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# Effects of locally and systemically administered cholinoceptor antagonists on the secretory response of human eccrine sweat glands to carbachol

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**1** Eleven healthy male volunteers participated in a study comparing the effects of locally and systemically administered cholinoceptor antagonists on the secretory response of sweat glands to intradermally injected carbachol chloride.
**2** Atropine sulphate administered locally into the skin antagonised the response to carbachol: the dose-response curve for carbachol was shifted to the right without any depression of the maximum of the curve. The nicotinic receptor antagonists hexamethonium bromide and (+)-tubocurarine chloride, however, had little effect on the response to carbachol.
**3** Atropine sulphate, administered systemically by intramuscular injection, caused a non-surmountable antagonism of the response to carbachol: the maximum of the dose-response curve was depressed with little change in the value of $ED_{50}$.
**4** Atropine methonitrate (a mixed muscarinic/nicotinic receptor antagonist), and hexamethonium bromide (a nicotinic receptor antagonist), both with poor access to the central nervous system, were injected intramuscularly: both caused non-surmountable antagonism of the response to carbachol.
**5** It is concluded that the response to carbachol is mediated by muscarinic rather than nicotinic receptors. The effect of atropine sulphate on the response to carbachol depends on the route of administration: while locally applied atropine sulphate appears to act as a competitive antagonist, systemically applied atropine sulphate, like atropine methonitrate and hexamethonium bromide, appears to act in a non-competitive manner.
**6** It is suggested that the systemically administered cholinoceptor antagonists reduce the response to carbachol by interacting with cholinoceptors in sympathetic ganglia: such an interaction would reduce the impulse flow in sudomotor fibres resulting in decreased sensitivity of the sweat glands to carbachol.

**Keywords** atropine sulphate atropine methonitrate hexamethonium (+)-tubocurarine carbachol sweat glands

## Introduction

Human eccrine sweat glands are innervated largely, if not exclusively, by cholinergic sympathetic fibres and they readily respond to intradermal injections of cholinomimetic substances

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(Dobson & Sato, 1972). Furthermore, there is a growing body of evidence that the pharmacological sensitivity of the sweat glands is influenced by the activity in the innervating sympathetic fibres: procedures which decrease sympathetic tone have been shown to cause hyposensitivity and conversely procedures which increase sympathetic tone have been shown to produce hypersensitivity. Thus it has been known for many years that destruction of the sympathetic innervation results in hyposensitivity of sweat glands to cholinoceptor stimulants (Randall & Kimura, 1955), and more recently it has been reported that sweat glands become more sensitive to intradermally injected cholinomimetic drugs in states of elevated sympathetic nerve activity (as seen in pathological anxiety (Maple *et al.*, 1982) or in the presence of thermal or psychological stressors (van den Broek *et al.*, 1984)).

The secretory response of sweat glands to intradermally injected cholinoceptor stimulants can be antagonized by systemic administration of drugs with muscarinic receptor blocking properties such as atropine sulphate (Clubley *et al.*, 1978) and amitriptyline (Szabadi *et al.*, 1980). Although the most likely explanation for these observations is an interaction between the cholinoceptor antagonists and muscarinic receptors on the sweat glands themselves, the possibility cannot be excluded that the systemically administered antagonists may also affect muscarinic receptors in the central and/or peripheral nervous system, which in turn could result in a decrease in sympathetic outflow and hence in a decreased sensitivity of sweat glands to cholinoceptor agonists.

The experiments reported here were carried out in an attempt to gain further information about the mechanism of action of muscarinic receptor antagonists on sweat gland function.

A preliminary report of some of these results has been presented to the British Pharmacological Society (Longmore *et al.*, 1984).

## Methods

### *Subjects*

Eleven healthy, drug-free male volunteers, aged between 19 and 38 years participated in the experiments. The subjects were informed of the nature and purpose of the study and all gave written consent. The experiments were approved by the Ethics Committees of Manchester Royal Infirmary and Withington Hospital, Manchester.

### *Pharmacological technique*

Sweat gland activity on the volar surface of the forearm was assessed using a plastic paint impression method (Clubley *et al.*, 1978; Lamb *et al.*, 1983). This technique involves coating an area of skin (3.5 × 2.5 cm) with a quick drying opaque plastic paint. When dry the paint is removed using Sellotape and mounted on a 35 mm slide holder. Active glands appear as holes in the paint and these may be counted when the slide is projected. In the present study, holes with diameters greater than 25 μm were regarded as representing active sweat glands.

In each experimental session, 'spontaneous' sweat gland activity was first assessed and then sweat gland activity evoked by six intradermal injections of drug solutions, three injections being placed on each forearm. Drugs were dissolved in sterile isotonic sodium chloride solution (0.154 M). Injections were administered using a 1.0 ml syringe and a 26-gauge hypodermic needle; the volume injected was always 0.05 ml. Two minutes were allowed between the termination of each injection and application of the plastic paint (Maple *et al.*, 1982).

### *Procedure*

The subjects rested in the experimental room for 20 min prior to administration of the intradermal injections of carbachol. The ambient temperature ranged between 21°C and 24°C and the relative humidity ranged between 40% and 75%.

*Preliminary experiment: Comparison of the effects of locally administered muscarinic and nicotinic receptor antagonists on the response to carbachol* A single male subject (one of the investigators) participated in three experimental sessions separated by at least 48 h. In each session the subject received six intradermal injections. In Session 1 he received injections of a standard concentration ($10^{-3}$M) of carbachol chloride either alone (one injection) or mixed with one of the five following concentrations of atropine sulphate (one injection of each mixture): $10^{-8}$ M, $10^{-7}$ M, $10^{-6}$ M, $10^{-5}$ M, $10^{-4}$ M. In sessions 2 and 3 the procedure was repeated using hexamethonium bromide and (+)-tubocurarine chloride in place of atropine sulphate.

*Experiment 1: Investigation of the effect of locally administered atropine sulphate on the dose-response relationship for carbachol* Five subjects participated in two experimental sessions separated by at least 48 h. In one session they

received intradermal injections of six different concentrations of carbachol chloride ($5 \times 10^{-6}$ M, $10^{-5}$, $5 \times 10^{-5}$ M, $10^{-4}$ M, $5 \times 10^{-4}$ M, $10^{-3}$ M), and in the other session they received injections of the same six concentrations of carbachol chloride; however, on this occasion it was mixed with atropine sulphate ($10^{-6}$ M).

*Experiment 2: Investigation of the effect of systemically administered atropine sulphate on the dose-response relationship for carbachol* Five subjects participated in two experimental sessions separated by at least 48 h. In both sessions the subjects received injections of the six concentrations of carbachol chloride (see Experiment 1). In one of the sessions (selected at random), 0.6 mg atropine sulphate was injected intramuscularly 1 h before the administration of carbachol.

*Experiment 3: Investigation of the effect of systemically administered atropine methonitrate on the dose-response relationship for carbachol* Five subjects participated in two experimental sessions separated by at least 48 h. The procedure was the same as in Experiment 2 only in this case atropine methonitrate 0.6 mg) was injected intramuscularly.

*Experiment 4: Investigation of the effect of systemically administered hexamethonium bromide on the dose-response relationship for carbachol* Six subjects participated in two experimental sessions separated by at least 48 h. The procedure was the same as in Experiment 2 only in this case hexamethonium bromide (10 mg) was injected intramuscularly.

*Analysis of data*

The response to each injection of carbachol was derived by subtracting the number of spontaneously active sweat glands from the total number of glands detected following the injection. Best-fit hyperbolic functions were derived for the data obtained from each group of subjects using an iterative procedure based on the method of Wilkinson (1961). This method provides estimates (± s.e. estimate) of the maximum number of sweat glands activated ($E_{max}$) and the concentration required to evoke the half maximal response ($ED_{50}$). The index of determination ($p^2$) was calculated for each curve; $p^2$ expresses the proportion of the variance in the $y$-values which can be accounted for in terms of $x$ in a curvilinear function (Lewis, 1960).

The data were also analyzed using two-factor analysis of variance, with repeated measures on both factors. Each experiment was analyzed separately. The two factors were (a) drug treatment and (b) dose of carbachol chloride.

## Results

*Preliminary experiment: Comparison of the effects of locally administered muscarinic and nicotinic receptor antagonists on the response to carbachol* The results are shown in Figure 1. It is apparent that while atropine sulphate reduced the response to carbachol, hexamethonium bromide and (+)-tubocurarine chloride, in the same concentration range, had little effect.

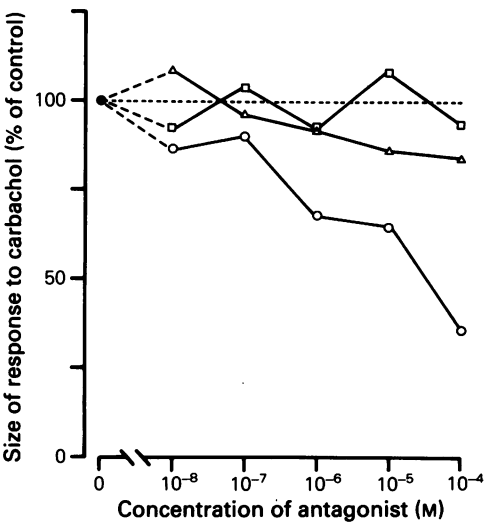


**Figure 1** Effect of atropine sulphate (○), hexamethonium bromide (□) and (+)-tubocurarine chloride (△) on responses to carbachol in a single male subject. *Ordinate:* number of active sweat glands detected, expressed as percent of number of glands detected following injection of carbachol chloride alone; *abscissa:* molar concentration of antagonist on logarithmic scale. Each set of connected points shows the effect of increasing doses of the antagonist on the response to a standard dose of carbachol (0.05 ml, $10^{-3}$ M).

*Experiment 1: Effect of locally administered atropine sulphate on the dose-response relationship for carbachol* The results are shown in Figure 2 and Table 1. It is apparent that locally administered atropine sulphate antagonized the response to carbachol, this being reflected in a shift to the right of the dose-response curve and an increase in the value of $ED_{50}$. The antagonism was surmountable: there was little change in the

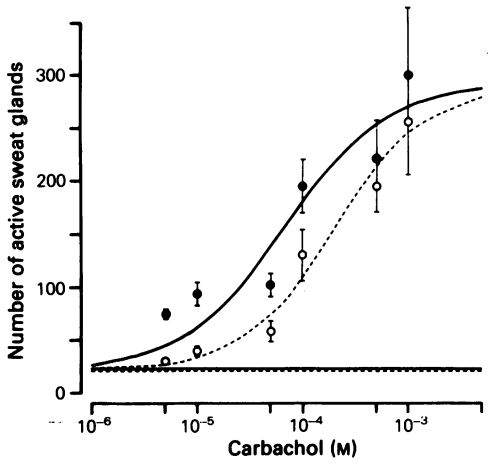


**Figure 2** Dose-response relationship for carbachol in the absence (●) and presence (○) of locally administered atropine sulphate $10^{-6}$ M. *Ordinate:* number of sweat glands detected; *abscissa*: molar concentration of carbachol (volume injected 0.05 ml) on logarithmic scale. Points show mean values ($n$ = 5); vertical bars indicate ± s.e. mean. Horizontal lines indicate number of active sweat glands detected under resting conditions. Curves are semi-logarithmic transformations of best-fit rectangular hyperbolic functions.

maximum of the dose-response curve in the presence of the antagonist. Analysis of variance showed that the antagonism was statistically significant (Table 2).

*Experiment 2: Effect of systemically administered atropine sulphate on the dose-response relationship for carbachol* The results are shown in Figure 3 and Table 1. It is apparent that systemically administered atropine sulphate antagonized the response to carbachol, this being reflected in a depression of the maximum of the dose-response curve with little change in the value of $ED_{50}$. Analysis of variance showed that the antagonism was statistically significant and that there was a significant interaction between drug treatment and the dose of carbachol chloride (see Table 2)

*Experiment 3: Effect of systemically administered atropine methonitrate on the dose-response relationship for carbachol* The results are shown in Figure 4 and Table 1. It is apparent that systemically administered atropine methonitrate antagonized the response to carbachol, this being reflected in a depression of the maximum of the dose-response curve, with little change in the value of $ED_{50}$. Analysis of variance showed that the antagonism was statistically significant and that there was a significant interaction between drug treatment and the dose of carbachol chloride (See Table 2).

*Experiment 4: Effect of systemically administered hexamethonium bromide on the dose-response relationship for carbachol* The results are shown in Figure 5 and Table 1. It is apparent that systemically administered hexamethonium

**Table 1** Parameters of the dose-response curves for carbachol

| | | *Estimate of parameter (± s.e. estimate)* | | |
|---|---|---|---|---|
| | | $E_{max}$ *Number of* | $ED_{50}$ *Concentration* | $p^2$ |
| | *Antagonist* | *sweat glands* | *(x $10^{-5}$ M)* | |
| Experiment 1 | | | | |
| Locally administered atropine sulphate | Absent | 264.2 ± 37.4 | 6.7 ± 3.7 | 0.852 |
| ($n$ = 5) | Present | 265.8 ± 27.3 | 19.7 ± 6.3 | 0.973 |
| Experiment 2 | | | | |
| Systemically administered atropine sulphate | Absent | 327.9 ± 38.9 | 5.2 ± 2.5 | 0.862 |
| ($n$ = 5) | Present | 165.0 ± 14.3 | 2.6 ± 1.1 | 0.899 |
| Experiment 3 | | | | |
| Systemically administered atropine methonitrate | Absent | 322.5 ± 51.2 | 11.3 ± 6.3 | 0.974 |
| ($n$ = 5) | Present | 143.3 ± 22.3 | 10.7 ± 5.9 | 0.887 |
| Experiment 4 | | | | |
| Systemically administered hexamethonium bromide | Absent | 248.0 ± 27.2 | 4.6 ± 2.1 | 0.876 |
| ($n$ = 6) | Present | 179.4 ± 10.7 | 1.6 ± 4.8 | 0.934 |

**Table 2** *F*-ratios obtained from analyses of variance

| | *Drug treatment (d.f. = 1, 4 except Experiment 5 where d.f. = 1, 5)* | *Concentration of intradermally injected carbachol (d.f. = 5, 20 except Experiment 5 where d.f. = 5, 25)* | *Interaction (d.f. = 5, 20 except Experiment 5 where d.f. = 5, 25)* |
|---|---|---|---|
| Experiment 1 Locally administered atropine sulphate | 19.72* | 16.16*** | 0.94 |
| Experiment 2 Systemically administered atropine sulphate | 32.26** | 37.93*** | 10.29*** |
| Experiment 3 Systemically administered atropine methonitrate | 708.41*** | 25.61*** | 24.29*** |
| Experiment 4 Systemically administered hexamethonium bromide | 8.04* | 48.81*** | 8.09*** |

Asterisks denote statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

bromide antagonized the response to carbachol, this being reflected in a depression of the maximum of the dose-response curve, with little change in the value of $ED_{50}$. Analysis of variance showed that this antagonism was statistically significant and that there was a significant interaction between drug treatment and the dose of carbachol chloride (see Table 2).

## Discussion

In the preliminary experiment, in which the cholinoceptor antagonists were administered locally, the secretory response of sweat glands to a standard dose of intradermally injected carbachol was antagonized by atropine sulphate but not by the nicotinic receptor antagonists, hexamethonium and +-tubocurarine. This suggests that the response to carbachol is mediated by muscarinic rather than nicotinic receptors.

The effect of atropine sulphate on the dose-response relationship for carbachol (Experiments 1 and 2), appeared to be dependent on the route of administration. When administered locally (Experiment 1), atropine sulphate antagonized the response to carbachol in a surmountable fashion: there was a shift to the right in the dose-response curve accompanied by an approximately threefold increase in the value of $ED_{50}$, whereas there was little if any change in the maximum of the curve. This pattern of antagonism is consistent with a competitive interaction between atropine and carbachol at muscarinic receptors on the sweat glands. However, when administered systemically (Experiment 2) atropine sulphate appeared to act as a non-competitive antagonist: there was no shift in the position of the dose-response curve, whereas the maximum of the curve was reduced by approximately 50%. The difference between the patterns of antagonism produced by locally and systemically administered atropine sulphate may reflect the fact that systemically administered atropine sulphate acts not only on muscarinic

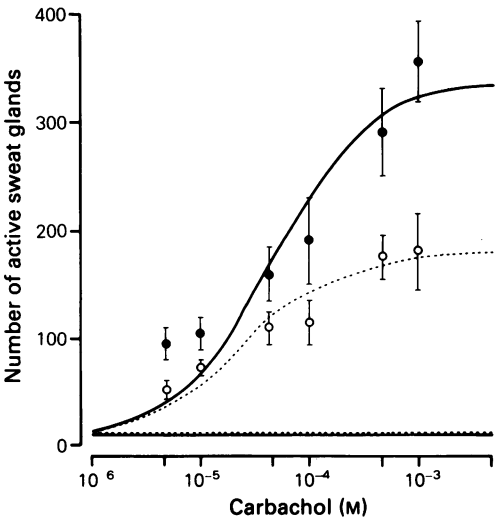


**Figure 3** Dose-response relationship for carbachol in absence (●) and presence (○) of systemically administered atropine sulphate 0.6 mg i.m. ($n = 5$). Conventions as in Figure 2.

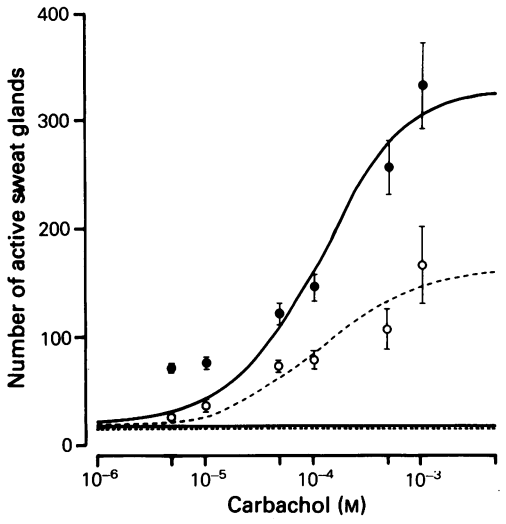


**Figure 4** Dose-response relationship for carbachol in absence (•) and presence (○) of systemically administered atropine methonitrate 0.6 mg i.m. ($n$ = 5). Conventions as in Figure 2.

receptors on the sweat glands, but also on muscarinic receptors in the central nervous system and sympathetic ganglia (Inch & Brimblecombe, 1974). Interestingly, systemically administered atropine methonitrate, a quarternary derivative of atropine which does not have ready access to the brain (Inch & Brimblecombe, 1974), produced the same unsurmountable antagonism as systemically administered atropine sulphate. It is possible that the effects of systemically given atropine methonitrate were mediated by blockade of muscarinic receptors located on sympathetic ganglia (Volle & Hancock, 1970). The effect of this drug may also have been mediated via blockade of ganglionic nicotinic receptors, since this derivative of atropine has affinity not only for muscarinic receptors but also for nicotinic receptors (Gyermek & Nádor, 1957). The possible involvement of ganglionic nicotinic receptors was further confirmed by the use of hexamethonium, a selective ganglionic nicotinic receptor blocking agent (Volle & Hancock, 1970) which, like atropine sulphate and atropine methonitrate, produced unsurmountable antagonism of carbachol-evoked sweating. The effect of hexamethonium is unlikely to reflect blockade of nicotinic receptors on sweat glands since, when administered locally, this drug did not affect the response of the sweat glands to intradermally injected carbachol (see preliminary experiment).

The different patterns of antagonism (surmountable *vs* non-surmountable) exerted by the locally and systemically administered drugs is confirmed by the analysis of variance (*c.f.* Table 2). The antagonistic effect of locally administered atropine sulphate was revealed in a significant main effect of the presence/absence of the antagonist, and there was no significant interaction term. In contrast, in the case of each of the systemically administered drugs, the significant main effect of the drug treatment was accompanied by a significant interaction term, indicating that the degree of antagonism varied as a function of the concentration of carbachol. Thus the results of the analysis of variance confirm the conclusions reached by comparison of the curve-fitting parameters (*c.f.* Table 1) that the effect of locally administered atropine sulphate was surmountable, whereas the effects of all three systemically administered antagonists were not.

In conclusion, systemically administered atropine sulphate, atropine methonitrate and hexamethonium bromide may have affected the response of the sweat glands to locally injected carbachol via the blockade of cholinoceptors in sympathetic ganglia. The most likely link between ganglionic cholinoceptors and the pharmacological responsiveness of sweat glands is the nervous activity in postganglionic sympathetic neurones: the blockade of ganglionic cholinoceptors would reduce impulse flow in sudomotor fibres, which in turn could result in a decreased sensitivity of the sweat gland to locally injected carbachol. Indeed, it has been reported that sympathetic denervation renders sweat glands hyporesponsive to locally applied cholinomi-

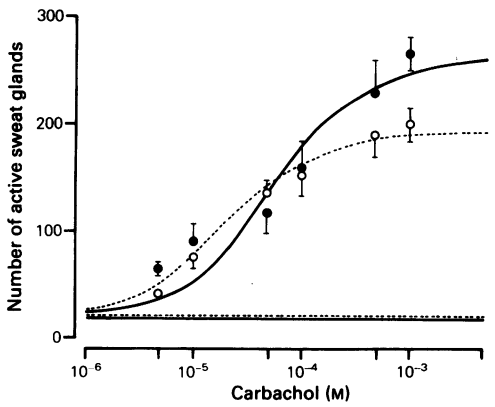


**Figure 5** Dose-response relationship for carbachol in absence (•) and presence (○) of systemically administered hexamethonium bromide 10 mg i.m. ($n$ = 6). Conventions as in Figure 2.

metics (Randall & Kimura, 1955). It is of interest that variables that are likely to increase sympathetic discharge (such as anxiety neurosis (Maple *et al.*, 1982), increased ambient temperature and mental arithmetic (van den Broek *et al.*, 1984)) produce an effect which is opposite to that seen after the administration of the ganglion receptor blockers: sweat glands became hyperresponsive to intradermally injected carbachol, and this is reflected in an elevation of the value of $E_{max}$ of the dose-respone curve. The present results, therefore, provide further evidence for the role of the sympathetic innervation in modulating the pharmacological responsiveness of sweat glands

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