

Neuromuscular Blocking Agents' Differential Bronchoconstrictive Potential in Guinea Pig Airways

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Background: Neuromuscular blocking agents are designed to antagonize nicotinic cholinergic receptors on skeletal muscle but also antagonize muscarinic receptors. Several muscle relaxants have the potential to promote bronchoconstriction due to unintended effects exemplified by histamine release of atracurium or mivacurium and detrimental interactions with muscarinic receptors by rapacuronium. Although interactions of muscle relaxants with muscarinic receptors have been extensively characterized *in vitro*, limited information is available on their potential interactions with airway tone *in vivo*.

Methods: Changes in pulmonary inflation pressures and heart rates induced by vagal nerve stimulation and intravenous acetylcholine were measured in the absence and presence of increasing doses of gallamine, pancuronium, mivacurium, vecuronium, cisatracurium, rocuronium, or rapacuronium in guinea pigs. Mivacurium's and rapacuronium's potential of inducing bronchoconstriction by histamine release was also evaluated.

Results: Rapacuronium potentiated both vagal nerve-stimulated and intravenous acetylcholine-induced increases in airway pressures, which were totally blocked by atropine but not pyrilamine. Vecuronium, rocuronium, mivacurium, and cisatracurium were devoid of significant airway effects. Mivacurium, at high doses, increased pulmonary inflation pressures, which were attenuated by pyrilamine.

Conclusion: Rapacuronium was unique among muscle relaxants evaluated in that it potentiated both vagal nerve- and intravenous acetylcholine-induced bronchoconstriction with no evidence of histamine release. The dual detrimental interactions of rapacuronium with muscarinic receptors previously demonstrated *in vitro* correlate with *in vivo* muscarinic receptor mechanisms of bronchoconstriction and may account for the profound bronchoconstriction seen with its clinical use. These findings may establish pharmacologic characteristics to avoid with new muscle relaxants intended for clinical use.

NEUROMUSCULAR blocking agents are designed to antagonize nicotinic cholinergic receptors on skeletal muscle to facilitate intubation of the trachea, certain surgical procedures, and mechanical ventilation. Acetylcholine is the endogenous ligand for the nicotinic ligand-gated ion channel as well as for the G protein-coupled muscarinic receptors. Therefore, it is not surprising that agents designed to be antagonists at nicotinic receptors may have affinities for muscarinic receptors.^{1,2} Many neuromuscular blocking agents have significant clinical side effects on the cardiac and respiratory systems, including tachycardia due to M2 muscarinic receptor antagonism,

or bronchoconstriction due to vagal nerve preganglionic M2 muscarinic receptor antagonism, positive allosterism with acetylcholine at the postjunctional smooth muscle M3 muscarinic receptors, or histamine release.

Five subtypes of muscarinic receptors are known, with three subtypes expressed on airway structures. Acetylcholine activation of M3 muscarinic receptors expressed on airway smooth muscle initiates airway smooth muscle contraction. M2 muscarinic receptors are expressed on at least two airway structures. They classically function as negative feedback autoreceptors on the presynaptic side of postganglionic parasympathetic nerves, where they inhibit further release of acetylcholine. In addition, M2 muscarinic receptors are expressed on airway smooth muscle, where their activation is thought to inhibit airway smooth muscle relaxation. M1 muscarinic receptors have been identified in parasympathetic ganglia, where they facilitate neurotransmission.^{3,4} Conceptually, activation of these M1 receptors would potentiate vagally induced bronchoconstriction, whereas antagonizing them would decrease acetylcholine release.

The upper airways are heavily innervated by parasympathetic nerves and are a key component in an irritant reflex arc that controls airway caliber. Irritation of the upper airway by a foreign body (e.g., endotracheal tube) results in the liberation of acetylcholine from parasympathetic nerves causing bronchoconstriction. Typically, ongoing acetylcholine release is attenuated by the activation of presynaptic M2 muscarinic receptors, thus limiting vagally induced bronchoconstriction. However, selective antagonism of these presynaptic M2 muscarinic receptors by any agent (e.g., a muscle relaxant with M2 antagonistic affinity) during a period of heightened parasympathetic activity (e.g., intubation) would block this protective negative feedback effect, leading to enhanced vagally induced bronchoconstriction. Likewise, M2 muscarinic receptor antagonism in the heart results in tachycardia due to this functional vagolytic effect. Current and previously used neuromuscular blocking agents such as pancuronium and gallamine have well-characterized M2 muscarinic receptor antagonism⁵ and are known to cause tachycardia and enhance vagally induced bronchoconstriction.⁶ Pancuronium's failure to elicit clinically significant bronchoconstriction is likely due to its coincident potent M3 muscarinic receptor antagonism^{6,7} on airway smooth muscle limiting the bronchoconstrictive effect of any liberated acetylcholine.

We recently showed a novel mechanism by which a clinically relevant concentration of the neuromuscular blocking agent rapacuronium could allosterically en-

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hance acetylcholine's effect at M3 muscarinic receptors.⁸ Although neuromuscular blocking agents are prototypical allosteric agents at some muscarinic receptor subtypes,^{9,10} never before had clinically relevant concentrations of a neuromuscular blocking agent been linked to such potent bronchoconstriction. Despite our extensive characterization of currently used neuromuscular blocking agents with muscarinic receptors *in vitro*, most currently used neuromuscular blocking agents, including vecuronium, rocuronium, and cisatracurium, have not been characterized for their interaction with airway muscarinic receptors *in vivo*. Moreover, it is not known whether the demonstrated *in vitro* detrimental effects of rapacuronium at airway muscarinic receptors translate into enhanced bronchoconstriction *in vivo*. Therefore, the current study was undertaken to demonstrate that the previously described *in vitro* interactions of muscle relaxants with muscarinic receptors correlated with *in vivo* detrimental effects on airway tone. Such a correlation would establish the importance of evaluating newly designed muscle relaxants' functional interaction with muscarinic receptors as one measure of their clinical safety.

Materials and Methods

Male Hartley guinea pigs weighing 400 ± 10.1 g were used. Guinea pigs were handled in accordance with the standards established by the US Animal Welfare Acts set forth in the National Institutes of Health guidelines, and all protocols were approved by the Columbia University Animal Care and Use Committee (New York, New York).

Animal Preparation

We used a well-established guinea pig model of airway responses that has been used for more than 25 yr for the measurement of changes in airway tone.^{5,6,11-16} This model uses intraperitoneal urethane (1.7 ± 0.2 g/kg) as the anesthetic technique because this anesthetic is known to not effect pulmonary nerve function and the anesthetic effect is known to have a duration of at least 10 h.¹⁷ Depth of anesthesia was monitored by changes in respiratory rate and response to foot pinch before paralysis. Surgical intervention did not begin until there was an absence of response to foot pinch (withdrawal). A 20% increase in respiration rate before paralysis was taken as a potential indication of inadequate anesthesia at which time incremental doses of urethane (0.2 g/kg intraperitoneal) were given until respiratory rate was unresponsive to stimulation.

The trachea was cannulated with a short (1-inch) 16-gauge intravenous catheter, and the animal was ventilated by a positive-pressure, constant-volume animal ventilator (model 683; Harvard Apparatus Co., South Natick, MA; tidal volume 4.0 ml, 60 breaths/min). Peak pulmo-

nary inflation pressure (Ppi) was measured just proximal to the cannula through a side port *via* rigid plastic extension tubing connected independently to two pressure modules with differing ranges of sensitivity (TSD160B 0–12.5 cm H₂O and TSD160C 0–25 cm H₂O; Biopac Systems, Inc., Goleta, CA). Peak Ppi has been used in this model for many years as a reflection of changes in airway tone.^{5,6,11-16} Paralysis standardizes chest wall compliance and a constant volume ventilator standardizes inspiratory volume such that changes in airway tone are reflected in changes in peak Ppi.

Both jugular veins were cannulated with PE-50 tubing for the administration of drugs, at which time the animals were paralyzed with succinylcholine ($170 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), which has been shown previously to have no effect on baseline Ppi, heart rate, or blood pressure and to have no effect on vagally induced increases in airway pressures.^{6,11} The carotid artery was cannulated with PE-50 tubing and connected to a pressure transducer in line with a Biopac TSD104A module for monitoring heart rate and blood pressure. Throughout the experiments, sympathetically mediated changes in hemodynamics (hypertension, tachycardia) were monitored as an indicator of the depth of anesthesia after muscle paralysis. A 20% increase in heart rate or systolic blood pressure was treated with incremental urethane (0.2 g/kg intraperitoneal). Both vagus nerves were tightly tied but not cut, and the distal ends were placed on shielded electrodes immersed in a pool of liquid mineral oil.^{5,6,11-16} All pressure transducers were connected to a Biopac MP100A acquisition system, and data were continuously captured using Acknowledge software, version 3.7.3 (Biopac Systems, Inc.).

Physiologic Measurements

Basal airway pressure was produced by positive-pressure ventilation of the guinea pigs' lungs. The increase in Ppi over the basal inflation pressure produced by the ventilator is used as a measure of bronchoconstriction^{18,19} because in a paralyzed animal with constant tidal volume, increases in Ppi reflect primarily an increase in lung resistance with little change in dynamic compliance.^{6,20-22} Electrical stimulation of both vagus nerves (10–25 Hz, 0.2-ms pulse duration, 10- to 12-s train duration) produced transient increases in Ppi and bradycardia.^{5,23} A single voltage was selected for each animal within a range of 10–25 V to yield similar increases in airway pressure between animals. The nerves were stimulated at approximately 10-min intervals alternating with interval injections of intravenous acetylcholine (4–24 $\mu\text{g}/\text{kg}$ in a volume of 0.15 ml lactated Ringer's solution) approximately every 10 min to directly stimulate postjunctional muscarinic receptors on the heart and airway smooth muscle. The dose of intravenous acetylcholine was chosen to yield similar increases in airway pressure between animals and similarly to the vagal

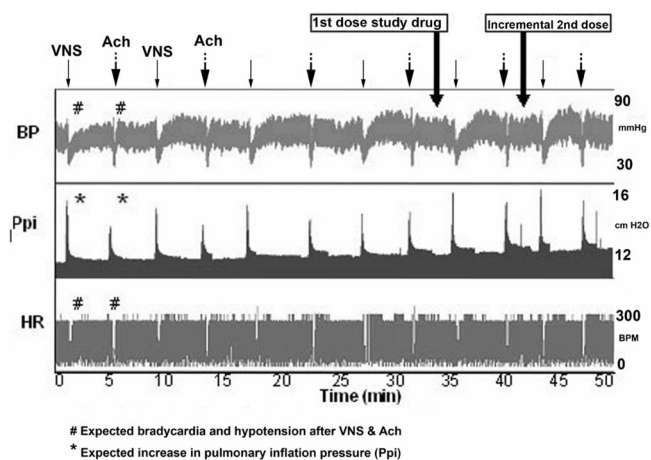


Fig. 1. Study design illustrating initial alternating challenges with vagal nerve stimulation (VNS) (solid arrows) and intravenous acetylcholine (ACh) administration (dashed arrows) and the effects on blood pressure (BP), pulmonary inflation pressure (Ppi), and heart rate (HR). VNS and intravenous ACh independently induce transient increases in Ppi (*) and bradycardia (#) with resultant decrease in BP (#). After consistent responses, incrementally increasing doses of muscle relaxants are administered intravenously with repeat challenges with VNS and intravenous ACh.

nerve stimulation (VNS)-induced increase in Ppi. The experimental design is shown in figure 1. Continuous measurements of peak Ppi, heart rate, and blood pressure were recorded. Initial studies were performed in eight animals without muscle relaxants to ensure that at least eight repetitive challenges with VNS or intravenous acetylcholine resulted in consistent changes in Ppi and heart rate over time.

In animals studied using muscle relaxants, repetitive cycles of alternating VNS followed 5 min later by intravenous acetylcholine were given to establish stable baseline responses of peak Ppi and heart rate (typically four or five measurements of each) (fig. 1). Subsequently, cumulative doses of muscle relaxants (each 0.15 ml in lactated Ringer's solution) were administered intravenously, with each dose followed approximately 3 min later by a VNS and then 5 min later by an intravenous acetylcholine stimulus with an experimental duration of 74.7 ± 10 min. Independent experiments were performed for each muscle relaxant in 3–10 animals. Cumulative doses of pancuronium (0.01–3.0 mg/kg; $n = 3$), mivacurium (0.01–5.0 mg/kg; $n = 7$), rocuronium (0.01–3.0 mg/kg; $n = 10$), vecuronium (0.01–0.5 mg/kg; $n = 6$), gallamine (0.01–10 mg/kg; $n = 3$), cisatracurium (0.01–1.5 mg/kg; $n = 6$), or rapacuronium (0.1–8 mg/kg; $n = 9$) were administered.

The effects of each dose of each muscle relaxant on airway and cardiac M2 muscarinic receptor function were assessed by the effect on the magnitude of the vagally induced increase in Ppi and the inhibition of bradycardia, respectively. The effect of each dose of each muscle relaxant on postjunctional muscarinic receptors (*i.e.*, M3 muscarinic receptors on airway smooth muscle) was assessed

by the effect on the magnitude of intravenous acetylcholine-induced increase in Ppi. Airway effects are expressed as the percent change in Ppi from Ppi measured before the injection of the initial dose of each muscle relaxant. Effects on heart rate are expressed as the percent change of bradycardia induced by VNS after a dose of each muscle relaxant *versus* resting heart rate, which was defined as the heart rate immediately before the VNS stimulus. At the end of each experiment, intravenous atropine (0.5 mg/kg) was given to confirm that vagally induced and intravenous acetylcholine-induced responses were mediated *via* muscarinic receptors.

Bronchoconstriction by high concentrations of mivacurium has been previously shown to be mediated by histamine release.^{24,25} In independent experiments, we exploited this model by testing for histamine-mediated effects of mivacurium and rapacuronium. We confirmed that large doses (5 mg/kg intravenous) of mivacurium alone (in the absence of VNS or intravenous acetylcholine) increased Ppi. Animals were then treated with intravenous atropine (1 mg/kg intravenous) before a repeat challenge with 5 mg/kg intravenous mivacurium. Subsequently, animals were pretreated with the histamine-1 receptor antagonist pyrilamine (5 mg/kg intravenous) before a repeat challenge with 5 mg/kg intravenous mivacurium. In separate animals, Ppi was measured after large doses of rapacuronium (8 mg/kg intravenous) followed by intravenous acetylcholine. Subsequently, animals were pretreated with pyrilamine (5 mg/kg intravenous) before a repeat challenge with intravenous rapacuronium and acetylcholine. Finally, animals were pretreated with atropine (1 mg/kg intravenous) before a repeat challenge with intravenous rapacuronium and acetylcholine.

Materials

Urethane, pyrilamine, atropine, pancuronium, gallamine, and acetylcholine were purchased from Sigma (St. Louis, MO). Rapacuronium, rocuronium, cisatracurium, vecuronium, and mivacurium were clinical formulations and were diluted in lactated Ringer's solution. Appropriate vehicle controls for each clinical formulation were tested (as vehicle controls) before the injection of any muscle relaxants. Vehicle controls were formulated according to the manufacturers' inserts for each muscle relaxant and were intravenously injected in 0.15-ml volumes (in lactated Ringer's solution).

Statistics

All data were expressed as mean \pm SEM. Dose-response curves of muscle relaxants effects on changes in Ppi (after vagally induced or intravenous acetylcholine-induced increases in Ppi) or vagally induced bradycardia were analyzed by analysis of variance with repeated measures using a Bonferroni post test comparison, and a *P* value of less than 0.05 was considered significant.

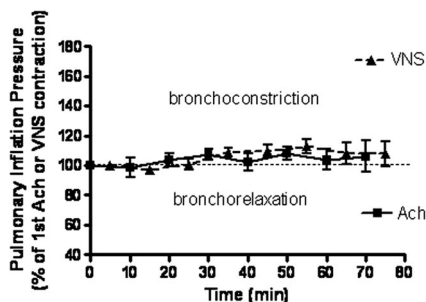


Fig. 2. Repetitive vagal nerve stimulation (VNS) or intravenous acetylcholine (ACh) yields stable effects over time on pulmonary inflation pressures or bradycardia in the absence of muscle relaxants. Alternating stimuli were given every 5 min (see fig. 1) for a total of eight cycles. Responses are expressed as a percentage of the pulmonary inflation pressure increase induced by the first ACh and VNS challenge or the degree of bradycardia induced by the first VNS. The magnitude of the pulmonary inflation pressure or bradycardic responses did not differ from the initial response ($P > 0.05$; $n = 8$).

Results

Baseline Ppi (10.3 ± 0.3 cm H₂O), heart rate (304 ± 6.2 beats/min), and blood pressure ($78 \pm 2.8/38 \pm 2.2$ mmHg) were not different among the animals ($P > 0.05$). Ppi was transiently and repetitively increased to a similar magnitude by either intravenous acetylcholine or electrical stimulation of the vagus nerves. At the conclusion of experiments, these responses were abolished by intravenous atropine (0.5 mg/kg), indicating that these responses were mediated by muscarinic receptors. In the heart, both VNS and intravenous acetylcholine caused transient bradycardia.

We initially established that the transient increase in Ppi and transient bradycardia after VNS or intravenous acetylcholine were stable over time. Figure 2 illustrates transient and repetitive challenges in eight animals with continuous measurement of peak Ppi and heart rate. Through eight repetitive and alternating challenges with VNS and intravenous acetylcholine, increases in Ppi and decreases in heart rate were consistent over time ($P > 0.05$ for each time point compared with initial challenge; $n = 8$).

Consistent with a previous study,⁶ pancuronium dose-dependently inhibited acetylcholine's increase in Ppi and potentiated vagally induced increases in Ppi ($P < 0.05$; $n = 3$; fig. 3A and table 1). However, at supraclinical doses (> 1 mg/kg), pancuronium inhibited vagally induced increases in inflation pressures.

Gallamine^{6,26,27} consistently potentiated vagally induced increases in Ppi but had no significant effect on the bronchoconstrictive effects of intravenous acetylcholine ($n = 3$; fig. 3B and table 1).

Mivacurium had no significant effect on either VNS- or intravenous acetylcholine-induced increases in Ppi ($n = 7$; fig. 3C and table 1). In independent experiments, a high dose of mivacurium (5 mg/kg) given alone (*i.e.*, without VNS or intravenous acetylcholine) increased baseline Ppi ($n = 7$; table 2). Pretreatment with the histamine-1 recep-

tor antagonist pyrilamine (5 mg/kg) completely prevented this increase in Ppi, whereas pretreatment with the muscarinic antagonist atropine (1 mg/kg) inhibited the increase in Ppi by 25% ($n = 7$; table 2).

Rapacurium's effects in the lung were unique in that it potentiated both vagally induced and intravenous acetylcholine-induced increases in Ppi by 40% within clinically relevant concentrations ($P < 0.05$; $n = 9$; fig. 3D and table 1). At high concentrations, rapacurium's potentiation of vagally induced increases in Ppi was dose-dependently attenuated (> 2.5 mg/kg) ($P < 0.05$; $n = 9$; fig. 3D and table 1). The potentiation of intravenous acetylcholine-induced increases in Ppi by high concentrations of rapacurium was partially reversed ($\pm 28\%$) by pyrilamine but was completely reversed by atropine ($P < 0.05$; $n = 9$; table 3). Administration of even large doses of rapacurium alone (8 mg/kg) had no effect on Ppi or heart rate.

Vecuronium had no significant effect on either vagally induced or intravenous acetylcholine-induced increases in Ppi within clinically relevant concentrations ($n = 6$; fig. 4A and table 1). However, higher concentrations of vecuronium (0.25 mg/kg), above those used clinically, dose-dependently potentiated intravenous acetylcholine's effect on Ppi ($P < 0.01$; $n = 6$; fig. 4A and table 1), with no potentiation of VNS induced increase in Ppi ($n = 6$; fig. 4A and table 1). Vecuronium administration alone had no effect on airway pressures or heart rate.

Similarly, rocuronium had no significant effect on either vagally induced or intravenously acetylcholine-induced increases in Ppi within clinically relevant concentrations ($n = 10$; fig. 4B and table 1). However, at concentrations above those likely encountered clinically (> 2.5 mg/kg), rocuronium potentiated intravenous acetylcholine-induced increases in Ppi ($P < 0.05$; $n = 10$; fig. 4B). Rocuronium given alone had no effect on airway pressures or heart rate.

Cisatracurium had no significant effect on either VNS- or intravenous acetylcholine-induced increase in Ppi ($n = 6$; fig. 4C and table 1). Cisatracurium administration alone had no effect on airway pressures or heart rate (table 1).

Stimulation of the vagus nerve induces bradycardia and the inhibition of this vagally induced bradycardia is frequently used in this animal model as a measure of M2 muscarinic receptor antagonism. In the heart, pancuronium potently inhibited vagally induced bradycardia ($P < 0.05$; $n = 3$; fig. 5A and table 1) with complete blockade at doses of 0.4 mg/kg or greater. Likewise, gallamine consistently inhibited vagally induced bradycardia ($n = 3$; fig. 5B and table 1), whereas mivacurium inhibited vagally induced bradycardia only at high doses (> 1 mg/kg) ($n = 7$; fig. 5A and table 1). Similar to pancuronium and gallamine, rapacurium dose-dependently inhibited vagally induced bradycardia ($P < 0.05$; $n = 9$; fig. 5C and table 1). Vecuronium had no effect on vagally induced bra-

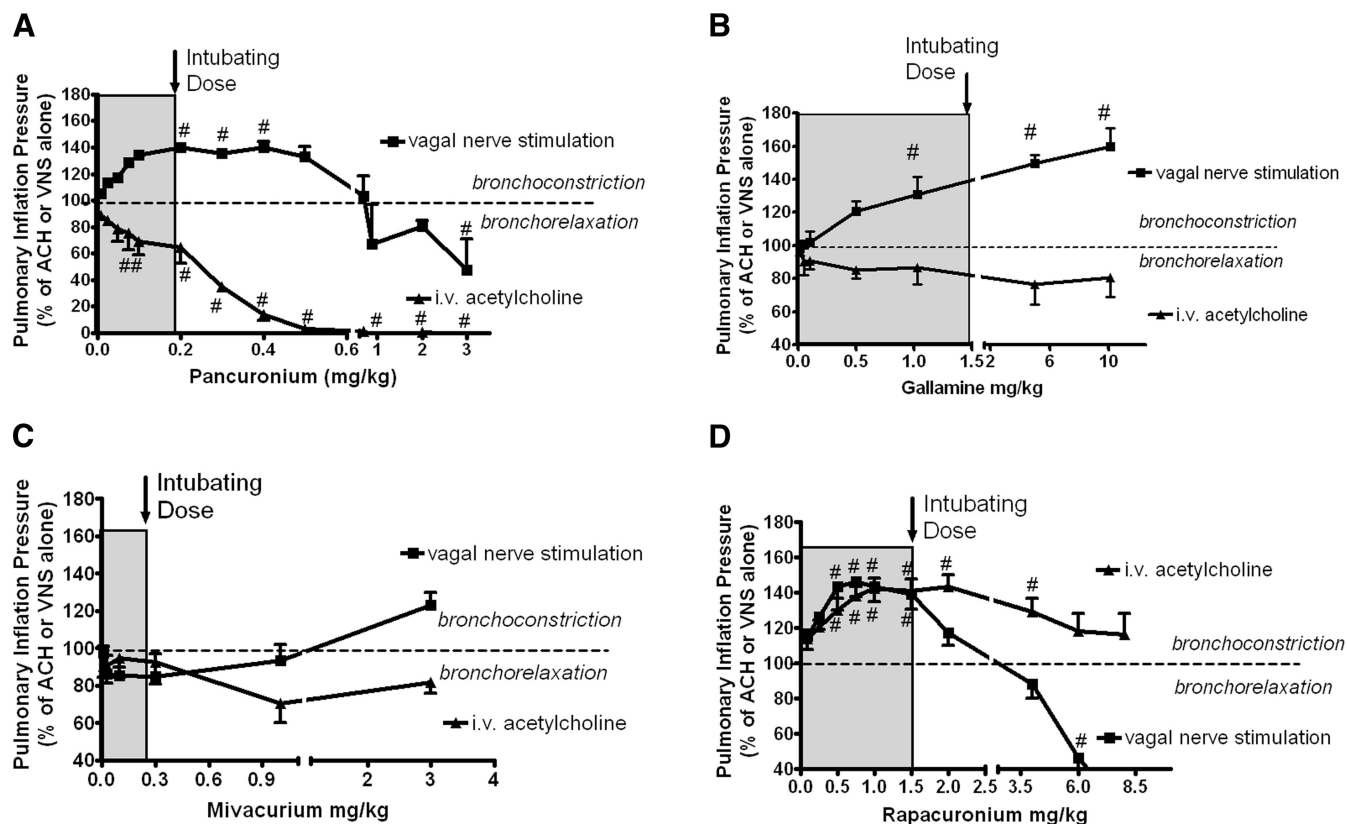


Fig. 3. Neuromuscular blocking agents' effects on pulmonary inflation pressures induced by either vagal nerve stimulation (VNS) or intravenous acetylcholine (ACh). (A) Clinically relevant concentrations of pancuronium potentiate vagal nerve-induced bronchoconstriction (M2 muscarinic receptor antagonism) but at higher doses inhibits vagally induced bronchoconstriction (M3 muscarinic receptor antagonism predominating at higher concentrations). Clinically relevant concentrations of pancuronium inhibit intravenous ACh-induced bronchoconstriction (M3 muscarinic receptor antagonism) ($P < 0.05$ compared with baseline; $n = 3$). (B) Clinically relevant concentrations of gallamine potently potentiates vagal nerve-induced increases in pulmonary inflation pressures but is without significant effect on intravenous ACh-induced bronchoconstriction ($P < 0.05$ compared with baseline; $n = 3$). (C) Mivacurium had no significant effect on VNS- or intravenous ACh-induced increases in pulmonary inflation pressure ($n = 6$). (D) Rapacurium uniquely potently potentiated both vagal nerve- (M2 muscarinic receptor antagonism) and intravenous ACh-induced (consistent with positive M3 muscarinic receptor allosterism) increases in pulmonary inflation pressures within clinically relevant doses ($P < 0.05$ compared with baseline; $n = 9$). ↓ = Human intubating dose.

dyscardia throughout the range of doses tested ($n = 6$; fig. 5A and table 1), and large doses of rocuronium were required to partially inhibit vagally induced bradycardia ($n = 9$; fig. 5B and table 1). Cisatracurium did not prevent vagally induced bradycardia ($n = 6$; fig. 5C and table 1).

Discussion

The primary finding of the current study is that the previously described detrimental interaction of muscle relaxants with muscarinic receptors *in vitro*^{1,2,8} accurately predicted their detrimental airway effects *via* mus-

Table 1. Summary of Muscle Relaxants Direct Airway Effects and Effects on VNS- and Acetylcholine-induced Increase in Ppi and Bradycardia

Muscle Relaxant	Airway: Increase in Ppi Secondary to:		Cardiac: VNS-induced Bradycardia	
	VNS + Muscle Relaxant	ACh + Muscle Relaxant	VNS + Muscle Relaxant	
Pancuronium	↔	↑*	↓ ↓	
Gallamine	↔	↑ ↑	↓ ↓	
Mivacurium	↑ ↑	↔	↔ †	
Rapacurium	↔	↑ ↑	↓ ↓	
Vecuronium	↔	↔ ‡	↔	
Rocuronium	↔	↔ §	↓	
Cisatracurium	↔	↔	↔	

* Pancuronium (> 1 mg/kg): ↓ vagal nerve stimulation (VNS)-induced increase in pulmonary inflation pressure (Ppi). † Mivacurium (> 1 mg/kg): ↓ VNS-induced bradycardia. ‡ Vecuronium (> 0.25 mg/kg): ↑ acetylcholine (ACh)-induced increase in Ppi. § Rocuronium (> 2.5 mg/kg): ↑ ACh-induced increase in Ppi.

Table 2. Mivacurium's Effect Alone on Pulmonary Inflation Pressures

Mivacurium Dose, mg/kg	Increase in Ppi, mean \pm SEM, mm H ₂ O
0.01	0.0 \pm 0
0.1	0.0 \pm 0
5	17.4 \pm 1.4*
5 + Atropine (0.5 mg/kg)	12.3 \pm 0.9*
5 + Pyrilamine (5 mg/kg)	0.0 \pm 0†

Increased pulmonary inflation pressures (Ppi) by mivacurium (5 mg/kg) were completely blocked by an antihistamine (pyrilamine) and only partially blocked (25%) by an antimuscarinic (atropine).

* $P < 0.05$ compared with baseline, † $P < 0.05$ compared with 5 mg/kg mivacurium alone; $n = 6$.

carinic receptors *in vivo*. Rapacuronium was the only muscle relaxant evaluated that potentiated both vagal nerve-stimulated and intravenous acetylcholine-induced increases in Ppi within clinically relevant concentrations. These results are consistent with mechanisms previously proposed *in vitro*: antagonism of presynaptic parasympathetic M2 muscarinic receptors^{1,2} (potentiating vagal nerve induced acetylcholine release) and allosteric potentiation of acetylcholine's effect at postsynaptic M3 muscarinic receptors,⁸ respectively.

Presynaptic muscarinic receptors on airway postganglionic parasympathetic nerves are of the M2 subtype and normally function in an inhibitory auto-feedback mode to prevent the further release of acetylcholine.¹¹ M1 receptors appear on the parasympathetic ganglia, where they can facilitate cholinergic transmission.²⁸ Muscarinic receptors on the airway smooth muscle are of the M2 and M3 subtypes, which function to inhibit relaxation and facilitate contraction, respectively.²⁹ Irritation of the well-innervated upper trachea (e.g., by the introduction of an endotracheal tube) initiates a neural reflex that results in the release of acetylcholine from parasympathetic nerves that acts on M2 and M3 muscarinic receptors in airway smooth muscle, resulting in bronchoconstriction. Normally, the release of acetylcholine is terminated by activation of the inhibitory auto-

Table 3. Rapacuronium's Effect on Increased Pulmonary Inflation Pressures in the Presence of Acetylcholine

Rapacuronium Dose, mg/kg, + 8 μ g/kg Acetylcholine	Increase in Pulmonary Inflation Pressure as % of Acetylcholine, Mean \pm SEM
0 (Acetylcholine alone)	100 \pm 0
0.25	128.6 \pm 3.7*
1	154.4 \pm 2.5*
8	156.2 \pm 23.7*
8 + Pyrilamine (5 mg/kg)	120.9 \pm 25*
8 + Atropine (0.5 mg/kg)	0.0 \pm 0†

Rapacuronium's (8 mg/kg intravenous) potentiation of intravenous acetylcholine (8 μ g/kg) was completely blocked by atropine and only partially blocked (28%) by pyrilamine.

* $P < 0.05$ compared with baseline, † $P < 0.05$ compared with 8 mg/kg rapacuronium + acetylcholine; $n = 9$.

feedback M2 muscarinic receptors on the parasympathetic nerves. The administration of a muscle relaxant that has selective M2 muscarinic receptor antagonist affinities during a period of parasympathetic nerve activation (e.g., intubation) creates a scenario where negative feedback inhibition of acetylcholine release is blocked resulting in unopposed acetylcholine release. If this were coincident with allosteric augmentation of acetylcholine's contractile effect at the muscle M3 muscarinic receptor (e.g., rapacuronium), airway tone would be further increased. In contrast, coincident M3 muscarinic receptor blockade (e.g., pancuronium) would decrease airway tone regardless of any enhanced acetylcholine release. Activation of cardiac M2 muscarinic receptors induces bradycardia, which can be attenuated by neuromuscular blocking agents that exhibit M2 muscarinic receptor blockade.

In the current study, using an established guinea pig *in vivo* model,^{5,6,11-16} we measured the ability of muscle relaxants to (1) enhance vagal nerve induced increases in Ppi by prejunctional M2 muscarinic receptor antagonism,^{5,18,19,30} (2) prevent vagally induced bradycardia due to cardiac M2 muscarinic receptor antagonism, (3) antagonize or enhance intravenous acetylcholine's effect on Ppi *via* postjunctional M3 muscarinic receptor effects, or (4) promote an increase in Ppi *via* the release of histamine.

Potential interaction of muscle relaxants with M1 muscarinic receptors on airway parasympathetic ganglia could not be excluded but are unlikely to account for the findings in the current study. M1 muscarinic receptors expressed in parasympathetic ganglia are facilitators of acetylcholine release. Therefore, a muscle relaxant would need to be an agonist or have positive allosteric effects at the M1 muscarinic receptor to facilitate vagally induced bronchoconstriction. No muscle relaxants studied to date are known to be orthosteric agonists at any muscarinic receptor subtype. Moreover, it has been difficult to identify any compound with positive allosteric effects at the M1 muscarinic receptor despite aggressive research motivated by the hypothesis that allosteric enhancement of acetylcholine's effects at the M1 muscarinic receptor could have therapeutic benefit in Alzheimer disease.³¹

In contrast to the lack of agonist or positive allosteric effects at the M1 muscarinic receptor by any muscle relaxant, gallamine demonstrates orthosteric antagonism and negative allosteric effects at M1 muscarinic receptor.^{27,32} This effect would be theoretically airway protective, and indeed, a selective M1 blocker was shown to partially block cholinergic reflex bronchoconstriction without an effect on inhaled methacholine, nocturnal asthma, or mild chronic obstructive pulmonary disease.³³⁻³⁵ Nonetheless, the possibility remains that a drug (and even a muscle relaxant) could have a positive allosteric effect at the M1 muscarinic receptor and facilitate neurally induced bronchoconstriction.

To validate our laboratory's use of this extensively

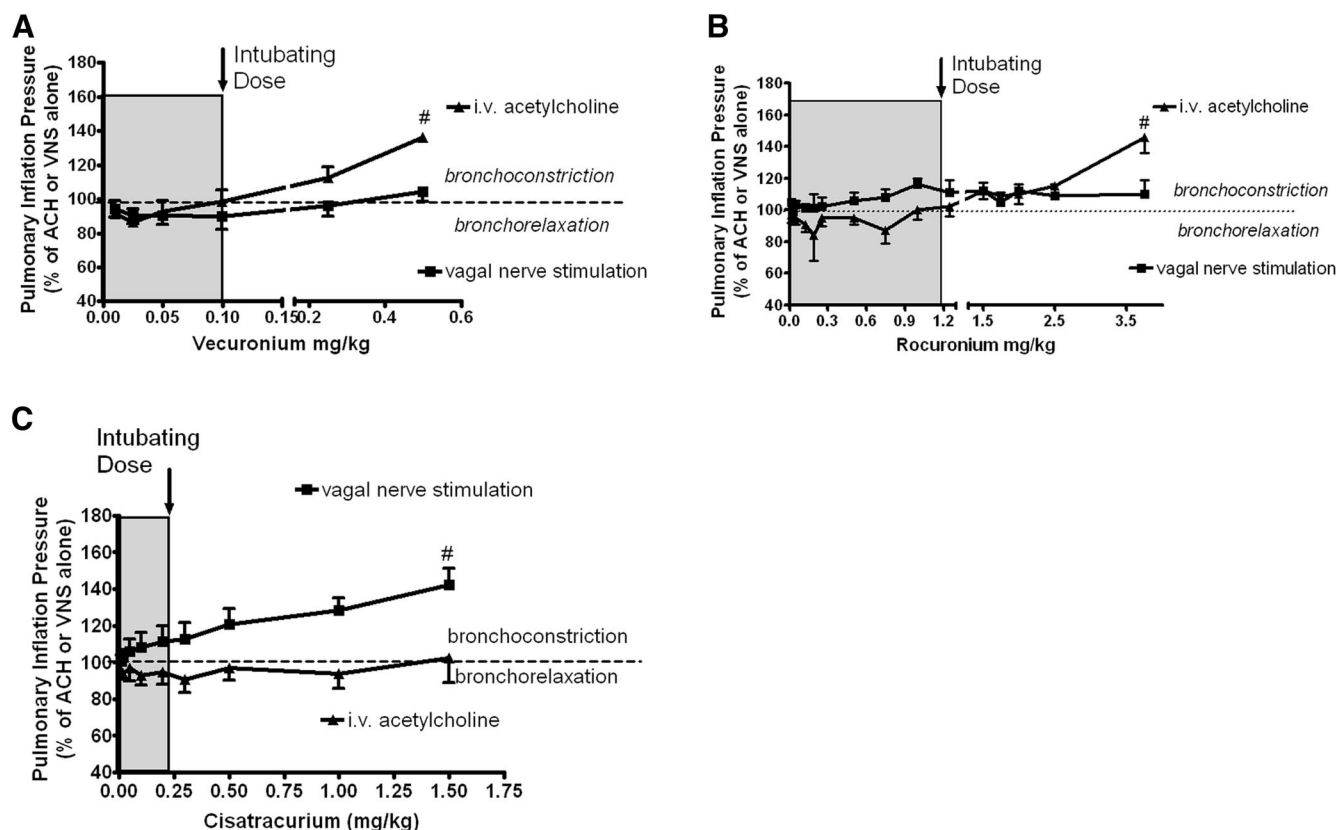


Fig. 4. Neuromuscular blocking agents' effects on pulmonary inflation pressures induced by either vagal nerve stimulation (VNS) or intravenous acetylcholine (ACh). (A) Clinically relevant doses of vecuronium are without significant effect on vagal nerve- or intravenous ACh-induced increases in pulmonary inflation pressures. Supraclinical doses of vecuronium significantly potentiated intravenous ACh-induced increases in pulmonary inflation pressures ($\# P < 0.05$ compared with baseline; $n = 6$). (B) Clinically relevant doses of rocuronium are without significant effect on vagal nerve- or intravenous ACh-induced increases in pulmonary inflation pressures ($\# P < 0.05$ compared with baseline; $n = 9$). Supraclinical doses of rocuronium significantly potentiated intravenous ACh-induced increases in pulmonary inflation pressures ($\# P < 0.05$ compared with baseline; $n = 10$). (C) Clinically relevant concentrations of cisatracurium are without significant effects on vagal nerve- or intravenous ACh-induced increases in airway pressures ($\# P < 0.05$ compared with baseline; $n = 6$). \downarrow = Human intubating dose.

characterized *in vivo* guinea pig model, we first repeated preliminary studies with gallamine, pancuronium, and mivacurium, which had previously been shown in this model to exhibit M2 muscarinic receptor antagonism, M2 and M3 muscarinic receptor antagonism, or histamine release, respectively.⁶ Subsequently, we measured the ability of previously uncharacterized muscle relaxants to interact with muscarinic receptors or induce histamine release *in vivo*.

The current study confirms that gallamine and pancuronium exhibit potent blockade of the M2 muscarinic receptor resulting in a potentiation of vagal nerve-induced increase in Ppi and blockade of vagally induced bradycardia. In addition, pancuronium exhibited potent M3 muscarinic receptor blockade resulting in blockade of intravenous acetylcholine-induced increase in Ppi consistent with previous studies in guinea pigs^{5,6} and dogs.³⁰ In further agreement with previous studies in this model,⁶ mivacurium was devoid of significant muscarinic receptor affinities within clinically significant doses but at larger doses exhibited pyrilamine-sensitive increases in Ppi indicative of histamine release. This increase in Ppi occurred with the administra-

tion of mivacurium alone, independent of vagally induced or intravenous acetylcholine-induced increases in Ppi. A small component of this mivacurium effect was blocked by atropine, which could be explained by two mechanisms. First, it is well described that a component of histamine-induced bronchoconstriction is mediated by the neural release of acetylcholine,^{36,37} and thus atropine would block this component of a histamine induced contraction. Second, it is well known that antimuscarinics, such as atropine, are weak antagonists at histamine receptors.^{38,39} This effect is generally small, represented by low pA₂ values for atropine at histamine receptors, but may explain this small decrease in Ppi. However, mivacurium's effect on Ppi was completely attenuated by pyrilamine (histamine antagonist), confirming the principal role of histamine release in the airway effect of high-dose mivacurium.

Our *in vivo* findings with rapacuronium were unique among muscle relaxants evaluated in that it potentiated both vagal nerve-stimulated and intravenous acetylcholine-induced increases in Ppi within clinically relevant concentrations. The potentiation of vagally induced bronchoconstriction is consistent with our previous studies *in vitro*

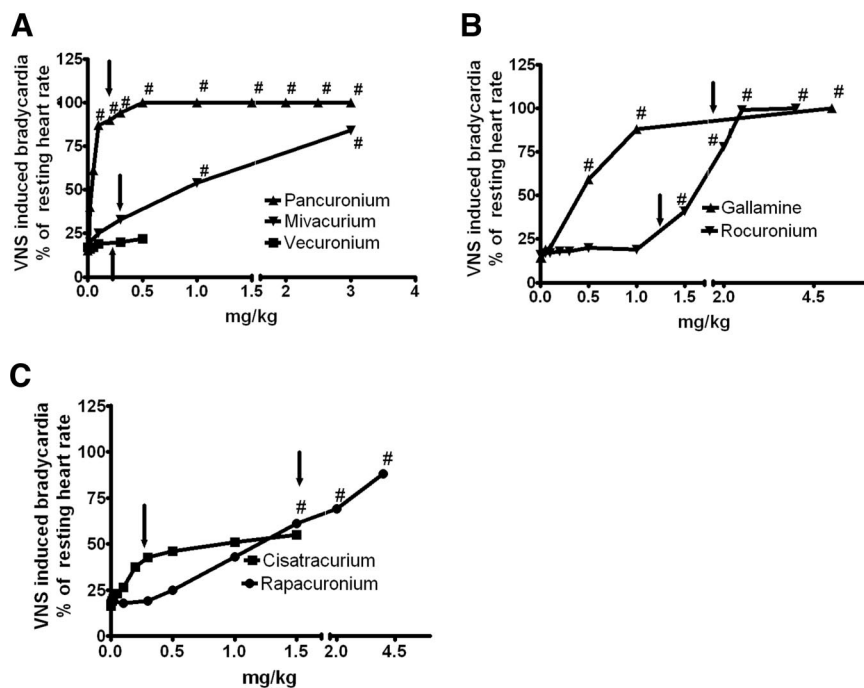


Fig. 5. Neuromuscular blocking agents' ability to block vagal nerve stimulation (VNS)-induced bradycardia by M2 muscarinic receptor antagonism expressed as a percentage of baseline induced bradycardia. (A) Within clinically relevant doses, pancuronium potentially attenuated vagal nerve-induced bradycardia ($\# P < 0.05$ compared with baseline; $n = 3$), and mivacurium also attenuated vagal nerve-induced bradycardia, but only at very large doses ($\# P < 0.05$ compared with baseline; $n = 6$), whereas vecuronium was without effect at all concentrations evaluated ($P > 0.05$; $n = 6$). (B) Gallamine potentially inhibited vagal nerve-induced bradycardia within clinically relevant concentrations ($\# P < 0.05$ compared with baseline; $n = 6$). Rocuronium was without effect except at supra-clinical concentrations ($\# P < 0.05$ compared with baseline; $n = 10$). (C) Within clinically relevant doses, rapacuronium potentially inhibited vagal nerve-induced bradycardia ($n = 9$), whereas cisatracurium was without effect ($\# P < 0.05$ compared with baseline; $n = 6$). \downarrow = Human intubating dose.

demonstrating selective presynaptic M2 muscarinic receptor blockade.² This M2 muscarinic receptor blocking potential is further demonstrated by the ability of rapacuronium to inhibit vagally induced bradycardia by preventing the acetylcholine released by the vagal stimulus from binding to the cardiac M2 muscarinic receptors. Furthermore, the potentiation of intravenous acetylcholine-induced bronchoconstriction is consistent with our previously reported positive allosteric interaction of rapacuronium at the M3 muscarinic receptor *in vitro*.⁸ These combined detrimental effects at airway muscarinic receptors at clinically relevant concentrations may account for the profound bronchoconstriction associated with rapacuronium.^{40–44} Interestingly, consistent with our previous *in vitro* studies,^{2,8} although clinically relevant concentrations of rapacuronium potentiate both vagally induced and intravenous acetylcholine-induced increases in Ppi, larger doses of rapacuronium actually attenuated the vagally induced and intravenous acetylcholine-induced increases in Ppi. This effect of large doses is consistent with M3 muscarinic receptor antagonism that predominates at these very high doses. As the concentrations of rapacuronium is increased above concentrations achieved clinically, the attenuation of Ppi occurs earlier with VNS- versus acetylcholine-induced increases (fig. 3D). This is likely due to a less sustained airway constriction with VNS compared with intravenous acetylcholine, making VNS-induced increases in Ppi easier to antagonize. This same pattern is seen with pancuronium but at lower and clinically achieved doses.

Importantly, the potentiation of intravenous acetylcholine-induced Ppi by rapacuronium was only partially (28%) reversed by the histamine receptor antagonist (pyrilamine) but was completely reversed by the muscarinic receptor

antagonism (atropine), confirming our previous *in vitro* studies that this potentiation was muscarinic receptor mediated rather than as a result of histamine release. Although a histamine effect cannot be completely excluded, the partial inhibition by pyrilamine may be explained by the fact that classic antihistamine drugs are well known to have weak antimuscarinic effects^{45–47} and that this partial inhibition of increased airway tone is a result of direct antimuscarinic activity. Moreover, unlike mivacurium, high concentrations of rapacuronium given alone did not cause increased Ppi but required the coincident administration of intravenous acetylcholine to demonstrate an airway effect. This further argues against a histamine-releasing mechanism and further supports an M3 muscarinic receptor positive allosteric effect of rapacuronium on airway constriction. Furthermore, rapacuronium's additive effect on acetylcholine's increased Ppi was completely attenuated by atropine, confirming the principal role of muscarinic receptors in this potentiation. These findings are consistent with clinical studies where increased airway tone during the maintenance phase of general anesthesia was attributed to selective M2 muscarinic receptor antagonism^{48,49} and a study of seven adult patients who developed bronchospasm while receiving rapacuronium and had no increases in serum histamine levels.⁵⁰

Neuromuscular blocking agents are known to have the potential for both orthosteric^{1,51} and allosteric interactions with muscarinic receptors.^{26,32,52,53} Gallamine and alcuronium, previously used neuromuscular blocking agents, are the most extensively characterized muscle relaxants in terms of muscarinic allosteric interactions, and gallamine serves as the prototypical muscarinic allosteric ligand.⁵⁴ Similarly, alcuronium has been shown to enhance binding

of muscarinic *antagonists*,⁵⁵ but neither gallamine nor alcuronium have been shown to enhance the binding of *agonists* at the muscarinic receptor.^{56,57}

We have previously shown *in vitro*² and now demonstrate *in vivo* that gallamine, pancuronium, and rapacuronium exhibit significant blockade of the M2 muscarinic auto-inhibitory receptor within clinically achieved concentrations. This raises the question as to why clinically relevant bronchospasm is or was not more of a clinical problem seen with use of pancuronium or gallamine. In the case of pancuronium, potent blockade of postjunctional M3 muscarinic receptors at clinically relevant concentrations likely protects against significant bronchoconstriction. Gallamine has a slow onset of action and was not widely used as an intubating drug. Therefore, it was not administered at a time of heightened parasympathetic tone and was less likely to potentiate significant acetylcholine release from activated parasympathetic nerves. Perhaps more importantly, gallamine does not potentiate acetylcholine effects at M3 muscarinic receptors. Therefore, the crucial interpretation of these findings that may explain the unique clinical experiences with rapacuronium is that it exhibits dual detrimental airway muscarinic receptor effects and that the potentiation at the M3 muscarinic receptor (by rapacuronium) has more deleterious effects in the airway than antagonism of prejunctional M2 muscarinic receptors (by gallamine or rapacuronium). Alternatively, the combination of prejunctional M2 antagonism and postjunctional M3 positive allosterism may synergize to account for the detrimental airway effects seen with rapacuronium.

Vecuronium at clinically used concentrations exhibited no affinity for M2 or M3 muscarinic receptors reflected in no potentiation of vagal nerve-induced increases in Ppi, no blockade of vagally induced bradycardia, and no effect on acetylcholine-induced increase in Ppi. Interestingly, as previously demonstrated *in vitro*, at supraclinical concentrations (> 0.3 mg/kg), vecuronium potentiated intravenous acetylcholine-induced increases in Ppi consistent with a positive allosteric effect at the M3 muscarinic receptor. Furthermore, when vecuronium was administered alone, it had no effect on airway pressures, suggesting that vecuronium even at high doses did not induce histamine release. This shared effect of increasing airway pressures in the presence of acetylcholine at high concentrations of vecuronium or with clinically achieved concentrations of rapacuronium is not surprising because rapacuronium is a vecuronium analog. However, the dose of rapacuronium was 15–25 times the dose of vecuronium on a mg/kg basis, likely resulting in much higher tissue concentrations of rapacuronium such that sufficient tissue concentrations of rapacuronium were achieved to allosterically enhance acetylcholine acting on M3 muscarinic receptors on airway smooth muscle.

Rocuronium, which has a very similar structure to that of vecuronium, also had minimal muscarinic airway ef-

fects. It did, however, also potentiate intravenous acetylcholine's airway effects at high concentrations (> 2 mg/kg), consistent with an allosteric effect at the M3 muscarinic receptor. At these higher concentrations, it was also able to partially inhibit vagally induced bradycardia. This is interesting in that it seems to have M2 blocking effects in the heart but does not potentiate vagally induced airway effects. This may indicate that the cardiac effects are a more sensitive means of detecting M2 muscarinic antagonism supported by the findings with the known M2 muscarinic receptor antagonists pancuronium and gallamine where they blocked vagally induced bradycardia at doses lower than that required to potentiate vagally induced increase in Ppi. As was the case with vecuronium, rocuronium had no effect on Ppi when rocuronium was administered alone, suggesting that rocuronium even at high doses did not induce histamine release.

Cisatracurium had no significant effects on vagally induced increases in Ppi, vagal nerve-induced bradycardia, or intravenous acetylcholine-induced increases in Ppi, illustrating its lack of significant interaction with muscarinic receptors *in vivo*. Cisatracurium exhibited no airway or heart rate effects when administered alone, suggesting that cisatracurium even at high doses did not induce histamine release.

In summary, the previously characterized interaction of neuromuscular blocking agents with M2 and M3 muscarinic receptors *in vitro*⁶ was consistently predictive of *in vivo* airway and heart rate responses. Many neuromuscular blocking agents in wide clinical use today, including vecuronium, cisatracurium, rocuronium, and mivacurium, when used within the clinically suggested dose ranges, are free of significant interactions with muscarinic receptors. However, certain aminosteroid drugs, including vecuronium and rocuronium, do potentiate acetylcholine effects at the M3 muscarinic receptor at doses higher than doses typically used clinically, as opposed to rapacuronium, which illustrated these effects at doses well within doses used clinically.

A least one new nondepolarizing muscle relaxant is in early clinical development.^{58–60} We believe that it is prudent to evaluate this and all new neuromuscular blocking agents for detrimental interactions with airway muscarinic receptors at concentrations of these drugs likely to be achieved clinically. Our *in vivo* confirmation of our previous *in vitro* studies of the mechanism of interaction of rapacuronium with airway muscarinic receptors establishes screening criteria that should be used for all such drugs under development.

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