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Pharmacological characterization of muscarinic receptors involved in McN-A-343-induced effects on intestinal motility and heart rate in conscious dogs

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1 Intravenous injection of the muscarinic agonist, McN-A-343, in conscious dogs equipped with an ileal Thiry fistula produced a dose-related inhibition of intestinal phasic contractile activity, and an increase in heart rate.

2 The inhibitory action of McN-A-343 on motility was antagonized with different potencies by antimuscarinic drugs. The non-selective drug, N-methylatropine, blocked the McN-A-343 effect as well as the reflex phasic activity. The M_1-selective compound, pirenzepine (1–30 $\mu g\,kg^{-1}$), was a potent antagonist of the McN-A-343 effect, whereas the cardioselective M_2-antagonist, AF-DX 116, and the smooth muscle selective compound, 4-diphenylacetoxy-N-methyl piperidine (4-DAMP), were completely ineffective at the doses tested.

3 The McN-A-343-induced inhibition of intestinal motility was blocked by locally applied lignocaine, suggesting the involvement of a neural inhibitory pathway. The resistance to hexamethonium and (α_1-, α_2- and β-) adrenoceptor blocking drugs excluded transmission through a nicotinic synapse or release of catecholamines.

4 McN-A-343-induced tachycardia was also the result of muscarinic receptor activation. It was very sensitive to antagonism by 4-DAMP, while being completely unaffected by AF-DX 116. Pirenzepine displayed an intermediate profile, reducing tachycardia at doses fully active in reversing the agonist-mediated effect on intestinal motility. Propranolol partially reduced McN-A-343 tachycardia, suggesting catecholamine release.

5 The two McN-A-343 effects investigated in the present study appear to be mediated by different muscarinic receptor subtypes. While the inhibitory action on intestinal motility results from stimulation of M_1-muscarinic receptors, the tachycardia is mediated by receptors blocked selectively by 4-DAMP.

Introduction

Acetylcholine is regarded as the major excitatory transmitter in the gastrointestinal tract. When released from neurones or administered exogenously, it increases gut motility by activating nicotinic and muscarinic receptors present in great abundance in the intestine (Furness & Costa, 1982).

There are, however, scattered observations describing inhibition of intestinal motility after stimulation of muscarinic receptors. For example, administration of acetylcholine (Youmans *et al.*, 1940) or of the muscarinic agonist McN-A-343 (Smith, 1966; Carlson *et al.*, 1970) were shown to cause intestinal relaxation in the conscious dog and anaesthetized cat. More recently, Fox *et al.* (1985) described an anaesthetized dog model in which intra-arterial injection of acetylcholine or McN-A-343 reduced the intestinal phasic activity evoked by either field stimulation or motilin. In the above-mentioned studies, the behaviour of McN-A-343 was somewhat different from that of acetylcholine, since the inhibitory effect evoked by the compound was not preceded by a contraction. On the basis of its sensitivity to atropine, the inhibition of motility described was attributed to stimulation of muscarinic receptors (Fox *et al.*, 1985). It is of interest that the existence of muscarinic-mediated inhibitory pathways has been described in other tracts of the gastrointestinal system, in different species (Goyal &

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Rattan, 1978; Gilbert *et al.*, 1984; Micheletti *et al.*, 1988).

In light of these findings and of the prevailing notion of multiple muscarinic receptor subtypes (Birdsall & Hulme, 1985; Eglen & Whiting, 1986) we thought it of interest to reinvestigate the muscarinic-mediated inhibition of intestinal motility with the aim of characterizing the receptor subtype involved.

Some of the present results have been briefly reported elsewhere (Schiavone *et al.*, 1987).

Methods

Five beagle dogs of either sex (9–13 kg b.w.) were prepared with an ileal Thiry fistula (Thiry, 1864). Anaesthesia was induced with sodium thiopentone (Pentothal, 30 mg kg^{-1}, i.v.) and maintained with halothane (Fluothane) by means of a respiratory pump (Soxil). After laparotomy, a 20–25 cm segment of terminal ileum was excised from the intestine, preserving the integrity of its vascular and extrinsic nervous supplies. The isolated loop was closed aborally while the oral edge was fixed in ileostomy to the abdominal wall. An end-to-end anastomosis was made to restore small bowel continuity.

Recording sessions were started 10 days after surgery and were conducted while the dogs lay comfortably on the right side. The animals were deprived of food, but not water, for 12–15 h before each study. Contractions of the ileal Thiry fistula were elicited and recorded by means of a latex balloon inserted into the fistula and distended with enough water (2–4 ml) to attain a basal pressure of 15–20 mmHg. The balloon was connected to a pressure transducer (Statham P 23 dB), and the signal recorded on a polygraph (Devices MX 2P-62). Heart rate was continuously measured by means of a ratemeter triggered by the ECG signal (lead II) and recorded on the same polygraph. Each dog was used at 4 day intervals.

McN-A-343 (10–300 μg kg^{-1}) was injected intravenously. In a first series of experiments each dog received all doses of McN-A-343 randomly, during a single recording session. In a second series of experiments antagonist potency was evaluated against McN-A-343 at 100 μg kg^{-1}; 60 min after a control response to McN-A-343, vehicle or antagonist were injected intravenously, 2 min before agonist challenge.

Lignocaine was administered 15 min before McN-A-343 injection into the fistula lumen, a modified Foley catheter being used to elicit and record motility; this allowed administration beyond the balloon tip, and prevented leakage through the stoma.

The effect of McN-A-343 on intestinal motility was quantified as duration (min) of complete suppression of phasic contractions, while that on heart rate was reported as Δ beats min^{-1} (b.p.m.) over basal. In each recording session the effect of antagonist administration on the action of McN-A-343 was compared to the pre-drug response.

Statistical analysis was performed by paired Student's t test; P values < 0.05 were regarded as significant.

Drugs

The following drugs were used: pirenzepine dihydrochloride, AF-DX 116 (11[[2-[(diethyl-amino)-methyl] - 1 - piperidinyl]acetyl] - 5, 11 - dihydro - 6H - pyrido[2, 3 - b][1, 4] benzodiazepine - 6 - one) (K. Thomae, W. Germany); hexamethonium chloride (Fluka AG, Switzerland); propranolol hydrochloride, lignocaine hydrochloride (Gianni, Italy); prazosin hydrochloride (Pfizer Laboratories, U.S.A.); yohimbine hydrochloride, atropine methyl bromide (Sigma, U.S.A.); McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl trimethyl ammonium chloride) and 4-DAMP (4-diphenylacetoxy-N-methyl piperidine methiodide) were synthesized by Dr M. Gil, Istituto De Angeli, Italy. All compounds were dissolved in 1 ml 0.9% NaCl, except prazosin, which was dissolved in 2 ml of 30% glycerin-formal/water solution. Doses refer to the base.

Results

Intraluminal distension of the Thiry fistula elicited a phasic contractile activity (Figure 1) that persisted for at least 5 h; each recording session was generally completed within 2–3 h.

McN-A-343 (10–300 μg kg^{-1}; 31.5–946 nmol kg^{-1}) produced a dose related suppression of such phasic contractions (Figure 1, Table 1). The onset of McN-A-343 effect was immediate, being evident 15–20 s after drug administration. No excitatory effect on intestinal motility could be detected; return to pre-drug conditions was achieved in less than 10 min.

Other effects induced by McN-A-343 were: (a) a dose-dependent, rapid rise in heart rate of short duration (Table 1); (b) an increase in saliva secretion (not quantified); (c) vomiting (limited to the dose of 300 μg kg^{-1}); this dose was therefore discontinued and the effect of antagonists was studied against the dose of 100 μg kg^{-1}. Responses to McN-A-343 were reproducible at 30 min intervals (results not shown).

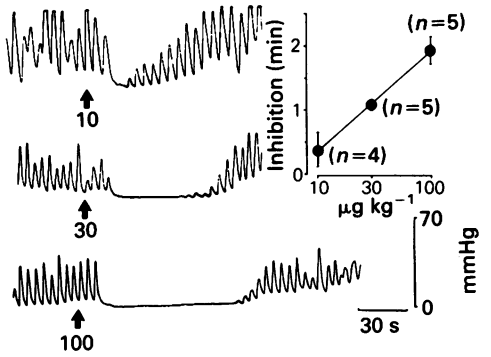

Figure 1 Representative responses of the ileal Thiry fistula to various doses of McN-A-343, in conscious dog. Phasic motility was induced by intraluminal distension. Administration of compound (intravenous route, $\mu g\,kg^{-1}$) is indicated by arrows. The inset depicts the dose-response effect of McN-A-343 (mean ± s.e. mean; $n = 4$–5) evaluated as duration of inhibition of reflex phasic contractions.

Muscarinic blockade

Pretreatment with the muscarinic M_1-antagonist, pirenzepine (1–30 $\mu g\,kg^{-1}$; 2.8–85 $nmol\,kg^{-1}$) counteracted, in a dose-related fashion, the McN-A-343-induced suppression of intestinal contractions without affecting basal activity (Table 2, Figure 2). On the other hand, McN-A-343-induced tachycardia was significantly reduced by pirenzepine only at 30 $\mu g\,kg^{-1}$, a dose maximally active on motility (Table 2).

The M_2-cardioselective antagonist, AF-DX 116 (500 $\mu g\,kg^{-1}$; 1186 $nmol\,kg^{-1}$), did not affect inhibition of motility produced by McN-A-343. AF-DX 116 increased heart rate *per se*, from 63.3 ± 6.6 to 181 ± 31.5 b.p.m. (mean ± s.e. mean; $P < 0.05$; $n = 4$); nevertheless, McN-A-343 was still able to exert its full tachycardiac effect (Table 2).

4-DAMP (3 $\mu g\,kg^{-1}$; 6.6 $nmol\,kg^{-1}$), an antagonist selective for smooth muscle muscarinic receptors, was virtually ineffective in antagonizing the effect of McN-A-343 on intestinal motility (Table 2). Its behaviour on McN-A-343-evoked tachycardia was different. It almost completely suppressed the increase in heart rate induced by McN-A-343 (Table 2).

Table 1 Effect of McN-A-343 on intestinal motility and heart rate

Dose ($\mu g\,kg^{-1}$ i.v.)	n	*Suppression of intestinal contractions* (min)	*Heart rate* (*b.p.m.*) *Basal*	*Treated*
10	4	0.38 ± 0.27	79.7 ± 10.5	104.1 ± 17.9
30	5	1.07 ± 0.08	75.3 ± 8.7	131.2 ± 15.9*
100	5	1.93 ± 0.21	76.8 ± 10.1	151.2 ± 9.6**

Values are mean ± s.e. mean.
n = number of replications.
* $P < 0.05$; ** $P < 0.01$; significantly different from basal values (paired Student's *t* test).

Table 2 Effect of antimuscarinic drugs on McN-A-343 (100 $\mu g\,kg^{-1}$, i.v.) responses in conscious dog

Drug	*Dose* ($\mu g\,kg^{-1}$ i.v.)	*Duration of McN-A-343-induced inhibition* (min) *Control*	*Treated*	Δ *heart rate* (b.p.m.) *Control*	*Treated*	n
N-methylatropine	3	2.6 ± 0.6	1.5 ± 0.3*	54 ± 6.4	31 ± 9.8*	5
	30	1.9 ± 0.3	n.c.	80 ± 4	0*	3
Pirenzepine	1	2.0 ± 0.6	1.9 ± 0.9	79 ± 15.6	83 ± 15.1	4
	3	2.1 ± 0.2	0.9 ± 0.3*	91 ± 10.2	69 ± 19.7	4
	10	2.0 ± 0.2	0.4 ± 0.2*	65 ± 6.4	41 ± 10.2	4
	30	2.4 ± 0.4	0.1 ± 0.1*	93 ± 9.4	67 ± 5.2*	4
AF-DX 116	500	2.1 ± 0.5	2.0 ± 0.8	56 ± 10.9	56 ± 15.4	4
4-DAMP	3	2.0 ± 0.2	1.8 ± 0.2	98 ± 10.0	11 ± 11.2*	4

Values are mean ± s.e. mean.
n = number of replications.
n.c. = not calculable. See text for details.
* Significantly different from control ($P < 0.05$; paired Student's *t* test).

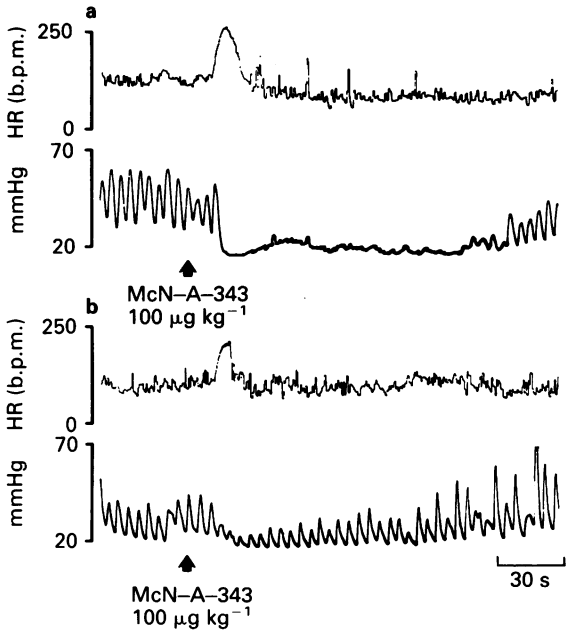

Figure 2 Representative effect of 100 $\mu g\,kg^{-1}$ McN-A-343 alone (a), and in the presence of 30 $\mu m\,kg^{-1}$ pirenzepine (b), on heart rate and intestinal contractility.

The non-selective antimuscarinic, N-methylatropine, was also investigated. At 3 $\mu g\,kg^{-1}$ (7.8 $nmol\,kg^{-1}$), it reduced both responses to McN-A-343 (Table 2) by about 40%; a ten fold higher dose substantially inhibited the reflex intestinal contractility, therefore preventing the assessment of McN-A-343 effect; the same dose increased heart rate from 75.0 ± 13.2 to 213.3 ± 14.5 b.p.m. (mean ± s.e. mean; $P < 0.05$; $n = 3$) and fully antagonized McN-A-343-induced tachycardia (Table 2).

Nicotinic blockade

Administration of hexamethonium (10 $mg\,kg^{-1}$; 58 $\mu mol\,kg^{-1}$) did not significantly modify the effect of McN-A-343 on motility, although a trend toward prolonged duration of agonist-induced inhibition was noted (Table 3). Hexamethonium produced a sustained increase in heart rate (from 66.7 ± 4.4 to 150.0 ± 20.8 b.p.m.; mean ± s.e. mean; $P < 0.05$; $n = 3$), that did not prevent the tachycardia induced by McN-A-343 (Table 3).

Adrenoceptor blockade

The effect of selective blockade of α- and β-adrenoceptors is reported in Table 3. Separate antagonism of α_1- or α_2-adrenoceptors effected with prazosin (0.1–1 $mg\,kg^{-1}$; 261–2610 $nmol\,kg^{-1}$) and yohimbine (100 $\mu g\,kg^{-1}$; 282 $nmol\,kg^{-1}$), respectively, did not inhibit either of the effects of McN-A-

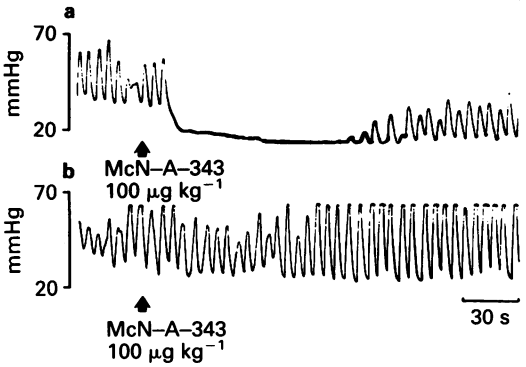

Figure 3 Inhibitory effect of McN-A-343 on ileal Thiry fistula in the absence (a) and in the presence (b) of 100 mg lignocaine applied into the fistula 15 min before McN-A-343 injection.

Table 3 Effect of various antagonists on McN-A-343 (100 $\mu g\,kg^{-1}$ i.v.) responses

Drug	*Dose* ($mg\,kg^{-1}$, i.v.)	*Duration of McN-A-343-induced inhibition* (min) *Control*	*Treated*	Δ *heart rate* (b.p.m.) *Control*	*Treated*	n
Hexamethonium	10	1.9 ± 0.2	3.7 ± 1.7	74 ± 1.0	49 ± 6.7	3
Propranolol	0.5	2.1 ± 0.5	2.7 ± 0.6	95 ± 10.1	40 ± 9.2*	3
	2	2.4 ± 0.3	2.9 ± 0.4	72 ± 12.4	36 ± 6.9*	4
Prazosin	0·1	2.0 ± 0.7	2.6 ± 1.1	60 ± 11.5	88 ± 4.5	3
	1.0	2.8 ± 0.3	3.3 ± 0.5	79 ± 6.1	120 ± 17.2	3
Yohimbine	0.1	2.3 ± 0.4	2.3 ± 0.5	82 ± 29.4	71 ± 17.1	4
Lignocaine	100§	2.4 ± 0.5	0.1 ± 0.1*	62 ± 11.0	60 ± 2.7	4

Values are mean ± s.e. mean.
n = number of replications.
* significantly different from control ($P < 0.05$; paired Student's t test).
§ dose per animal.

343. After prazosin, an increase in agonist-induced tachycardia, that did not reach statistical significance, was observed. Administration of propranolol (0.5–2 mg kg^{-1}; 1.9–7.7 μmol kg^{-1}) to block β-adrenoceptors, significantly reduced only the tachycardia (Table 3).

Nerve conduction blockade

Lignocaine (100 mg), topically applied into the fistula lumen, increased the amplitude of phasic contractions (data not shown) and almost completely abolished the inhibition of intestinal contractility elicited by McN-A-343, leaving unaffected the agonist-induced tachycardia (Figure 3, Table 3).

Discussion

The results of the present study confirm and extend previous findings of an inhibitory effect on intestinal motility regulated by muscarinic receptors (Youmans *et al.*, 1940; Smith, 1966; Fox *et al.*, 1985). Furthermore, they provide a characterization of the receptor subtype involved.

The heterogeneity of muscarinic receptors is a well established concept. Indications of the existence of two subtypes, M_1 and M_2, (Hammer *et al.*, 1980; Hammer & Giachetti, 1982; Giachetti *et al.*, 1986; Hammer *et al.*, 1986) have been confirmed by genetic recombinant techniques (Kubo *et al.*, 1986a; Peralta *et al.*, 1987). There is growing evidence from pharmacological studies for the existence of a third subtype, present in exocrine glands and smooth muscle. This is recognized with low affinity by the cardioselective M_2-antagonist, AF-DX 116 (Hammer *et al.*, 1987; Korc *et al.*, 1987; Micheletti *et al.*, 1987) and with high affinity by compounds such as 4-DAMP (Barlow *et al.*, 1976; Louie & Owyang, 1986) and hexahydrosiladiphenidol (Mutschler & Lambrecht, 1984).

In the light of the existence of multiple subtypes, the observation that muscarinic agonists, beside increasing intestinal motility (Furness & Costa, 1982), can also inhibit it (Youmans, *et al.*, 1940; Smith, 1966; Fox *et al.*, 1985) may be interpreted as due to different receptor populations.

This study attempts to investigate the muscarinic receptors responsible for inhibition of intestinal motility, by employing selective antagonists.

McN-A-343 was employed as agonist (Roszkowski, 1961) since it has proved to be a useful tool in discriminating among different muscarinic responses (Goyal & Rattan, 1978; Hammer & Giachetti, 1982; Micheletti *et al.*, 1988). Although not selective in classical terms, as it displays equal affinities for the different muscarinic subtypes (Eglen *et al.*, 1987; Giraldo, personal communication), McN-A-343 seems capable of discriminating on the basis of efficacy. Evidence that efficacy may be the basis of selectivity is provided by results of the present study, showing that the compound stimulated only the receptors mediating inhibition of motility, without exciting those responsible for smooth muscle contraction. In addition, we were able to evaluate the effect of McN-A-343 on a parameter totally unrelated to intestinal motility, i.e. heart rate.

Both McN-A-343 responses were due to stimulation of muscarinic receptors, as demonstrated by their sensitivity to treatment with antimuscarinic drugs. N-methylatropine inhibited the intestinal and the cardiac actions of McN-A-343 with similar potencies, as expected for a non-selective antagonist, whereas all the other antimuscarinic compounds assayed displayed some degree of selectivity.

The muscarinic receptor-mediated inhibition of motility was extremely sensitive to the M_1-antagonist pirenzepine, which appeared at least equipotent with N-methylatropine. This finding, together with the lack of efficacy shown by AF-DX 116 and 4-DAMP on this parameter, at doses highly active on heart rate, suggests that McN-A-343 inhibits intestinal motility by activating an M_1-receptor subtype.

Simultaneous comparison of antagonist potencies in inhibiting McN-A-343 intestinal and cardiac effects strengthens this notion. In fact, the most potent antagonist of McN-A-343-induced tachycardia was 4-DAMP, which induced a near-maximal inhibition of the response to McN-A-343 at a dose totally ineffective on motility. In contrast, the threshold dose for pirenzepine in preventing McN-A-343 tachycardia was at least 10 times greater than that found on motility. AF-DX 116 was unable to antagonize either the actions of McN-A-343, although the dose employed was highly effective in inducing tachycardia (see results; Giachetti *et al.*, 1986).

Taken together, these findings indicate that the muscarinic receptor responsible for McN-A-343 tachycardiac effect does not belong to the M_1 or the M_2 subtype, but rather to the subtype recognized with high affinity by 4-DAMP. Since the myocardium contains a predominant M_2-receptor population (Hammer *et al.*, 1980; Kubo *et al.*, 1986b; Peralta *et al.*, 1987), the receptor activated by McN-A-343 has presumably an extra-cardiac location; the effectiveness of propranolol indicates that the receptor operates, at least partially, through catecholamine release.

Considering the problems inherent in using *in vivo* models to characterize receptors and the moderate selectivity afforded by the muscarinic antagonists

available, the evidence emerging from the present experiments with McN-A-343 adequately demonstrates that different receptor subtypes may mediate the increase in heart rate and the inhibition of intestinal motility.

Our results provide a partial insight into the mechanism through which M_1-receptors inhibit motility. The sensitivity of the McN-A-343 effect to lignocaine and its resistance to hexamethonium, indicate that the agonist activates a nervous pathway devoid of nicotinic synapses.

The results obtained after blockade of α- and β-adrenoceptors, definitely exclude a catecholamine component in McN-A-343 motility response. Fox *et al.* (1985) found that the inhibitory action of McN-A-343 in anaesthetized dogs, was partly sensitive to adrenergic and nicotinic antagonism. The discrepancy may depend on the substantial differences in the experimental conditions employed in our studies: while in the former experiments, motility was stimulated electrically or with motilin, in the present protocol it was reflexly activated by distension of the intestinal wall.

By analogy with findings obtained in the opossum lower oesophageal sphincter (Goyal & Rattan, 1978; Gilbert *et al.*, 1984) and in the rat duodenum (Micheletti *et al.*, 1988), where the muscarinic M_1-receptor excites inhibitory non-adrenergic non-cholinergic neurones, it is conceivable that the same mechanism operates in the dog intestine.

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