A Mechanism for Rapacuronium-induced Bronchospasm

M2 Muscarinic Receptor Antagonism

Edmund Jooste, M.B.Ch.B.,* Farrah Klafter, B.A.,† Carol A. Hirshman, M.D.,‡ Charles W. Emala, M.D.§

Background: **A safe and effective ultra-short-acting nondepolarizing neuromuscular blocking agent is required to block nicotinic receptors to facilitate intubation. Rapacuronium, which sought to fulfill these criteria, was withdrawn from clinical use due to a high incidence of bronchospasm resulting in death. Understanding the mechanism by which rapacuronium induces fatal bronchospasm is imperative so that newly synthesized neuromuscular blocking agents that share this mechanism will not be introduced clinically. Selective inhibition of M2 muscarinic receptors by muscle relaxants during periods of parasympathetic nerve stimulation (*e.g.*, intubation) can result in the massive release of acetylcholine to act on unopposed M3 muscarinic receptors in airway smooth muscle, thereby facilitating bronchoconstriction.**

Methods: **Competitive radioligand binding determined the binding affinities of rapacuronium, vecuronium, cisatracurium, methoctramine (selective M2 antagonist), and 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP; selective M3 antagonist) for M2 and M3 muscarinic receptors.**

Results: **Rapacuronium competitively displaced ³H-QNB from the M2 muscarinic receptors but not from the M3 muscarinic receptors within clinically relevant concentrations. Fifty percent inhibitory concentrations (mean ± SE) for rapacuronium were as follows: M2 muscarinic receptor, 5.10 ± 1.5 μM (n = 6); M3 muscarinic receptor, 77.9 ± 11 μM (n = 8). Cisatracurium and vecuronium competitively displaced ³H-QNB from both M2 and M3 muscarinic receptors but had affinities at greater than clinically achieved concentrations for these relaxants.**

Conclusions: **Rapacuronium in clinically significant doses has a higher affinity for M2 muscarinic receptors as compared with M3 muscarinic receptors. A potential mechanism by which rapacuronium may potentiate bronchoconstriction is by blockade of M2 muscarinic receptors on prejunctional parasympathetic nerves, leading to increased release of acetylcholine and thereby resulting in M3 muscarinic receptor–mediated airway smooth muscle constriction.**

NEUROMUSCULAR blocking agents are needed to facilitate tracheal intubation and to maintain muscle relaxation during many surgical procedures. Due to the numerous undesirable side effects of succinylcholine, the

<div>This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.</div>

* Resident in Anesthesiology, † Technician, ‡ Professor of Anesthesiology, § Associate Professor of Anesthesiology.

Address reprint requests to Dr. Emala: Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, P & S Box 46, New York, New York 10032. Address electronic mail to: cwe5@columbia.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

search has continued for a nondepolarizing muscle relaxant that can rapidly achieve optimal intubation conditions and that has a rapid termination of action in the event of difficulties in managing the airway.

Rapacuronium, a nondepolarizing muscle relaxant to be used in large doses during intubation as a substitute for succinylcholine, was introduced and then subsequently withdrawn from clinical practice because of a high incidence of bronchospasm[1] and at least five fatalities[2] that have been attributed to irreversible bronchoconstriction. However, the mechanism(s) by which rapacuronium potentiates bronchoconstriction are unknown. Defining this potential mechanism for muscle relaxant–induced bronchospasm is critical, as additional nondepolarizing muscle relaxants will continue to be introduced into clinical practice to replace succinylcholine.

Bronchospasm during induction of general anesthesia is a potentially life-threatening event. Histamine release by drugs is one known risk factor for bronchospasm. Instrumentation of the well-innervated upper trachea initiates an irritant reflex that results in the release of acetylcholine from parasympathetic nerves that act on M2 and M3 muscarinic receptors in airway smooth muscle, resulting in bronchoconstriction. Normally the release of acetylcholine is terminated by acetylcholine acting on M2 muscarinic auto-feedback receptors present in the presynaptic terminals of postganglionic parasympathetic nerves. However, nondepolarizing muscle relaxants are known to antagonize muscarinic receptors.[3] Moreover, nondepolarizing muscle relaxants have different affinities for subtypes of muscarinic receptors.[3] Agents that have a higher affinity for the M2 muscarinic receptor than the M3 muscarinic receptor can block these presynaptic parasympathetic M2 receptors, allowing for the augmented release of acetylcholine to act on unopposed M3 muscarinic receptors in airway smooth muscle and thereby resulting in enhanced bronchoconstriction.[4] Therefore, the characteristics of a muscle relaxant that together could potentiate vagally induced bronchoconstriction include (1) a higher affinity for M2 *versus* M3 muscarinic receptors, (2) an affinity for M2 muscarinic receptors but not for M3 muscarinic receptors within a clinically obtained concentration range, and (3) use of large doses of this muscle relaxant during a period of heightened parasympathetic tone (*e.g.*, intubation). Therefore, we questioned whether rapacuronium exhibits a higher affinity for M2 *versus* M3 muscarinic receptors, which would increase its potential to precipitate bronchospasm during anesthesia.

Materials and Methods

Reagents

Rapacuronium and vecuronium were purchased from Organon (West Orange, NJ), cisatracurium was purchased from GlaxoSmithKline (Research Triangle Park, NC), and methoctramine and 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) were purchased from Sigma (St. Louis, MO). The muscarinic receptor antagonist ^{3}H-QNB was purchased from Amersham Life Science (Arlington Heights, IL). All drugs were dissolved in deionized water. Chinese hamster ovary (CHO) cells stably transfected with complementary DNA encoding the rat M3 muscarinic receptor were purchased from the American Type Culture Collection (Rockville, MD). CHO cells stably transfected with complementary DNA encoding the human M2 muscarinic receptor were provided by Norman Lee, Ph.D. (The Institute for Genome Research, Rockville, Maryland).

Cell Culture

Chinese hamster ovary cells stably transfected with either the M2 or the M3 muscarinic receptor were grown in Dulbecco's modified essential medium (DMEM) containing 10% fetal bovine serum and antibiotic agents (100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, 0.25 μg/ml amphotericin B, and 100 U/ml nystatin). Cells were cultured in T500 flasks (500 cm^2) at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air and were harvested at confluence.

Preparation of Chinese Hamster Ovary Cell Membranes

At confluence, culture media were removed from the flasks. The cells were incubated in lysis buffer (10 mM HEPES, 2 mM EDTA, 100 μM phenyl-methane-sulfonyl fluoride; pH, 8.0) at 37°C in a carbon dioxide incubator until detached (20–40 min). Lysed cells were centrifuged at 48,000g (Sorvall RC-5B with SS-34 rotor; Sorvall, Newton, CT) for 20 min at 4°C. Cold HEPES buffer (100 mM; pH, 7.4) was used to resuspend the pellet after the removal of the supernatant. The lysates were washed two additional times, and the final pellet was resuspended in 6 ml HEPES buffer at 2–5 mg/ml and stored at −70°C until used for radioligand binding assays.

Competitive Radioligand Binding Assays

Twenty-five micrograms of CHO cell membrane protein was incubated in triplicate tubes with ^{3}H-QNB (0.18 nM) and muscle relaxant of increasing concentrations ($10^{-9.5} − 10^{-3}$ M) in binding buffer (40 mM KH$_2$PO$_4$, 160 mM K$_2$HPO$_4$ in 50 mM NaCl; pH, 7.4). All radioligand experiments were incubated for 2 h at room temperature in a final volume of 0.25 ml. Preliminary experiments confirmed that the 2-h incubation period was adequate to achieve equilibrium binding. All binding experiments were terminated by filtration through GF/B glass fiber fil-
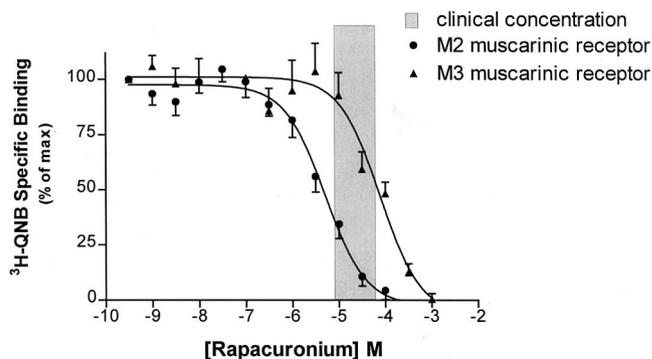

Fig. 1. Competitive displacement of ^{3}H-QNB by rapacuronium in membranes prepared from Chinese hamster ovary cells expressing the M2 or M3 muscarinic receptor. The affinity of rapacuronium for the M2 muscarinic receptor was higher than its affinity for the M3 muscarinic receptor and was within clinically relevant concentrations. (n = 6 for M2, and n = 8 for M3.)

ters and were washed three times with 5 ml cold NaCl, 0.9%, using a cell harvester (Brandell, Gaithersburg, MD). Filters were immersed in 5 ml Econo scintillation fluid, stored overnight, and counted in a scintillation counter (Beckman LS 5000 TD; Beckman, Fullerton, CA) with an efficiency of 45–50%. Methoctramine, a selective M2 muscarinic receptor antagonist, and 4-DAMP, a selective M3 muscarinic receptor antagonist, also were used in increasing concentrations ($10^{-9.5} − 10^{-3.5}$ M) to confirm the M2 and M3 muscarinic receptor expression in our CHO cell preparations. The chosen radioligand concentration (0.18 nM) for the competition experiments was 3.6 times the equilibration constant (K_d) of the M2 muscarinic receptor and 1 times the K_d of the M3 muscarinic receptor. The competitive displacement of ^{3}H-QNB by increasing concentrations of muscle relaxants was analyzed by nonlinear regression. A reiterative curve-fitting program, Prism 3.0 (Graph Pad, San Diego, CA), was used to calculate the relative binding affinity (50% inhibitory concentration [IC$_{50}$]) values using a four-parameter logistic equation (log scale) with the slope factor set to −1.

Statistical Analysis

Comparisons between IC$_{50}$ values for the M2 *versus* the M3 muscarinic receptor for each muscle relaxant were made using unpaired, two-tailed Student t test.

Results

All of the muscle relaxants studied (rapacuronium, cisatracurium, and vecuronium) displaced ^{3}H-QNB from M2 and M3 muscarinic receptors in a dose-dependent manner (figs. 1–3). In competitive binding experiments using muscle relaxants and ^{3}H-QNB, the IC$_{50}$ values of each muscle relaxant at the M2 and M3 muscarinic receptors were determined by nonlinear regression analysis (table 1). There is a wide variation in the affinity of muscle relaxants for both M2 and M3 muscarinic recep-
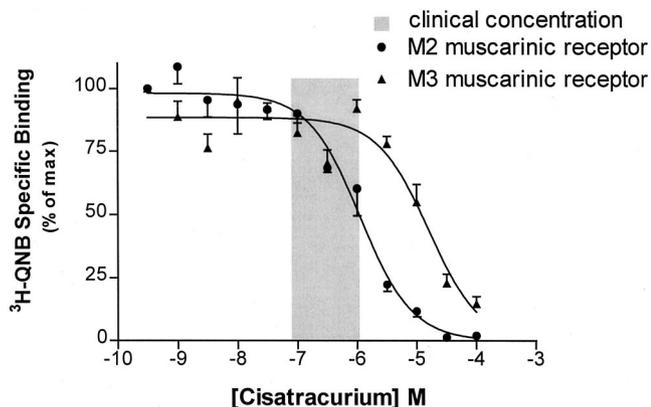
Fig. 2. Competitive displacement of ^{3}H-QNB by cisatracurium in membranes prepared from Chinese hamster ovary cells expressing the M2 or M3 muscarinic receptor. Cisatracurium has a higher affinity for the M2 *versus* the M3 muscarinic receptor. However, the 50% inhibitory concentration value for the M2 muscarinic receptor falls to the far right of reported plasma concentrations. (n = 3 for M2 and M3, respectively.)

tors, and, in general, the affinity for the M3 muscarinic receptor is lower than the affinity for the M2 muscarinic receptor.

Methoctramine, a selective M2 muscarinic receptor antagonist, and 4-DAMP, a selective M3 muscarinic receptor antagonist, also were used in competitive binding experiments to confirm specific expression of M2 and M3 muscarinic receptors in our CHO cell membranes and to allow a comparison between the affinities of the muscle relaxants and classic M2 and M3 antagonists. As expected, methoctramine had a 26-fold higher affinity for receptors expressed in CHO-M2 cells as compared with receptors expressed in CHO-M3 cells (fig. 4), whereas 4-DAMP had a fourfold higher affinity for receptors expressed in CHO-M3 cells as compared with receptors expressed in CHO-M2 cells (fig. 5; table 1). These differential affinities are consistent with those previously
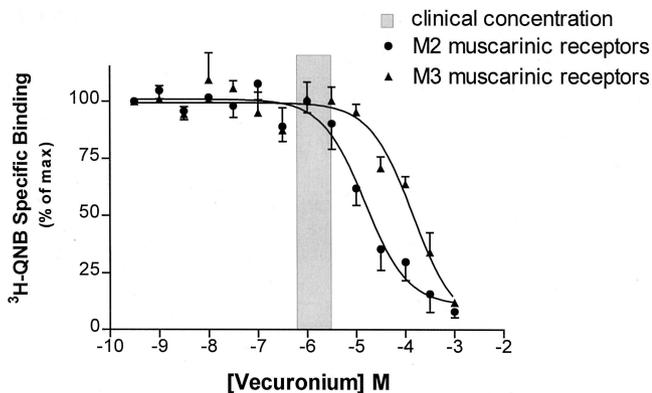

Fig. 3. Competitive displacement of ^{3}H-QNB by vecuronium in membranes prepared from Chinese hamster ovary cells expressing the M2 or M3 muscarinic receptor. Vecuronium has a higher affinity for the M2 *versus* the M3 muscarinic receptor; however, the 50% inhibitory concentration value for both of these receptor subtypes was greater than clinically relevant concentrations. (n = 5 for M2, and n = 3 for M3.)

reported for the M2 and M3 muscarinic receptor subtypes expressed in CHO cells.[5,6]

Discussion

The primary finding of this study is that although rapacuronium was found to be an antagonist at both M2 and M3 muscarinic receptors, it had an approximately 15-fold greater affinity for blocking the M2 *versus* the M3 muscarinic receptor. Moreover, the IC_{50} of rapacuronium for the M2 muscarinic receptor, but not for the M3 muscarinic receptor, was within a clinically relevant concentration range (fig. 1; table 1).[7–10] Thus, a mechanism that could account for severe bronchospasm resulting from a large intubating dose of rapacuronium is preferential blockade of presynaptic M2 muscarinic receptors on parasympathetic nerves. This would result in the enhanced release of acetylcholine from activated parasympathetic nerves by airway irritant receptors, leading to potent activation of M3 muscarinic receptors on airway smooth muscle and thereby producing bronchoconstriction.

In human airways, parasympathetic innervation predominates in the more central airways, and parasympathetic tone is heightened by the presence of endotracheal tubes or other irritating substances introduced into the airway,[11] mediating rapid reflex changes in airway caliber. Irritant receptors are found just beneath the tight junctions of the epithelial lining of the airway. The afferent and efferent connections of these receptors travel in the vagus nerve. Acetylcholine administered exogenously or released from parasympathetic postganglionic nerves induces airway constriction by activating muscarinic receptors on airway smooth muscle.[12] Airway smooth muscle expresses both M2 and M3 muscarinic receptors. In airway smooth muscle, M3 muscarinic receptors initiate contraction, while M2 muscarinic receptors inhibit relaxation. The massive increase in acetylcholine release and binding of acetylcholine to M3 muscarinic receptors on the muscle overrides the inhibition of M2 muscarinic receptors on the muscle itself in every model studied, and the net effect is constriction.[13] Conversely, blockade of M3 muscarinic receptors on the airway smooth muscle inhibits both vagally induced and exogenously administered acetylcholine-induced airway constriction. Studies in animal models show that pancuronium and gallamine, at clinically relevant concentrations, can potentiate bronchospasm in the setting of vagally induced acetylcholine release.[14,15] Antagonism of presynaptic M2 muscarinic receptors was thought to be the mechanism of action.

The clinical experience with rapacuronium has been unique because of the frequency and severity of pulmonary complications. In premarketing studies, bronchospasm was reported in 3.2% of patients who received rapacuronium compared with 2.1% who received succi-

Table 1. Comparative Binding Affinities of Muscle Relaxants and Selective Muscarinic Receptor Antagonists to M2 and M3 Muscarinic Receptors

Muscle Relaxant	IC$_{50}$ at M2 Receptors, μM	IC$_{50}$ at M3 Receptors, μM	Difference in M2 vs. M3 Affinity	Reported Plasma Concentrations, μM
Rapacuronium	5.10 (n=6)	77.9 (n=8)	$P < 0.0001$	7.39–59.1
Cisatracurium	1.12 (n=3)	16.0 (n=3)	$P < 0.05$	0.08–1.13
Vecuronium	14.2 (n=4)	140 (n=3)	$P < 0.05$	0.63–3.14
Methochtramine	0.249 (n=3)	6.54 (n=3)	$P < 0.05$	—
4-DAMP	0.057 (n=3)	0.013 (n=3)	$P < 0.05$	—

4-DAMP = 4-diphenylacetoxy-*N*-methylpiperidine methiodide.

nylcholine. Subsequent independent studies documented an incidence of bronchospasm of 10.7% with rapacuronium *versus* 4.1% with succinylcholine.[12] During its short period of clinical use, a number of cases of severe bronchospasm were reported.[1,16–19] The bronchospasm was so severe in these cases that no end-tidal carbon dioxide was detected. The mechanism of the bronchospasm was unclear from these accounts but had the following characteristics.

1. Bronchospasm most frequently occurred after intubation but also occurred in the absence of intubation.
2. Bronchospasm lasted 8–15 min.
3. Attempted treatments included positive pressure ventilation, nebulized albuterol, increased concentrations of volatile anesthetics, antihistamines, and epinephrine; however, no one treatment seemed to be effective, and if the bronchospasm resolved, it appeared to do so on its own.
4. A higher incidence of bronchospasm occurred in patients with reactive airway disease.
5. Only one patient had erythema, possibly denoting histamine release.[16–18]

Although most cases of bronchospasm were associated with intubation, at least one of the reported cases occurred in the absence of intubation, and two recent studies demonstrated an increase in peak airway inflating pressures[20] or a decrease in maximal expiratory flow[21] with the administration of rapacuronium during steady state anesthesia in intubated patients. This suggests that, during general anesthesia, rapacuronium is still capable of facilitating acetylcholine release from postganglionic parasympathetic nerves, leading to increases in airway tone but not overt bronchospasm. Alternatively, rapacuronium may increase airway tone by other mechanisms, such as histamine release. However, neither of these two sets of investigators reported other signs of histamine release, and both proposed selective M2 muscarinic receptor antagonism as a mechanism to account for their findings.[20,21]

If M2 muscarinic receptor inhibition with rapacuronium is an important mechanism in eliciting bronchospasm, other muscle relaxants with a similar profile should also induce bronchospasm during anesthesia. Gallamine has a profile similar to that of rapacuronium, yet bronchospasm seldom was reported with its use.[22] Gallamine is the most selective M2 muscarinic receptor antagonist available,[3] but it is no longer used as a muscle relaxant because of its inhibition of cardiac M2 muscarinic receptors, which induces tachycardia. Gallamine was not used frequently for intubation due to its slow onset of action. It is plausible that if intraoperative studies had been performed with gallamine using the same techniques as those used to measure airway tone with rapacuronium, similar results would have been found.

An alternative mechanism to account for rapacuronium-induced bronchospasm is the release of histamine
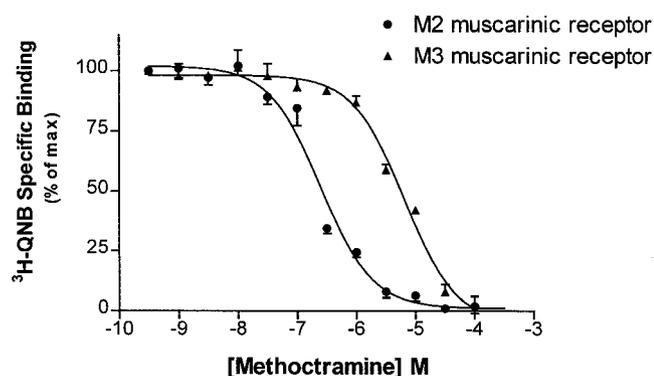

Fig. 4. Methochtramine competitively displaces ^{3}H-QNB with a higher affinity for the M2 *versus* the M3 muscarinic receptor. (n = 3 for M2 and M3, respectively.)
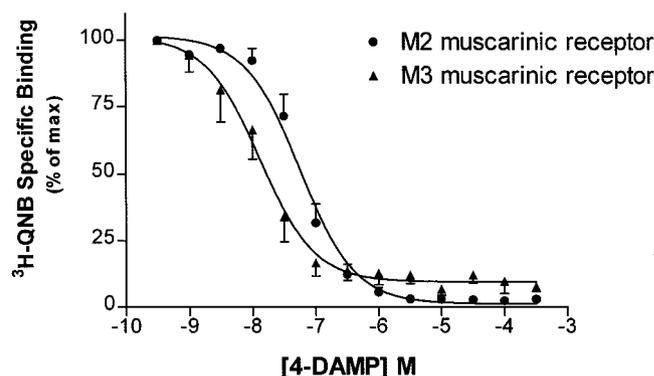

Fig. 5. 4-Diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) competitively displaces ^{3}H-QNB with a higher affinity for the M3 *versus* the M2 muscarinic receptor. (n = 3 for M2 and M3, respectively.)

from mast cells lining the vessels into which the drug is injected. Histamine release has been described following the administration of several nondepolarizing muscle relaxants, including curare, atracurium, and mivacurium.[23–26] However, in a study of 47 adult patients receiving rapacuronium during elective general anesthesia, 7 developed bronchospasm without increases in plasma histamine levels.[19] Therefore, it does not appear that histamine release can account for rapacuronium-induced bronchospasm. Although β_2-adrenoceptor antagonism is another potential mechanism precipitating bronchospasm, there is also no current evidence suggesting that rapacuronium or any other clinically used muscle relaxants behave as β_2-adrenoceptor antagonists.

In the present study, we measured the affinity of muscle relaxants for M2 and M3 muscarinic receptors in CHO cells stably transfected to express pure populations of these receptor subtypes. These cells are a widely used model in which to study binding affinities of receptor agonists or antagonists of the muscarinic receptor family.[5,6,27–30] Radioligand binding studies in cells expressing a pure population of receptors eliminate the confounding effects of multiple receptor subtypes competing with differential affinities for the same ligand. This occurs in tissues that express a mixed population of muscarinic receptors, such as airway smooth muscle.[31] Furthermore, CHO cells expressing muscarinic receptors are often used as a standard for defining an unknown receptor subtype in a tissue.[29]

Muscle relaxants can be grouped into four different categories according to their muscarinic receptor–blocking capabilities in clinical concentration ranges. The first group is composed of muscle relaxants that, at clinically relevant concentrations, bind to the M2 muscarinic receptor with a much greater affinity than to the M3 muscarinic receptor and, thus, could potentiate bronchoconstriction during a period of parasympathetic stimulation (*e.g.*, rapacuronium, gallamine, cisatracurium). In the current study, cisatracurium also was found to have a higher affinity for the M2 *versus* the M3 receptor. However, unlike rapacuronium, the clinical use of cisatracurium has not resulted in a large number of reports of isolated bronchospasm. This may be due to the fact that the M2 affinity of cisatracurium is to the far right of the reported concentration range, whereas that of rapacuronium falls to the left of even the lowest reported concentration range. Most reports of bronchospasm following the administration of cisatracurium have been associated with an anaphylactoid reaction,[32–34] which likely involves histamine release. Muscle relaxants that block both M2 and M3 muscarinic receptors at clinically relevant concentrations comprise the second group. Pancuronium and rocuronium are also potent M2 muscarinic antagonists,[3] but their use has not been associated with bronchospasm because both are also potent antagonists for the M3 muscarinic recep-

tor, particularly at clinically used doses. Muscle relaxants in the third group only have affinities for the M3 muscarinic receptor (*e.g.*, mivacurium) and, thus, lack the potential to produce bronchospasm *via* this reflex; in fact, these muscle relaxants may protect against the effect of acetylcholine on M3 muscarinic receptors on airway smooth muscle. However, their ability to release histamine in animals[15,26] and in humans[25] may overwhelm any potential protective effects on M3 muscarinic receptors and may account for the clinical reports of bronchospasm associated with these muscle relaxants.[35] The majority of muscle relaxants are in the fourth and final category, having affinities for both M2 and M3 muscarinic receptors but only at concentrations higher than those typically achieved with routine clinical dosing. Vecuronium, pipecuronium, and doxacurium are examples of such relaxants.[3]

Despite the removal of rapacuronium from clinical practice, it is important to understand the mechanism by which it apparently contributed to fatal bronchospasm. All of the muscle relaxants developed to date have affinities for muscarinic receptors, and it is likely that the next generation of muscle relaxants that are introduced to replace succinylcholine also will have affinities for muscarinic receptors. Therefore, if the higher affinity of rapacuronium for M2 *versus* M3 muscarinic receptors accounts for induced bronchospasm, it seems prudent that all newly designed muscle relaxants be evaluated for their potential to selectively inhibit M2 muscarinic receptors at concentrations used clinically—especially if the intended use of the muscle relaxant is to facilitate tracheal intubation, which requires large doses coinciding with the activation of vagal reflexes by intubation of the trachea.

References

1. Goudsouzian NG: Rapacuronium and bronchospasm. ANESTHESIOLOGY 2001; 94:727–8
2. Adams C: Anesthesia drug is being pulled from the market after five deaths. Wall Street Journal. March 30, 2001;B3
3. Hou VY, Hirshman CA, Emala CW: Neuromuscular relaxants as antagonists for M2 and M3 muscarinic receptors. ANESTHESIOLOGY 1998; 88:744–50
4. Bowman WC: Non-relaxant properties of neuromuscular blocking drugs. Br J Anaesth 1982; 54:147–60
5. Buckley NJ, Bonner TI, Buckley CM, Brann MR: Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. Science 1989; 238:672–5
6. Barras M, Coste A, Eon MT, Guillot E: Pharmacological characterization of muscarinic receptors implicated in rabbit detrusor muscle contraction and activation of inositol phospholipid hydrolysis in rabbit detrusor and parotid gland. Fundam Clin Pharmacol 1999; 13:562–70
7. Fisher DM, Dempsey GA, Atherton DP, Brown R, Abengochea A, Hunter JM: Effect of renal failure and cirrhosis on the pharmacokinetics and neuromuscular effects of rapacuronium administered by bolus followed by infusion. ANESTHESIOLOGY 2000; 93:1384–91
8. Reynolds LM, Infosino A, Brown R, Hsu J, Fisher DM: Pharmacokinetics of rapacuronium in infants and children with intravenous and intramuscular administration. ANESTHESIOLOGY 2000; 92:376–86
9. Duvaldestin P, Slavov V, Rebufat Y: Pharmacokinetics and pharmacodynamics of rapacuronium in patients with cirrhosis. ANESTHESIOLOGY 1999; 91:1305–10
10. Fisher DM, Kahwaji R, Bevan D, Bikhazi G, Fragen RJ, Angst MS, Ornstein E, Matteo RS: Factors affecting the pharmacokinetic characteristics of rapacuronium. ANESTHESIOLOGY 1999; 90:993–1000

11. Widdicombe JG: Regulation of tracheobronchial smooth muscle. Physiol Rev 1963; 43:1–37

12. Roffel AF, Meurs H, Elzinger CRS, Zaagsma J: Characterization of the muscarinic receptor subtype involved in phosphoinositide metabolism in bovine tracheal smooth muscle. Br J Pharmacol 1990; 99:293–6

13. Fryer AD, Maclagan J: Pancuronium and gallamine are antagonists for pre- and post-junctional muscarinic receptors in the guinea pig lung. Naunyn Schmiedebergs Arch Pharmacol 1987; 335:367–71

14. Fryer AD, Maclagan J: Muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. Br J Pharmacol 1984; 83:973–8

15. Okanlami OA, Fryer AD, Hirshman C: Interaction of nondepolarizing muscle relaxants with M_2 and M_3 muscarinic receptors in guinea pig lung and heart. ANESTHESIOLOGY 1996; 84:155–61

16. Kron SS: Severe bronchospasm and desaturation in a child associated with rapacuronium. ANESTHESIOLOGY 2001; 94:923–4

17. Naguib M: How serious is the bronchospasm induced by rapacuronium? ANESTHESIOLOGY 2001; 94:924–5

18. Meakin GH, Pronske EH, Lerman J, Orr R, Joffe D, Savaree AM, Lynn AM: Bronchospasm after rapacuronium in infants and children. ANESTHESIOLOGY 2001; 94:926–7

19. Levy JH, Pitts M, Thanopoulos A, Szlam F, Bastian R, Kim J: The effects of rapacuronium on histamine release and hemodynamics in adult patients undergoing general anesthesia. Anesth Analg 1999; 89:290–5

20. Tobias JD, Johnson JO, Sprague K, Johnson G: Effects of rapacuronium on respiratory function during general anesthesia: A comparison with cis-atracurium. ANESTHESIOLOGY 2001; 95:908–12

21. Fine GF, Motoyama EK, Brandom BW, Fertal KM, Mutich R, Davis PJ: The effect on lung mechanics in anesthetized children with rapacuronium: A comparative study with mivacurium. Anesth Analg 2002; 95:56–61

22. Okazaki K, Saito T, Wakisaka K, Hirano T, Kozu K: Bronchospasm possible due to gallamine. A case report. Tokushima J Exp Med 1969; 16:9–14

23. Naguib M, Samarkandi AH, Bakhamees HS, Magboul MA, el Bakry AK: Histamine-release haemodynamic changes produced by rocuronium, vecuronium, mivacurium, atracurium and tubocurarine. Br J Anaesth 1995; 75:588–92

24. Takeda J, Ueda E, Takahashi J, Fukushima K: Plasma N-methylhistamine concentration as an indicator of histamine release by intravenous d-tubocurarine in humans: Preliminary study in five patients by radioimmunoassay kits. Anesth Analg 1995; 80:1015–7

25. Koppert W, Blunk JA, Petersen LJ, Skov P, Rentsch K, Schmelz M: Different patterns of mast cell activation by muscle relaxants in human skin. ANESTHESIOLOGY 2001; 95:659–67

26. Habre W, Babik B, Chalier M, Petak F: Role of endogenous histamine in altered lung mechanics in rabbits. ANESTHESIOLOGY 2002; 96:409–15

27. Smith CM, Wallis RM: Characterisation of [3H]-darifenacin as a novel radioligand for the study of muscarinic M3 receptors. J Recept Signal Transduct Res 1997; 17:177–84

28. Ehlert FJ, Oliff HS, Griffin MT: The quaternary transformation products of N-(3-chloropropyl)-4-piperidinyl diphenylacetate and N-(2-chloroethyl)-4-piperidinyl diphenylacetate (4-DAMP mustard) have differential affinity for subtypes of the muscarinic receptor. J Pharmacol Exp Ther 1996; 276:405–10

29. Akici A, Karaalp A, Iskender E, Christopoulos A, el Fakahany EE, Oktay S: Further evidence for the heterogeneity of functional muscarinic receptors in guinea pig gallbladder. Eur J Pharmacol 2000; 388:115–23

30. Lysikova M, Havlas Z, Tucek S: Interactions between allosteric modulators and 4-DAMP and other antagonists at muscarinic receptors: potential significance of the distance between the N and carboxyl C atoms in the molecules of antagonists. Neurochem Res 2001; 26:383–94

31. Emala CW, Aryana A, Levine MA, Yasuda RP, Satkus SA, Wolfe BB, Hirshman CA: Expression of muscarinic receptor subtypes and M2-muscarinic inhibition of adenylyl cyclase in lung. Am J Physiol 1995; 268:L101–7

32. Krombach J, Hunzelmann N, Koster F, Bischoff A, Hoffmann-Menzel H, Buzello W: Anaphylactoid reactions after cisatracurium administration in six patients. Anesth Analg 2001; 93:1257–9

33. Newman PJ, Quinn AC, Grounds RM, Hunter JM, Boyd AH, Eastwood NB, Pollard BJ, Pearson AJ, Harper NJ, Beale RJ, Sutjarittam M, Elliot JM, Bion JF: A comparison of cisatracurium (51W89) and atracurium by infusion in critically ill patients. Crit Care Med 1997; 25:1139–42

34. Briassoulis G, Hatzis T, Mammi P, Alikatora A: Persistent anaphylactic reaction after induction with thiopentone and cisatracurium. Paediatr Anaesth 2000; 10:429–34

35. Loan PB, Elliott P, Mirakhur RK, Bunting HF, Bhanumurthy S, McMurray TJ: Comparison of the haemodynamic effects of mivacurium and atracurium during fentanyl anaesthesia. Br J Anaesth 1995; 74:330–2