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Presynaptic muscarinic receptors mediating inhibition of neurogenic contractions in rabbit vas deferens are of the ganglionic M_1-type

Manfrid Eltze *, Gernot Gmelin [1], Jürgen Wess [2], Carsten Strohmann [3], Reinhold Tacke [3], Ernst Mutschler [2] and Günter Lambrecht [2]

Department of Pharmacology, Byk Gulden Pharmaceuticals, D-7750 Konstanz, F.R.G., [1] Sandoz Ltd., CH-4002 Basel, Switzerland, [2] Department of Pharmacology, University of D-6000 Frankfurt/M, and [3] Institute of Inorganic and Analytical Chemistry, Technical University, D-3300 Braunschweig, F.R.G.

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The present study was designed to further characterize the presynaptic muscarinic M_1-receptor responsible for the inhibition of neurogenic contractions in the isolated rabbit vas deferens. Electrically induced twitch contractions of this preparation were inhibited by the M_1-agonist, McN-A-343, and by some of its analogs: 4-chloro-phenyl derivative > McN-A-343 > trans-olefinic analog > cis-olefinic analog. The same rank order of potency was observed for these agonists to raise the blood pressure of pithed rats by stimulation of M_1-receptors in sympathetic ganglia. A highly significant correlation was found between the antimuscarinic potencies of atropine, pirenzepine and a series of 9 antagonists structurally related to the ganglionic $M_{1\beta}$-receptor selective compounds, hexocyclium and hexahydro-difenidol, to antagonize the McN-A-343-induced inhibition of twitch contractions in rabbit vas deferens or the muscarine-induced depolarization in rat isolated superior cervical ganglia. It is suggested that the presynaptic muscarinic receptor that mediates inhibition of neurogenic contractions in rabbit vas deferens is of the ganglionic $M_{1\beta}$-type.

Muscarinic receptor subtypes; Vas deferens (rabbit); Pithed rat; Ganglia (rat);
Muscarinic acetylcholine receptor agonists; Muscarinic acetylcholine receptor antagonists; McN-A-343 analogs

1. Introduction

It has been shown recently that rabbit vas deferens is endowed with muscarinic M_1- and M_2-receptors which inversely modulate neurotransmission in this preparation (Eltze, 1988a,b). Activation of the M_1-receptors, e.g. by McN-A-343, inhibits neuronally evoked contractions, whereas activation of the M_2-receptors, e.g. by carbachol, leads to a potentiation of electrically induced twitch contractions. McN-A-343 and carbachol have no effect on tension in unstimulated preparations, whereas contractions elicited by exogenously applied ATP, KCl and noradrenaline are potentiated by carbachol, but remain unaffected by McN-A-343. Therefore, it has been postulated that the M_1-receptor is located presynaptically, and its activation inhibits transmitter (presumably ATP) release, whereas the M_2-receptors are located postsynaptically.

The postsynaptic M_2-receptor in rabbit vas deferens is of the cardiac $M_{2\alpha}$-type (Eltze, 1988b), whereas preliminary studies with analogs of McN-A-343 suggest that the presynaptic M_1-receptor in this organ is pharmacologically similar to the ganglionic M_1-subtype (Eltze, 1988a). It has become

* To whom all correspondence should be addressed: Department of Pharmacology, Byk Gulden Pharmaceuticals, D-7750 Konstanz, F.R.G.

evident recently that M_1-receptors, similar to M_2-receptors (Eglen and Whiting, 1986; Lambrecht et al., 1987; Melchiorre et al., 1987), can be further subdivided by functional methods (Lambrecht et al., 1987, 1988a). The most primising tools for this were the muscarinic antagonists hexahydro-difenidol and hexocyclium. The antagonists display considerably higher affinity (159- and 63-fold, respectively) for the M_1-receptors of rat isolated superior cervical ganglia, which mediate depolarization, than for those present in rat hippocampal pyramidal cells, which mediate an increase in the spike rate (Lambrecht et al., 1988b). Based on these antagonist potencies and on the results obtained with the agonist, McN-A-343 (see Discussion), it was proposed that M_1-receptors should be subclassified into two subtypes (Lambrecht et al., 1987, 1988b; Mutschler et al., 1987, 1988): $M_{1\alpha}$ (hippocampal type) and $M_{1\beta}$ (ganglionic type).

To further characterize the presynaptic M_1-receptors in rabbit vas deferens, the affinities of hexocyclium, hexahydro-difenidol and several analogs (fig. 2) for the M_1-receptors in this organ were compared with those for the receptors in rat isolated superior cervical ganglia. We also compared the agonistic potencies of McN-A-343 and some analogs (fig. 1) to inhibit neurogenic contractions in rabbit vas deferens with their potencies to raise blood pressure in pithed rats.

2. Materials and methods

2.1. Isolated rabbit vas deferens

Male New Zealand white rabbits (2.5-3.0 kg) were killed by exsanguination after the animals had been anaesthetized with pentobarbitone sodium (60 mg/kg i.v.) and the vasa deferentia were removed. The organs were carefully dissected free of surrounding tissue and divided into four segments, two prostatic portions (1 cm) and two epididymal portions (approximately 1.5 cm). Each segment was folded in two and fixed vertically in

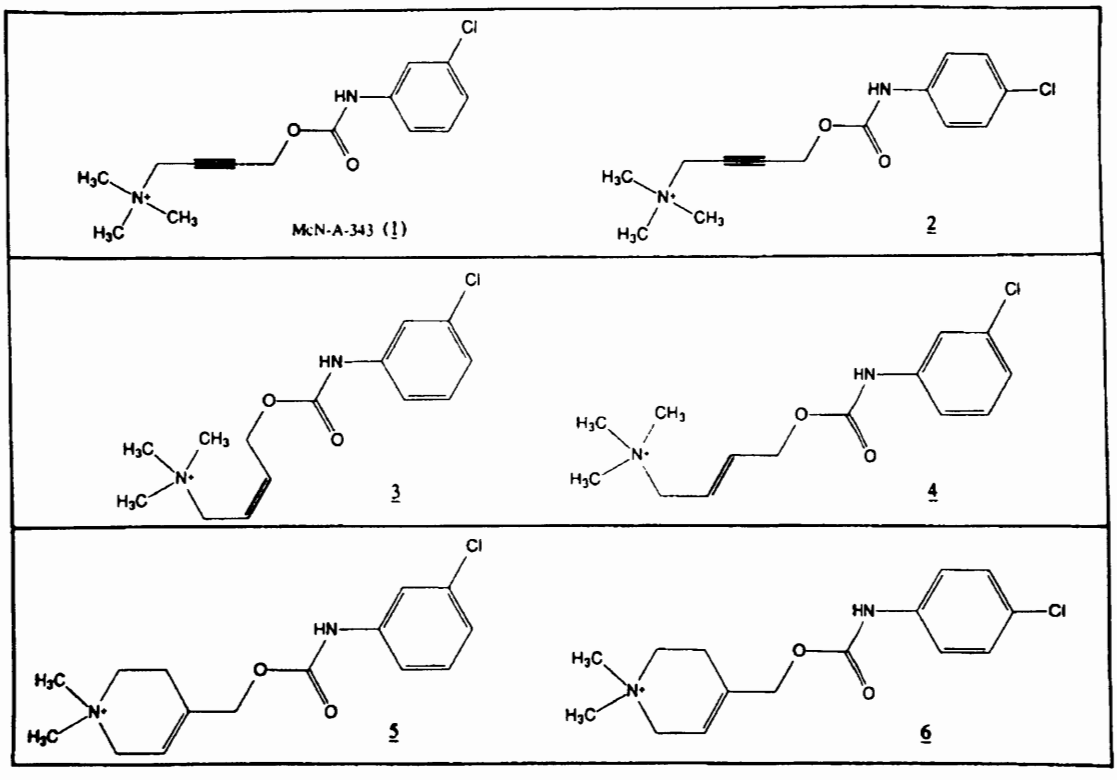

Fig. 1. Chemical structure of McN-A-343 (**1**) and analogs **2-6**.

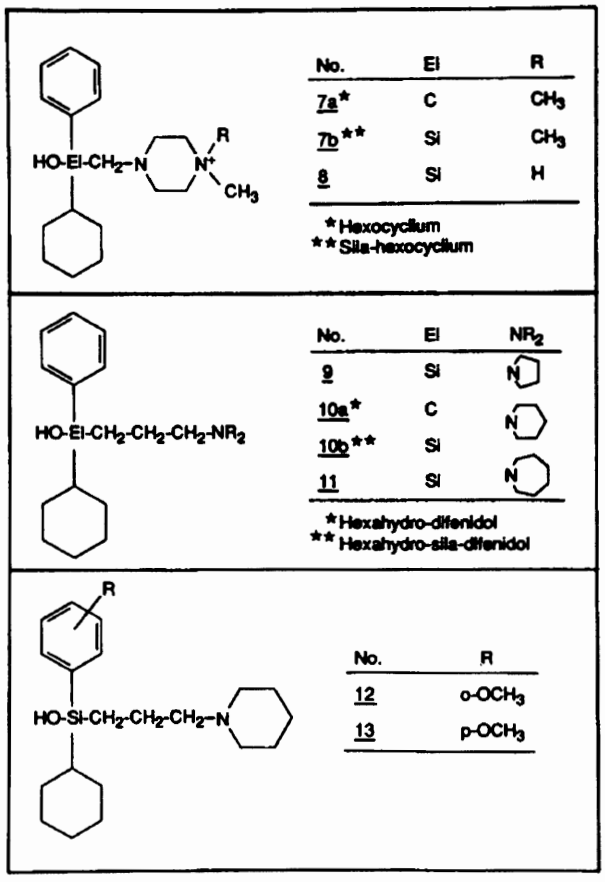

Fig. 2. Chemical structure of antagonists of the hexocyclium (**7a**) and hexahydro-difenidol (**10a**) type.

a 10 ml water-jacketed organ bath by means of a hook-shaped platinum electrode and a cotton thread connected to a force-displacement transducer. A second platinum ring electrode was placed at the top of the bathing fluid which consisted of (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.1; 10^{-6} M yohimbine was included to block α_2-adrenoceptors.

The bath solution was maintained at 31° C and was continuously bubbled with 95% O_2-5% CO_2. The preparations were preloaded with 0.75 g and were left to equilibrate for 30 min before starting the continuous field stimulation (0.5 ms, 30 V, 0.05 Hz). The contractions of eight preparations, run in parallel, were measured isometrically (K-30, Hugo Sachs Elektronik) and recorded on multichannel recorders (Kipp and Zonen, BD 9).

2.1.1. Agonist potencies

Following the stabilization of the contractile response to electrical field stimulation, cumulative concentration-response curves to muscarinic receptor agonists were made. The concentration of agonist was increased as soon as a stable response to the previous concentration was achieved. The apparent potency of an agonist was expressed by its $-\log EC_{50}$ value, i.e. the $-\log$ of the molar concentration that induced a response that was 50% of the individual maximal effect.

2.1.2. Antagonist affinities

Eight preparations were used to determine two or three reproducible concentration-response curves for McN-A-343 (**1**) (10^{-7}-2×10^{-6} M). Subsequent curves were then obtained at 45 min intervals, the antagonist being added 15 min before the agonist. Schild plots were constructed from the dose-ratios of the agonist obtained for three to five different antagonist concentrations to estimate the pA_2 value with confidence limits and the slope of the regression line (Arunlakshana and Schild, 1959). The experimental data were fitted by a conventional least-squares method (Waud and Parker, 1971).

2.2. Isolated superior cervical ganglion of the rat

Experiments on ganglia were performed as described by Brown et al. (1980). Superior cervical ganglia were excised from male Sprague-Dawley rats (200-300 g) that had been anaesthetized with urethane (1.2 g/kg i.p.). Each ganglion was desheathed, suspended vertically in a separate heated chamber (36° C) and superfused with oxygenated (95% O_2-5% CO_2) Krebs solution (1 ml/min) which consisted of (mM): NaCl 124.0, KCl 3.0, $NaHCO_3$ 26.0, NaH_2PO_4 1.25, $CaCl_2$ 2.0, $MgCl_2$ 2.0 and glucose 10.0. The muscarine-induced depolarization ($pD_2 = 7.4$) was recorded differentially, via two calomel electrodes, between the ganglion and its postganglionic trunk. The DC potentials were amplified by microvoltmeters (KEITHLEY 177) and were monitored on a chart recorder. To obtain dose-response curves, single doses of muscarine were applied at 20-45 min intervals, followed by a washout phase until the baseline was reached.

To determine the antagonistic effects of the drugs, dose-response curves for muscarine were obtained before and after the addition of an antagonist which was allowed to equilibrate for 30 min. Two or three different concentrations of each antagonist were tested and the dose ratios for the antagonist-induced parallel shifts of the muscarine dose-response curves were estimated. The pA_2 values were calculated from individual dose ratios according to Arunlakshana and Schild (1959).

2.3. Pithed rat preparation

Male Wistar rats (200-350 g) were anaesthetized with pentobarbitone sodium (60 mg/kg i.p.). The left jugular vein was cannulated for the administration of drugs. Arterial blood pressure was measured from the cannulated right common carotid artery by means of a Statham pressure transducer connected to a Hellige amplifier and a Rikadenki recorder. After cannulation of the trachea, the rats were pithed by introducing a steel rod into the spinal canal. The rats were artificially respirated with room air by means of a Braun-Melsungen pump (1 ml/100 g body weight; 60 strokes/min). Heparin (150 I.U./kg i.v.) was given to prevent coagulation of the blood. Body temperature was kept at $37 \pm 1°$ C throughout the experiment by means of an overhead heating lamp.

2.3.1. Experimental protocol

All agonists were dissolved in saline (0.9% w/v) and increasing doses were injected i.v. in a volume of 0.1 ml/100 g at 10-15 min intervals until an increase in mean arterial pressure of about 80-100 mm Hg was obtained. As a measure of agonist potencies, the doses that caused a blood pressure increase of 70 mm Hg (ED_{70} values) were determined graphically from the dose-response curves.

2.4. Drugs

The following drugs were used: heparin sodium (Promonta); pentobarbitone sodium (Abott); pirenzepine dihydrochloride (Boehringer Ingelheim); McN-A-343 (**1**), 4-[m-chlorophenylcarbamoyloxy]2-butynyltrimethylammonium chloride (RBI, Wayland, USA); Atropine sulfate, d,l-muscarine chloride, yohimbine hydrochloride (Sigma). McN-A-343 analogs 3 and **4** were kindly donated by Dr. W.L. Nelson, University of Washington, USA. Compounds **2** and **5-13** were synthesized in our laboratories (Lambrecht et al., 1986: **2**, **5**, **6**; Zaugg et al., 1958: **7a**; Tacke et al., 1988: **7b**, **8**; Tacke et al., 1985: **10a**, **10b**; compounds **9** and **11-13** were synthesized by a method similar to that used to prepare the parent compound **10b**, unpublished results).

3. Results

3.1. Response of the rabbit vas deferens to muscarinic agonists

Electrical field stimulation of the isolated rabbit vas deferens elicited individual phasic contractions of the 'rapid twitch' type, which were reproducible for more than 6 h. McN-A-343 (**1**) and the analogs **2-6** caused a dose-dependent inhibition of these twitch responses (fig. 3). However, only McN-A-343 (**1**) and its 4-chloro-phenyl derivative **2** completely inhibited the twitch contractions at high concentrations (fig. 3, table 1). Whereas **2** proved to be 2.3 times more potent than McN-A-343 itself, the olefinic derivatives **3** and **4** showed a relative potency (compared to McN-A-343) of 0.13 and 0.52, respectively (fig. 3, table 1). The maximal inhibition of twitch responses achieved by the cis-isomer **3** (36% inhibition) was considerably lower than that obtained with the trans-analog **4** (84% inhibition). The cyclic isoarecolinol derivatives **5** and **6** proved to be weak partial agonists, whereby the 4-chloro derivative **6** showed a somewhat greater efficacy. The twitch-inhibiting effect of McN-A-343 (**1**) and analogs **2-6** was readily reversible on washing. All agonists tested had no effect on the base-line tension of the stimulated or quiescent organs at concentrations that markedly inhibited the twitch contractions.

3.2. Effect of muscarinic antagonists in rabbit vas deferens

Atropine, pirenzepine and compounds **7-13** inhibited the McN-A-343-induced decrease in con-

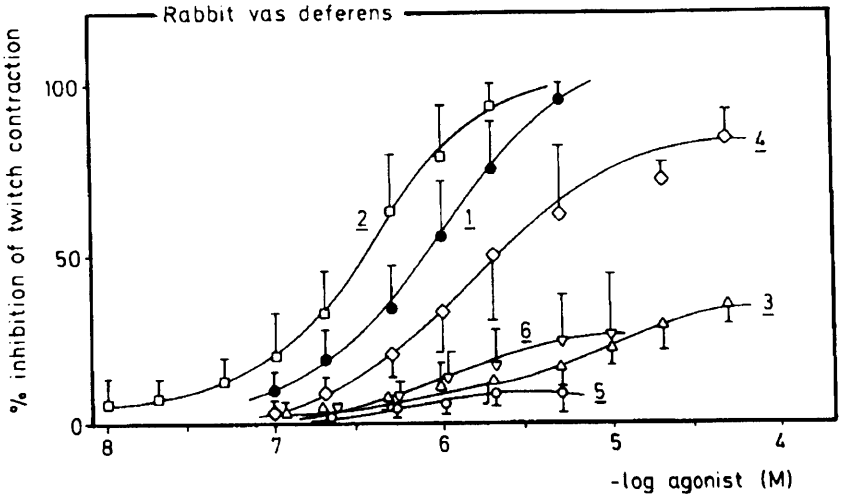

Fig. 3. Concentration-response curves for the inhibitory effects of McN-A-343 (**1**) and analogs **2-6** on contractions induced by field stimulation in the rabbit vas deferens (means ± S.D., n = 12-22).

traction amplitude in the field-stimulated rabbit vas deferens. All antagonists shifted the McN-A-343 dose-response curves to the right in a parallel fashion and did not depress the maximal inhibition caused by the agonist (data not shown), thus indicating competitive antagonism. However, in some cases (atropine, **7a**, **7b**, **10a**, **11**, **12**), the slopes of the Schild plots were found to be significantly different from unity (table 2). As shown in table 2, the affinities of atropine, pirenzepine and compounds **7-13** for muscarinic M_1-receptors in rabbit vas deferens differed by more than two orders of magnitude.

TABLE 1

Potency of McN-A-343 (**1**) and analogs **2-6** to inhibit twitch contractions in the field-stimulated rabbit vas deferens.

Agonist	$-\log EC_{50}$ [a] Mean (95% conf. lim.)	Maximal effect [b] (mean ± S.D.)	Potency [c]	n
McN-A-343 (**1**)	6.10 (5.53; 6.66)	1.00	1.00	18
2	6.46 (5.83; 7.10)	1.00	2.3	22
3	5.22 (4.57; 5.81)	0.36 ± 0.07	0.13	12
4	5.82 (5.33; 6.31)	0.84 ± 0.08	0.52	14
5	-	0.09 ± 0.06	-	19
6	≈ 6.0 (-; -)	0.26 ± 0.19	0.79	17

[a] Negative log molar concentration producing 50% of the maximum response. [b] Maximal inhibition obtained in relation to the maximal effect of McN-A-343 (= 1.00). [c] Relative potency was calculated from the antilog of differences between the $-\log EC_{50}$ values for the respective agonist and for McN-A-343 which has been arbitrarily assigned a value of 1.00.

3.3. Effect of muscarinic antagonists in rat superior cervical ganglia

The muscarine-induced depolarization of the ganglia was antagonized by atropine, pirenzepine

TABLE 2

The affinities of the antagonists for muscarinic M_1-receptors in rabbit vas deferens, related to their inhibition of the McN-A-343-induced decrease of contraction to field stimulation. The pA_2 values (mean with 95% confidence limits) and slopes of regression lines (mean ± S.D.) were calculated from Schild plots.

	pA_2	Slope	n
Atropine	9.16 (8.22; 10.21) [a]	1.69 ± 0.20 [b]	12
Pirenzepine	7.64 (7.13; 8.19) [a]	1.22 ± 0.09	16
7a [c]	8.99 (8.35; 9.63)	1.44 ± 0.08 [b]	19
7b [d]	8.95 (8.37; 9.53)	1.34 ± 0.23 [b]	20
8	7.60 (7.06; 8.14)	1.20 ± 0.11	10
9	7.76 (7.04; 8.51)	1.07 ± 0.04	12
10a [e]	7.75 (7.21; 8.28)	1.22 ± 0.06 [b]	12
10b [f]	7.88 (7.42; 8.38) [a]	1.09 ± 0.03	19
11	7.02 (6.37; 7.67)	1.23 ± 0.07 [b]	12
12	6.77 (6.15; 7.39)	1.21 ± 0.06 [b]	10
13	6.72 (6.20; 7.25)	1.06 ± 0.04	11

[a] Data taken from Eltze (1988b). [b] Slope significantly different from unity ($P < 0.05$). [c] Hexocyclium. [d] Sila-hexocyclium. [e] Hexahydro-difenidol. [f] Hexahydro-sila-difenidol.

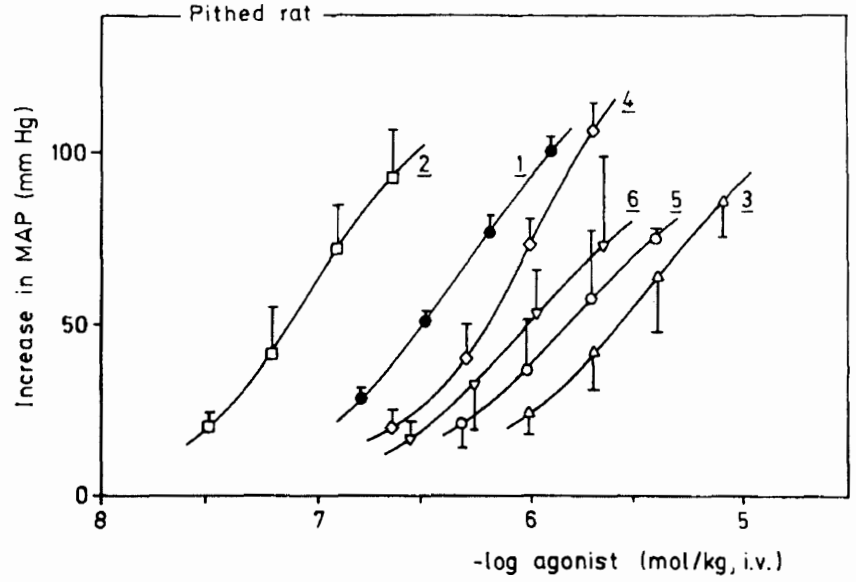

Fig. 4. Dose-response curves for the increase in mean arterial pressure (MAP) caused by McN-A-343 (**1**) and analogs **2-6** in the pithed rat (means ± S.D., n = 2-7).

and compounds **7-13**. All antagonists produced a parallel shift of the muscarine dose-response curve, and none of these drugs consistently reduced the maximum response to muscarine, obviously indicating competitive antagonism. As shown in table 3, compound **7-13** showed quite wide variations in their affinity for ganglionic muscarinic M_1-receptors. Their pA_2 values differed by up to three orders of magnitude.

TABLE 3

The affinities of the antagonists for muscarinic M_1-receptors in rat superior cervical ganglia, related to their inhibition of the muscarine-induced depolarization. The mean pA_2 values and their 95% confidence limits are given. n indicates the number of independent experiments.

	pA_2	n
Atropine	9.1 (9.0; 9.2)	10
Pirenzepine	8.3 (8.2; 8.4) [a]	12
7a [b]	8.8 (8.5; 9.1)	6
7b [c]	9.6 (9.2; 10.0)	12
8	7.6 (7.3; 7.9)	15
9	7.7 (7.5; 7.9)	15
10a [d]	7.9 (7.6; 8.2)	6
10b [e]	7.3 (7.2; 7.4)	12
11	6.9 (6.6; 7.2)	8
12	6.7 (6.4; 7.0)	9
13	6.4 (6.2; 6.6)	10

[a] Data taken from Lambrecht et al. (1988a). [b] Hexocyclium. [c] Sila-hexocyclium. [d] Hexahydro-difenidol. [e] Hexahydro-sila-difenidol.

3.4. Pressor effects of muscarinic agonists in the pithed rat

The initial mean arterial pressure of the pithed rats prior to drug treatment was 50 ± 9 mm Hg (mean ± S.D., n = 22). McN-A-343 (**1**) and the analogs **2-6** caused a dose-dependent rise in the mean arterial pressure of the pithed rat (fig. 4). All pressor responses were highly sensitive to blockade by low doses (100-300 μg/kg i.v.) of the

TABLE 4

The pressor activity of McN-A-343 (**1**) and analogs **2-6** in pithed rats. The $-\log ED_{70}$ values refer to the doses of the agonists that cause an increase of 70 mm Hg in the mean arterial blood pressure. The values are presented as the means with 95% confidence limits (n = 2-7).

Agonist	$-\log ED_{70}$ (mol/kg i.v.)
McN-A-343 (**1**)	6.27 (6.23; 6.31)
2	6.93 (6.73; 7.13)
3	5.35 (5.18; 5.52)
4	6.04 (5.98; 6.10)
5	5.60 (5.31; 5.89)
6	5.89 (5.69; 6.09)

selective M_1-receptor antagonist, pirenzepine (data not shown). As a measure of the agonist potencies of these compounds, $-\log ED_{70}$ values (ED_{70} = agonist dose corresponding to an increase in mean arterial pressure by 70 mm Hg) are listed in table 4. The 4-chlorophenyl derivative **2** ($ED_{70} = 0.12$ μmol/kg i.v.) was ca. 4.5 times more potent than McN-A-343 ($ED_{70} = 0.53$ μmol/kg i.v.). Whereas the trans-olefinic analog **3** ($ED_{70} = 0.91$ μmol/kg i.v.) showed a relative potency of about 0.5 (compared to McN-A-343), the cis-olefinic isomer **4** ($ED_{70} = 4.63$ μmol/kg i.v.) was about one order of magnitude less potent than the parent compound. The two cyclic derivatives **5** and **6** ($ED_{70} = 2.77$ and 1.36 μmol/kg i.v., respectively) were less active than McN-A-343 by a factor of about 5 and 3.5, respectively.

3.5. Comparison of agonist potencies in rabbit vas deferens and pithed rats

A significant correlation was found between the $-\log EC_{50}$ values of the agonists **1-4** and **6** for the inhibition of twitch contractions in the rabbit vas deferens (table 1) and the $-\log ED_{70}$ values for the rise in mean arterial blood pressure in pithed rats (table 4). This correlation ($r = 0.95$, slope = 0.75, $P < 0.01$) is shown in fig. 5.

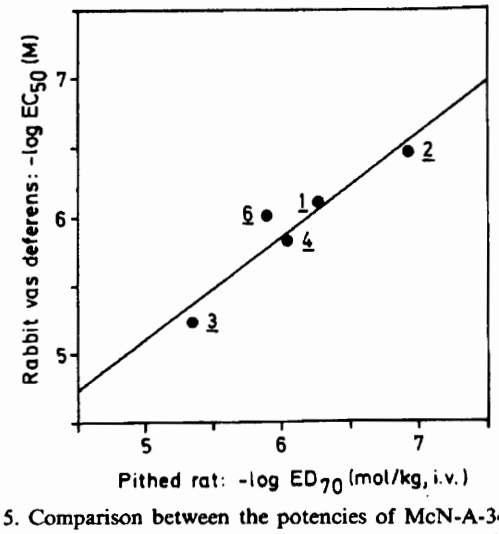

Fig. 5. Comparison between the potencies of McN-A-343 (**1**), analogs **2-4** and **6** to inhibit twitch contractions in rabbit vas deferens ($-\log EC_{50}$, M) and their ability to increase mean arterial blood pressure by 70 mm Hg ($-\log ED_{70}$, mol/kg, i.v.) in pithed rats.

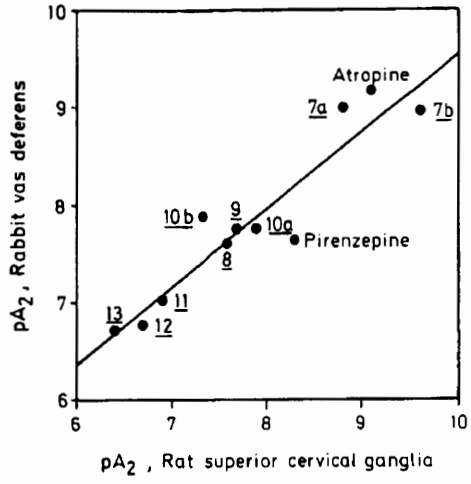

Fig. 6. Comparison between the affinities (pA_2 values) of different antimuscarinic drugs listed in table 2 and 3 for muscarinic M_1-receptors in rabbit vas deferens and rat superior cervical ganglia.

3.6. Comparison of antagonist affinities in rabbit vas deferens and rat superior cervical ganglia

A significant correlation ($r = 0.94$, slope = 0.80, $P < 0.001$; fig. 6) was found between the pA_2 values for atropine, pirenzepine and compounds **7-13** obtained in rabbit vas deferens (table 2) and those obtained in rat ganglia (table 3).

4. Discussion

It has been shown recently (Eltze, 1988a,b) that the McN-A-343-induced inhibition of neurogenic twitch contractions of the isolated rabbit vas deferens is mediated by stimulation of presynaptic muscarinic heteroreceptors of the M_1-type. The transmitter released by field stimulation appears to be ATP (Eltze, 1988b; Sneddon et al., 1984).

Recent evidence suggests that M_1-receptors do not form a homogeneous population, but may be further subclassified into at least two subtypes: $M_{1\alpha}$- (hippocampal type) and $M_{1\beta}$- (ganglionic type) receptors (Lambrecht et al., 1987; 1988b; Mutschler et al., 1987; 1988). In the present study, we have investigated the actions of a series of agonists and antagonists at M_1-receptors in the isolated rabbit vas deferens and compared these actions with their effects at M_1-receptors in rat

sympathetic ganglia in vitro and in vivo. The principal conclusion we draw from these experiments is that the presynaptic M_1-receptors present in the rabbit vas deferens are pharmacologically very similar to those that mediate depolarization in rat sympathetic ganglia.

McN-A-343 (**1**) and analogs **2-6** produced a concentration-dependent inhibition of twitch contractions in the electrically stimulated rabbit vas deferens (fig. 3). However, classical muscarinic agonists, such as carbachol or oxotremorine, caused a potentiation of twitch contractions via activation of postsynaptic $M_{2\alpha}$- (M_2 cardiac type) receptors (Eltze, 1988a,b). As the inhibitory effects of McN-A-343 in the rabbit vas deferens have been shown to be mediated by the activation of presynaptic M_1-receptors (Eltze, 1988a,b), these findings further support the view that McN-A-343 and several of its analogs may be regarded as selective M_1-receptor stimulants (Hammer and Giachetti, 1982; Lambrecht et al., 1986; Micheletti et al., 1988).

In the pithed rat, McN-A-343 and the analogs **2-6** produced dose-dependent pressor effects (fig. 4) which are known to be mediated by stimulation of M_1-receptors in sympathetic ganglia (Hammer and Giachetti, 1982; Wess et al., 1984; Lambrecht et al., 1986). Interestingly, the agonist potencies of compounds **1-6** on the vas deferens and in pithed rats were strikingly similar, as measured by their EC_{50} and ED_{70} values (table 1 and 4), both individually and in rank order. A highly significant correlation was found between these values (fig. 5). This implies that the structural and stereochemical demands on compounds **1-6** made by the M_1-receptors of the vas deferens and sympathetic ganglia are similar. 4-Chloro substitution of both McN-A-343 (**1**) and the quaternary isoarecolinol derivative **5** led to an increase in potency (compounds **2** and **6**). Thus, the 4-chloro McN-A-343 analog **2** represents the most potent M_1-agonist known so far. The trans-olefinic analog **4** was found to be nearly as potent as McN-A-343 (**1**) in rabbit vas deferens and pithed rats, whereas the cis-isomer **3** was about one order of magnitude less potent in both preparations. This observation might be explained by the fact that certain low energy conformations of McN-A-343 can be closely approximated by the trans-stereoisomer **4**, in which the quaternary nitrogen atom and the carbamate ether oxygen are about 0.57 nm apart in a nearly fully extended conformation (fig. 1). In contrast, the cis-isomer **3** cannot be fit to such a pattern (Nelson et al., 1973).

Atropine, pirenzepine and compounds **7-13** caused a concentration-dependent blockade of muscarinic M_1-receptors in the rabbit vas deferens and rat superior cervical ganglia. The concentration-response curves for the agonists, McN-A-343 and muscarine, were shifted to the right in a parallel fashion and the maximum effects of the agonists were not depressed. However, in some cases the slopes of the Schild plots obtained from the rabbit vas deferens experiments were found to be significantly greater than unity. Thus, the respective pA_2 values might be underestimated slightly. An insufficient equilibration time might account for this phenomenon (Kenakin, 1982).

Compounds **7-13** showed quite wide variations in their affinities for the M_1-receptors in both vas deferens and ganglia (table 2 and 3), their pA_2 values differing by nearly three orders of magnitude. The pA_2 values determined for pirenzepine (7.64 and 8.3, respectively) were close to the M_1-affinities found previously in binding studies with calf sympathetic and human stellate ganglia ($-\log K_i = 8.0$ and 7.85, respectively) (Giraldo et al., 1985; Watson et al., 1984). Moreover, the sensitivities of the M_1-receptors in the rabbit vas deferens and in rat superior cervical ganglia to atropine and compounds **7-13** were also strikingly similar, as shown by their pA_2 values (table 2 and 3; fig. 6), again both individually and in rank order. This means that the structural demands on the antagonists made by the M_1- receptors are very similar in both tissues.

Taking into account that most of the antagonists used in this study (**7a**, **7b**, **8**, **9**, **10a**, **10b** and **12**) possess a higher affinity for ganglionic $M_{1\beta}$- (this study) than for hippocampal $M_{1\alpha}$-receptors (Lambrecht et al., 1987, 1988b) by factors of 10-160, these data indicate that the muscarinic inhibition of neurogenic contractions in rabbit vas deferens is mediated by presynaptic $M_{1\beta}$-receptors (M_1 ganglionic type). This view is further supported by the observation that the M_1-agonist,

McN-A-343 (**1**), is highly potent at ganglionic $M_{1\beta}$-receptors in vitro (Palacios et al., 1986) and at M_1-receptors in the rabbit vas deferens but has only very weak, if any, stimulating properties at hippocampal $M_{1\alpha}$-receptors (Gmelin, 1985).

In conclusion, we have provided pharmacological evidence that the muscarinic presynaptic heteroreceptors in rabbit vas deferens that mediate inhibition of neurogenic contractions are similar to the $M_{1\beta}$-receptors in rat sympathetic ganglia in terms of agonist potencies and antagonist affinities. Presynaptic M_1-receptors have also been found in chicken heart (Jeck et al., 1988), different guinea-pig and rat brain areas (Belleroche and Gardiner, 1985; Marchi et al., 1986; Williams and Constanti, 1988) as well as in guinea-pig and rat small intestine (Micheletti et al., 1988; Schwörer and Kilbinger, 1988). However, it remains to be elucidated whether all these presynaptic muscarinic receptors are pharmacologically identical or whether they belong to different M_1-receptor subclasses.

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