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Actions of methoctramine, a muscarinic M_2 receptor antagonist, on muscarinic and nicotinic cholinoceptors in guinea-pig airways *in vivo* and *in vitro*

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1 The effects of the muscarinic M_2 receptor antagonist methoctramine, on contractions of airway smooth muscle induced by cholinergic nerve stimulation and by exogenously applied acetylcholine (ACh), have been investigated *in vivo* and *in vitro* in guinea-pigs.

2 Stimulation of the preganglionic cervical vagus nerve in anaesthetized guinea-pigs, caused bronchoconstriction and bradycardia which were mimicked by an intravenous dose of ACh. The muscarinic M_2 antagonist, methoctramine (7–240 nmol kg^{-1}), inhibited the bradycardia induced by both vagal stimulation and ACh (ED_{50}: 38 ± 5 and 38 ± 9 nmol kg^{-1}, respectively). In this dose-range, methoctramine facilitated vagally-induced bronchoconstriction (ED_{50}: 58 ± 5 nmol kg^{-1}), despite some inhibition of ACh-induced bronchoconstriction (ED_{50}: 81 ± 11 nmol kg^{-1}). The inhibition of ACh-induced bronchoconstriction and hypotension was dose-dependent, but was not statistically significant until doses of 120 nmol kg^{-1} and 240 nmol kg^{-1} respectively.

3 In the guinea-pig isolated, innervated tracheal tube preparation, methoctramine (0.01–1 μM) caused facilitation of contractions induced by both pre- and postganglionic nerve stimulation, whereas contractions induced by exogenously applied ACh were unaffected. Higher concentrations of methoctramine ($\geq$ 10 μM), reduced responses to both nerve stimulation and exogenous ACh, indicating blockade of postjunctional muscarinic M_3 receptors.

4 ACh caused a slow maintained increase in tone of the tracheal tube and at the same time reduced the contractions induced by nerve stimulation. This inhibitory effect of ACh on neuronally mediated responses was antagonized by methoctramine (0.01–1 μM) in a concentration-dependent manner. However, the ACh-induced tone change was unaffected by methoctramine in this concentration-range, indicating a lack of muscarinic M_3 receptor antagonist activity in this concentration-range.

5 The effect of methoctramine on responses induced by pre- and postganglionic nerve stimulation was not identical. At concentrations of methoctramine of 1 μM and greater, preganglionic stimulation-induced contractions were reduced when compared to those induced by postganglionic stimulation, suggesting an inhibitory effect of methoctramine on ganglionic transmission. This ganglion blocking action of methoctramine was not due to its reported M_1 receptor antagonist activity (blocking facilitatory M_1 receptors in the ganglia) since pirenzepine was without effect in this preparation. We believe that the ganglionic blocking action of metoctramine is due to its nicotinic receptor antagonist properties, since the concentration of methoctramine inhibiting ganglionic transmission in the tube preparation (1 μM) was shown to inhibit contractions induced by the nicotinic agonist, 1,1-dimethyl-4-phenyl-piperazine in tracheal strips.

6 These results show that methoctramine is able to demonstrate adequately the presence of autoinhibitory receptors functionally both *in vivo* and *in vitro* and confirms their pre-junctional location on pulmonary cholinergic nerve terminals and their classification as muscarinic M_2 subtypes. These results also indicate that while methoctramine is a potent muscarinic M_2 receptor antagonist, it does not possess the required selectivity to discriminate between cholinoceptor subtypes in preparations, such as the airways, where mixed populations of muscarinic and nicotinic cholinoceptors exist.

Keywords: Methoctramine; muscarinic and nicotinic cholinoceptors; guinea-pig airways

Introduction

The parasympathetic nerves supplying the lung and trachea provide the dominant neuronal control of airway smooth muscle tone. The events leading to nerve-induced contraction of airway smooth muscle involve the interaction of acetylcholine (ACh) at a number of different cholinoceptors (Barnes *et al.*, 1988). Transmission through the parasympathetic ganglia involves release of ACh from preganglionic nerve terminals onto nicotinic receptors on the cell bodies of the postganglionic nerve fibres (Hawkins & Paton, 1958). This is the main pathway for transmission through parasympathetic ganglia, but in some species, including man and rabbit, a role for facilitatory muscarinic M_1 receptors in parasympathetic ganglia is suggested (Bloom *et al.*, 1987; Lammers *et al.*, 1989). Activation of nicotinic receptors by ACh leads to the propagation of an action potential in the postganglionic nerve fibre and the release of ACh onto muscarinic M_3 receptors located on airway smooth muscle (Roffel *et al.*, 1988). Muscarinic receptors have also been identified on the terminals of pulmonary parasympathetic nerves where they have an autoinhibitory role. Activation of these receptors by the neurotransmitter inhibits the outflow of ACh from these postganglionic nerve terminals (Fryer & Maclagan, 1987; Kilbinger *et al.*, 1991) thereby controlling the degree of neuronally induced airway contraction. Autoinhibitory muscarinic receptors have been found in a number of species including guinea-pig, cat, dog, man and rat, (Blaber *et al.*, 1985; Ito & Yoshitomi, 1988; Minette & Barnes, 1988; Aas & Maclagan, 1990). Blockade of these receptors with the muscarinic receptor antagonist, gallamine, leads to a facilitation of neuronally-induced bronchoconstriction while having little or no effect on bronchoconstriction induced by exogenous ACh (Fryer & Maclagan, 1984).

The results obtained with gallamine suggested that the prejunctional receptors are of the M_2 subtype and this possibility

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has been further investigated in the present study by use of a novel potent and selective muscarinic M_2 receptor antagonist, methoctramine (Melchiorre *et al.*, 1987). This antagonist has been shown to have a high degree of selectivity for muscarinic M_2 receptors present in atria when compared to the muscarinic M_3 receptors found in tracheal smooth muscle (Giraldo *et al.*, 1988). Clearly such an antagonist provides a useful experimental tool for investigating the actions of the autoinhibitory M_2 receptors on pulmonary parasympathetic nerves, since both M_2 and M_3 receptors are present in airways on opposite sides of the neuroeffector junction and their activation exerts opposing effects. Methoctramine has also been reported to have antagonist properties at two other subtypes of cholinoceptors (Melchiorre *et al.*, 1987) which are found to be present in the airways, namely nicotinic and muscarinic M_1 receptors. Nicotinic receptors are found in parasympathetic ganglia (Hawkins & Paton, 1958) and facilitatory M_1 receptors have been demonstrated in rabbit and human airways (Bloom *et al.*, 1987; Lammers *et al.*, 1989).

The work described here investigates the actions of methoctramine *in vivo* and *in vitro*, on bronchoconstrictor responses elicited by stimulation of parasympathetic nerves innervating airway smooth muscle in the guinea-pig. Similar work with the M_2 antagonist, gallamine, has previously been performed in this laboratory (Fryer & Maclagan, 1987) and we wished to determine what advantages methoctramine has over gallamine. Ganglionic mechanisms were also studied *in vitro* by comparing responses elicited by pre- and postganglionic stimulation and revealed ganglionic nicotinic receptor antagonist properties of methoctramine. Abstracts of this work have been presented to the British Pharmacological Society (Watson *et al.*, 1989; 1990a) and the XIth International Congress of Pharmacology (Watson *et al.*, 1990b).

Methods

In vivo *preparation*

In vivo experiments were performed according to the protocol described by Fryer & Maclagan (1987). Blood pressure was recorded via a Statham P23 pressure transducer and expressed as the mean arterial blood pressure (BP) given by the equation: BP-Diastolic pressure + 1/3 Pulse pressure, where the pulse pressure is the difference between systolic and diastolic pressures. Bronchoconstriction was induced alternately by stimulation of the peripheral end of the cut right vagus nerve (30 Hz, 0.2 ms, 10–40 V, for 5 s every 180 s) and by intravenous administration of acetylcholine (5–10 $\mu g\,kg^{-1}$, 90 s after each vagal stimulation). Bronchoconstriction was recorded as an increase in pulmonary inflation pressure (Ppi) over the basal inflation pressure change produced by the pump, before and during cumulative i.v. administration of methoctramine.

In vitro *vagally-innervated tracheal tube preparation*

In vitro experiments using the vagally-innervated guinea-pig tracheal tube preparation (Blackman & McCaig, 1983), were performed according to the protocol described by Faulkner *et al.* (1986). Indomethacin (5 μM) and propranolol (1 μM) were present in the Krebs-Henseleit buffer solution (composition mM: NaCl 118.4, KCl 4.9, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $CaCl_2\,6H_2O$ 2.5, glucose 11.1) throughout all the experiments to eliminate prostaglandin-induced tone and noradrenergic nerve stimulation during transmural stimulation (TMS), respectively. A ganglion blocking concentration of hexamethonium (75 μM) was also present during TMS to ensure that responses recorded were due to stimulation of postganglionic nerve fibres. Contractions of the trachea were measured as an increase in intraluminal pressure (ILP), and were recorded on a polygraph Grass Model 7D.

After mounting in the organ bath, tissues were left to equilibrate for at least 60 min during which time the vagus nerves were stimulated regularly for 5 s at 40 s intervals (30 V; 30 Hz; 0.2 ms preganglionic stimulation (PGS)). Once stable responses were established after this period, cumulative concentration-response curves to ACh (0.1–100 μM) were performed during PGS, in the absence and presence of single concentrations of methoctramine (0.01–1 μM). Each tissue acted as its own control, thus enabling paired analysis of the results. In a second set of experiments, cumulative concentrations of methoctramine (0.01–10 μM) were given during either PGS or TMS, in the absence of exogenous ACh. In a further set of experiments, pirenzepine (1–300 nM) was applied to the preparation during PGS in a cumulative manner. Cumulative concentrations of ACh were added to the organ bath in the absence and then the presence of either pirenzepine or methoctramine, to determine postjunctional actions of both these antagonists.

In vitro *tracheal strip preparation*

Guinea-pigs were killed by cervical dislocation. The trachea was removed, cut open longitudinally along its ventral surface and divided into six sections, each containing 4–5 cartilaginous rings. The resultant tracheal strips were then suspended in 10 ml organ baths, at a resting tension of 2 g, which was found in preliminary experiments to produce optimal contractions to ACh. Isometric contractions of the tracheal strips were measured with Grass FT03 force displacement transducers and recorded on a polygraph Grass Model 7D. The organ baths contained Krebs Henseleit buffer solution maintained at 37°C and oxygenated with 95% O_2: 5% CO_2. Indomethacin (5 μM) was present in the Krebs solution to eliminate prostaglandin-induced tone.

After equlibration for 60 min, cumulative concentrations of ACh were added to the organ bath. The tissues were then washed 6–8 times over a further 60 min period, until a stable baseline was re-established. Reproducible, consecutive responses to the nicotinic agonist 1,1-dimethyl-4-phenyl-piperazine (DMPP) could not be obtained because of tachyphylaxis, so tissues were paired according to their responsiveness to ACh in order that one should act as the control while the other received methoctramine. Vehicle or a single concentration of methoctramine in the range 0.1–1.0 μM were administered to each pair of tissues, 20 min before the addition of cumulative concentrations of DMPP. In parallel experiments, the addition of 1 μM tetrodotoxin and 1 μM atropine to two separate strips confirmed the neuronal, cholinergic nature of the DMPP-induced contractions.

Drugs

The following drugs were used: acetylcholine bromide, histamine acid phosphate and atropine sulphate were obtained from BDH Ltd., (Dagenham, Essex). Hexamethonium bromide, indomethacin, succinylcholine, urethane ethyl carbamate, tetrodotoxin and 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) were from Sigma Chemical Co. Ltd. (Poole, Dorset). Pirenzepine (Boots Chemicals Ltd., Nottingham) and propranolol hydrochloride (Imperial Chemical Industries Ltd,. Andover, Hants) were also used. Methoctramine was a generous gift from Dr C. Melchiorre, University of Camerino, Italy.

All drugs were made up fresh each day in 0.9% (w/v) saline, with the exception of DMPP which was made up in distilled water and of indomethacin which was made up in a buffer of composition (mM): KH_2PO_4 19.77 and Na_2HPO_4 118.34, adjusted to pH 7.8 with NaOH, and sonicated for 3 min. Ascorbic acid (1 μM) was added to the histamine solutions to prevent oxidation.

Statistical analysis of data

The Wilcoxon matched-pairs signed-ranks test was used to determine the level of significance of differences resulting from treatments within the same preparation and the Mann-Whitney U-test was used to determine the level of significance of differences between treatments in different preparations. All

results are expressed as mean values ± standard error of the mean (s.e.mean).

Results

Effect of methoctramine in vivo

Stimulation of the peripheral end of the cut right vagus nerve during the control period, produced an increase in pulmonary inflation pressure of 8 ± 1 mmH_2O, from a basal value of 100 ± 6 mmH_2O and a fall in heart rate of 205 ± 11 beats per min (b.p.m.), from a basal rate of 327 ± 13 b.p.m. A bronchoconstriction of similar magnitude to that induced by vagal nerve stimulation (6 ± 1 mmH_2O, from the same basal Ppi) could be produced by i.v. ACh and this was associated with a fall in heart rate of 170 ± 31 b.p.m. (from the same basal rate of 327 ± 13 b.p.m.). While both vagus nerve stimulation and i.v. ACh produced a fall in mean arterial blood pressure, the effect was only accurately measurable when induced by ACh (fall of 9 ± 2 mmHg, from a basal value of 36 ± 3 mmHg) since that induced by vagal nerve stimulation was smaller and shorter in duration.

Vagal nerve stimulation and i.v. ACh were given alternately at 90s intervals, while methoctramine was given in a cumulative manner by i.v. injections every 9 min. Preliminary experiments had shown that the peak effects of methoctramine in the lung were obtained within 6–9 min of i.v. injection and that recovery of control responses could be achieved within 15–20 min.

The effects of methoctramine on vagally- and ACh-induced bronchoconstriction and bradycardia are summarised in Figure 1. Methoctramine caused a dose-dependent inhibition of bradycardia induced by either vagal nerve stimulation (ED_{50}: 38 ± 5 nmol kg^{-1}) or i.v. ACh (ED_{50}: 38 ± 9 nmol kg^{-1}). In contrast, vagally-induced bronchoconstriction was facilitated by methoctramine despite some inhibition of ACh-induced bronchoconstriction. Inhibition of ACh-induced bronchoconstriction was statistically significant at doses of methoctramine of 120 nmol kg^{-1} and greater.

Effect of methoctramine on ACh-induced hypotension

ACh-induced hypotension was not significantly affected by methoctramine at doses up to 120 nmol kg^{-1}, but at the highest dose of 240 nmol kg^{-1} there was approximately a 30% inhibition, which was statistically significant (Figure 2).

Effect of methoctramine on the in vitro *innervated tracheal tube preparation*

In the absence of prostaglandin-induced tone the tracheal tube preparation had a low constant intraluminal pressure (ILP). Contractions of the tube induced by nerve stimulation or spasmogen added to the organ bath, were recorded as an increase in ILP. Exogenously applied ACh produced a slow contraction which was insensitive to tetrodotoxin (1 μM) but inhibited by atropine (1 μM), while stimulation of either pre- or postganglionic cholinergic nerves for 5 s caused rapid contractions both of which were blocked by tetrodotoxin and atropine.

Application of exogenous ACh, during nerve stimulation, caused a slow, concentration-dependent increase in basal tone of the trachea, while at the same time inhibited the contractions induced by PGS (Figure 3a). Similar tone changes induced by the spasmogen histamine (1–10 μM) did not cause inhibition of nerve-induced contractions. At concentrations of ACh up to 10 μM the sum of the slow increase in pressure caused by ACh plus the rapid increase in pressure caused by neuronally released ACh was much smaller than the pressure increase achieved with the maximum concentration of ACh used (0.3 mM). The reduction in PGS-induced contractions caused by ACh was antagonized by methoctramine (Figure 3b 0.01 μM and c 1 μM), producing a rightward shift in the concentration-effect curves for ACh. These concentrations of

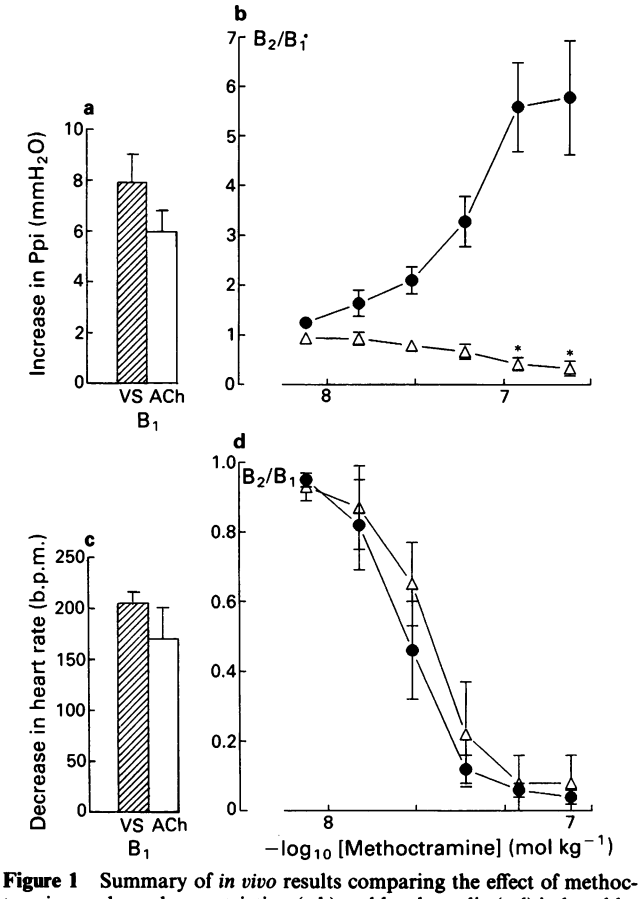

Figure 1 Summary of *in vivo* results comparing the effect of methoctramine on bronchoconstriction (a,b) and bradycardia (c,d) induced by either vagal nerve stimulation (VS: 30 Hz, 0.2 ms, 5 s, 10–40 V, solid circles and hatched columns) or by intravenous acetylcholine (ACh: 5–10 μg kg^{-1} open triangles and open columns). The control responses, before methoctramine (B_1), are shown (a) and (c) in absolute units. Results (b) and (d) are expressed as the ratio B_2/B_1 where B_2 is the response after methoctramine. All points are the mean with s.e.mean shown by vertical lines; $n = 8$; * $P < 0.05$ for inhibition of ACh-induced bronchoconstriction.

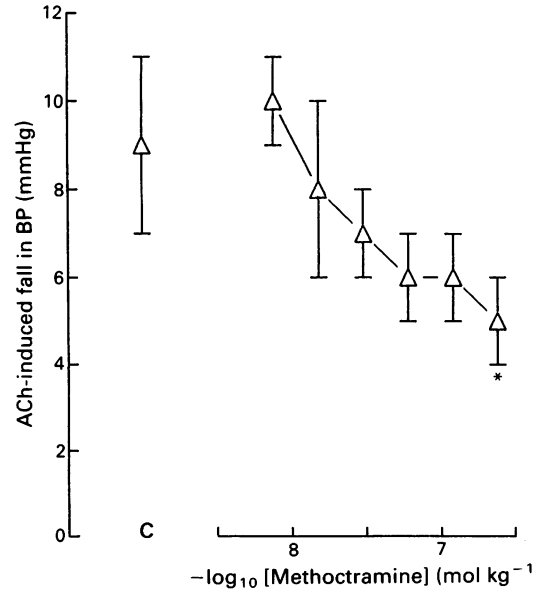

Figure 2 The effect of cumulative doses of methoctramine on the fall in mean arterial blood pressure (BP, mmHg) induced by intravenous acetylcholine (ACh, 5–10 μg kg^{-1}), in anaesthetized guinea-pigs. C indicates the mean control value before addition of methoctramine. All points are the mean with s.e.mean shown by vertical bars, $n = 8$; * $P < 0.005$, Wilcoxon-paired sign-ranked test compared with C.

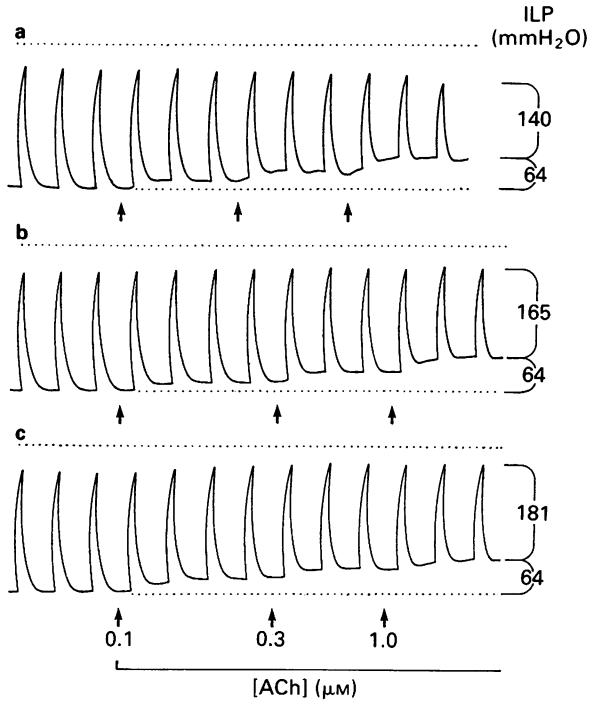

Figure 3 Contractions of the isolated tracheal tube elicited by preganglionic nerve stimulation (PGS: 30 V, 30 Hz, 0.2 ms, for 5 s every 40 s), recorded as an increase in intraluminal pressure (ILP) in mmH_2O. Acetylcholine (ACh) was added cumulatively to the organ bath during PGS, in the absence (a) and presence of methoctramine (0.01 μM (b) and 1 μM (c)) in the same preparation. The size of the stimulation-induced contraction (204 mmH_2O) before addition of methoctramine, was the same in (a), (b) and (c). The distance between the dotted lines on each panel, indicates the maximum contractile response (274 mmH_2O) attained to 0.1 mM ACh.

methoctramine had no effect on the contractions induced by exogenously applied ACh, though at the highest concentration of methoctramine used (10 μM) there was significant inhibition of the ACh concentration-effect curve (Figure 4). Table 1 summarises the results showing that methoctramine (0.01, 0.1 and 1 μM) caused a concentration-dependent antagonism of the inhibitory actions of ACh on PGS-induced contractions.

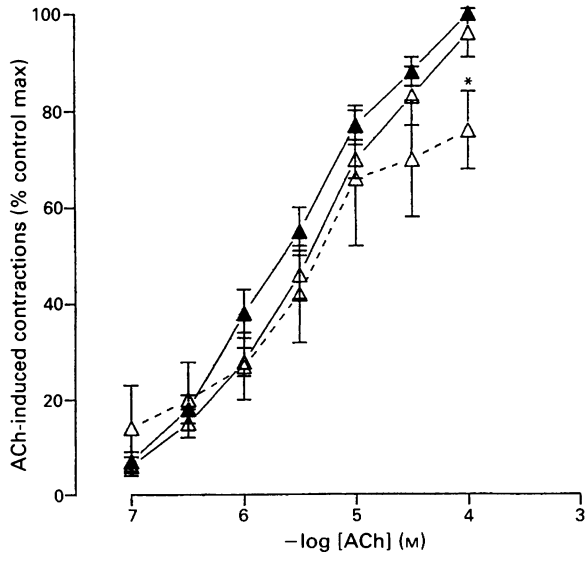

Figure 4 Contractions of the guinea-pig tracheal tube preparation induced by cumulative concentrations of acetylcholine (ACh) in the absence (▲—▲) and presence of methoctramine 1 μM (△—△) and 10 μM (△----△). Results are expressed as a % of the maximum contraction to ACh in the absence of methoctramine (control) and are the mean (s.e.mean shown by vertical lines) of at least 5 data points (* $P < 0.05$).

Table 1 Antagonism of acetylcholine (ACh)-induced inhibition of preganglionic stimulation-induced contractions of the guinea-pig isolated trachea, by methoctramine

	% *inhibition by ACh* (1 μM)		
Methoctramine (μM)	*Control* A	*+Methoctramine* B	*Difference* (A − B)
0.01	26 ± 8	16 ± 8	10 ± 1]
0.1	27 ± 6	10 ± 9	17 ± 5 *
1.0	30 ± 7	0 ± 8	30 ± 8]
	**		

Effect of methoctramine (0.01, 0.1 and 1 μM) on the inhibitory effect of ACh on contractions induced by PGS, compared at a concentration of ACh of 1 μM. This concentration of ACh produced an increase in tone which did not contribute to the inhibition observed to PGS. (Results are expressed as mean ± s.e.mean, $n = 6$. ** $P < 0.025$, according to Wilcoxon-paired sign-ranked test; * $P < 0.05$, according to a Mann-Whitney U test).

In contrast, when the effect of methoctramine (0.01–1 μM) was studied on contractions elicited by nerve stimulation, in the absence of exogenously applied ACh, facilitation of the rapid contractions was observed. This facilitation was the same using either PGS or TMS in the concentration-range of methoctramine 0.01–0.3 μM. An additional effect was seen at concentrations of methoctramine of 1 μM and greater, when a significant inhibition of PGS-induced contractions occurred when compared to TMS-induced contractions (Figure 5). At the highest concentration of methoctramine used (10 μM) both PGS and TMS-induced contractions were inhibited, as were contractions induced by exogenously applied ACh (Figures 4 and 5).

The effect of pirenzepine on the in vitro *innervated tracheal tube preparation*

The M_1 antagonist, pirenzepine in the concentration range 1–100 nM, had no effect on PGS-induced contractions (30 V; 30 Hz; 0.2 ms). At the highest concentration of pirenzepine used (300 nM) some degree of inhibition of both PGS-induced contractions and ACh-induced contractions was observed.

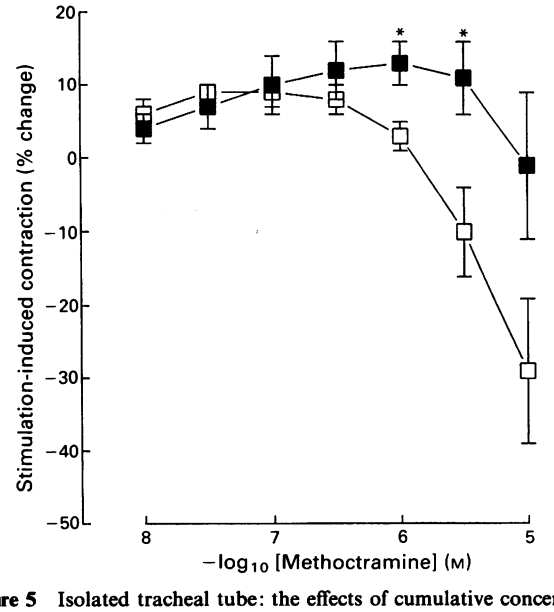

Figure 5 Isolated tracheal tube: the effects of cumulative concentrations of methoctramine on preganglionic stimulation (PGS)-induced contractions (□) and transmural stimulation (TMS)-induced contractions (■) are shown. Results are expressed as the percentage change in response from control values before addition of the antagonist. All points are the mean with s.e.mean shown by vertical lines, $n \geq 5$; * $P < 0.05$, between contractions induced by PGS compared to TMS, according to a Mann-Whitney U statistical test.

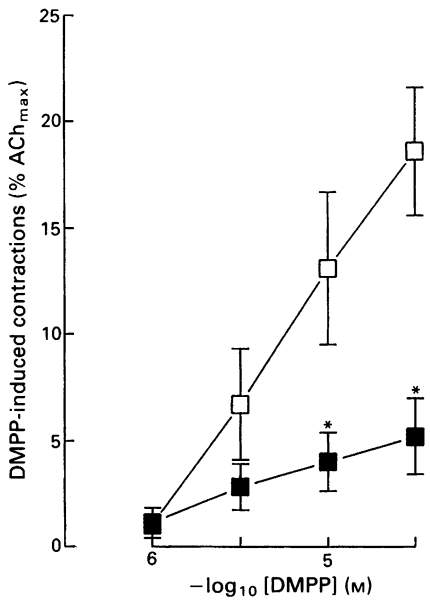

Figure 6 The effect of methoctramine (1 μM) on contractions of strips of guinea-pig trachea induced by the nicotinic cholinoceptor agonist, 1,1-dimethyl-4-phenyl-piperazine (DMPP). Contractions are expressed as a % of the maximum response to acetylcholine (ACh) (0.3 mM), since tissues were paired according to their responsiveness to ACh. Control contractions induced by DMPP in the absence of antagonist (□); response in the presence of 1 μM methoctramine (■). Results are the mean with s.e.mean shown by vertical lines, $n = 8$; * $P < 0.05$, according to a Mann-Whitney U test.

Effect of methoctramine on the in vitro *tracheal strip preparation*

In tracheal strip preparations, which had been paired according to their responsiveness to ACh, methoctramine (1 μM) caused an inhibition of contractions induced by cumulative concentrations of the nicotinic receptor agonist, DMPP (Figure 6). Tetrodotoxin 1 μM and atropine 1 μM completely inhibited the contractions induced by DMPP.

Discussion

The results presented in this paper, confirm the presence and location of the autoinhibitory muscarinic receptors on pulmonary parasympathetic nerves (Faulkner *et al.*, 1986) and their classification as muscarinic M_2 receptors (Fryer & Maclagan, 1987; Del Monte *et al.*, 1990; Doelman *et al.*, 1991; Kilbinger *et al.*, 1991). The results also demonstrate for the first time, that methoctramine is an antagonist for ganglionic nicotinic cholinceptors in the airways.

The *in vivo* data presented here with methoctramine show very similar results to those previously reported with the M_2 receptor antagonists, gallamine and pancuronium (Fryer & Maclagan, 1987). Unlike gallamine, which has a long lasting effect in guinea-pigs *in vivo*, methoctramine achieved peak pulmonary effects within 6 to 9 min of i.v. administration and responses returned to control levels within 15–20 min. There appears to be very little difference in the relative muscarinic M_2/M_3 receptor selectivities of these two compounds *in vivo*, therefore for *in vivo* work there seems little to choose from between these drugs apart from their differing time course of action.

Facilitation of stimulation-induced bronchoconstriction *in vivo*, occurred at all doses of methoctramine despite evidence from the data on ACh-induced bronchoconstriction, of significant postjunctional effects at doses of methoctramine of 120 nmol kg^{-1} and greater. An altenative measure of the potency of methoctramine for M_3 receptors is provided by its effect on ACh-induced hypotension, which is believed to be mediated via muscarinic receptors on the vascular endothelium with a similar profile to the M_3 subtype (Eglen *et al.*, 1988; McCormak *et al.*, 1988). Methoctramine (15–240 nmol kg^{-1}) produced a dose-related inhibition of the ACh-induced hypotension, but this did not reach statistical significance until the highest dose of methoctramine (240 nmol kg^{-1}). These data taken in conjunction with the effects of methoctramine on ACh-induced bronchoconstriction suggest that at best, *in vivo*, methoctramine in our hands is able to show about a 20 fold separation between M_2 and M_3 receptor antagonist activity. This is much less than the 100 fold separation previously reported by Melchiorre *et al.* (1987) and the reasons for this difference are unclear. There also appears to be some controversy in the literature as to the precise degree of selectivity of this drug. Studies by Eglen *et al.* (1988) on the effects of methoctramine on M_2 receptors on the atria and M_3 receptors on tracheal smooth muscle and blood vessel epithelium, indicate approximately a 60 fold difference in selectivity.

In vitro, experiments can more easily be used to determine the exact location of these inhibitory M_2 receptors than *in vivo* experiments, since stimulation of pulmonary parasympathetic nerves at both the pre- and postganglionic level can be achieved within the same preparation.

Application of exogenous ACh *in vitro*, to preparations during nerve stimulation, results in an inhibition of nerve-induced contractions via activation of the autoinhibitory M_2 receptors. However, since ACh is also an agonist for the postjunctional M_3 receptors on airway smooth muscle, this inhibition of stimulation-induced contractions is associated with an increase in tracheal tone, seen as an increase in the base line intraluminal pressure. At high concentrations of ACh ($\geq 10\,\mu$M) this increase in pressure in the tube reduces the contraction elicited by nerve stimulation because the tissue is unable to contract further. However, at concentrations of ACh less than 10 μM, inhibition of stimulation-induced contractions cannot be explained in this way and is probably due to the actions of ACh on the autoinhibitory M_2 receptors. Evidence for this comes from the fact that (i) at these concentrations of ACh ($< 10\,\mu$M), the sum of the slow increase in pressure caused by exogenously applied ACh plus the rapid increase in pressure caused by neurally released ACh, is much smaller than the pressure increase induced by the maximal concentration of ACh use (0.3 mM); (ii) the inhibitory effect of this concentration-range of ACh ($< 10\,\mu$M) on nerve-induced contractions can be antagonized by blockade of the autoinhibitory receptors by the M_2 receptor antagonist, methoctramine; and (iii) other spasmogens including the stable thromboxane analogue U46619 (Faulkner *et al.*, 1986) and histamine can produce similar increases in basal tone without inhibiting nerve-induced contractions.

The interpretation of results from these types of functional experiments, is complicated by the dual effect of ACh on pre- and postjunctional muscarinic receptors. At low concentrations of ACh these complications can be minimized and an assessment of M_2 receptor activity made by use of the selective M_2 receptor antagonist methoctramine in conjunction with ACh. However, accurate determination of a pA_2 value (Arunlakshana & Schild, 1959) for methoctramine on these autoinhibitory M_2 receptors must await the availability of a selective muscarinic M_2 receptor agonist.

Methoctramine facilitated contractions induced by both pre- and postganglionic nerve stimulation *in vitro*. This finding confirms the previous reports that the autoinhibitory M_2 receptors are located postganglionically on the prejunctional cholinergic nerve terminals (Faulkner *et al.*, 1986; Doelman *et al.*, 1991). A comparison of the effect of high concentrations of methoctramine ($\geq 1\,\mu$M), on contractions induced by these two methods of stimulation however, revealed an unexpected ganglionic blocking action of this drug. This ganglionic blocking action of methoctramine was not due to inhibition of facilitatory muscarinic M_1 receptors in the ganglia, since ganglionic blockade could not be reproduced using the muscarinic antagonist pirenzepine at concen-

trations known to block muscarinic M_1 receptors. Therefore, although M_1 receptors have been demonstrated to cause facilitation of pulmonary, ganglionic transmission in man and rabbit (Lammers *et al.*, 1989; Bloom *et al.*, 1987), they do not appear to be involved in ganglionic transmission in guinea-pig pulmonary cholinergic nerves.

The concentration of methoctramine, which in the vagally innervated tracheal tube preparation had been shown to have differential effects on contractions induced by stimulation of pre- or postganglionic nerves (1 μM), significantly antagonized contractions of tracheal strips induced by the nicotinic agonist, DMPP. This is not due to a postjunctional action of methoctramine, since contractions induced by exogenous ACh were not significantly altered by 1 μM methoctramine. Therefore, methoctramine shows significant antagonist activity at nicotinic receptors in pulmonary parasympathetic ganglia as well as its previously reported actions at nicotinic receptors in the frog rectus abdominis (Melchiorre *et al.*, 1987). The concentrations of methoctramine associated with this nicotinic receptor antagonist activity are in the micromolar range for both muscle and ganglionic nicotinic receptors.

While our *in vivo* results were unable to show the high degree of M_2/M_3 receptor selectivity previously reported for methoctramine by Melchiorre *et al.* (1987), these results indicate that despite this, methoctramine is at least as effective as gallamine in demonstrating autoinhibitory M_2 receptors on the pulmonary parasympathetic nerve *in vivo*. There appears to be little difference between the actions of these two drugs *in vivo*, apart from their different durations of action. Our *in vitro* results suggest that methoctramine is a potent muscarinic M_2 receptor antagonist with around a 100 fold separation between concentrations facilitating contractions induced by postganglionic nerve stimulation (0.01 μM) and those inhibiting contractions induced by ACh (10 μM). However, its usefulness may be limited by the fact that it also demonstrates significant ganglionic nicotinic receptor antagonist properties at micromolar concentrations. It is clear that like several other cholinoceptor antagonists, methoctramine has antagonist activity at nicotinic and muscarinic M_1 receptors in addition to its well-documented M_2 and M_3 antagonist properties. In preparations of airway smooth muscle, these other properties of methoctramine are important since nicotinic cholinoceptors are responsible for transmission through parasympathetic ganglia (Hawkins & Paton, 1958) and these ganglia are embedded within the airway walls. In addition, a modulatory role for facilitatory muscarinic M_1 receptors has been suggested in transmission in parasympathetic ganglia in man and rabbit (Bloom *et al.*, 1987; Lammers *et al.*, 1989) however, the present experiments were unable to demonstrate the presence of functional muscarinic M_1 receptors in guinea-pig parasympathetic ganglia.

Therefore while methoctramine is a potent muscarinic M_2 receptor antagonist both *in vivo* and *in vitro*, caution should be exercised when it is used in preparations with mixed cholinoceptor populations since methoctramine, in common with many other cholinoceptor antagonists, does not possess the required selectivity to discriminate effectively between muscarinic and nicotinic cholinoceptor subtypes under these circumstances.

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