The Neuromuscular Junction: Pharmacologic Applications During Anesthesia

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Introduction

The neuromuscular junction and neuromuscular transmission are well-studied phenomenon. The system is designed to allow translation of an electrical impulse into a motor action potential and subsequent muscular contraction. While at first glance relatively simple in design, an appreciation of its complexity has added to the understanding and development of drugs used to modulate its function. That being said, there is increasing evidence that while monitors of depth of neuromuscular block lessen the degree of postoperative residual neuromuscular block, patients continue to arrive to the postanesthesia care unit with unacceptable levels of neuromuscular block and are prone to an increased incidence of critical respiratory events. A recent survey of practices of attitudes toward the use of neuromuscular blockers and use of neuromuscular blockers indicated that while approximately 20% of respondents felt that postoperative NMB was a significant problem, 66% of practitioners do not routinely administer an anticholinesterase and when they don't approximately 40% base their decisions on clinical tests of residual neuromuscular block and absence of visible or palpable fade in response to train-of-four monitoring with conventional nerve stimulators. Quantitative monitors are available in only 5% of practices and only 10% feel that they should be part of minimal monitoring standards.

The Neuromuscular Junction: Prejunctional Components

The nerve terminal contains the synaptic vesicles in which acetylcholine is stored and, while they can be found throughout the motor nerve terminal, they tend to be concentrated around active zones on the nerve terminal membrane. The synaptic vesicles dock at the active zones, fuse with the cell membrane, release acetylcholine into the synaptic cleft and then are reused or recycled. They do not become permanently incorporated into the cell membrane of the motor nerve terminal.

The motor nerve terminal lies in the junctional cleft of the membrane of the muscle cell. The Schwann cells of the axon form a cover at the junction of the motor nerve terminal and the muscle fiber – essentially enclosing the neuromuscular junction. The basement membrane fills the junctional cleft, which is the space between the axon and the muscle fiber. It acts as a support, anchoring the acetylcholinesterase found at the neuromuscular junction. It also has a physiologic role, allowing for the rapid diffusion of acetylcholine. Much of the acetylcholinesterase of the neuromuscular junction is found within the basement membrane.

Both nicotinic and muscarinic receptors are found presynaptically on the motor nerve terminal. These prejunctional receptors are involved in modulation of the release of acetylcholine into the neuromuscular junction and they have been attributed with both excitatory and inhibitory roles. Prejunctional nicotinic receptors are activated by acetylcholine and function in a positive feedback control system that serves to maintain availability of acetylcholine when demand for it is high. They are involved in the mobilization of acetylcholine, but not the process of acetylcholine release. Block of these receptors by nondepolarizing neuromuscular blockers may explain the fade seen with tetanic and TOF stimulation and, perhaps, the fade in neuromuscular response during a stress protocol following recovery to the train-of-four to 1.0.

The presynaptic nicotinic receptor is structurally distinct from the postsynaptic acetylcholine receptor. While it, like the postsynaptic receptor, is also comprised of 5 subunits, it contains only α and β subunits (α_3 β_2). This may account for the different interaction of the pre- and postjunctional receptors with compounds such as α bungarotoxin, d-tubocurarine and decamethonium. Synergism between nondepolarizing neuromuscular blocking agents of different structures has been attributed, in part, to their different effects on the presynaptic nicotinic receptors, reducing the release of acetylcholine**. T**hese binding sites have varied interactions with different nondepolarizing neuromuscular blocking agents. Vecuronium and d-tubocurarine are the most potent inhibitors at this receptor and mivacurium is the least potent. There appears to be a noncompetitive as well as a competitive aspect to block induced by d-tubocurarine and vecuronium.

The Neuromuscular Junction: Postjunctional Components

The muscle at the motor end plate forms a recess into which the motor nerve terminal fits. This recess is characterized by multiple clefts, called secondary junctional clefts. The distance between the motor nerve unit and the top of the junctional cleft is 60 nm. Nicotinic acetylcholine receptors are found throughout the muscle membrane but, in healthy muscle cells, are concentrated at the tops of the secondary junctional clefts.

Acetylcholine receptors are synthesized in the muscle cell as one of two isoforms, a junctional or mature form and an extrajunctional or immature form. The five subunits that comprise the mature form of the acetylcholine receptor consist of two α, one β, one δ and one ε subunits. The α subunits contain the major portion of the acetylcholine receptor site. There are two binding sites on each acetylcholine receptor, one at the α-δ and one at the

α-ε subunit interface. Each of these binding sites has different affinities for the neuromuscular blocking agents and the α-δ binding site is the more dominant one in determining the affinity of the wild type receptor for pancuronium, vecuronium and cisatracurium.

Neuromuscular Transmission: Prejunctional Events
While acetylcholine is present throughout the cytoplasm of the nerve terminal, it is that which is found in synaptic vesicles that is involved in neuromuscular transmission. Synchronized release of acetylcholine from the synaptic vesicles of the nerve terminal, in an amount adequate to generate a muscle action potential, occurs in response to an electrical impulse. The rapid release of acetylcholine once an impulse arrives at the motor nerve terminal indicates that only those vesicles close to the membrane of the nerve terminal can participate in the process of exocytosis. Repetitive stimulation results in multiple electrical impulses. These cause vesicles to move toward the motor nerve terminal for subsequent release – allowing for the posttetanic potentiation observed during neuromuscular block. The increased release of acetylcholine allows for a transient increase in apparent muscle strength as the relative concentration of available acetylcholine increases relative to the nondepolarizing neuromuscular blocking agent.

The release of acetylcholine is a calcium dependent process resulting in the release of the contents of 50-100 vesicles of acetylcholine. The connection between the impulse, depolarization and the rise in intracellular calcium is a voltage-gated calcium channel. This calcium channel allows for intracellular movement of calcium in response to an electrical impulse. In response to an increase in intracellular calcium, the synaptic vesicles move to release sites on the membrane of the nerve terminal. They then attach to these sites and exocytosis begins and the contents of the vesicle are released into the extracellular space.

Neuromuscular transmission: Postjunctional Events
Once released, acetylcholine moves across the junctional cleft to the motor end plate where it binds to the α subunits of the acetylcholine receptor. Once both α subunits are occupied simultaneously by agonist, the channel of the receptor opens allowing the influx of calcium and sodium and subsequently the efflux of potassium, as the ions move along their concentration gradients.

Nondepolarizing neuromuscular blocking agents exert their effect by occupying one or both of the acetylcholine receptors. The competitive block of the nondepolarizing agents is terminated by an increase the ratio of acetylcholine to the neuromuscular blocking agent. This can occur by either or both of two mechanisms. As plasma concentrations of the agent decreases, it moves from the neuromuscular junction to the plasma, where it is broken down, eliminated through hepatic or renal mechanisms or moved to storage sites. Alternatively, inhibition of acetylcholinesterase by administration of an anticholinesterase, allows acetylcholine released into the neuromuscular junction to remain available to bind to the acetylcholine receptor.

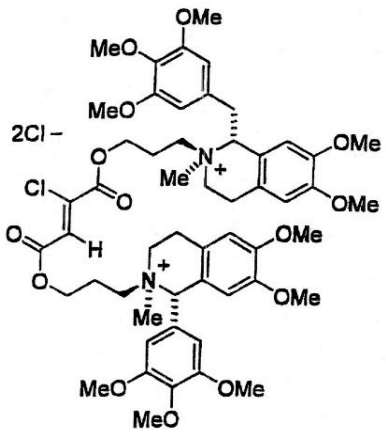
Generation of a muscle contraction
Activation of the acetylcholine channels is not, in and of itself, enough to generate a muscle action potential. Following a nerve impulse, thousands of acetylcholine receptors are activated and an end plate potential is generated. When an adequate number of end plate potentials are generated, adjacent gated-sodium channels in the muscle membrane are opened and a muscle action potential, which activates muscle contraction, is started.

Channel block, different than competitive block at the acetylcholine receptor, can occur at these sodium channels. It is caused by any number of agents including local anesthetics, gallamine, pancuronium, d-tubocurarine – and acetylcholine. When animals are given neostigmine after complete recovery from vecuronium—induced block, upper airway volume is decreased as is diaphragmatic function. Similarly, patients given neostigmine, 50 μg/kg, after recovery of the train-of-four ratio to > 0.9 developed a decrement in response to neuromuscular stimulation.

New Approaches to Manipulation of Neuromuscular Transmission
In spite of the relatively large number of compounds that have been introduced into clinical practice, pharmacologic research in the area of neuromuscular blockade and its antagonism is ongoing. None of the clinically available neuromuscular blocking agents fulfills the criteria defined by Savarese and Kitz in 1975 for a short-acting compound with a rapid onset of effect. This, coupled with the lack of use of quantitative monitors of residual neuro-muscular block, concerns regarding their efficacy and the adverse effects of inadequate recovery of neuromuscular function led to the identification of two new compounds. One is a series of neuromuscular blockers and the other a selective relaxant binding-agent that may, once introduced into clinical practice, allow for a revision of the paradigm of what has long been considered acceptable levels of recovery of neuromuscular function for extubation and the time course required to achieve these levels.

Fumarates: Gantacurium

Gantacurium is one of several enantiomeric bisquarternary compounds, identified as an asymmetrical chlorofumarate. This class of compound incorporates 1-benzyl and 1-phenyltetrahydroisoquinolinium groups in the same molecule. Gantacurium was first identified from this series of neuromuscular blockers as having an ultra-short duration of action and a safety margin for cardiovascular effects similar to that of mivacurium. This compound is characterized as a single isomer. Its stereochemistry is derived from its orientation about each of its six asymmetric centers.



In Rhesus monkeys, the potency of gantacurium is identical to that of mivacurium, 0.06 mg/kg. Its onset, however, is significantly faster and its recovery shorter that that of mivacurium. Hemodynamic changes indicative of histamine release are observed at doses of 3.2 mg/kg. Smaller doses caused less than a 10% change in mean arterial pressure and heart rate. Based on this data, the margin of safety for histamine release was determined to be 53 (ED for histamine release/ED95 neuromuscular block). The margin of safety for histamine release associated with administration of mivacurium was 13.

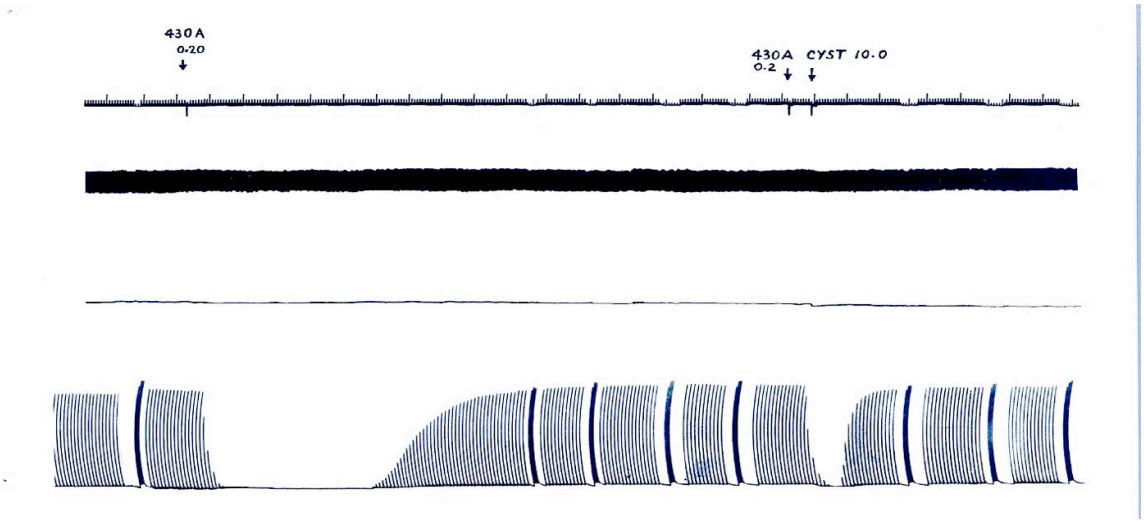
In volunteers, gantacurium has pharmacodynamic properties that are very similar to those of succinylcholine. A study in anesthetized human volunteers evaluated the onset and recovery profiles of 430A in the thumb and larynx. The pattern of blockade resembled that of succinylcholine, with fully paralyzing doses (2–3 x ED95 or 0.38–0.54 mg/kg) producing 100 percent block of TOF within 60 to 70 seconds in the larynx (Table 1). To date there has been one published trial of gantacurium in human volunteers receiving a nitrous oxide-opioid anesthetic. In this study of 11 individuals, the ED95 of the compound was 0.19 mg/kg. Administration of 2 times the ED95 caused 100% neuromuscular at the adductor pollicis within 1.7 minutes and administration of 3 times the ED95 caused 100% block within 1.5 minute. Complete recovery to a train-of-four ratio of 90% following administration of an ED95 dose occurs within 10 minutes and within 12 to 15 minutes following administration of doses as large as 0.72 mg/kg (4 x ED95). Recovery intervals were not lengthened by increasing the dose of gantacurium. Recovery was accelerated by administration of edrophonium.

Gantacurium is degraded by two chemical mechanisms neither of which is enzymatic. The chlorine in the molecule allows for a unique form of inactivation. Preliminary studies with gantacurium in human blood indicate that the major metabolite of the compound is mixed-onium thiazolidine. This compound is formed through the adduction of cysteine, a nonessential amino acid, to the compound at the site of the chlorine molecule. The adduction process occurs rapidly. The second process of inactivation occurs more slowly and involves hydrolysis of the ester bond adjacent to the chlorine substitution. This process yields inactive hydrolysis products.

Table 1. Onset of Maximal Block and Spontaneous Recovery to 25% or 95% Twitch Height following the Administration of Gantacurium or Succinylcholine (Mean ± S.D.)

		Min to Max Block	Min to T1 = 25%	Min to T1 = 95%
Gantacurium 0.36 mg/kg	**LA**	1.1 ± 0.3	7.2 ± 1.1	12.9 ± 2.1
	AP	1.7 ± 0.2	7.0 ± 0.5	12.2 ± 1.3
Gantacurium 0.54 mg/kg	**LA**	0.9 ± 0.2	9.3 ± 2.9	16.1 ± 4.1
	AP	1.5 ± 0.3	9.3 ± 1.5	15.2 ± 3.0
Succinylcholine 1 mg/kg	**LA**	0.8 ± 0.3	6.1 ± 1.7	11.3 ± 1.9
	AP	1.5 ± 0.2	8.5 ± 1.5	12.1 ± 2.0

Intravenous administration of cysteine to monkeys rapidly reverses gantacurium-induced block. Administration 10 mg/kg, 2 minutes following 8x ED95 gantacurium shortens the 5-95% recovery interval by 2 ½ minutes and the total duration of block (the time from administration of gantacurium to a train-of-four ratio ≥ 0.9) by 6 ½ minutes. A shown in the figure above, cysteine, 10 mg/kg, will facilitate complete recovery of neuromuscular function even when administered within one minute of gantacurium.



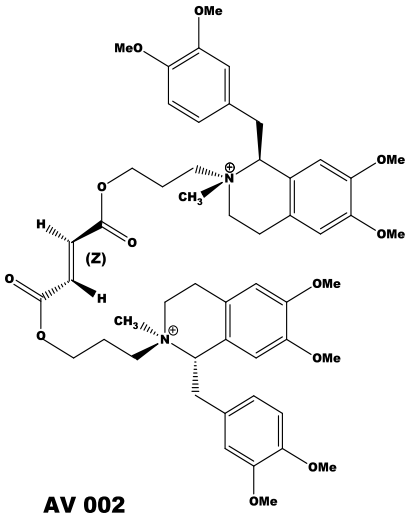
Fumatares: CW002

The structure of CW002 is similar to that of gantacurium with the primary difference being that there is no chlorine at the fumarate double bond. Additionally, there are fewer methoxy groups on the 1-benzyl substitutions on each side of the fumarate bond. Like gantacurium, CW002 is inactivated by cysteine. The structural changes in CW002, relative to gantacurium, slow the process of cysteine adduction, so that it has an intermediate duration of action.

CW002 is more potent in monkeys than is gantacurium. It has an ED95 of approximately 0.05 mg/kg.[1] The time required for onset of maximal effect is fast. Administration of three times the ED95 of CW002 to anesthetized monkeys results in 100% block within one minute. Following administration of this dose, complete spontaneous recovery to a train-of-four ratio ≥ 95% occurs in approximately 30 minutes. The 25-75% and 5-95% percent recovery intervals are 5.6 and 13.8 minutes, respectively.

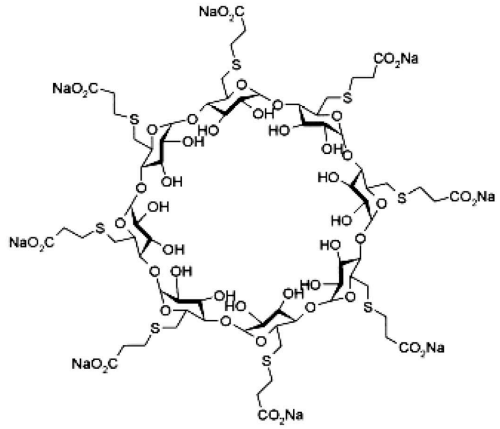
Neostigmine (50 mcg/kg) shortens the total duration of action (administration to TOFR ≥ 95%) of CW002 minimally. This same interval, though, is almost halved when cysteine is administered one minute after CW002. The total duration of action is decreased from 30 minutes to 5 minutes.

The safety ratio for vagal blockade (ED50 vagal blockade/ED95 neuromuscular blockade) with CW002 is 31 and for sympathetic blockade (ED50 sympathetic blockade/ED95 neuromuscular blockade), it is more than 50 – indicating that effects in patients will be minimal.



Sugammadex

The structure of sugammadex, a new reversal agent,. is novel in that is a cyclodextrin. Cyclodextrins are crystalline, water soluble oligosaccharides built up from 6, 7 or 8 glucopyranose units. They have long been used in industry to speed up solubilization, solubilize lipophilic compounds and stabilize reactive compounds. Sugammadex (ORG 25969) is a γ-cyclodextrin that is highly water soluble with a hydrophobic cavity to encapsulate steroidal neuromuscular blocking drugs. The γ-cyclodextrins are more potent in reversing rocuronium-induced neuromuscular block than the α- and β-cyclodextrins



because they have the largest body cavity. Their cavity of 7.5 – 8.3 Å allows the cyclodextrin to encapsulate the steroidal muscle relaxant. By encapsulating rocuronium, the muscle relaxant is rendered unavailable to interact with the postsynaptic nicotinic receptors of the neuromuscular junction.

When administered to volunteers who had not received a neuromuscular blocking agent, sugammadex, 0.1 – 8.0 mg/kg, had a clearance of 120 ml/min, an elimination half-life of 100 min and a volume of distribution of 18 L. Approximately 75% of a dose was eliminated through the urine. Kinetics were not significantly different when administration followed a single dose of rocuronium. The plasma concentration of rocuronium is higher following administration of sugammadex than it is following the administration of placebo. The mean plasma concentration of rocuronium increases as the dose of sugammadex increased. Recovery of muscle strength can occur in spite of increasing plasma concentrations of rocuronium as the efficacy of sugammadex does not rely on renal excretion of the cyclodextrin-relaxant complex. More rocuronium is eliminated in the urine of subjects receiving a sugammadex than in those who receive a placebo.

Sugammadex is an effective reversal agent following administration of steroidal neuromuscular blocking agents. One of the features that makes it pharmacodynamically so different than the available anticholinesterases is that it works more quickly and efficaciously than either neostigmine or edrophonium. It readily reversed rocuronium-induced block that has been maintained throughout a surgical procedure.

Larger doses of sugammadex may be required for reversal of profound levels of neuromuscular block. There has been one report of a study patient, who received 0.5 mg/kg to reverse residual rocuronium-induced block when she had recovered to a post-tetanic count of 1, whose muscle strength decreased after an initial increase. Following a rapid recovery of the train-of-four ratio to approximately 60%, the train-of-four decreased to approximately 30% and did not recover to 60% until more than 40 minutes after the initial administration of sugammadex. Ultimately, train-of-four ratio recovered to approximately 80% 55 minutes after administration of sugammadex. This phenomenon has not been observed in other subjects receiving small doses of sugammadex to antagonize profound levels of neuromuscular block.

Sugammadex does not antagonize benzylisoquinoline-induced neuromuscular block. Even amongst the steroidal nondepolarizing agents, its potency varies. It more effectively reverses rocuronium-induced block than vecuronium-induced block and it is more effective reversing vecuronium- than pancuronium-induced block.

Sugammadex is tolerated well at all doses. There appears to be no adverse event related to sugammadex dose or increase in frequency of adverse events with increasing dose of the reversal agent. Adverse effects potentially related to sugammadex include: hiccups, hypotension, dizziness, paresthesias, tachycardia, bradycardia, prolonged emergence, movement and coughing, erythema, abdominal discomfort, nausea and vomiting, increased creatinine phosphokinase and increased β2-microglobinuria. These adverse effects have been reported with varying frequency in the many people who have received the drug .

Summary

Both the neuromuscular junction and neuromuscular transmission have been studied extensively. They remain, though, incompletely understood. Ongoing research continues to elucidate details regarding the specifics of neuromuscular transmission. Studies with blocking agents and compounds that reverse their effects, capitalize on these details. We can anticipate that future studies will further modify our understanding of the complex nature of neuromuscular transmission and the drugs that impact it.

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