

Pharmacological differences between two muscarinic responses of the rat superior cervical ganglion *in vitro*

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1 Pharmacological differences have been observed between the muscarinic agonist-induced depolarizing and hyperpolarizing responses of the rat isolated superior cervical ganglion.

2 Pirenzepine (0.3 μ M) selectively reduced the depolarizing response and unmasked the hyperpolarizing response. No such selectivity was observed with a concentration of N-methylatropine which was equipotent with pirenzepine in antagonizing the depolarizing response.

3 The neuromuscular blocking agents gallamine (10 μ M) and pancuronium (3 μ M) exhibited the opposite selectivity to pirenzepine, both dramatically reduced the hyperpolarization but only slightly antagonized the depolarization.

4 The potencies of a range of agonists in evoking the depolarizing and hyperpolarizing responses, the latter in the presence of 0.3 μ M pirenzepine, have been determined. Methylfurmethide failed to hyperpolarize the ganglion at concentrations which evoked maximal depolarizations.

5 The muscarinic hyperpolarization did not appear to be mediated by the secondary release of catecholamines.

6 It was concluded that the two muscarinic responses on the rat superior cervical ganglion, the slow depolarization and faster hyperpolarization, are mediated by different muscarinic receptor subtypes.

Introduction

Atropine-sensitive (muscarinic) receptors can be classified into at least two subtypes: M_1 and M_2 (Hirschowitz *et al.*, 1984; Levine *et al.*, 1986). The antagonist potency of the anti-ulcer drug pirenzepine is particularly relevant for this classification (Hammer *et al.*, 1980; Hammer & Giachetti, 1982), although pharmacological differences between muscarinic responses were previously indicated by the cardioselective action of gallamine (Riker & Wescoe, 1951; Clark & Mitchellson, 1976).

The muscarinic actions of cholinomimetics on sympathetic ganglia have been known for many years (Volle, 1966). The most studied response is an excitation associated with a small slow depolarization, but hyperpolarizing responses have often been reported. These potential changes may be analogous to the synaptic slow excitatory postsynaptic potential (e.p.s.p.) and slow inhibitory postsynaptic potential (i.p.s.p.) (Eccles & Libet, 1961), respectively, as the

application of acetylcholine will mimic both potentials (Takeshige & Volle, 1964a). The inhibition of a resting potassium current probably underlies the depolarization (Kobayashi & Libet, 1968; Weight & Votava, 1970; Brown & Adams, 1980), whereas, in contrast, an increase in potassium permeability appears to be responsible for the hyperpolarization (Dodd & Horn, 1983; Cole & Shinnick-Gallagher, 1984; but see Brown & Selyanko, 1985).

Two potentials are evoked by muscarine on the isolated superior cervical ganglion of the rat. The pharmacology of the depolarizing response has been studied in detail (Brown *et al.*, 1980a,b), but that of the smaller hyperpolarization has received less attention. The purpose of our experiments was to compare the pharmacology of the two potentials and to examine the possible involvement of catecholamines in the mediation of the hyperpolarizing response (see Weight & Padjen, 1973; Cole & Shinnick-Gallagher, 1980; Ivanov & Skok, 1980; Ashe & Libet, 1982; Rafuse & Smith, 1986). A preliminary account of some of this work has been published (Newberry *et al.*, 1985).

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Methods

Superior cervical ganglia were excised from male Sprague-Dawley rats, weighing 150–200 g, which had been killed by a blow to the thorax. Each ganglion was desheathed under a microscope and then submerged in a three compartment bath. The ganglion body was situated in the central compartment and the preganglionic (cervical sympathetic) and postganglionic (internal carotid) trunks protruded through greased slots into the outer two chambers. The central compartment (volume approx 0.5 ml) was continually perfused ($2\text{--}2.5\text{ ml min}^{-1}$) with medium at 25°C , pre-equilibrated with 5% carbon dioxide in oxygen, whereas the medium in the outer compartments was static. The medium was an aqueous solution containing (mM): NaCl 125, KCl 5, KH_2PO_4 1, CaCl_2 2.5, MgSO_4 1, NaHCO_3 25 and dextrose 10.

Ganglionic potential changes, induced by drug perfusion, were recorded using Ag/AgCl electrodes between the chambers containing the ganglion body (earthed) and the postganglionic trunk. Concentration-response relationships were determined using 10 min intervals between 1 min perfusions of muscarinic agonists (cf. Brown *et al.*, 1980a). Longer intervals or application times did not affect the EC_{50} values obtained for muscarine. The EC_{50} value for an agonist was determined on each of a number of ganglia, with at least five agonist applications (ascending in semilogarithmic molar units), and the negative logarithms of the EC_{50} values (pEC_{50}) were analysed statistically. The maximum depolarization evoked by an agonist was compared with the depolarization to $1\text{ }\mu\text{M}$ muscarine. The 'antagonists' were perfused for at least 30 min before the muscarine EC_{50} was re-determined. The maximum response of an agonist in the presence of an antagonist was compared to that previously measured in the absence of the antagonist.

The pharmacology of the depolarizing muscarinic response was studied in the standard medium. Under these conditions (2.5 mM CaCl_2) overt muscarinic hyperpolarizing responses were, when evident, small. The calcium chloride in the medium was reduced to 0.1 mM , with no substitution, to help the study of the hyperpolarization (Figure 1b): the facilitatory action of low calcium on ganglionic muscarinic responses has previously been demonstrated (see Figure 3 of Brown *et al.*, 1980a). Possible complications due to action potentials and their afterhyperpolarizations were minimized by the inclusion, in most experiments, of $0.1\text{ }\mu\text{M}$ tetrodotoxin in the medium.

The constituent salts of the medium were all of analytical reagent grade. The drugs used were (\pm)-muscarinic chloride (Sigma lot 122F-0908), pirenzepine dihydrochloride (Boots), pancuronium bromide (Organon) and idazoxan (Reckitt and Colman); acetylcholine chloride, pilocarpine nitrate,

gallamine triethiodide, (\pm)-noradrenaline hydrochloride, dopamine hydrochloride, physostigmine sulphate, carbamylcholine (carbachol) chloride, N-methylatropine bromide and tetrodotoxin were obtained from Sigma. The sample of 5-methylfurmethide (hydroiodide salt) was prepared on site by Dr David Billington.

Results

Acetylcholine evoked three responses on the rat isolated superior cervical ganglion (Figure 1a): an initial, muscarinic hyperpolarization followed by fast, nicotinic, and slower muscarinic depolarizations (see Takeshige & Volle, 1964a; Brown, 1966). The two muscarinic responses are the subject of this paper.

The muscarinic depolarization

Applications of muscarine depolarized the ganglion in a reproducible manner. An example of the concentration-response relationship to muscarine is shown in Figure 1c. The potencies and maximum responses, compared to muscarine, of a range of muscarinic agonists are shown in Table 1. A faster, larger and N-methylatropine-resistant (nicotinic) depolarization was evoked by relatively high concentrations ($3\text{ }\mu\text{M}$ or greater) of carbachol and acetylcholine. Since the concentration of these compounds in evoking muscarinic depolarizations was lower than that needed for the nicotinic action, it was just possible to determine the maximum of the muscarinic depolarization before the nicotinic depolarization dominated. This was not an ideal situation but was unavoidable since, as has been described previously (Brown *et al.*, 1980a), the ganglionic nicotinic antagonist hexamethonium (2.5 mM) could not be used to suppress selectively the nicotinic response since it also possessed muscarinic antagonist properties (muscarine EC_{50} ratios of 12 and 16, $n = 2$). A further complication arose when using the acetylcholinesterase inhibitor physostigmine ($10\text{ }\mu\text{M}$) in attempting to determine the potency of acetylcholine, namely that physostigmine evoked an N-methylatropine-sensitive, slow depolarization of the ganglion (20–40% of the response to $1\text{ }\mu\text{M}$ muscarine). The potency of muscarine was decreased in the presence of this compound (see Table 1). The direct depolarization precluded a detailed investigation of the latter effect, but it should be noted that muscarinic antagonist properties of cholinesterase inhibitors have been described previously (Brown *et al.*, 1982; Kenakin & Beck, 1985).

The agonist-induced muscarinic depolarizations of the ganglion were characteristically slower than the nicotinic depolarizations (see Figure 1a). When induced by different agonists they were qualitatively

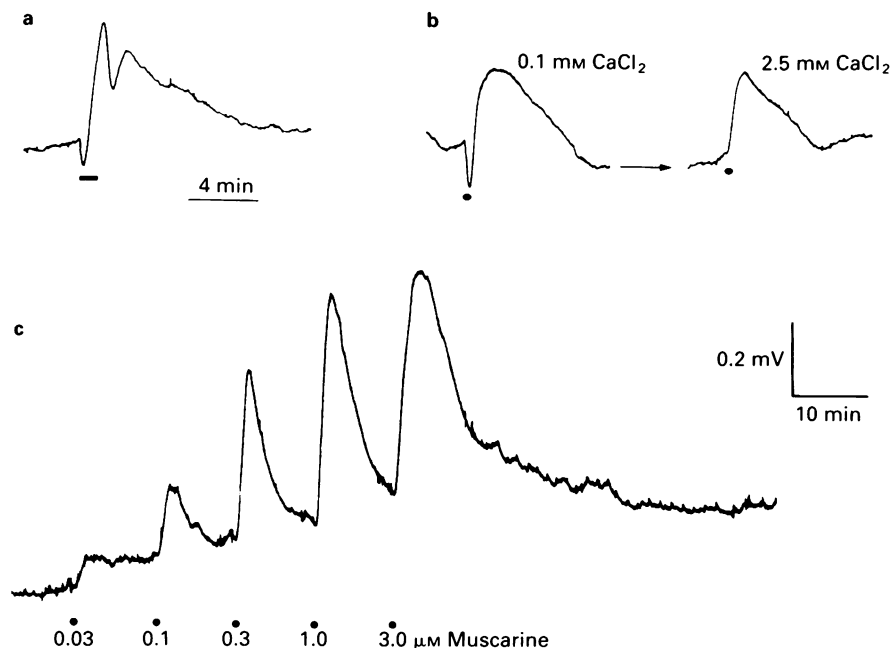


Figure 1 Records of the presence of three isolated superior cervical ganglia to cholinergic agonists. (a) Acetylcholine ($10\ \mu\text{M}$) evoked an initial hyperpolarization followed by a fast and a slower depolarization (medium contained $0.1\ \text{mM}\ \text{CaCl}_2$, $10\ \mu\text{M}$ physostigmine, $0.1\ \mu\text{M}$ tetrodotoxin). (b) A low calcium medium facilitates the observation of the hyperpolarizing muscarinic response evoked by $1\ \mu\text{M}$ carbachol. (c) The concentration-response relationship of a ganglion to (\pm)-muscarine chloride in standard medium ($2.5\ \text{mM}\ \text{CaCl}_2$). Depolarization is upward and the bar and (●) signify the time and duration of drug application. All records are at the same gain. The time calibration for (b) is the same as (c).

Table 1 The potencies and relative maxima of agonists on the depolarizing and hyperpolarizing responses of the isolated superior cervical ganglion of the rat

Compound	Depolarization		Hyperpolarization	
	pEC_{50}	Relative maximum	pEC_{50}	Relative maximum
Muscarine	$6.8 \pm 0.2 (60)^D$	$1.0, 1.0-1.6 (60)^F$	$5.7 \pm 0.3 (14)$	$2.4, 1.1-4.4 (7)^H$
Carbachol	$6.7 \pm 0.1 (8)^D$	$0.9, 0.8-1.0 (8)$	$6.2 \pm 0.4 (11)^G$	$1.5, 1.0-2.4 (11)^H$
Methylfurmethide	$7.1 \pm 0.2 (21)$	$1.7, 1.2-1.9 (14)$	B	B
Pilocarpine	$6.0 \pm 0.2 (8)^E$	$1.3, 0.8-1.5 (8)^F$	C	C
Muscarine in physostigmine ($10\ \mu\text{M}$)	$5.0 \pm 0.1 (4)$	A	ND	ND
Carbachol in physostigmine ($10\ \mu\text{M}$)	ND	ND	$6.3 \pm 0.5 (10)^G$	$1.4, 1.1-2.0 (10)^H$
Acetylcholine in physostigmine ($10\ \mu\text{M}$)	$6.2 \pm 0.3 (6)^E$	A	$7.1 \pm 0.3 (6)$	$0.9, 0.8-1.0 (6)$

Potencies (means \pm s.d. (n)) at depolarizing the ganglion were determined in medium containing $2.5\ \text{mM}\ \text{CaCl}_2$. To study the hyperpolarizing responses the medium contained $300\ \text{nM}$ pirenzepine and $0.1\ \text{mM}\ \text{CaCl}_2$. Maximum depolarizations (median, range (n)) were related to the response to $1\ \mu\text{M}$ muscarine. Maximum hyperpolarizations were related to $1\ \mu\text{M}$ carbachol because the initial pharmacology had been done using this concentration (e.g. Figure 4). A: not determined as physostigmine reduced response to $1\ \mu\text{M}$ muscarine. B and C: not obtainable (see text). All parameters within a column were significantly different ($P < 0.05$) unless indicated by a letter (D to H) indicating similarity. pEC_{50} values and relative maxima were statistically compared by use of Newman-Keuls and Kruskal-Wallis, respectively (criteria for parametric analysis were not met when comparing the maxima). ND: not determined.

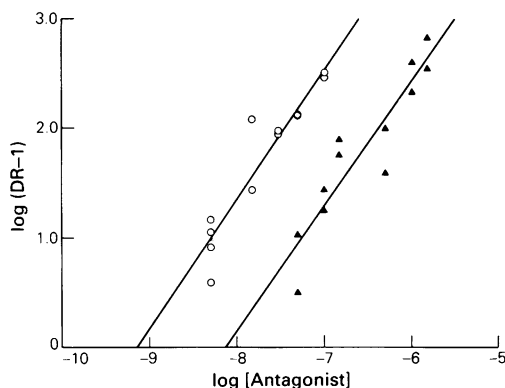


Figure 2 Concentration-dependent antagonism of the depolarization to muscarine. Arunlakshana-Schild plots for N-methylatropine (O) and pirenzepine (▲). Least squares fits to data reveal pA_2 values of 9.14 and 8.13 with slopes ($\pm 95\%$ confidence intervals) of 1.18 ± 0.30 and 1.14 ± 0.34 , respectively. The slopes are not significantly different from unity (at $P = 0.05$ level). pA_2 values calculated from individual dose-ratios by $pA_2 = -\log [B] + \log (DR - 1)$ resulted in means \pm s.d. (n) of 9.40 ± 0.23 (12) and 8.36 ± 0.28 (12).

similar, except that the response to pilocarpine was prolonged and the response to methylfurmethide was faster to rise and decay (Figure 6). The larger maximum depolarization to methylfurmethide was not a result of activation of nicotinic receptors since it was abolished by $1 \mu\text{M}$ N-methylatropine ($n = 4$).

Muscarinic antagonists

The muscarine-induced depolarization of the ganglion was antagonized by the 'M₁ selective' compound pirenzepine. The action of pirenzepine was compared with that of the quarternary analogue of atropine, N-methylatropine. Neither of these antagonists consistently reduced the maximum response to muscarine and the slopes of the Arunlakshana-Schild (1959) plots were not significantly different from unity (Figure 2). The skeletal neuromuscular antagonist gallamine, which antagonizes cardiac muscarinic responses (Clark & Mitchelson, 1976), had a complex action on the muscarine-evoked depolarization of the ganglion. At $10 \mu\text{M}$ it slightly antagonized muscarine (mean $\log [EC_{50} \text{ ratio}] = 0.28 \pm 0.18$, $n = 6$), but also increased its maximum response (median relative increase = 1.64, range 1.20–1.77, $n = 6$, Figure 3a). Pancuronium ($3 \mu\text{M}$) had a similar action to gallamine (mean $\log [EC_{50} \text{ ratio}] = 0.66 \pm 0.06$, $n = 4$, median increase in maximum = 1.89, 1.75–2.03, $n = 4$, cf. Dalton & Tyers, 1982). In the presence of the neuromuscular blocking agents the muscarinic agonist

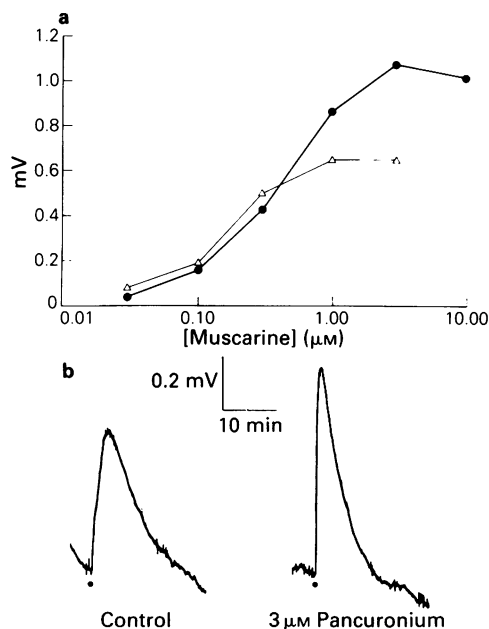


Figure 3 The effect of neuromuscular blocking agents on the muscarinic depolarizing response. (a) The effect of gallamine 10 mM (●), superfused for 30 min, on the concentration-response relationship to muscarine. (Δ) Control responses to muscarine. (b) Records of the carbachol ($1 \mu\text{M}$)-evoked depolarization of the ganglion showing the effect of pancuronium (30 min). Note the faster rise-time, increased response amplitude and faster decay-rate in the presence of the neuromuscular blocker. The latter is difficult to reconcile with the observation that gallamine decreases the dissociation rates of muscarinic ligands from the receptor (Stockton *et al.*, 1983). (a) and (b) are from two separate ganglia, medium contained 2.5 mM CaCl_2 and 0.1 mM CaCl_2 in (b).

responses were 'sharpened' (see Figure 3b), such that they looked more like those of methylfurmethide. The concentrations of gallamine and pancuronium used in these experiments were selected to inhibit cardiac-like (M₂) receptors but have minimal actions on cortical-like (M₁) receptors (Dalton & Tyers, 1982; Stockton *et al.*, 1983; Nedoma *et al.*, 1985).

Muscarinic hyperpolarization

The initial hyperpolarization was more often observed with carbachol compared with muscarine, and was never seen with pilocarpine or methylfurmethide. This response was not observed on all ganglia, even to carbachol, in the standard (2.5 mM Ca^{2+}) medium. A medium containing less calcium (0.1 mM) facilitated the observation of the hyperpolarization (Figure 1b) –

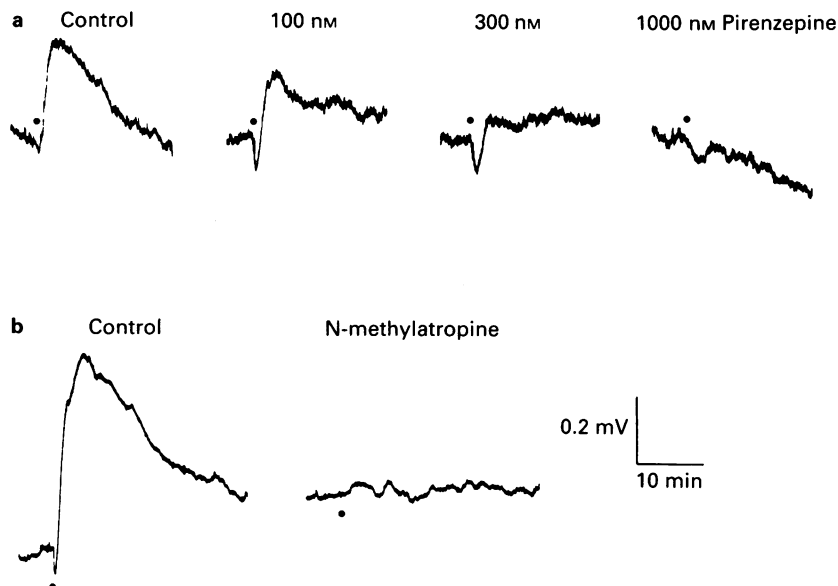


Figure 4 The effect of antagonists on the biphasic response to $1\ \mu\text{M}$ carbachol in medium containing $0.1\ \text{mM}$ CaCl_2 . (a) Increasing concentrations of pirenzepine, each superfused for half an hour, resulted in the progressive decline of the depolarizing response and the unmasking of the hyperpolarizing response. (b) No such selective effect was seen after 1 h in $30\ \text{nM}$ N-methylatropine, this concentration was equipotent with $300\ \text{nM}$ pirenzepine against the depolarization. Calibration applies to all records.

but even then it was not always visible (e.g. Figure 3b). In this medium muscarine was marginally, but consistently, more potent at activating the depolarization (mean $\log [\text{EC}_{50}\ \text{ratio}] = -0.12 \pm 0.09$, $n = 4$) and its maximum response was greater (median = 1.27, range 1.05–1.42, $n = 4$). Pharmacological distinctions between the two muscarinic responses were readily made in this medium but they were also observed in the standard medium. The perfusion of pirenzepine (100 and $300\ \text{nM}$) caused a reduction in the depolarizing muscarinic response and a concomitant increase in the amplitude of the hyperpolarization ($n = 10$). However, at concentrations of $1\ \mu\text{M}$ ($n = 3$) or greater, pirenzepine antagonized the hyperpolarization (see Figure 4a). A concentration of $300\ \text{nM}$ pirenzepine, therefore, seemed optimal for observing the muscarinic hyperpolarization. The hyperpolarization response was always observed under these conditions ($0.1\ \text{mM}$ Ca^{2+} , $300\ \text{nM}$ pirenzepine) whether or not it had been previously visible. N-methylatropine did not selectively antagonize the depolarization since $30\ \text{nM}$, which antagonized the depolarization response to the same extent as $300\ \text{nM}$ pirenzepine, abolished both potentials ($n = 8$, Figure 4b). Gallamine ($10\ \mu\text{M}$) exhibited the opposite selectivity to pirenzepine, since it reduced the initial hyperpolarizing response ($n = 6$, Figure 5). This property was shared by pancuronium

($3\ \mu\text{M}$, $n = 3$). It should be noted that this action of the neuromuscular blocking agents occurred at concentrations which had only slight antagonist actions on the depolarizing response (see earlier). As in the standard medium, these compounds increased the amplitude of the depolarizing response, reduced the latency to peak and reduced the duration of the response (Figure 3b).

Concentration-response curves for agonist-induced hyperpolarizations were determined, in $0.1\ \text{mM}$ Ca^{2+} and $300\ \text{nM}$ pirenzepine (see Table 1), in order to compare the agonists, but the conditions were not ideal for two reasons. Firstly, the maximum hyperpolarizations evoked by acetylcholine and carbachol were limited by physiological antagonism, due to activation of a nicotinic depolarization (with concentrations greater than $3\ \mu\text{M}$); secondly, a false maximum could result when the antagonist action of pirenzepine, against the depolarizing response, was surmounted. In contrast to its action on the muscarine-induced depolarization, physostigmine ($10\ \mu\text{M}$) did not change the pEC_{50} for the carbachol-induced hyperpolarization determined in $0.3\ \mu\text{M}$ pirenzepine, although the amplitudes of individual responses were slightly reduced under these conditions.

Methylfurmethide and, to a lesser extent, pilocarpine appeared to activate selectively the depolarizing

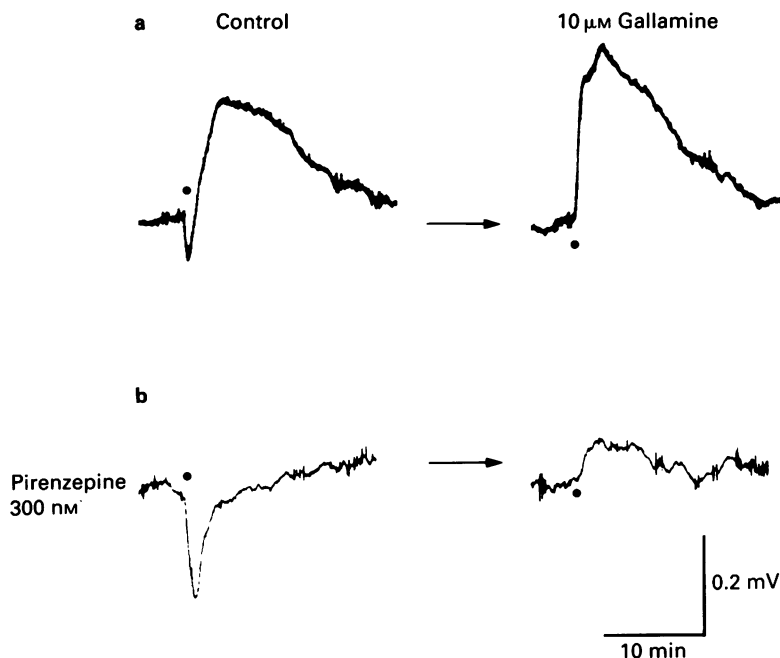


Figure 5 The effect of gallamine on the muscarinic hyperpolarization evoked by 1μ M carbachol. Two experiments: one in the absence of pirenzepine (a) and one in its presence (b). Gallamine was superfused for 30 min in each experiment. Medium contained 0.1 mM CaCl_2 . Calibration applies to all records.

response, since hyperpolarizing responses were rarely observed at concentrations previously determined to be maximally effective for the depolarizing response (1 and $10 \mu\text{M}$, respectively). Small hyperpolarizations were seen on only 2 out of 10 ganglia with pilocarpine and were not observed on 13 ganglia with methylfurmethide (eg Figure 6). The depolarizing response to physostigmine enabled us to confirm the selectivity of methylfurmethide ($0.3 \mu\text{M}$, $n = 5$) and pilocarpine ($1 \mu\text{M}$, $n = 2$, e.g. Figure 6).

It seems unlikely that the muscarinic hyperpolarization of the rat superior cervical ganglion was due to the secondary, extracellular calcium-dependent release of catecholamines (Tosaka *et al.*, 1968) for several reasons: (1) 0.1 mM CaCl_2 abolished the postsynaptic compound action potential recorded from the internal carotid nerve following the stimulation of the cervical sympathetic trunk (our unpublished observations), whereas the amplitude of the muscarinic hyperpolarization was enhanced in this medium; (2) the hyperpolarization was resistant to tetrodotoxin ($0.1\text{--}1 \mu\text{M}$); and (3) idazoxan ($0.1\text{--}1.0 \mu\text{M}$) antagonized noradrenaline ($1 \mu\text{M}$)- and dopamine ($10 \mu\text{M}$)-induced hyperpolarizations of the ganglion ($n = 4$ ganglia, for each catecholamine) but not the muscarine-induced hyperpolarization ($n = 8$, Figure 7).

Discussion

The principal finding of this study is that the depolarizing and hyperpolarizing responses evoked by muscarinic agonists on the isolated superior cervical ganglion of the rat can be pharmacologically distinguished: (1) Pirenzepine antagonized the depolarizing response, to muscarine and carbachol, and unmasked a gallamine- and pancuronium-sensitive hyperpolarizing response; (2) methylfurmethide potently depolarized the ganglion but failed to evoke a hyperpolarization at comparable concentrations. The receptors responsible for these responses may therefore be different, a conclusion consistent with the demonstration of two distinct muscarinic binding sites in mammalian sympathetic ganglia (Giraldo *et al.*, 1985).

The pA_2 determined for pirenzepine (Figure 2) against the depolarization to muscarine is similar to those previously found on this ganglion (Brown *et al.*, 1980a; Palacios *et al.* 1986) and on myenteric neurones (North *et al.*, 1985). These data, together with the potency of N-methylatropine, indicate that the muscarinic depolarization of the rat superior cervical ganglia is mediated by M_1 receptors. Pirenzepine (300 nM) selectively reduced the depolarizing response and unmasked the hyperpolarization. This selective

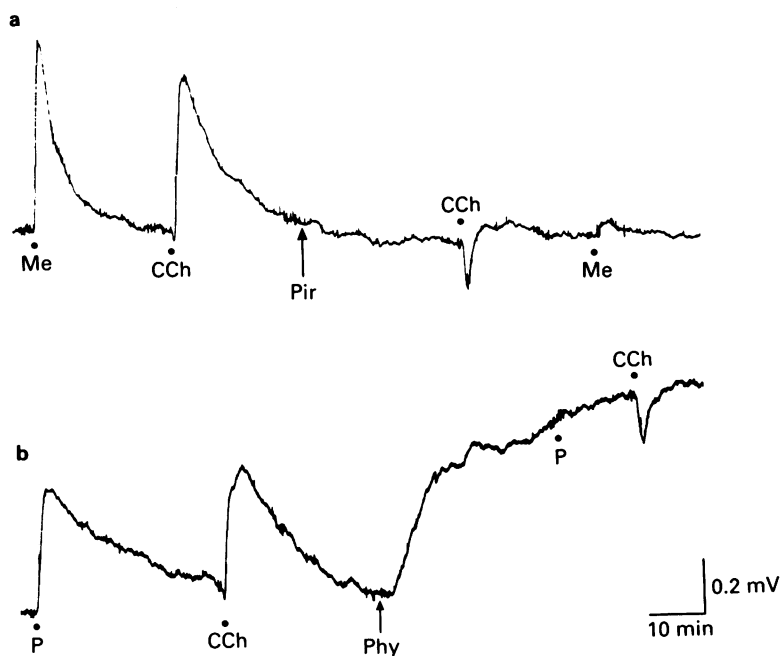


Figure 6 Selective activation of the depolarizing response by agonists. Methylfurmethide ($0.3 \mu\text{M}$, Me) and pilocarpine ($1 \mu\text{M}$, P) failed to evoke a hyperpolarizing response either before or following the superfusion of 300 nM pirenzepine (Pir in (a)) or $10 \mu\text{M}$ physostigmine (Phy in (b)). Under either condition, (started at arrow) carbachol (CCh, $1 \mu\text{M}$) was able to hyperpolarize the ganglion. The concentrations of methylfurmethide and pilocarpine used were chosen to give similar depolarizations of the ganglion to those of carbachol (before pirenzepine or physostigmine). The records are from 2 experiments and in both medium contained 0.1 mM CaCl_2 . Calibration applies to both continuous records. (It should be noted that the depolarization to physostigmine was delayed in onset, compared with the other agonists, and its response was often larger in this medium cf. medium containing 2.5 mM CaCl_2).

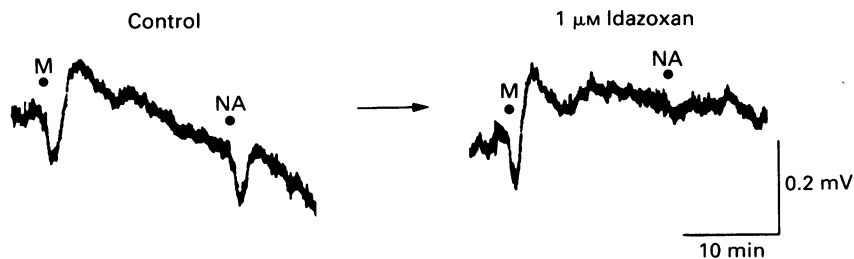


Figure 7 Pharmacological distinction between the muscarine (M, $1 \mu\text{M}$)- and noradrenaline (NA, $1 \mu\text{M}$)-induced hyperpolarizing responses of the ganglion. Idazoxan (RX 781094) was superfused for 30 min. Medium contained 0.1 mM CaCl_2 , 300 nM pirenzepine and 0.5 mM ascorbic acid.

action of pirenzepine was confirmed when an equipotent concentration of N-methylatropine antagonized both responses. N-methylatropine has a similar partition coefficient to pirenzepine and hence the different action of these antagonists is less likely to be artefactual (cf. Black & Shankley, 1985).

The selective action of pirenzepine indicates that the hyperpolarization was probably not mediated by M_1 receptors. Its sensitivity to gallamine and pancuronium suggests that it may be mediated by an ' M_2 ' receptor more similar to that in the atrium than in the ileum (Nedoma *et al.*, 1985). The muscarinic antagonism by these neuromuscular drugs is believed to be allosteric (Clark & Mitchelson, 1976; Stockton *et al.*, 1983). However, the competitive cardio-selective antagonist AFDX-116 (Hammer *et al.*, 1986; Giachetti *et al.*, 1986) also antagonized the hyperpolarizing response (Connolly & Newberry, unpublished observations) consistent with the suggestion of M_2 -receptor involvement. The increase in the amplitude of the depolarizing response in the presence of gallamine and pancuronium, which was seen in both calcium concentrations, may be explained if the hyperpolarizing response, whether overt or covert, physiologically attenuated the depolarizing response. The fact that methylfurmethide consistently evoked a larger maximum response than muscarine may be explained by its inability to evoke the hyperpolarizing response. Methylfurmethide appears to be a full agonist on many peripheral preparations (Grana *et al.*, 1986) and hence its selectivity is probably not a consequence of differences in spare receptors (see Kenakin, 1984). In support of this, it has been found that methylfurmethide is slightly selective for cortical compared to cardiac muscarinic binding sites (Birdsall & Hulme, 1986). In contrast, however, the maximum response of methylfurmethide in inducing inositol phospholipid breakdown in cortical slices is less than that of carbachol (Jacobson *et al.*, 1985). Pilocarpine also seemed to activate selectively the depolarizing response on the ganglion, as previously demonstrated (Takeshige & Volle, 1964b), but its selectivity was not as profound as methylfurmethide. Carbachol was the most potent cholinesterase-resistant agonist (of those listed in Table 1) at evoking the hyperpolarizing response. The difference in its potencies for producing the hyperpolarizing and depolarizing responses (compared to muscarine in Table 1) may explain why it could evoke overt hyperpolarization responses in the absence of

pirenzepine. Significantly, it has been suggested that this agonist displays cardioselectivity when compared over a range of peripheral tissues (Grana *et al.*, 1986).

In order to obtain more meaningful potencies for acetylcholine, we superfused the cholinesterase inhibitor physostigmine. This compound depolarized the ganglion and reduced the potency of muscarine to depolarize the ganglion. It may be argued, therefore, that the pEC_{50} values for acetylcholine to depolarize the ganglion should be adjusted accordingly to obtain its true potency. In contrast, the potency of carbachol to hyperpolarize the ganglion (in the presence of pirenzepine) was not affected by physostigmine. The pEC_{50} determined for the hyperpolarization evoked by acetylcholine may therefore be more accurate than that for the depolarization.

The effects of the selective antagonists described above are similar to their action on the slow i.p.s.p. and slow e.p.s.p. in sympathetic ganglia of other species (Gardier *et al.*, 1978; Ashe & Yarosh, 1984). Hence, the receptors involved in the muscarinic agonist responses and slow synaptic potentials of this ganglion may be similar. In mammalian ganglia the release of an endogenous catecholamine has been suggested to mediate the hyperpolarizing synaptic response (Ashe & Libet, 1982), possibly by an action involving α_2 -adrenoceptors (Brown & Caulfield, 1979). In our experiments, the selective α_2 -antagonist idazoxan (Doxey *et al.*, 1983) failed to antagonize the muscarinic hyperpolarization (see also Brown *et al.*, 1980a), whereas it did reduce the catecholamine-induced hyperpolarizations (see Figure 7). Consequently, the agonist-induced muscarinic hyperpolarization on this ganglion was probably the direct consequence of muscarinic receptor activation.

In conclusion, we have provided pharmacological evidence for differences between the receptors mediating muscarinic depolarizing and hyperpolarizing responses on the rat superior cervical ganglion *in vitro*. Given that these responses probably summate algebraically, the observed gross ganglionic potential induced by a given agonist may reflect its relative potency in activating these two opposing responses.

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References

- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14**, 48–58.
- ASHE, J.H. & LIBET, B. (1982). Pharmacological properties and monoaminergic mediation of the slow IPSP, in mammalian sympathetic ganglion. *Brain Res.*, **242**, 345–349.
- ASHE, J.H. & YAROSH, C.A. (1984). Differential and selective antagonism of the slow inhibitory postsynaptic potential and slow excitatory postsynaptic potential by gallamine

- and pirenzepine in the superior cervical ganglion of the rabbit. *Neuropharmacology*, **23**, 1321–1329.
- BIRDSALL, N.M.J. & HULME, E.C. (1986). Differences in the agonist binding properties of muscarinic receptor subpopulations in the rat cerebral cortex and myocardium. In *Advances in Behavioural Biology*, Vol. 29, *Alzheimer's and Parkinson's Diseases: Strategies For Research and Development* ed. Fisher, A., Hanin, I. & Lachman, C. New York and London: Plenum Press. pp. 565–574.
- BLACK, J.W. & SHANKLEY, N.P. (1985). Pharmacological analysis of muscarinic receptors coupled to oxyntic cell in the mouse stomach. *Br. J. Pharmacol.*, **86**, 601–607.
- BROWN, D.A. (1966). Effects of hexamethonium and hyoscine on the drug-induced depolarisation of isolated superior cervical ganglia. *Br. J. Pharmacol.*, **26**, 521–537.
- BROWN, D.A. & ADAMS, P.R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+ -current in a vertebrate neurone. *Nature*, **283**, 673–676.
- BROWN, D.A. & CAULFIELD, M.P. (1979). Hyperpolarizing ' α_2 '-adrenoceptors in rat sympathetic ganglia. *Br. J. Pharmacol.*, **65**, 435–445.
- BROWN, D.A., FATHERAZI, S., GARTHWAITE, J. & WHITE, R.D. (1980a). Muscarinic receptors in rat sympathetic ganglia. *Br. J. Pharmacol.*, **70**, 577–592.
- BROWN, D.A., FORWARD, A. & MARSH, S. (1980b). Antagonist discrimination between ganglionic and ileal muscarinic receptors. *Br. J. Pharmacol.*, **71**, 362–364.
- BROWN, D.A. & SELYANKO, A.A. (1985). Two components of muscarine-sensitive membrane current in rat sympathetic neurones. *J. Physiol.*, **358**, 335–363.
- BROWN, J.H., WETZEL, G.T. & DUNLAP, J. (1982). Activation and blockade of cardiac muscarinic receptors by endogenous acetylcholine and cholinesterase inhibitors. *J. Pharmacol. Exp. Ther.*, **223**, 20–24.
- CLARK, A.L. & MITCHELSON, F. (1976). The inhibitory effect of gallamine on muscarinic receptors. *Br. J. Pharmacol.*, **58**, 323–331.
- COLE, A.E. & SHINNICK-GALLAGHER, P. (1980). Alpha-adrenoceptor and dopamine receptor antagonists do not block the slow inhibitory postsynaptic potential in sympathetic ganglia. *Brain Res.*, **187**, 226–220.
- COLE, A.E. & SHINNICK-GALLAGHER, P. (1984). Muscarinic inhibitory transmission in mammalian sympathetic ganglia mediated by increased potassium conductance. *Nature*, **307**, 270–271.
- DALTON, D.W. & TYERS, M.B. (1982). A comparison of the muscarinic antagonist actions of pancuronium and alcuronium. *J. Auton. Pharmacol.*, **2**, 261–266.
- DODD, J. & HORN, J.P. (1983). Muscarinic inhibition of sympathetic C neurones in the bullfrog. *J. Physiol.*, **334**, 271–291.
- DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX 781094: a selective, potent and specific antagonist of α_2 -adrenoceptors. *Br. J. Pharmacol.*, **78**, 489.
- ECCLES, R.M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol. (Lond.)*, **157**, 484–503.
- GARDIER, R.W., TSEVDOS, E.J. & JACKSON, D.B. (1978). The effect of pancuronium and gallamine on muscarinic transmission in the superior cervical ganglion. *J. Pharmacol. Exp. Ther.*, **204**, 46–53.
- GIACHETTI, A., MICHELETTI, R. & MONTAGNA, E. (1986). Cardiosensitive profile of AF-DX 116, a muscarinic M_2 receptor antagonist. *Life Sci.*, **38**, 1663–1672.
- GIRALDO, E., MONFERINI, E. & HAMMER, R. (1985). Selective labelling of M_1 -receptors in autonomic ganglia with 3H -pirenzepine. *Arzn. Forsch/Drug Res.*, **35**, 325–328.
- GRANA, E., LUCHELLI, A., ZONTA, F., SANTAGOSTINO-BARBONE, M.G. & D'AGOSTINO, G. (1986). Comparative studies of the postjunctional activities of some very potent muscarinic agonists. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332**, 213–218.
- HAMMER, R., BERRIE, C.P., BIRDSALL, N.J.M., BURGEN, A.S.V. & HULME, E.C. (1980). Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature*, **283**, 90–92.
- HAMMER, R. & GIACHETTI, A. (1982). Muscarinic receptor subtypes: M_1 and M_2 biochemical and functional characterization. *Life Sci.*, **31**, 2991–2998.
- HAMMER, R., GIRALDO, E., SCHIAVI, G.B., MONFERINI, E. & LADINSKY, H. (1986). Binding profile of a novel cardioselective muscarine receptor antagonist, AF-DX 116, to membranes of peripheral tissues and brain in the rat. *Life Sci.*, **38**, 1653–1662.
- HIRSCHOWITZ, B.I., HAMMER, R., GIACHETTI, A., KEIRNS, J.J. & LEVINE, R.R. (ed) (1984). Subtypes of Muscarinic Receptors. *Trends Pharmacol. Sci.*, (Suppl.).
- IVANOV, A.Y. & SKOK, V.I. (1980). Slow inhibitory postsynaptic potentials and hyperpolarisation evoked by noradrenaline in the neurones of mammalian sympathetic ganglion. *J. Auton. Nerv. Syst.*, **1**, 255–263.
- JACOBSON, M.D., WUSTEMAN, M. & DOWNES, C.P. (1985). Muscarinic receptors and hydrolysis of inositol phospholipids in rat cerebral cortex and parotid gland. *J. Neurochem.*, **44**, 465–472.
- KENAKIN, T.P. (1984). The relative contribution of affinity and efficacy to agonist activity: organ selectivity of noradrenaline and oxymetazoline with reference to the classification of drug receptors. *Br. J. Pharmacol.*, **81**, 131–141.
- KENAKIN, T.P. & BECK, D. (1985). Self-cancellation of drug properties as a mode of organ selectivity: the antimuscarinic effects of ambenonium. *J. Pharmacol. Exp. Ther.*, **232**, 732–740.
- KOBAYASHI, H. & LIBET, B. (1968). Generation of slow postsynaptic potentials without increases in ionic conductance. *Proc. Natl. Acad. Sci. U.S.A.*, **60**, 1304–1311.
- LEVINE, R.R., BIRDSALL, N.J.M., GIACHETTI, A., HAMMER, R., IVERSEN, L.L., JENDEN, D.J. & NORTH, R.A. (1986). Subtypes of muscarinic receptors. II. *Trends Pharmacol. Sci.*, (Suppl.).
- NEDOMA, J., DOROFEEVA, N.A., TUCEK, S., SHELKOVNIKOV, S.A. & DANILOV, A.F. (1985). Interaction of the neuromuscular blocking drugs alcuronium, decamethonium, gallamine, pancuronium, ritebronium, tercuronium and d-tubocurarine with muscarinic acetylcholine receptors in heart and ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **329**, 176–181.
- NEWBERRY, N.R., PRIESTLEY, T. & WOODRUFF, G.N. (1985). Pharmacological distinction between two muscarinic responses on the isolated superior cervical ganglion of the rat. *Eur. J. Pharmacol.*, **116**, 191–192.
- NORTH, R.A., SLACK, B.E. & SUPRENANT, A. (1985). Muscarinic M_1 and M_2 receptors mediate depolarisation and presynaptic inhibition in guinea-pig enteric nervous system. *J. Physiol.*, **368**, 435–452.
- PALACIOS, J.M., BOLLIGER, G., CLOSSE, A., ENZ, A.,

- GMELIN, G. & MALANOWSKI, J. (1986). The pharmacological assessment of RS86 (2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide). A potent, specific muscarinic acetylcholine receptor agonist. *Eur. J. Pharmacol.*, **125**, 45–62.
- RAFUSE, P.E. & SMITH, P.A. (1986). α_2 -Adrenergic hyperpolarisation is not involved in slow synaptic inhibition in amphibian sympathetic ganglia. *Br. J. Pharmacol.*, **87**, 409–416.
- RIKER, W.F. & WESCOE, W.C. (1951). The pharmacology of flaxedil with observations on certain analogs. *Ann. N.Y. Acad. Sci.*, **54**, 373–394.
- STOCKTON, J.M., BIRDSALL, N.J.M., BURGEN, A.S.V. & HULME, E.C. (1983). Modification of the binding properties of muscarinic receptors by gallamine. *Mol. Pharmacol.*, **23**, 551–557.
- TAKESHIGE, C. & VOLLE, R.L. (1964A). A comparison of the ganglionic potentials and block produced by acetylcholine and tetramethylammonium. *Br. J. Pharmacol.*, **23**, 80–89.
- TAKESHIGE, C. & VOLLE, R.L. (1964B). Modification of ganglionic responses to cholinomimetic drugs following preganglionic stimulation, anticholinesterase agents and pilocarpine. *J. Pharmacol.*, **146**, 335–343.
- TOSAKA, T., CHICHIBU, S. & LIBET, B. (1968). Intracellular analysis of slow inhibitory and excitatory postsynaptic potentials in sympathetic ganglia of the frog. *J. Neurophysiol.*, **31**, 396–409.
- VOLLE, R.L. (1966). Modification by drugs of synaptic mechanisms in autonomic ganglia. *Pharmacol. Rev.*, **18**, 839–869.
- WEIGHT, F.F. & PADJEN, A.L. (1973). Acetylcholine and slow synaptic inhibition in frog sympathetic ganglion cells. *Brain Res.*, **55**, 225–228.
- WEIGHT, F.F. & VOTAVA, J. (1970). Slow synaptic excitation in sympathetic ganglion cells: evidence for synaptic inactivation of potassium conductance. *Science*, **170**, 755–758.

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