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Do Pipecuronium and Rocuronium Affect Human Bronchial Smooth Muscle?

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Background: **Muscle relaxants affect nicotinic and muscarinic receptors. Interaction of muscle relaxants with muscarinic receptors of human airways has been studied incompletely.**

Methods: **The effects of pipecuronium bromide (long-acting, nondepolarizing) and rocuronium bromide (intermediate-acting, nondepolarizing) on prejunctional and postjunctional muscarinic receptors were studied in 96 isolated human bronchial rings from 12 patients. Contractile isometric responses to electric field stimulation of pilocarpine-stimulated and nonstimulated M_2 muscarinic receptors were compared before and after incubation with the two muscle relaxants. The effect on postjunctional muscarinic receptors was studied by comparing acetylcholine concentration–response curves before and after incubation with the two muscle relaxants.**

Results: **Pipecuronium bromide, but not rocuronium bromide, inhibited pilocarpine-stimulated prejunctional M_2 muscarinic receptors. Neither pipecuronium bromide nor rocuronium bromide had significant inhibitory effects on nonstimulated M_2 muscarinic receptors and on postjunctional M_3 muscarinic receptors.**

Conclusions: **The inhibitory effect of pipecuronium bromide on pilocarpine-stimulated prejunctional M_2 muscarinic receptors occurred at clinical concentrations. (Key words: Airway smooth muscle; bronchoconstriction; pilocarpine.)**

MUSCLE relaxants affect not only nicotinic receptors of neuromuscular junctions, but also muscarinic receptors of airways.[1,2] There are several subtypes of muscarinic receptors in the airways. The M_3 muscarinic receptors, located on the surface of smooth muscle cells, initiate contraction if stimulated. Stimulation of M_2 muscarinic receptors, located on postganglionic nerve endings of cholinergic nerves, inhibits acetylcholine release.[3] M_2 muscarinic receptors, located on the surface of smooth muscle cells, have several functions, including inhibition of adenylyl cyclase activity and relaxation in response to β_2-adrenoceptor stimulation[4] and inhibition of large-conductance calcium-activated potassium channels, thus contributing to contractile responses to metacholine.[5] Interaction of muscle relaxants with muscarinic receptors of airways has been studied in anesthetized dogs[1] and guinea pigs,[2] but to our knowledge only the effect of gallamine has been studied in isolated human bronchial rings.[6] Because the distribution and relative abundance of receptors may vary between species,[7,8] the results of studies in animals cannot be extrapolated to human airways. Therefore, we decided to study the effect of two new muscle relaxants, the long-acting muscle relaxant pipecuronium bromide and the intermediate-acting agent rocuronium bromide (henceforth referred to as pipecuronium and rocuronium, respectively) on muscarinic receptors in human isolated bronchial rings.

Materials and Methods

Bronchi were obtained from 12 patients (aged 40–77 yr) who underwent operation for removal of lung cancer. All patients received general anesthesia for the surgical procedure (the choice of anesthetic drugs was made by attending anesthesiologists). Surgical specimens, remote from the cancerous lesions, were obtained from the surgical waste after tissue had been removed for microscopic examination. The specimens were immersed in chilled, aerated (95% O_2 and 5% CO_2) physiologic salt solution (PSS) of the following composition: NaCl, 110.5 μM; KCl, 3.4 μM; $CaCl_2$, 2.4 μM; $MgSO_4$, 0.8 μM; KH_2PO_4, 1.2 μM; $NaHCO_3$, 25.7 μM; and dextrose, 5.6 μM. They were transported to the laboratory and

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stored overnight in aerated PSS at 4°C. Eight bronchial rings (2–4 mm ID) were used from each patient.

Procedure

The bronchi were dissected from surrounding tissue without damaging the epithelium[9] and cut into rings of 4 to 5 mm. The rings were suspended between stirrups in 25-ml water-jacketed tissue baths containing aerated PSS with propranolol 10^{-6} M at 37°C. The lower stirrup was connected *via* a silk string to a stationary hook in the tissue bath; the upper stirrup was connected *via* a silk string to a force transducer (model FT 03 D; Grass Medical Instruments, Quincy, MA) mounted on a micromanipulator. Two platinum electrodes (1 × 4 cm) were placed on each side of the rings. The rings were stimulated by electric field stimulation (EFS). EFS was provided by a direct-current amplifier (Mayo Clinic, Section of Engineering, Rochester, MN) triggered by an electric stimulator (Model S 44; Grass Medical Instruments). Isometric forces were recorded continuously (TA 4000; Gould, Valley View, OH). The rings were stretched to a resting force of 1 ± 0.4 g, which corresponds to optimal length in human bronchi of this size.[9] The lengths of the rings were not changed during the study.

Effects of Pipecuronium and Rocuronium on Postjunctional Muscarinic Receptors

Pipecuronium and rocuronium were gifts from Organon Technika (Turnhout, Belgium). Four rings from each of the 12 patients (48 rings total) were incubated with 10^{-6} M tetrodotoxin for 30 min to block the effect of prejunctional stimulation of muscarinic receptors by acetylcholine. Acetylcholine concentration–response curves were then obtained by cumulatively increasing the concentration of acetylcholine from 10^{-9} to 10^{-2} M in half-log increments. After the acetylcholine concentration–response curves were completed, the rings were washed with PSS until the resting forces were reestablished. The rings were then reincubated with 10^{-6} M tetrodotoxin for 30 min. Three rings from each of 6 of 12 patients (18 rings total) were incubated for 30 min with pipecuronium 10^{-7} M (n = 6), 10^{-6} M (n = 6), or 10^{-5} M (n = 6). The remaining rings from each patient (six rings each) were not incubated with pipecuronium and served as controls. Complete sets of acetylcholine concentration–response curves were again obtained. The same procedure was used in 24 rings from the other six patients to study the effect of 10^{-7} M (n = 6), 10^{-6} M (n = 6), and 10^{-5} M (n = 6) rocuronium.

Effects of Pipecuronium and Rocuronium on Nonstimulated M_2 Muscarinic Receptors

Four other rings from each of the 12 patients (48 rings total) were stimulated for 30 s at 3-min intervals by EFS (25 Hz, 25 V, 0.5 ms) until three reproducible contractions were observed. Three rings from each of 6 of the 12 patients (18 rings total) were then incubated for 30 min with pipecuronium 10^{-7} M (n = 6), 10^{-6} M (n = 6), or 10^{-5} M (n = 6), and EFS was repeated. One ring from each patient (six rings total) was not incubated with pipecuronium and served as control. The same protocol was used in the 24 rings from the other six patients to study the effects of rocuronium, 10^{-7} M (n = 6), 10^{-6} M (n = 6), and 10^{-5} M (n = 6).

Effects of Pipecuronium or Rocuronium on Pilocarpine-stimulated M_2 Muscarinic Receptors

Following the same procedure, the same 48 rings were incubated for 3 min with 10^{-9} M pilocarpine, to stimulate M_2 muscarinic receptors. EFS (25 Hz, 25V, 0.5 ms), 30 s in duration, was then applied at 3-min intervals until the contractions became steady. The pilocarpine concentrations in the tissue bath were cumulatively increased in half-log increments up to a concentration of 10^{-4} M after contractile responses to EFS became constant. After the study, all rings were blotted dry and weighed.

Data Analysis

Active contractile forces (total contractile force minus resting force) in response to EFS or acetylcholine were corrected for the effect of time by assuming the effect of time in control rings to be equal to that in rings incubated with muscle relaxants.[7] Mean weights, maximal forces, and resting forces were compared by unpaired *t* tests. Contractile responses of nonstimulated M_2 muscarinic receptors before and after incubation with pipecuronium or rocuronium were compared by paired *t* tests.

Two-factor repeated-measure analysis of variance with the Newman–Keuls *post hoc* test was used for statistical analysis of the pilocarpine concentration–response curves and concentrations necessary for 50% inhibition of contraction (IC_{50}).

Data were considered to be significantly different if $P < 0.05$. All data are reported as the mean ± SD.

Drugs

Pilocarpine hydrochloride, DL-propranolol hydrochloride, acetylcholine chloride, and tetrodotoxin were purchased from Sigma (Milan, Italy). All drugs were dis-

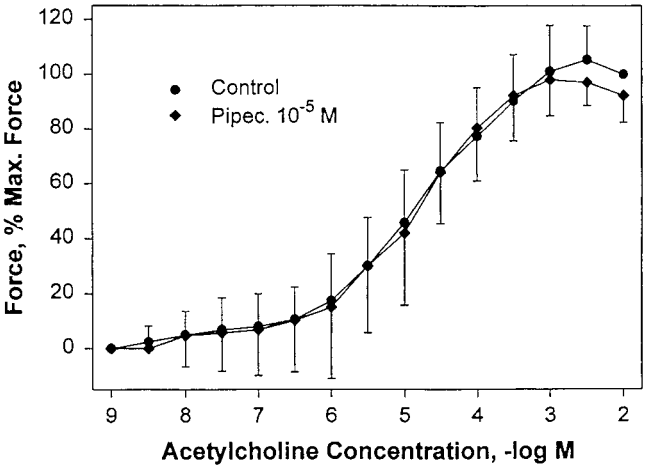

Fig. 1. Acetylcholine concentration–response curves for control bronchial rings and bronchial rings incubated with 10^{-5} M (n = 6) pipecuronium. There was no significant difference between the curves ($P = 0.82$), suggesting that pipecuronium had no effect on postjunctional M_3 muscarinic receptors.

solved in distilled water before use, and fresh solutions were prepared daily.

Results

Resting forces of the rings in which the effect of pipecuronium was studied were not significantly different from rings in which rocuronium was studied ($P = 0.09$). The maximal forces were significantly different between the two groups ($P < 0.03$), but the difference in mean ring weights did not achieve statistical significance ($P = 0.33$).

Effects of Pipecuronium and Rocuronium on Postjunctional Muscarinic Receptors

Pipecuronium (10^{-7}–10^{-5} M) and rocuronium (10^{-7}–10^{-5} M) had no significant effects on acetylcholine concentration–response curves ($P > 0.07$). Data for pipecuronium are shown in figure 1.

Effects of Pipecuronium and Rocuronium on Nonstimulated M_2 Muscarinic Receptors

Pipecuronium (10^{-7} to 10^{-5} M) and rocuronium (10^{-7} to 10^{-5} M) had no significant effects on contractile responses to EFS (table 1).

Effects of Pipecuronium and Rocuronium on Pilocarpine-stimulated M_2 Muscarinic Receptors

Pilocarpine reduced significantly contractile responses to EFS in a concentration-dependent manner ($P < 0.0001$) (fig. 2). Contractile responses to EFS were increased significantly after incubation with 10^{-7} M pipecuronium at pilocarpine concentrations of 3×10^{-6} and 10^{-5} M ($P < 0.05$), with 10^{-6} M pipecuronium at 10^{-7} to 3×10^{-6} M pilocarpine ($P < 0.03$), and with 10^{-5} M pipecuronium at 10^{-7} M to 3×10^{-5} M pilocarpine concentrations ($P < 0.01$). The IC_{50}s of the pilocarpine concentration–response curves were significantly reduced by pipecuronium 10^{-6} and 10^{-5} M ($P = 0.02$ and $P = 0.0004$, respectively), but not with 10^{-7} M pipecuronium ($P = 0.46$). Conversely, rocuronium (10^{-7} to 10^{-5} M) had no significant effect ($P > 0.05$) on the contractile responses to EFS at any pilocarpine concentration and did not significantly ($P > 0.15$) reduce the IC_{50} (table 2).

No significant ($P > 0.14$) differences in increases in resting forces between control rings and rings incubated with pipecuronium 10^{-7} or 10^{-6} M occurred (fig. 3). However, with 10^{-5} M pipecuronium, increases in resting forces were significantly smaller than those in control rings at pilocarpine concentrations larger than 3×10^{-7} M ($P < 0.002$).

Discussion

The two important findings of this study are that pipecuronium had an inhibitory effect on pilocarpine-stimulated prejunctional M_2 muscarinic receptors, but no effect on nonstimulated prejunctional M_2- or on postjunctional M_3 muscarinic receptors. Rocuronium had neither pre- nor postjunctional inhibitory effects on muscarinic receptors.

Limitations

One must be careful in extrapolating these results obtained in isolated human bronchi to humans *in vivo*. First, only bronchi with internal diameters of 2–4 mm were studied. The diameters of the studied bronchi may be important because relative abundance of receptors may vary among airway generations.[7,8] Second, in intact subjects, the response of airways to muscle relaxants may be modulated by circulating hormones and humoral substances carried in the blood. This may be of particular importance in those muscle relaxants releasing histamine from mast cells. Furthermore, the response of airway smooth muscles may be altered by stimuli from the central nervous system.

Stimulation of muscarinic receptors in airways may result in synthesis and release of prostaglandins,[10] which

Table 1. Effect of Pipecuronium and Rocuronium on EFS-induced Contraction of Isolated Human Bronchial Rings

	Concentrations of Pipecuronium and Rocuronium					
	10^{-7} M		10^{-6} M		10^{-5} M	
Drug	Control	After	Control	After	Control	After
Pipecuronium (g)	1.8 ± 2.1	1.7 ± 1.8	2.3 ± 2.2	2.2 ± 2.1	1.9 ± 1.4	1.9 ± 1.3
Rocuronium (g)	1.6 ± 0.8	1.6 ± 0.7	1.6 ± 1.2	1.6 ± 1.2	1.7 ± 1.1	1.9 ± 1.1

Values are mean ± SD; n = 6 for each concentration of pipecuronium and rocuronium. There were no significant differences (paired *t* test).
Control = before incubation of bronchial rings with pipecuronium or rocuronium; After = after incubation of bronchial rings with pipecuronium or rocuronium.

in turn inhibit release of acetylcholine from postganglionic prejunctional cholinergic fibers, thus reducing contractile response to EFS.[11] To inhibit the reduction in contractile response, synthesis and release of prostaglandins can be experimentally antagonized by incubation with indomethacin.[7,12] But indomethacin can inhibit prejunctional M_2 muscarinic receptor function in guinea pigs[13]; therefore, we decided not to incubate the bronchial rings with indomethacin.

Low concentrations of pilocarpine selectively stimulate prejunctional M_2 muscarinic receptors,[6,9] with no change in resting force. At higher concentrations postjunctional muscarinic M_2- and M_3 muscarinic receptors also are stimulated,[6,9] resulting in an increase in resting force. EFS stimulates not only cholinergic nerves, but also excitatory and inhibitory nonadrenergic noncholinergic (i-NANC) nerves. Human airways have few excitatory nonadrenergic noncholinergic nerves,[14] making it unlikely that pipecuronium enhanced contractile responses by stimulation of excitatory nonadrenergic noncholinergic nerves. But human airways have i-NANC nerves.[15] Inhibition of i-NANC nerves by pipecuronium could contribute to the increased contractile responses to EFS. To exclude this possibility, we determined in eight bronchial rings from two additional patients the effect of 10^{-7} to 10^{-5} M pipecuronium on i-NANC nerve stimulation and found no consistent effect, suggesting that inhibition of i-NANC nerves by pipecuronium did not contribute to the increased contractile responses to EFS.

All patients received a general anesthetic. To remove the anesthetic drugs from the tissue, all bronchi were stored overnight in 100 ml aerated PSS, and they were washed repeatedly for 2 h with PSS on the day of the study before measurements were begun. One cannot exclude the possibility that the drugs were not washed out completely.

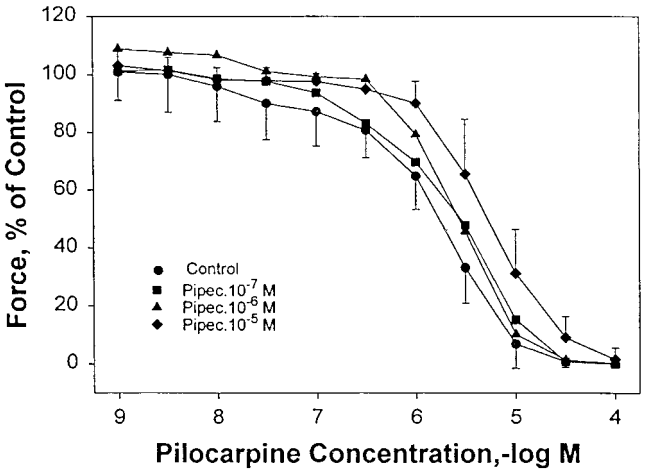

Fig. 2. Pilocarpine concentration–response curves for control bronchial rings and bronchial rings incubated with pipecuronium 10^{-7} M (n = 6), 10^{-6} M (n = 6), or 10^{-5} M (n = 6). With pipecuronium 10^{-7} M, contractile responses to EFS were significantly increased ($P < 0.05$) at 3×10^{-6} and 10^{-5} M pilocarpine, with pipecuronium 10^{-6} M at pilocarpine concentrations ranging from 10^{-7} to 3×10^{-6} M ($P < 0.03$), and with pipecuronium 10^{-5} M at pilocarpine concentrations ranging from 10^{-7} to 3×10^{-5} M ($P < 0.01$). For clarity only standard deviations for control measurements and for pipecuronium 10^{-5} M are shown.

Effects of Pipecuronium and Rocuronium on Postjunctional Muscarinic Receptors

Pipecuronium, 10^{-7} to 10^{-5} M, and rocuronium, 10^{-7} to 10^{-5} M, had no significant effects on acetylcholine concentration–response curves. Because the bronchial rings used for acetylcholine concentration–response curves were incubated with tetrodotoxin to interrupt

Table 2. IC_{50} of Pilocarpine Concentration–Response Curves

	Control	10^{-7} M	10^{-6} M	10^{-5} M
Pipecuronium	5.76 ± 0.17	5.66 ± 0.42	5.56 ± 0.23*	5.30 ± 0.26†
Rocuronium	5.83 ± 0.15	5.73 ± 0.22	5.86 ± 0.22	5.71 ± 0.49

Values are mean ± SD.
* Significantly different from control ($P = 0.02$).
† Significantly different from control ($P = 0.0004$).

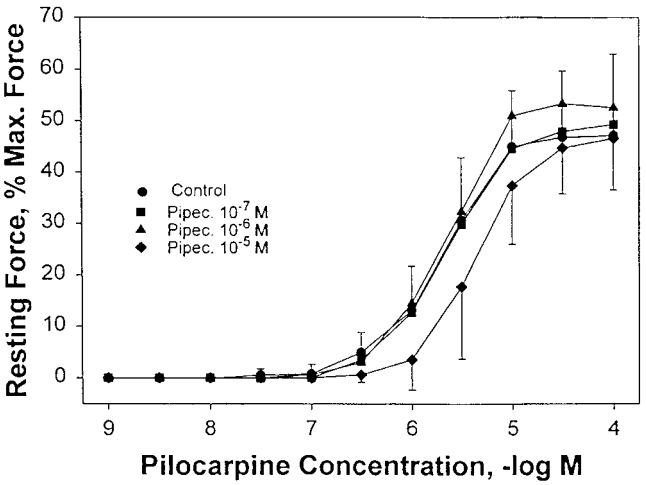

Fig. 3. No significant differences in increases of resting forces with pilocarpine between control rings and rings incubated with pipecuronium 10^{-7} or 10^{-6} M were found ($P > 0.14$). The increase in resting force, however, was significantly smaller in rings incubated with 10^{-5} M pipecuronium than in control rings at pilocarpine concentrations $> 3 \times 10^{-7}$ M ($P < 0.002$).

neuronal conduction, only postjunctional effects of acetylcholine could contribute to the contractile response. The unchanged acetylcholine concentration–response curves therefore suggest that pipecuronium and rocuronium did not inhibit postjunctional muscarinic receptors. In canine isolated trachealis muscle, the specific M_3 antagonist 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP) methiode attenuates the response to acetylcholine, suggesting that postjunctional M_3 muscarinic receptors primarily mediate the contractile response to acetylcholine.[16] Also, the characteristics of the antagonist effect of (P_{A_2}) 4-DAMP methiode on acetylcholine is consistent with M_3 receptors mediating contractile responses to acetylcholine.[16] By contrast gallamine, a specific M_2-receptor agonist, does not alter the contractile response to acetylcholine in canine isolated trachealis muscle,[16] suggesting that postjunctional M_3- and not M_2 muscarinic receptors mediate contractile responses to acetylcholine. Assuming human airways respond similarly, the data of this study suggest that pipecuronium and rocuronium had no effect on postjunctional M_3 muscarinic receptors.

But postjunctional M_2 muscarinic receptors can also contribute to contractile responses.[4] If pilocarpine stimulated postjunctional M_2 muscarinic receptors, inhibition of postjunctional M_2 muscarinic receptors by pipecuronium should reduce the resting force in response to pilocarpine. The increase in resting forces with pilocarpine was significantly less after incubation with 10^{-5} M pipecuronium than in control rings, suggesting an inhibitory effect on postjunctional M_2 muscarinic receptors by this large dose of pipecuronium.

Effects of Pipecuronium and Rocuronium on Nonstimulated M_2 Muscarinic Receptors

No consistent or convincing evidence for functional prejunctional M_2 muscarinic receptors in human airways using nonstimulated M_2 muscarinic receptors has been published.[6,8,17] We also did not find consistent or significant increases in contractile responses to EFS in bronchial rings incubated with pipecuronium.

However, functional prejunctional M_2 muscarinic receptors have been shown in human isolated bronchi using pilocarpine-stimulated M_2 muscarinic receptors.[6,9] More recently, measurement of acetylcholine release in response to vagus nerve stimulation before and after incubation with M_2 muscarinic antagonists[3] has provided more direct evidence for functioning prejunctional M_2 muscarinic receptors in human airways.

Effects of Pipecuronium and Rocuronium on Pilocarpine-stimulated M_2 Muscarinic Receptors

Low concentrations of pilocarpine (10^{-9} to 3×10^{-7} M) selectively stimulated M_2 muscarinic receptors. Contractile responses to EFS were increased by pipecuronium 10^{-6} and 10^{-5} M at low concentrations of pilocarpine, suggesting that pipecuronium inhibited prejunctional M_2 muscarinic receptors. Inhibition of prejunctional M_2-receptors has also been shown with gallamine in isolated human bronchi.[6] Rocuronium did not increase contractile responses to EFS at the three tested concentrations, suggesting that it had no inhibitory effects on prejunctional M_2 muscarinic receptors.

The conclusion of an inhibitory effect of pipecuronium on prejunctional M_2 muscarinic receptors agrees with observations by Okanlami *et al.*[2] in intact guinea pigs. These authors, however, suggested that the M_2-inhibitory effect occurred at doses larger than used clinically. However, plasma concentrations of pipecuronium as high as 1.3×10^{-6} M occur in humans after bolus injections of 0.07 mg/kg,[18] suggesting that pipecuronium may exert inhibitory effects on prejunctional M_2 muscarinic receptors during clinical practice.

In an elegant recent study, Hou *et al.*[19] determined the binding affinities of muscle relaxants in cells with either pure M_2- or M_3 muscarinic receptor populations. These authors found a higher binding affinity for rocuronium (IC_{50} = 3.0 μM) than for pipecuronium (IC_{50} = 5.8 μM) for M_2 muscarinic receptors. These results appear to be

inconsistent with the results of the current study, which found no effect of rocuronium on M_2 muscarinic receptors. However, in the study by Hou *et al.*[19] no statistical analyses for the binding affinities were included, and differences in binding affinities between pipecuronium and rocuronium were small compared with differences between pancuronium and pipecuronium or pancuronium and rocuronium.

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