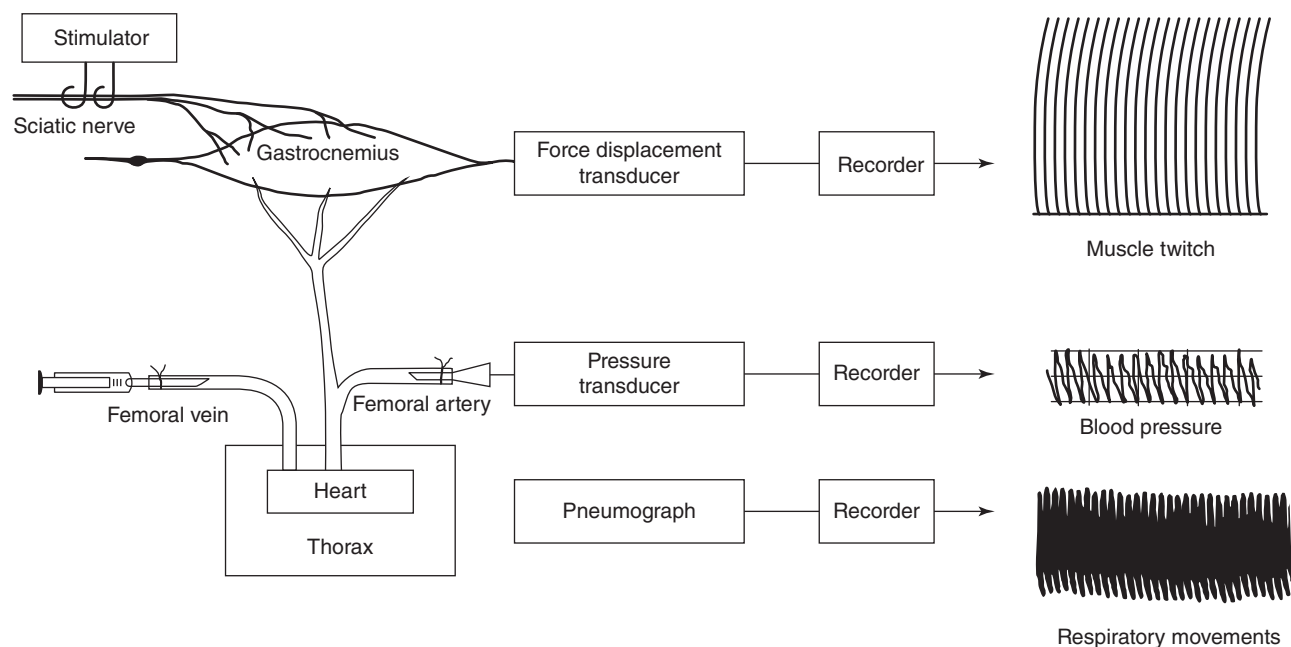


Figure 10.4 Schematic representation of a sciatic nerve-gastrocnemius muscle preparation in an anesthetized cat. Stimulation of the isolated and decentralized sciatic nerve evokes contraction (muscle twitch) of the gastrocnemius muscle. Femoral arterial blood pressure and respiratory movements can be measured concurrently. Neuromuscular blocking drugs can be administered intravenously and changes in muscle twitch height observed.

produce malignant hyperthermia-like episodes, and has the potential to increase intracranial pressure (Minton et al., 1986). Muscle pain, increases to serum creatine kinase, and myoglobinemia have all been reported in man following the drug's administration (Brodsky et al., 1979; McLoughlin et al., 1992).

Pharmacological Effects of Neuromuscular Blocking Agents

When administered, NMB agents are given only to anesthetized patients to provide relaxation of skeletal muscles. It should be remembered that NMB agents do not possess analgesic or amnesic properties and other drugs must be utilized for these effects. Because of a narrow therapeutic index, the clinical usage of NMB agents necessitates strict oxygen supplementation and assisted ventilation in conjunction with qualified supervision by experienced personnel. Said personnel must be thoroughly familiar with the indications, limitations, and adverse effects of NMB agents, principles of monitoring, as well as familiarity with the use of their reversal agents.

Skeletal Muscle

Competitive Neuromuscular Blocking Agents

Nondepolarizing curare-like drugs interact with nicotinic cholinergic receptors of skeletal muscle cells and

render them insensitive to the transmitter function of Ach in a dose-dependent fashion. Flaccid paralysis may ultimately occur. Neither axonal conductance nor response to direct stimulation of muscle is blocked by nondepolarizing NMB agents.

A schematic representation of an *in vivo* nerve-muscle preparation is shown in Figure 10.4. This sciatic nerve-gastrocnemius muscle preparation has been used to examine the actions and pharmacological interactions of NMB agents. In this preparation, electrical stimulation of the sciatic nerve causes contraction (kg of isometric tension) of the gastrocnemius muscle.

Figure 10.5 demonstrates the neuromuscular blocking effect of *d*-tubocurarine on the stimulated muscle twitch of the sciatic nerve-gastrocnemius muscle preparation of a cat, as described in Figure 10.4. Provided a consistent supramaximal stimulus is delivered at a frequency of no greater than once every 7–10 seconds, a depression in muscle twitch response is not seen until approximately 75% of nicotinic cholinergic receptors are blocked. Loss of muscle twitch in response to electrical stimulation requires 90–95% blockade of cholinergic receptors on the motor end-plate (Kelly and Brull, 1993). In this example, muscle twitch quickly decreases after IV injection of *d*-tubocurarine, reaches peak depression within a few minutes, and then gradually returns to normal in approximately 15 minutes. Tubocurarine does not evoke an initial increase in muscle twitch; this lack of facilitation is a consistent finding with nondepolarizing agents.

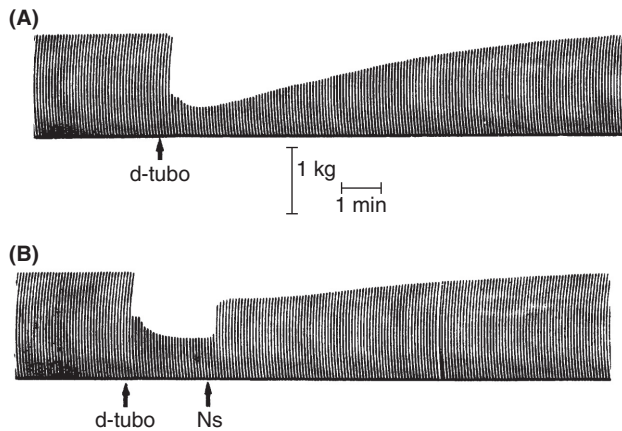


Figure 10.5 Neuromuscular blocking effect of *d*-tubocurarine (*d*-tubo) and reversal of the *d*-tubo by neostigmine (Ns) in a sciatic nerve-gastrocnemius muscle preparation of a cat. The cat was anesthetized with pentobarbital; muscle twitch was monitored as described in Figure 10.4. (A) Typical depression of muscle twitch by *d*-tubo (0.2 mg/kg) administered intravenously at designated arrow. (B) Antagonism of *d*-tubo (0.2 mg/kg) induced depression of muscle twitch by Ns (0.1 mg/kg). Agents were administered intravenously at arrow. Notice rapid antagonism of the neuromuscular blocking effect of *d*-tubo by Ns. Compare this with the lack of antagonism by Ns of the muscle twitch depressant effect of succinylcholine in Figure 10.6.

Antagonism of the neuromuscular blocking effects of *d*-tubocurarine by administration of a cholinesterase inhibitor, neostigmine, is shown in Figure 10.5. By comparing the two tracings in this figure, it is readily apparent that neostigmine markedly hastens recovery from muscle twitch depression caused by *d*-tubocurarine. This antagonistic interaction is primarily attributed to the anticholinesterase activity of neostigmine. Inhibition of cholinesterase delays the catabolic breakdown of ACh and allows its accumulation at receptor sites. Newly available ACh, now in increased concentration at the postsynaptic membrane, effectively competes with *d*-tubocurarine for the cholinergic receptors. ACh-mediated depolarization of the end-plate, muscle action potentials, and muscle contraction are restored; muscle twitch quickly returns to normal.

The sensitivity to the range of receptor blockade represented in the single twitch muscle preparation of Figure 10.4 is narrow and therefore limits its clinical usefulness as a measure of receptor blockade. In clinical situations, a TOF stimulation pattern is often used to determine the extent of blockade present when NMB agents are being used. Four supramaximal stimuli are delivered at a frequency of 2 Hz and, in normal muscle, four clearly separated twitch responses can be observed in the muscle, which ideally are quantified in amplitude via the use of mechanomyography or acceleromyography (Kelly and Brull, 1993). After administration of a nondepolarizing NMB agent, the TOF stimulation pattern will

elicit muscle responses that exhibit fade. The degree of fade is proportional to the extent of receptor blockade. The amplitude of the fourth twitch response (T4) compared to the amplitude of the first twitch response (T1) gives a T4/T1 ratio or TOF ratio. With no blockade the TOF ratio is 1.0. The T4 amplitude will start to decrease when 70–75% of receptors are blocked by a nondepolarizing NMB agent. If the T4 response is lost completely, approximately 80% of the motor end-plate cholinergic receptors are blocked. If T3 and T2 responses disappear, this represents 85% and then 85–90% of receptor occupancy. When 90–95% of receptors are occupied at the NMJ then T1 through T4 will have disappeared (Kelly and Brull, 1993). In order to assess recovery from neuromuscular blockade, a TOF ratio of 0.9 should be achieved before an animal is deemed suitably recovered to be extubated (Ali et al., 1970). Reversal of a nondepolarizing NMB agent can be safely administered when a TOF count is at least demonstrating two twitches or greater (Viby-Mogenson, 2000; Jones et al., 2015).

Depolarizing Neuromuscular Blocking Agents

Succinylcholine elicits transient muscle fasciculations prior to causing neuromuscular paralysis, due to initial depolarization of the motor end-plate and is characterized in the intact animal by asynchronous muscular contractions of the head, body trunk, and limbs.

The *in vivo* neuromuscular blocking effect of a small dose of succinylcholine in a cat nerve-muscle preparation is shown in Figure 10.6. Initially, there is a slight and transient facilitatory effect of succinylcholine on neuromuscular transmission; muscle twitch height momentarily increases by a small increment as a result of the initial depolarizing effect of the drug. Subsequently, however, muscle twitch rapidly decreases and within 1–2 minutes maximum depressant effect is obtained. Shortly thereafter, the neuromuscular effects of succinylcholine subside and muscle twitch returns to normal within an additional 5–8 minutes. The magnitude and duration of neuromuscular paralysis is dependent upon the dosage of succinylcholine. The relatively short duration of succinylcholine activity is from rapid biotransformation of this drug by plasma cholinesterase.

The effects of a cholinesterase inhibitor, neostigmine, on the neuromuscular paralysis produced by succinylcholine are demonstrated in Figure 10.6. By comparing the two tracings in this figure, it is apparent that neostigmine potentiated the muscle twitch depression evoked by succinylcholine and prolonged recovery from the effects of this agent. This synergistic interaction is primarily attributed to the anticholinesterase activity of neostigmine, resulting in decreased biotransformation of both succinylcholine and endogenous ACh. Thus succinylcholine and ACh are available at receptor sites for

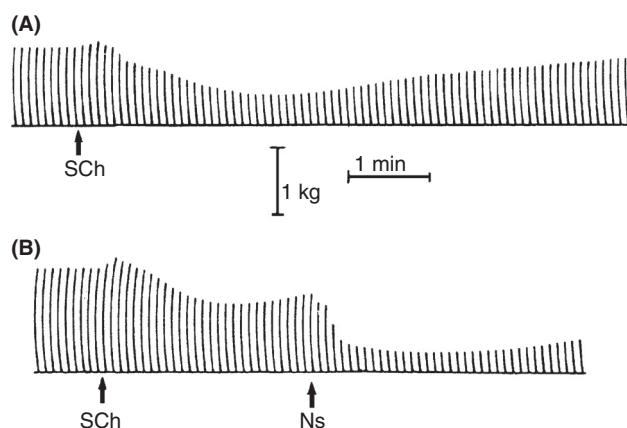


Figure 10.6 Neuromuscular blocking effect of succinylcholine (SCh) and augmentation of its effect by neostigmine (Ns) in a sciatic nerve-gastrocnemius muscle preparation of a cat. The cat was anesthetized with pentobarbital; muscle twitch was monitored as described in Figure 10.4. (A) Typical depression of muscle twitch by SCh (0.04 mg/kg) administered intravenously at designated arrow. (B) Augmentation of the muscle twitch depressant effect of SCh (0.04 mg/kg) by Ns (0.1 mg/kg). Agents were administered intravenously at designated arrows. Notice augmentation of the degree and duration of effect of SCh by Ns. Compare this with the antagonism by Ns of the neuromuscular blocking effect of *d*-tubo in Figure 10.5.

longer periods and the duration of depolarizing neuromuscular paralysis is prolonged.

The potency of neuromuscular effects of succinylcholine varies in different species (Hansson, 1958), as shown schematically in Figure 10.7, with bovine and canine species being quite sensitive to succinylcholine, whereas horses and pigs are considerably less responsive. This difference is probably dependent upon species differences in the activity of plasma cholinesterase, the enzyme that biotransforms succinylcholine (Radeleff and Woodard, 1956; Palmer et al., 1965). Cattle and sheep, for example, have considerably less detectable plasma

cholinesterase activity than horses and pigs. Administration of purified cholinesterase preparation to dogs increases resistance to succinylcholine (Hall et al., 1953).

Autonomic Effects

Synaptic transmission at autonomic ganglia involves activation by ACh of nicotinic receptors of the postganglionic nerve body (see Chapter 8). It is not surprising, therefore, that NMB agents may also alter ganglionic transmission.

Tubocurarine is an excellent example of a drug selected for site of action at nicotinic receptors of the somatic myoneural junction that as a side effect also acts at ganglionic nicotinic receptors. Tubocurarine interacts with ganglionic receptors, renders them inaccessible to ACh, and thereby increases the threshold of the postganglionic nerve to ACh. However, as a general rule, autonomic ganglia are less sensitive to curare than are the myoneural junctions. Ganglionic impulse transmission involves, at least partially, a muscarinic pathway (see Chapter 7); *d*-tubocurarine has little blocking effect on muscarinic receptors. Thus in most cases it would be anticipated that ganglionic transmission is functional during treatment with curare-like drugs. Nevertheless, hypotension believed to be partly dependent upon ganglionic blockade can occur after administration of *d*-tubocurarine.

Other NMB drugs, both competitive and depolarizing types, have been shown experimentally to alter ganglionic transmission, but in clinically insignificant amounts. Succinylcholine induces transient ganglionic stimulation prior to blockade evoked by larger doses. The former effect may partially explain hypertension that has occurred subsequent to succinylcholine administration.

Parasympathetic effects of NMB agents are usually minimal. Succinylcholine is approximately 1,000 times less potent than ACh in eliciting contraction of guinea pig ileum. In dogs, large doses of succinylcholine induce

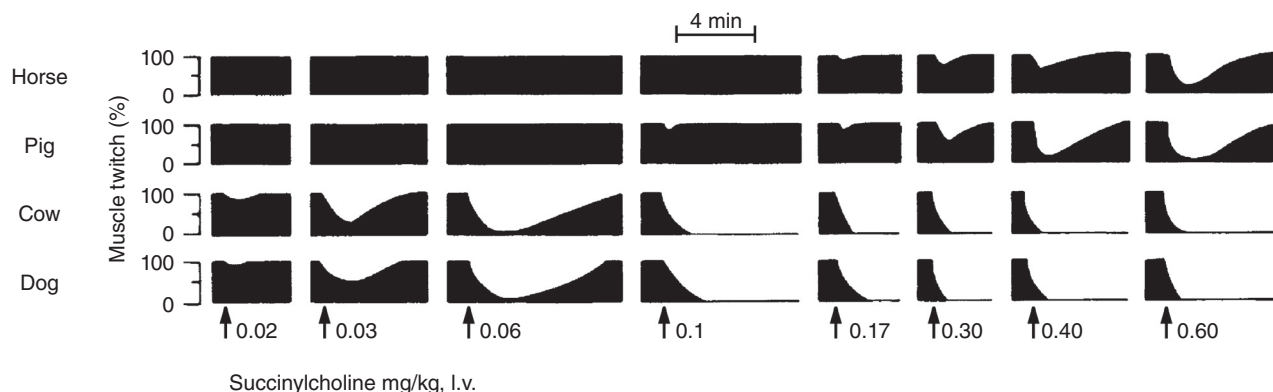


Figure 10.7 Schematic representations of the neuromuscular blocking effect of succinylcholine iodide in nerve-muscle preparations of different species during barbiturate anesthesia. Notice interspecies differences in degree and duration of paralysis caused by succinylcholine. Source: Modified from Hansson 1956, Lumb and Jones 1973.

salivation; this is antagonized by pretreatment with atropine (Hansson, 1956).

Histamine Release

d-Tubocurarine causes release of histamine. The magnitude of this response varies, depending on species, dosage, and rate and route of administration. Histamine-like wheals can be produced by subdermal and intraarterial administration of *d*-tubocurarine. In vivo, increased respiratory tract secretions and bronchospasm seen after administration of *d*-tubocurarine have been attributed to histamine release, as has the hypotensive effect of *d*-tubocurarine. Pretreatment with antihistamine drugs antagonizes these side effects; they are not inhibited by atropine or neostigmine. Succinylcholine and gallamine are very weak histamine-releasing agents.

Central Nervous System

Although synaptic transmission in the brain is altered by direct application of NMB drugs into the brain, CNS effects are nondetectable when these drugs are administered by other routes. NMB agents do not gain entry into the CNS to any appreciable extent because of the presence of the highly charged quaternary ammonium moieties. Therefore, neither CNS depression nor tranquilization is produced by NMB agents. Nonambulation results only from peripheral myoneural paralysis. This was decisively confirmed when Smith (Smith et al., 1947) allowed himself to be paralyzed with *d*-tubocurarine. At no time during the experiment did he experience hypnosis, tranquilization, amnesia, anesthesia, or analgesia. He simply could not voluntarily breathe or move, an experience described as quite frightful.

Cardiovascular Effects

As outlined above, *d*-tubocurarine often induces hypotension, particularly if rapidly administered to dogs. Slight increases in heart rate and cardiac output have been observed after administration of gallamine, apparently from a vagolytic effect on the heart (Longnecker et al., 1973). Others have reported no significant cardiovascular changes after IV administration of gallamine to anesthetized dogs (Evans et al., 1977). In cats, mild atropine-like effects on the heart were observed after injection of gallamine, pancuronium, and alcuronium (Hughes and Chapple, 1976).

Studies with pancuronium in humans and dogs indicated that this agent evokes slight increases in heart rate, blood pressure, and cardiac output during thiobarbiturate anesthesia (Coleman et al., 1972; Reitan and Warpin-ski, 1975). Cardiovascular effects of this agent were

absent if patients were pretreated with atropine. Others have reported no significant cardiovascular changes with pancuronium (Brown et al., 1973). Similarly, studies have indicated that neither pancuronium nor gallamine significantly altered heart rate or blood pressure in anesthetized horses (Klein et al., 1983). Atracurium (up to 0.6 mg/kg) and vecuronium (up to 0.2 mg/kg) were reported to have negligible effects on arterial blood pressure in dogs (Jones, 1985a).

Succinylcholine usually evokes minimal cardiovascular changes in horses or dogs if administered during general anesthesia; blood pressure remains fairly constant if mechanical ventilation is provided (Evans et al., 1977; Benson et al., 1979).

Subparalytic doses of succinylcholine increase the arrhythmogenicity of epinephrine during light halothane anesthesia in dogs (Tucker and Munson, 1975). In dogs not treated with succinylcholine, an average dose of 4.15 µg/kg epinephrine was required to evoke premature ventricular contractions, whereas an average dose of 1.6 µg/kg epinephrine was the arrhythmogenic dose in dogs pretreated with 0.25 mg/kg succinylcholine. However, *d*-tubocurarine provides a slight protection against epinephrine-induced arrhythmias. Mechanisms involved in these drug interactions have not been clarified. If deemed essential, catecholamines should be used cautiously in patients treated with depolarizing NMB agents. Also, succinylcholine has been reported to increase susceptibility to the myocardial irritant effects of digitalis preparations, and it has been suggested that succinylcholine may be contraindicated in digitalized patients (Dowdy et al., 1965).

Pronounced cardiovascular side effects have been reported in horses after administration of succinylcholine (Larson et al., 1959; Hofmeyer, 1960; Lees and Tavernor, 1969). In general, these effects seem to be more pronounced in unanesthetized and unsedated animals than during general anesthesia. Severe hypertension, initial bradycardia followed by tachycardia, atrioventricular conduction disturbances, and extrasystoles have been reported, and myocardial damage has been suspected. Early institution of positive pressure ventilation has been reported to block the blood pressure effect. The hypertensive response seems to be at least partially mediated by the succinylcholine-induced dyspnea and the accompanying arterial PO_2 - PCO_2 disturbances, causing a reflexogenic increase in blood pressure. Direct activation of autonomic ganglia by succinylcholine may also be involved.

It should be remembered that NMB agents do not depress the brain unless or until apnea-induced hypoxia actually causes syncope. Prior to hypoxic states, skeletal muscle paralysis affords no depression whatsoever of conscious centers of the brain of unanesthetized animals. It seems likely, then, that the novel sensations

experienced by conscious animals as they are being paralyzed evoke profound fright. This can cause activation of autonomic centers within the brain. Autonomic discharge may be altered markedly resulting in cardiovascular side effects. Autonomic blockade has been shown to substantially decrease the cardiovascular side effects of succinylcholine, supporting the argument for its association with a stress response in those animals.

Ocular Effects

Clinically important ocular effects may occur as a result of pronounced contracture of ocular muscles that occurs after treatment with depolarizing neuromuscular blockers. These agents are contraindicated in glaucoma, since intraocular pressure may be increased. There is no contraindication for nondepolarizing NMB agents in this instance.

Serum Potassium

Depolarizing NMB agents cause a release of K^+ from skeletal muscle. This change is typically small in magnitude, but could be of concern in patients that already have an elevation in serum potassium. Elevation of serum K^+ may be particularly significant, if repeated injections of the NMB are given. There could be a greater risk in animals with muscle disorders, such as Golden Retriever muscular dystrophy (Larach, 1997).

Drug Interactions

Various drugs influence the pharmacological effects of muscle relaxants. NMB agents themselves alter activity of other neuromuscular agents. As would be expected, competitive agents summate with each other. Similarly, depolarizing agents also interact synergistically with one another. However, tubocurarine decreases the muscle twitch depressant effects of succinylcholine. This is related to persistent occupation of a certain portion of receptors by tubocurarine, although muscle twitch may have recovered (see Section Margin of Safety of Neuromuscular Transmission). Depolarization of the end-plate by succinylcholine is partially impeded by the stabilizing effects of tubocurarine. Succinylcholine antagonizes the effects of curare as a result of the partial agonistic characteristics of the former agent. These complex antagonistic interactions, however, have no clinical application, since they depend upon complicated treatment and time and dosage schedules. During clinical situations, NMB agents should not be used in attempts to reverse the effects of other types of NMB agents, since potentiation may occur despite experimental results to the contrary.

The interaction of cholinesterase inhibitors with NMB agents has been discussed in Section Skeletal Muscle.

Cholinesterase inhibitors decrease responsiveness to the competitive agents, while they tend to increase intensity and duration of action of depolarizing agents (Sunew and Hicks, 1978). Organophosphate pesticides and anthelmintics, carbamates, and any other type of cholinesterase inhibitor will cause similar interactions.

Aminoglycoside antibiotics (neomycin, streptomycin, dihydrostreptomycin, kanamycin, gentamicin) decrease the release of ACh from the nerve and also the sensitivity of the end-plate to ACh (Pittinger and Adamson, 1972; Adams et al., 1976a). They do not cause depolarization. Their effects in many ways resemble those of low Ca^{++} or excess Mg^{++} . The presynaptic effect of antibiotics is believed to be due to interruption of Ca^{++} -dependent events at the axonal membrane (Adams, 1984). Cholinesterase inhibitors such as neostigmine antagonize the postsynaptic depressant effect of these antibiotics. Calcium antagonizes the presynaptic action and is usually more effective than neostigmine in reversing the neuromuscular paralyzing effects of aminoglycoside antibiotics. These antibiotics interact synergistically at the myoneural junction with NMB agents, anesthetics, and other antibiotics. The clinical significance of neuromuscular interactions of antibiotics and other drugs has been well established in humans and has been suggested in other species (Adams and Bingham, 1971). These subjects have been reviewed (Pittinger et al., 1970; Adams et al., 1976b; Keller et al., 1992).

Different disease states influence pharmacological effects of NMB agents. Hepatic synthesis of cholinesterase is decreased in the presence of liver disease. The duration of succinylcholine activity may be prolonged if the liver is seriously affected. Impairment of hepatic biotransformation may also cause prolongation of the effect of vecuronium. Renal dysfunction can delay excretion of *d*-tubocurarine, gallamine, doxacurium, and pancuronium.

Clinical Use

Muscle paralysis proceeds at different rates in different body regions after administration of a NMB agent. Usually, the extraocular muscles, the facial muscles, and those of the head and neck are affected first, often within 0.25–1 minute after injection. The tail is usually affected with the head and neck. Subsequently, muscles of the limbs are paralyzed, then the deglutition and laryngeal muscles (glottis). Abdominal muscles, intercostal muscles, and the diaphragm are then paralyzed in this order. Recovery usually proceeds in the reverse of this sequence (Hall, 1971).

Attempts have been made in clinical practice to use the sequential development of muscle paralysis by administering doses of NMB agents adequate to paralyze

ambulatory muscle but insufficient to affect the diaphragm. This rarely has proven to be effective, because respiratory insufficiency may still occur, although the diaphragm is seemingly spared. Therefore, it is imperative that apparatus for administering mechanical ventilation be available when NMB agents are used clinically. To circumvent the need for immediate establishment of an adequate airway and other emergency procedures, it is wise to routinely perform tracheal intubation and institute ventilation whenever a NMB agent is used.

Muscle relaxants have been used in clinical practice for several purposes: to facilitate tracheal intubation; to paralyze respiratory muscles so that mechanical ventilation can be easily controlled; to increase muscle relaxation to facilitate surgical access to difficult anatomic regions; to evoke muscle relaxation to facilitate orthopedic manipulations or improve ocular positioning for surgery and as part of balanced anesthesia procedures to reduce the amount of general anesthetic required.

Tracheal intubation may be performed in a unanesthetized animal immediately after a paralyzing dose of NMB agent has taken effect. Prior administration of sufficient sedation or tranquilization is advisable for humane reasons and to circumvent potential side effects that may be precipitated by fear reaction to paralysis.

A wide range of dosages of NMB agents have been reported for use of these drugs during anesthesia (Hanson, 1956; Tavernor, 1971; Lumb and Jones, 1973). Often this variance reflects differences in the procedures reported in the original studies, for example, the use of different anesthetics and sedatives, different salts of the NMB agent, and in some cases the use of unanesthetized subjects. NMB agents should be given to effect rather than by bolus administration of a set precalculated dose. It is advisable for these drugs to be administered by titration during anesthesia and to be continuously correlated with the extent of muscle paralysis in the patient. The use of neuromuscular monitoring techniques markedly improves the ability to accurately dose and safely terminate the effects of neuromuscular blockade in all animals. Care should always be taken during the use of NMB agents to ensure that the animal does not suffer from residual paralysis as it enters the recovery period postanesthesia.

Margin of Safety of Neuromuscular Transmission

The concept of a margin of safety of neuromuscular transmission bears discussion in relation to clinical use of these drugs. It has been estimated that a relatively large percentage of the cholinergic receptors must be occupied by a paralyzing agent before muscle twitch fails. In the cat diaphragm, for example, muscle twitch is not affected until about 80% of the receptors are blocked by

d-tubocurarine, and twitch is not completely abolished until about 90% of the receptors are occupied (Waud and Waud, 1972). Accordingly, for recovery of the diaphragm from the effects of a previous injection of *d*-tubocurarine, only a small percentage (5% in dogs, 18% in cats) of the receptors need to be free. Therefore, and most important, although to all outward signs recovery seems complete, over 80% of the receptors can still be blocked.

Recognition of this aspect becomes clinically important in the postoperative recovery room and should be considered in patients that have been exposed to NMB drugs and/or other myoneural depressants such as anesthetics. As a patient regains some control of voluntary muscles, spontaneous respiration returns and may seem completely normal. However, it must be remembered that at this time an extremely small margin of safety of neuromuscular transmission exists. That is, only a small percentage of the postsynaptic receptors are available for interaction with ACh; this small fraction of receptors is now responsible for maintaining muscle contraction. Therefore, if the patient is then exposed to another drug that as a side effect depresses neuromuscular function (even though it may be minimal or even nondetectable normally), disastrous complications may result. Anesthetic mortality has occurred in humans that can be attributed to such interactions. For example, Pridgen (1956) reported the anesthetic deaths of two children who were given neomycin immediately after completion of successful laparotomies under ether anesthesia. Initially, respiration was adequate, but within a short time after administration of the antibiotic, persistent apnea occurred. Death followed several hours later. It seems likely that the margin of safety of neuromuscular transmission was reduced in these infants by ether, resulting in marked augmentation of the neuromuscular blocking properties of neomycin. Pittinger et al. (1970) estimated a 9% death rate in human patients experiencing antibiotic-induced respiratory problems in conjunction with anesthetics and NMB agents. Apnea and eventual death in a traumatized dog were attributed to antibiotic (dihydrostreptomycin)-induced neuromuscular paralysis (Adams and Bingham, 1971).

These examples illustrate potential problems that may be inadvertently introduced in a patient that seemingly is recovering quite well from anesthesia and surgery. The margin of safety of neuromuscular transmission should be considered any time that anesthetics, NMB agents, or any other drug that depresses myoneural function are used in multiple drug regimens.

Clinical Reversal of Neuromuscular Paralysis

Treatment of persistent neuromuscular paralysis and/or treatment of inadvertent overdosage of NMB agents should be approached conservatively (Bevan et al., 1992).

The first step should be to initiate immediate (or to continue) positive pressure ventilation and to withdraw administration of the involved NMB agent. Often, ventilation will allow adequate time for the drug to be disposed of or metabolized by the patient's system. Exposure to other drugs that may synergistically interact with NMB agents should be avoided. However, if the effect of a neuromuscular blocker is no longer needed as the end of a procedure is approaching, reversal of the neuromuscular blockade from a nondepolarizing NMB agent is a good option. There are two classes of drugs used for reversal of neuromuscular blockade: the AChE inhibitors and the cyclodextrin, sugammadex (Jones et al., 2015).

Acetylcholinesterase Inhibitors

Acetylcholinesterase inhibitors, including edrophonium, neostigmine, and pyridostigmine, are available to reverse the action of nondepolarizing NMB drugs. As discussed in previous sections, AChE inhibitors are not routinely effective for the reversal of depolarizing NMB agents such as succinylcholine unless prolonged administration or repeat dosages have resulted in phase II block. Fortunately, recovery from succinylcholine is rapid and spontaneous because of plasma cholinesterases and the issue of drug reversal is rarely a clinical issue.

Acetylcholinesterase inhibitors act to inhibit the enzyme AChE thereby preventing the enzymatic hydrolysis of ACh into choline and acetic acid. This results in an increase in ACh that is not specific to the NMJ. While nicotinic effects occur at the NMJ and autonomic ganglia, muscarinic cholinergic effects also occur at the sinus node, smooth muscle, and glands. As nondepolarizing neuromuscular agents and ACh both compete for the same postsynaptic nicotinic receptor, an increase in ACh concentration in the synaptic cleft can tip the balance of competition in favor of ACh and neuromuscular transmission is restored.

Because of an increased ACh concentration at muscarinic cholinergic receptors, bradyarrhythmias, bronchoconstriction, nausea, vomiting, diarrhea, increased intestinal peristalsis, and salivation can adversely occur. In very rare incidences cardiopulmonary arrest has been reported. For this reason, anticholinergic drugs such as atropine or glycopyrrolate should always be available to treat excessive muscarinic side effects. Several human AChE inhibitor products come premixed with an anticholinergic and clinicians should confirm what product they have before drug administration. However, in veterinary medicine it is more common practice to administer an AChE inhibitor such as edrophonium and administer an anticholinergic drug only as needed based upon side effects observed.

Edrophonium, neostigmine, and pyridostigmine differ in how they inhibit AChE activity, in their onset and duration of action, and in their incidence of muscarinic

side effects. Edrophonium is a prosthetic inhibitor that produces a reversible inhibition by electrostatic attachment to the anionic site and by hydrogen bonding at the esteratic site on AChE. Neostigmine and pyridostigmine are oxydiaphoretic (acid transferring) inhibitors and inhibit AChE by forming a carbamyl-ester complex at the esteratic site of AChE to form a covalent bond. Edrophonium has the most rapid onset of action followed by neostigmine and then pyridostigmine. The muscarinic effects of edrophonium are generally mild compared to neostigmine or pyridostigmine and for this reason edrophonium is the drug most commonly selected for neuromuscular blockade reversal in veterinary medicine. The duration of action is similar for both edrophonium and neostigmine, whereas pyridostigmine is approximately 40% longer (Cronnelly et al., 1982; Morris et al., 1981). Acetylcholinesterase inhibitors undergo hepatic biotransformation and renal elimination. Patients with renal disease may have prolonged drug elimination.

Larger doses of AChE inhibitors should antagonize nondepolarizing NMB drugs more rapidly and more completely than smaller doses do. In dogs, 0.5 mg/kg IV edrophonium reversed 100% of vecuronium treated dogs while subjects receiving only 0.25 mg/kg IV required further reversal (Clutton, 1994). Neostigmine can be administered to small and large animals by slow IV injection at the dose of 0.022 mg/kg. Atropine (0.01 mg/kg large animals; 0.04 mg/kg small animals) should be administered prior to or in conjunction with neostigmine to circumvent the muscarinic effects of the latter drug (Klein et al., 1983; Jones et al., 2015).

While both metabolic and respiratory acidemia may augment the blockade induced by a nondepolarizing neuromuscular blocker, only respiratory acidemia inhibits drug reversal. Therefore, the effectiveness of AChE inhibitors may be reduced if a patient hypoventilates (Miller et al., 1974; Miller et al., 1978). Calcium channel blockers such as verapamil have been shown to potentiate nondepolarizing NMB drugs and may make achieving adequate reversal more difficult (Wali, 1986; Jones et al., 1985).

Sugammadex

Sugammadex (Org 25969) is a unique agent recently available to reverse the effects of nondepolarizing NMB agents. It is the first drug to be known as a selective relaxant binding agent (SRBA). Sugammadex is a modified γ -cyclodextran that when administered, tightly encapsulates aminosteroid-based nondepolarizing NMB agents. The cyclodextran structure has a hydrophobic cavity and a hydrophilic exterior because of the presence of polar hydroxyl groups. Hydrophobic interactions trap the NMB agent tightly in the cyclodextrin cavity and form a water-soluble guest-host complex which is

readily available for elimination. This creates a concentration gradient favoring movement of the remaining nondepolarizing NMB agent away from the NMJ and its site of action and into the plasma.

Sugammadex appears to be most effective in reversal of rocuronium, followed by vecuronium, and then pancuronium. Administered at a dose of 2–8 mg/kg IV in man, the drug appears to have excellent efficacy in the face of deep neuromuscular blockade when traditional reversal with AChE inhibitors might fail. Additionally, as sugammadex does not increase the presence of ACh, it is devoid of muscarinic signs and appears to be largely free of side effects. In phase I and II trials, the most frequently reported side effects were hypotension, coughing, nausea, and xerostomia. Although cost and widespread availability have yet to make the drug's use commonplace, sugammadex has been evaluated in dogs and appears to offer promising clinical benefit. In dogs undergoing

profound neuromuscular blockade with rocuronium or vecuronium, an 8 mg/kg IV dose of sugammadex results in a restoration of a TOF ratio to 0.9 in under 2 minutes with no adverse side effects.

Neostigmine or other cholinesterase inhibitors should not be used in attempts to reverse the effects of a depolarizing agent (Sunew and Hicks, 1978). Reliable chemical antidotes are not available for this group of agents. Mechanical ventilation may be required for a prolonged period. Injection of purified cholinesterase preparation has been shown to hasten recovery from the effects of succinylcholine (Scholler et al., 1977).

Because of the small therapeutic index of NMB agents, their clinical use should always be supervised by qualified experienced personnel who are thoroughly familiar with the indications, limitations, hazards, and methods of administration of these highly active drugs.

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