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Inhibition of field stimulation-induced contractions of rabbit vas deferens by muscarinic receptor agonists: selectivity of McN-A-343 for M_1 receptors

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Abstract

Inhibition of the field stimulation-induced twitch responses of the rabbit vas deferens by the muscarinic receptor agonist, McN-A-343, has been attributed to presynaptic muscarinic receptors of the M_1 subtype located on noradrenergic nerve terminals. Stimulation of these receptors causes inhibition of transmitter release and inhibition of the contractile response. However, the selectivity of McN-A-343 for M_1 receptors has been questioned and this throws doubt on whether the prejunctional receptors of the rabbit vas deferens are of the M_1 subtype. In this study we have undertaken a comprehensive re-evaluation of the inhibition of prostatic and epididymal portions of the rabbit isolated field-stimulated vas deferens by several agonists, including McN-A-343, and quantified the antagonism by M_1-selective antagonists, pirenzepine and telenzepine.

Prostatic and epididymal portions of vasa deferentia from New Zealand White rabbits were immersed in a low Ca^{2+} Krebs solution at 32 ± 0.5°C gassed with 5 % CO_2 in oxygen. Yohimbine (1.0mM) was present throughout to block prejunctional α_2-adrenoceptors. Field stimulation was applied by repeated application of single pulses (30 V, 0.05 Hz, 0.5 ms) and isometric contractions recorded. Carbachol and oxotremorine initially potentiated the epididymal contractions but at higher concentrations there was inhibition. In the prostatic portion, oxotremorine only inhibited. McN-A-343 produced inhibitory responses only in both epididymal and prostatic portions. Pirenzepine shifted the concentration–response curves for the inhibitory responses to oxotremorine to the right. However, the potentiation of the twitches also became more apparent with the lower concentrations of oxotremorine. Schild plots for the antagonism by pirenzepine yielded pA_2 values of 7.96 ± 0.004 and 7.7 ± 0.02 for the epididymal and prostatic portions, respectively. The concentration–response curves for the inhibition of twitches by McN-A-343 were displaced to the right in a parallel manner by pirenzepine in both prostatic and epididymal portions with no potentiation of the twitches. The Schild plot for this antagonism generated pA_2 values of 7.68 ± 0.01 and 8.07 ± 0.01, respectively. Telenzepine caused parallel shifts of the McN-A-343 concentration–response curves to the right in prostatic portions, the pA_2 value being 8.70 ± 0.13. Telenzepine (10^{-7}M) abolished the inhibitory effect of carbachol to reveal only concentration-dependent potentiation of the contractions. The Schild plot for antagonism of this contractile effect yielded a pA_2 value (7.07 ± 0.09) that was significantly less by almost two orders of magnitude (1.70) than the value for the antagonism by telenzepine of the McN-A-343-induced inhibitory response.

The pA_2 values of pirenzepine and telenzepine against the inhibitory responses of the rabbit vas deferens are consistent with the involvement of M_1 receptors. This leads to the conclusion that McN-A-343 causes inhibition through this receptor type. The doubts concerning the selectivity of McN-A-343 for M_1 receptors are therefore unfounded. The fact that McN-A-343 does not display a selective binding profile suggests that its selectivity does not arise from affinity differences but probably resides in its intrinsic efficacy.

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Introduction

Muscarinic receptors have now been subclassified by functional pharmacological tests and radioligand binding into four types, M_1 to M_4, with an additional cloned m5 receptor. The mRNA for an m5 receptor has been identified in the brain, but a functional M_5 receptor has yet to be identified (Caulfield 1993). The basis of subclassification has been the use of selective agonists and antagonists. M_1 muscarinic receptors have been identified by the use of pirenzepine which was shown to selectively bind to receptors in the cerebral cortex and hippocampus areas of the brain (Hammer et al 1980). One of the earliest agonists to be identified with potential selectivity for one of the subtypes was McN-A-343, since it was a selective stimulant of autonomic ganglia and increased blood pressure rather than lowering it, as would be the case with usual muscarinic receptor agonists such as acetylcholine (Roszkowski 1961). Stimulation of the rat superior cervical ganglion induced a slow excitatory postsynaptic potential (s-EPSP) in the postganglionic neurone, which was not mediated via nicotinic receptors but via muscarinic receptors since the response was inhibited by atropine and pirenzepine (Newberry & Priestley 1987). These muscarinic receptors have therefore been classified as M_1 receptors. McN-A-343 produces negligible responses of isolated atria mediated via M_2 receptors or contractions of smooth muscle in trachea, ileum or urinary bladder mediated via M_3 receptors (Eglen et al 1987).

McN-A-343 produces inhibition of the twitch responses of the rabbit vas deferens produced by field stimulation (Eltze 1988; Choo & Mitchelson 1990; Shannon et al 1993). This response has therefore been attributed to presynaptic muscarinic receptors of the M_1 subtype located on noradrenergic nerve terminals, stimulation of which causes inhibition of transmitter release and inhibition of contractile function (Grimm et al 1994). However, the selectivity of McN-A-343 for M_1 receptors has been questioned (Eglen et al 1987). Eglen et al (1987) suggested that the ability of McN-A-343 to contract the taenia caeci, without eliciting responses of guinea-pig ileum (M_3), atria (M_2), bladder (M_3) or trachea (M_3), was not due to the taenia caeci response being mediated via M_1 receptors. They suggested that the receptor type was identical to that in the ileum, bladder and trachea (M_3) but the response was produced because of a substantial receptor reserve in this tissue and a greater intrinsic efficacy. The basis of this conclusion is that the affinity of McN-A-343, measured as the dissociation constant for displacement of [^{3}H]-methylscopolamine binding, does not display selectivity between muscarinic receptor subtypes (Eglen et al 1987) and is at best only two-fold. This doubt concerning the M_1 receptor selectivity of McN-A-343 therefore has implications with regard to the classification of the prejunctional receptors in the rabbit vas deferens, which have been based on studies with McN-A-343. Further complications arise from the fact that some authors report differences in the magnitude of the inhibition of twitch height in the prostatic and epididymal portions of the vas deferens (Eltze 1988; Dorje et al 1991) whereas others have not (Shannon et al 1993). We have therefore undertaken a comprehensive re-evaluation of the inhibitory response of both the prostatic and epididymal portions of the rabbit isolated field-stimulated vas deferens using several agonists, including McN-A-343, and pirenzepine and telenzepine as antagonists. We have quantified and characterized the antagonist-induced shifts of concentration–response curves by classical Schild analysis.

Materials and Methods

Drugs

Carbamoylcholine chloride (carbachol), pirenzepine dihydrochloride and yohimbine hydrochloride were obtained from Sigma (Poole, Dorset, UK) and McN-A-343 [4-(4-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium iodide], oxotremorine sesquifumarate and telenzepine dihydrochloride were obtained from RBI (St Albans, UK). All drugs were dissolved in distilled water initially and dilutions made in Krebs solution.

Vas deferens preparations

Male New Zealand White rabbits (1.47–3.4 kg) were killed by a sharp blow to the back of the head and exsanguinated. Vasa deferentia were removed, dissected free of connective tissue and divided into prostatic and epididymal portions. Each segment was mounted on a tissue holder and passed through two ring electrodes (5 mm apart). They were immersed in a modified low Ca^{2+} Krebs solution, composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ (as the dihydrate) 1.8, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4 7H_2O$ 2.4, glucose 11.1 at 32 $\pm$ 0.5°C and gassed with 5% CO_2 in oxygen. Yohimbine (1.0 mM) was present throughout to block prejunctional α_2-adrenoceptors. The upper end of the tissue was attached by cotton thread to an isometric transducer (MLT050, ADInstruments). Tissues were left to equi-

librate for at least 45 min at passive force of 0.75–1 g. Field stimulation was then applied by repeated application of single pulses (30 V, 0.05 Hz, 0.5 ms). Isometric tension was recorded by computer at a sampling rate of 100 Hz, using Powerlab/200 (ADInstruments) software and MacLab bridge amplifiers.

Agonist concentration–response curves

Cumulative concentration–response curves for the muscarinic agonists were constructed following stabilization of the twitch responses to field stimulation. The concentration was increased in half logarithmic increments after the contraction, in the presence of each concentration, had plateaued. Steady-state contractions at each concentration were measured and expressed as a percentage of the baseline twitch height.

Effects of antagonists

A concentration–response curve to the test agonist was established in the absence of antagonist, and after achieving the maximum effect the agonist was washed from the bath to restore twitch contractions. Three further concentration–response curves were then obtained in the same manner at approximately 30 min intervals, with the antagonist being introduced to the bath 15 min before each of these subsequent curves. The concentrations of pirenzepine and telenzepine were added in ascending order (30, 100 and 300 nM or 100 nM, 300 nM and 1 μM, respectively). To allow for any time- and agonist exposure-dependent changes in sensitivity, control experiments were performed in which each tissue was exposed to four concentration–response curves but without the addition of antagonist.

Calculation of pA_2 values

Concentration–response curves in the absence and presence of antagonist were measured as described for the agonist studies. Where curves were biphasic, consisting of an initial increase in twitch height followed by the inhibition, the potentiation and inhibitory portions were measured separately and expressed as a percentage of the baseline twitch height prior to commencing the agonist addition. EC50 values were determined from individual curves as the molar concentration required for 50% inhibition of twitch height, by linear interpolation between points on either side of the 50% response. These were converted to the negative logarithm and the mean EC50 values $\pm$ s.e.m. were calculated.

The shifts in the concentration–response curves in the presence of antagonist compared with the absence of antagonist were expressed as the dose-ratios (DR) of the EC50 values. Any changes in EC50 values in control experiments were also expressed as control dose-ratios and the test dose-ratios corrected by dividing by the control ratios. pA_2 values were then determined from Schild analysis of plots of the mean corrected $-\log(DR-1)$ against log molar concentration of antagonist (Arunlakshana & Schild 1959). The slopes of the Schild plots were determined by linear regression and the pA_2 values determined from the intercept on the concentration axis (when $-\log(DR-1)$ is zero). pA_2 values were also determined from individual concentrations of antagonist by applying the equation:

$$pA_2 = \log(DR-1) - \log[B]$$

where B is the molar concentration of antagonist (Mackay 1978).

All results are presented as the mean $\pm$ s.e.m for at least four experiments. Statistical analysis of differences between two means were made by Student's *t*-test for unpaired observations, $P < 0.05$ was considered significant.

Results

Effects of agonists on unstimulated vas deferens

Neither carbachol nor McN-A-343 produced any change in tone of the unstimulated prostatic vas deferens up to concentrations of 20 μM.

Effects of agonists on field-stimulated epididymal and prostatic vas deferens

Carbachol produced potentiation of the twitch responses of the vas deferens, the degree of potentiation being significantly greater in the epididymal than the prostatic portion (Figure 1). Oxotremorine caused potentiation of the epididymal contractions with lower concentrations but at higher concentrations there was inhibition, which was complete at 30 μM. In the prostatic portion there was only inhibition (Figure 1). The EC50 value in the prostatic portion ($-\log$EC50 7.27 ± 0.15) was less than in the epididymal portion ($-\log$EC50 7.37 ± 0.26) but this difference was not significant ($P > 0.05$). McN-A-343 produced inhibitory responses only in both epididymal and prostatic portions (Figure 1).

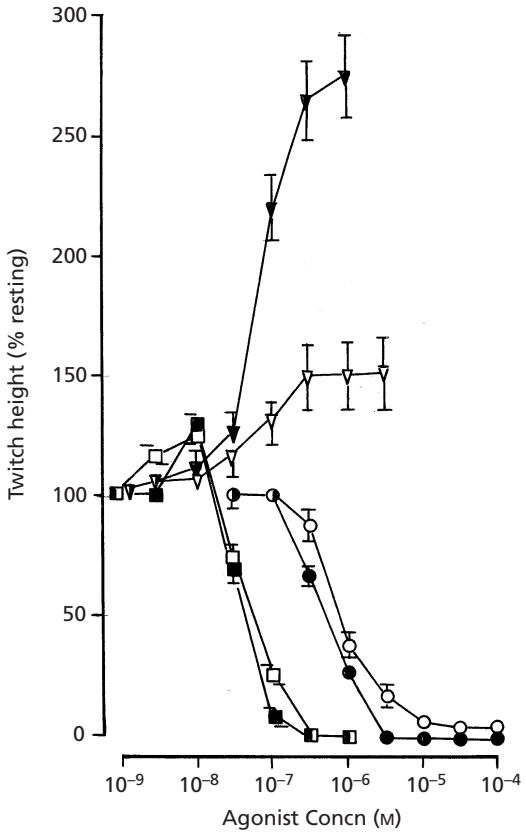

Figure 1 Effects of oxotremorine (■,□), McN-A-343 (●,○) and carbachol (▼, ▽) on the twitch contractions of rabbit vas deferens induced by field stimulation. Changes in twitch height are expressed as a percentage of the control contraction immediately before the cumulative addition of the agonist and the mean values ± s.e.m. are shown (n = 4–6). Concentration–response curves were obtained in prostatic (open symbols) or epididymal (closed symbols) portions of the vas deferens.

The sensitivity in the prostatic portion (−logEC50 6.18 ± 0.09) was slightly, but significantly greater than in the epididymal portion (−logEC50 6.3 ± 0.12).

Effects of pirenzepine on oxotremorine and McN-A-343

The maximum inhibitions of the twitch responses by oxotremorine and McN-A-343 were completely reversed by washout and this permitted the construction of four successive concentration–response curves for the agonist. These four curves were reproducible and virtually superimposable in both epididymal and prostatic portions (Figure 2).

In the presence of pirenzepine, there was a progressive shift to the right of the concentration–response curves for the inhibition of twitches by oxotremorine (Figure 3). However, the potentiation of the twitches also became more apparent with the lower concentrations of oxotremorine in both the prostatic and epididymal portions. This antagonism of the responses by pirenzepine was presented as Schild plots of $-\log(DR-1)$ against molar concentration of pirenzepine (Figure 4A). The slopes of the regression lines did not differ from unity in the case of the epididymal portions but were significantly greater than unity for the prostatic vas deferens (Table 1). The pA_2 value for the epididymal portion derived from the Schild plot was significantly ($P < 0.05$) greater than for the prostatic end (Table 1). The concentration − response curves for the inhibition of twitches by McN-A-343 were displaced to the right in a parallel manner by pirenzepine in both prostatic and epididymal portions (Figure 5). On this occasion, no potentiation of the twitches was revealed in the presence of pirenzepine. The Schild plots for this antagonism of the responses showed a slope of unity in the epididymal portion but significantly greater than unity ($P < 0.05$) in the prostatic end (Figure 4B; Table 1). The antagonism was greater in the epididymal portion than in the prostatic portion, the pA_2 value being significantly greater ($P < 0.05$) (Table 1).

Effects of telenzepine on McN-A-343 and carbachol

Telenzepine was examined against the inhibition of twitch contractions by McN-A-343 and the potentiation by carbachol using the prostatic portions of the vas deferens. In this series of experiments, after the potentiation of the contractions by lower concentrations of carbachol, there was an inhibition of the twitches at higher concentrations. In the control experiments with no antagonist included, the four concentration–response curves to either carbachol or McN-A-343 were virtually superimposable. Telenzepine caused parallel shifts of the McN-A-343 concentration–response curves to the right (Figure 6A) which were used for generating the Schild plot (Figure 7). From this, the pA_2 value and slope were obtained (Table 1), the latter being significantly greater than unity ($P < 0.05$). In view of the high slope of the Schild plot, pA_2 values were also calculated for each concentration of telenzepine by the Mackay (1978) equation. The values for 10^{-8}, 3×10^{-8} and 10^{-7}M were 8.97, 8.89 and 9.23, respectively.

Telenzepine (10^{-7} M) abolished the inhibitory effect of higher concentrations of carbachol to reveal only concentration-dependent potentiation of the contractions. The concentration–response curves for the potentiation

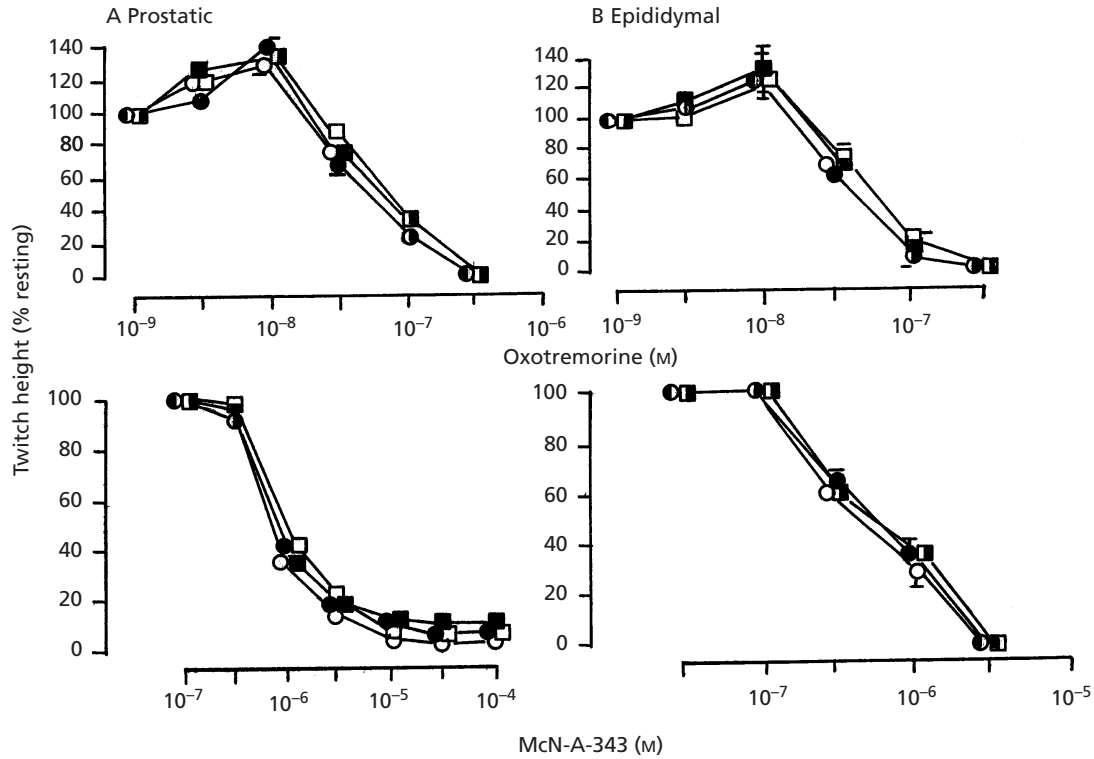

Figure 2 Control concentration–response curves for the inhibition by oxotremorine (upper panels) and McN-A-343 (lower panels) of contractions of rabbit vas deferens induced by field stimulation. Four consecutive concentration–response curves (curve 1, ●; curve 2, ○; curve 3, ■; curve 4, □) were produced in prostatic (A) and epididymal (B) portions of the vas deferens, with washout between. Changes in twitch height are expressed as a percentage of the control contraction immediately before commencing each cumulative concentration–response curve and the mean values ± s.e.m. are shown (n = 4–6).

of the twitches were therefore examined in the absence and presence of telenzepine (10^{-7}, 3×10^{-7} and 10^{-6}M). There was a progressive shift of the curves to the right but the maximum contraction was also raised to 285 ± 23% above the control contractions compared with 61 ± 14% in the absence of telenzepine (Figure 6B). The Schild plot constructed for this antagonism (Figure 7) yielded a slope not different from unity. The pA_2 value was significantly less by almost two orders of magnitude (1.70) than the value for the antagonism by telenzepine of the McN-A-343-induced inhibitory response (Table 1).

Discussion

McN-A-343 and oxotremorine caused concentration-related inhibition of contractions of the rabbit vas deferens induced by field stimulation. The key conditions that were employed to reveal this response were the low freqency of stimulation (0.05 Hz), relatively low levels of Ca^{2+} (1.8 mM) and the presence of yohimbine (1 mM) in the bathing medium, to inhibit the pre-junctional negative feedback via α_2-adrenoceptors. These results confirm observations made by others (Eltze 1988; Dörje et al 1991; Shannon et al 1993). The contractions to field stimulation were potentiated by carbachol and by low concentrations of oxotremorine. The potentiation by both carbachol and oxotremorine was greater in the epididymal than the prostatic portions, which agrees with the observation of Eltze (1988). It is at variance with Shannon et al (1993) who found little potentiating effects even with carbachol. In our study, carbachol only inhibited in the second series of experiments and at higher concentrations. The sensitivity of epididymal portions of vas deferens to the inhibitory effects of muscarinic agonists has been found to be either greater than for the prostatic end (Eltze 1988) or approximately equivalent (Shannon et al 1993). In this study, McN-A-343 was more effective in the epididymal portion. However, there was no difference in potency with oxotremorine. This may have been due to

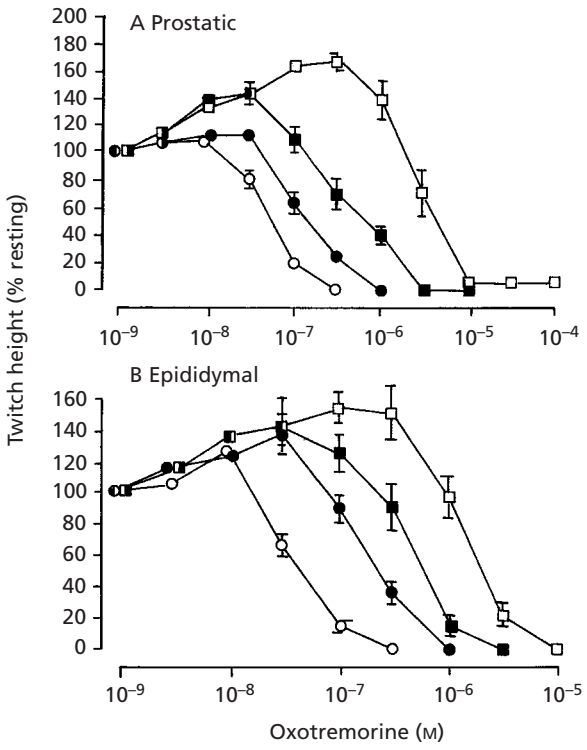

Figure 3 Effects of pirenzepine on the concentration–response curves for the inhibition by oxotremorine of field stimulation-induced contractions of prostatic (A) and epididymal (B) portions of rabbit vas deferens. Concentration–response curves were obtained in the absence (○) and then in the presence of 30 nM (●), 100 nM (■) and 300 nM (□) pirenzepine, with washout after each curve. Changes in twitch height are expressed as a percentage of the control contraction immediately before commencing each cumulative concentration–response curve and the mean values ± s.e.m. are shown (n = 4).

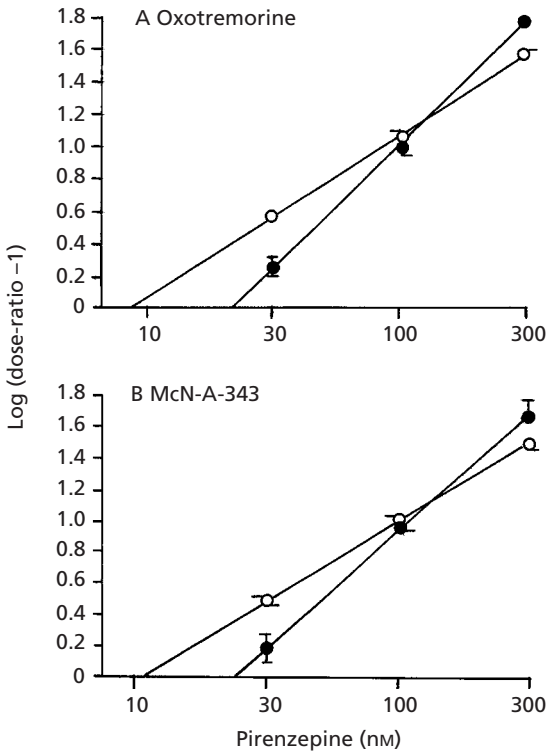

Figure 4 Schild plots for the antagonism by pirenzepine of the inhibitory responses to oxotremorine (A) and McN-A-343 (B) of field stimulation-induced contractions of rabbit vas deferens. Antagonism in the prostatic (●) and epididymal (○) portions of vas deferens are plotted. Regression lines are plotted of mean log(dose-ratio − 1) values ± s.e.m. calculated at the EC50 of each concentration–response curve against log molar concentration of pirenzepine.

the counteracting potentiating effects of this agonist, which were also greater on the epididymal portion.

The inhibition of twitches by McN-A-343 and oxotremorine were readily reversed by washout and reproducible concentration–response curves could be repeated on at least three further occasions. This permitted the estimation of antagonism by pirenzepine and telenzepine by the classical approach of constructing agonist concentration–response curves in the presence of increasing concentrations of antagonist. All curves in the presence of antagonist were constructed to the maximum effect, thereby demonstrating the surmountability of the antagonism. This differs from previous studies with telenzepine which used an inverse procedure of adding increasing concentrations of antagonist to tissues in which the twitches were inhibited by a single concentration of the agonist (Eltze 1988; Schudt et al 1989). Minor corrections were therefore necessary to adjust the dose-ratios of shifts in the curves by antagonist for time- and exposure-dependent changes in sensitivity.

The Schild plots derived from these antagonism experiments had unity slopes for the antagonism of McN-A-343 and oxotremorine by pirenzepine in the epididymal vas deferens, which indicates competitive antagonism (Kenakin 1993). The slopes for the antagonism in the prostatic portions, however, were generally greater than unity. This has been observed previously for atropine and telenzepine (Eltze 1988). A possible explanation for telenzepine is that equilibrium conditions are reached extremely slowly with this antagonist (more than 1 hour), so that the 15 min equilibrium used here may not have been sufficient. Failure to reach equilibrium would have a proportionately greater effect on lower concentrations of antagonist, steepening the Schild plot and underestimating the pA_2 value derived therefrom. Telenzepine exists as two relatively

Table 1 pA_2 values and slopes of Schild plots for the antagonism of the responses of the rabbit vas deferens to muscarinic agonists by pirenzepine and telenzepine.

Antagonist	Agonist	Prostatic		Epididymal	
		pA_2	slope	pA_2	slope
pirenzepine	McN-A-343	7.68 ± 0.01	1.50†	8.07 ± 0.01*	1.02
	oxotremorine	7.7 ± 0.02	1.49†	7.96 ± 0.004*	0.99
telenzepine	McN-A-343	8.70 ± 0.13	1.26†	ND	
	carbachol	7.07 ± 0.09	0.99	ND	

Response to McN-A-343 and oxotremorine are the inhibitions of the twitch contractions to field stimulation while the response to carbachol is the potentiation of the twitch contractions. *Significantly different from the value in the prostatic vas deferens, †Significantly different from unity slope, ND, not determined.

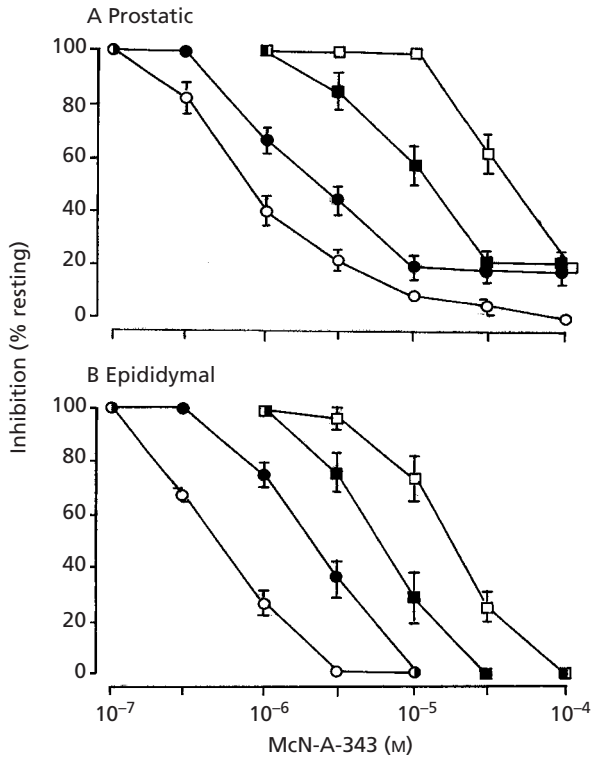

Figure 5 Effects of pirenzepine on the concentration–response curves for the inhibition by McN-A-343 of field stimulation-induced contractions of prostatic (A) and epididymal (B) portions of rabbit vas deferens. Concentration–response curves were obtained in the absence (○) and then in the presence of 30 nM (●), 100 nM (■) and 300 nM (□) pirenzepine, with washout after each curve. Changes in twitch height are expressed as a percentage of the control contraction immediately before commencing each cumulative concentration–response curve and the mean values ± s.e.m. are shown (n = 4).

stable enantiomers in the racemate, each having different kinetic properties (Schudt et al 1989). (−)-Telenzepine ($t_{\frac{1}{2}} = 3$ min) will reach equilibrium well before (+)-telenzepine ($t_{\frac{1}{2}} = 23$ min) but whether this could affect the equilibrium of the racemate is unclear. An alternative explanation is that the presence of opposing effects of the agonists mediated via different receptor types can influence the simple competitive relationships between agonist and antagonist. This would seem unlikely, however, because the potentiating actions were more apparent in the epididymal portion yet the slopes were of unity in these portions.

The pA_2 values for pirenzepine in the epididymal vas deferens with unity slopes of the Schild plots were approximately 8 and in the prostatic portions were 7.7, which as described above may have been an underestimate. These values were identical for McN-A-343 and oxotremorine as agonists and compare with values obtained previously in the rabbit vas deferens (7.64, Eltze 1988; 8.82, Shannon et al 1993; 7.85, Dörje et al 1991), rat superior cervical ganglion (8.36, Brown et al 1980) and for contraction of canine saphenous and femoral veins (8.1 and 8.0, Eglen & Whiting 1990). The value is also comparable with the affinity of [^{3}H]-pirenzepine binding to muscarinic receptors in rat hippocampal membranes (8.31, Shannon et al 1993). The muscarinic receptors mediating these responses and found in these tissues have been assigned to the M_1 subtype. The pA_2 values for antagonism by pirenzepine of responses mediated via other receptor subtypes have been found to be at least an order of magnitude lower, for example, in guinea-pig atria (6.82, Dörje et al 1990), guinea-pig ileum (6.88, Dörje et al 1990), rat ileum (6.99, Brown et al 1980), rat atria (6.31, Lazareno & Roberts 1989) and for endothelium-dependent relaxations of rat and rabbit aorta, rat pulmonary artery and rabbit ear artery (7.0, 6.8, 7.0 and 6.7, respectively, Eglen & Whiting 1990). For a comprehensive list of pA_2 values for pirenzepine see Broadley (1996). Thus, the antagonism by pirenzepine of the inhibition of the twitch

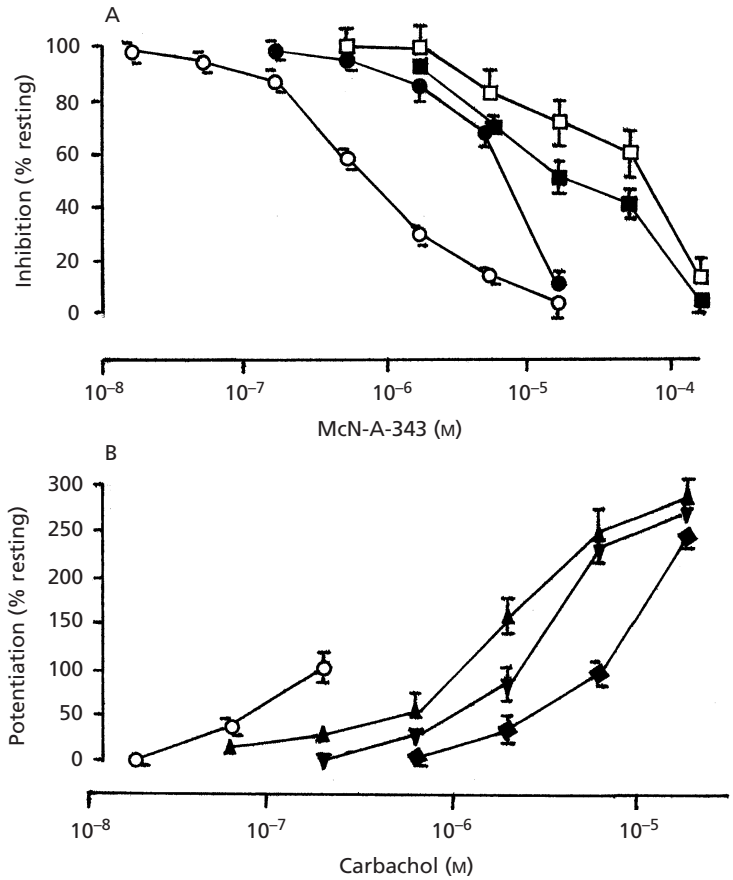

Figure 6 Effects of telenzepine on the concentration–response curves for the inhibition by McN-A-343 (A) and potentiation by carbachol (B) of field stimulation-induced contractions of rabbit prostatic vas deferens. Concentration–response curves were obtained in the absence (○) and then in the presence of 30 nM (●), 100 nM (■) and 300 nM (□) telenzepine against McN-A-343 or 100 nM (▲), 300 nM (▼) and 1 μM (◆) telenzepine against carbachol, with washout after each curve. Changes in twitch height are expressed as a percentage of the control contraction immediately before commencing each cumulative concentration–response curve and the mean values ± s.e.m. are shown (n = 4).

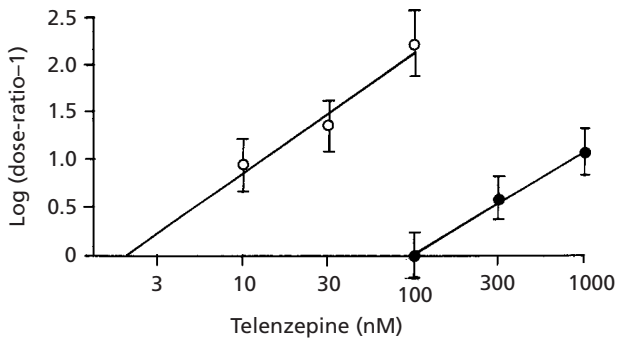

Figure 7 Schild plots for the antagonism by telenzepine of the inhibitory responses to McN-A-343 (○) and potentiation responses to carbachol (●) of field stimulation-induced contractions of rabbit prostatic vas deferens. Regression lines are plotted of mean log(dose-ratio − 1) values ± s.e.m., calculated at the EC50 of each concentration–response curve, against log molar concentration of pirenzepine.

responses of the rabbit vas deferens is consistent with this response being mediated via receptors of the M_1 subtype.

The pA_2 values for telenzepine, whether determined from the Schild plot (8.70) or from the individual concentrations (8.97, 8.89 and 9.23) were also comparable with previous estimates in the rabbit vas deferens (8.61, Eltze 1988). Our value is more than one order of magnitude greater than for responses mediated via receptors other than the M_1 subtype, such as the negative inotropy of rat atria (7.32), and contraction of rat stomach strip (7.35, Eltze et al 1985). Thus, telenzepine also allows us to classify the inhibitory response to muscarinic agonists of the vas deferens as being mediated via M_1 receptors. The potentiation of the twitches by carbachol was also antagonized by telenzepine, but the pA_2 value (7.07) for this antagonism was significantly

less than for the antagonism of the inhibitory response to McN-A-343. This is somewhat lower than a value of 7.39 reported previously which was determined under reverse antagonism conditions (Eltze 1988). The slope of the Schild plot was unity and thus the antagonism can reliably by assumed to be competitive and measured under equilibrium conditions. This would further overcome the earlier arguments that telenzepine had not reached equilibrium. The low pA_2 value allows us to conclude that the receptor type involved in the potentiated twitch response is not of the M_1 subtype. The high potency of the selective M_2 receptor antagonist, AF-DX116, in this tissue (pA_2 7.39) compared with its activity for M_3 receptor-mediated responses (6.0–6.5, Broadley 1996) suggests that the potentiation is due to activation of M_2 receptors (Eltze 1988). Although carbachol potentiated the twitch contractions to field stimulation, it did not exert a contractile effect in the unstimulated preparation. The fact that carbachol enhances the contractions to noradrenaline, KCl and ATP (Eltze 1988) suggests that the potentiation of the responses seen here is due to activation of postjunctional M_2 receptors on the smooth muscle rather than prejunctional receptors that facilitate transmitter release. The lack of a direct contractile action suggests that there may be few M_2 receptors or the coupling may be poor so that the tissue needs to be already activated to reveal their activity.

In conclusion, the activity of the two antagonists against the inhibitory responses of the rabbit vas deferens strongly implicates M_1 receptor involvement in this response. The conclusion arising from this is that McN-A-343 must cause inhibition through this receptor type. The doubts concerning the selectivity of McN-A-343 for M_1 receptors are therefore unfounded. The fact that McN-A-343 does not display a selective binding profile (Eglen et al 1987) suggests that its selectivity does not arise from affinity differences. The tissue-related factors for agonist activity are the total receptor population and the function (f) relating stimulus and response, while the drug-related factors are the dissociation constant (affinity, K_A), the concentration and the intrinsic efficacy (ϵ) (Kenakin 1993). Thus, the source of the selectivity of McN-A-343 probably resides in its intrinsic efficacy. McN-A-343 has been shown to have activity in certain tissues where the response has been characterized as non-M_1 receptor mediated on the basis of antagonist selectivity, such as the taenia caecum (Eglen et al 1987) and guinea-pig ileum and urinary bladder (Rubinstein & Cohen 1992). This can be explained by a high receptor reserve and efficient stimulus–response coupling in these tissues so that McN-A-343 can exert a response via non-M_1 receptors by virtue of having sufficient activity at these receptors.

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