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# Effects of several muscarinic agonists on cardiac performance and the release of noradrenaline from sympathetic nerves of the perfused rabbit heart

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**Summary**

1. The effects of several muscarinic agonists on atrial tension development, ventricular rate and noradrenaline release from terminal sympathetic fibres evoked by electrical nerve stimulation (SNS) and 1,1-dimethyl-4-phenylpiperazinium (DMPP) were measured in isolated perfused rabbit hearts.

2. Hexamethonium, in a concentration which almost abolished the release of noradrenaline by DMPP, had no effect on the release produced by SNS, confirming that the stimulation was postganglionic.

3. The order of potency for inhibition of atrial tension development was *N*-methyl-1,2,5,6, tetrahydro-nicotinic acid prop-2-yne ester (MH-1)>oxotremorine > acetylcholine > methacholine > carbachol > furtrethonium > pilocarpine>4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343)>*N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602). All effects were abolished by atropine ($1{\cdot}4 \times 10^{-6}$M).

4. Each compound was more potent relative to acetylcholine in inhibiting ventricular rate than atrial tension. With the exception of carbachol, the order of potency was the same.

5. Both AHR 602 and McN-A-343 facilitated the release of noradrenaline by SNS and inhibited that by DMPP. The effects were atropine-resistant and hence non-muscarinic.

6. The muscarinic compounds (except AHR 602 and McN-A-343) each produce atropine-sensitive inhibition of noradrenaline release evoked both by SNS and DMPP although it is likely that furtrethonium and pilocarpine have additional non-muscarinic inhibitory activity against DMPP. The order of potency on both parameters and the potencies relative to acetylcholine were in good agreement with those for inhibition of atrial tension.

7. The results suggest that similar muscarinic receptors mediate inhibition of atrial tension development, ventricular rate and neuronal noradrenaline release caused by SNS and DMPP.

8. In terms of the two muscarinic sites known to be present in the superior cervical ganglion, the receptors of the terminal fibres mediating inhibition of noradrenaline release are more likely to correspond to those mediating hyperpolarization than to those mediating depolarization, for which AHR 602 and McN-A-343 show specificity.

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## Introduction

In 1967, Löffelholz, Lindmar & Muscholl demonstrated that muscarinic receptors were present on the terminal sympathetic fibres of the rabbit heart. Agonists at these receptors inhibited the noradrenaline release evoked either by infusions of nicotinic drugs (Lindmar, Löffelholz & Muscholl, 1968) or electrical nerve stimulation (Löffelholz & Muscholl, 1969). The existence of such receptors is not unexpected since both excitatory and inhibitory atropine-sensitive sites have been identified on the soma-dendritic membrane of the rabbit superior cervical ganglion (Eccles & Libet, 1961; Libet, 1967; Kosterlitz, Lees & Wallis, 1968; Libet & Tosaka, 1969).

The present investigation had two objectives. First to characterize further the receptors of the terminal fibres by comparing the potencies of several compounds with different muscarinic affinities as inhibitors of noradrenaline release with their potencies on other cardiac muscarinic receptor sites. Secondly, to investigate whether the terminal muscarinic receptors correspond to the receptors mediating hyperpolarization or to those mediating depolarization of the superior cervical ganglion. Since no selective antagonist is available at the present time, we have used two compounds which are reported to be selective agonists at the depolarizing receptors of the ganglion (Volle, 1966; Trendelenburg, 1967). These are *N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602) (Franko, Ward & Alphin, 1963) and 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) (Roszkowski, 1961).

A preliminary account of these experiments has been given to the British Pharmacological Society (Fozard & Muscholl, 1971b).

## Methods

### *Perfusion of the heart*

Rabbits of either sex weighing 1·6 to 2·4 kg were stunned by a blow to the head and bled. Hearts were rapidly removed with the right sympathetic nerves attached (Huković & Muscholl, 1962) and perfused according to the Langendorff technique with modified Tyrode solution at 35·5° C. The Tyrode solution (concentrations in g/l.: NaCl 8·0; KCl 0·2; $CaCl_2$ 0·2; $MgCl_2$ 0·1; $NaHCO_3$ 1·0; $NaH_2PO_4$ 0·05; glucose 1·0; ascorbic acid 0·01) was gassed with a mixture of 95% $O_2$ and 5% $CO_2$. Perfusion pressure was maintained at 60 cm water.

Right ventricular and atrial tensions were recorded transversely as described by Beckett (1970) and Fozard & Muscholl (1971a). Briefly, right ventricular tension was recorded by a transducer (Grass model FT.03) attached to a thread tied into the wall of the right ventricle. The heart was secured and prevented from rotating by two retaining threads placed in the ventricular septal tissue on both sides of the heart. Right atrial tension was recorded by means of an additional thread stitched into the tip of the right atrium and passed to a second transducer. Tension records were displayed on two channels of a Hellige Programm 19 pen recorder. The third channel was used to display the ventricular rate measured by a ratemeter triggered from the ventricular tension record.

*Design of the experiments*

Concentration-effect curves on atrial tension development and ventricular rate were established by perfusion with increasing concentrations of each compound for 1 min at 10 min intervals. The maximum percentage difference between the atrial tension or ventricular rate recorded immediately preceding drug perfusion and that recorded during perfusion was taken as the response.

In separate experiments noradrenaline release from the hearts was evoked either by electrical stimulation of the right sympathetic nerves leaving the stellate ganglion (600 rectangular pulses, 1 ms, 10 Hz, supramaximal voltage—SNS) or by a 3 min infusion of 1,1-dimethyl-4-phenylpiperazinium ($9{\cdot}6 \times 10^{-5}$M—DMPP). The design of these experiments is illustrated in Figure 1. In control experiments, three periods of SNS separated by intervals of 10 min were followed after 30 min by two periods of DMPP separated by 15 min (Fig. 1A). The procedure in the test experiments was similar except that muscarinic drugs were infused 1 min before and during the second periods of SNS or DMPP (Fig. 1B). In experiments with atropine the antagonist was perfused from 8 min before the first period of SNS until the end of the third, or, in the case of DMPP, from 8 min before until the end of the second infusion period.

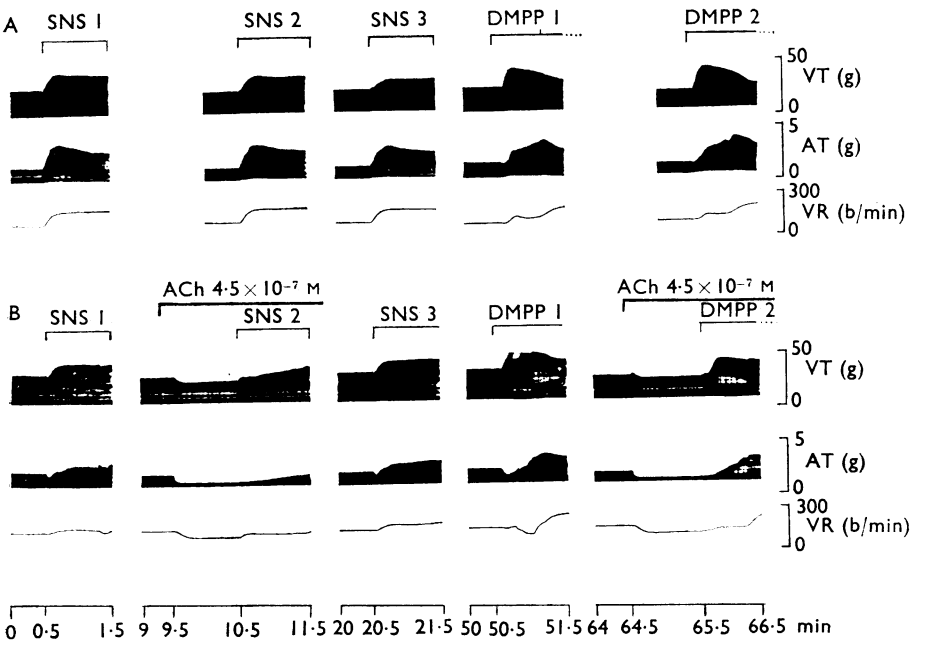


FIG. 1. Illustration of the experimental design adopted to measure the effects of muscarinic compounds on noradrenaline release from the sympathetic nerves of the isolated perfused rabbit heart. SNS=electrical stimulation of the sympathetic nerves (600 pulses, 1 ms, 10 Hz, supramaximal voltage). DMPP=3 min perfusion with 1,1-dimethyl-4-phenylpiperazinium ($9{\cdot}6 \times 10^{-5}$M). VT=ventricular tension in g; AT=atrial tension in g; VR=ventricular rate in beats per minute. The scale along the base shows the time course of the experiments in minutes. Upper panel, A, control experiment. Lower panel, B, test experiment in which acetylcholine (ACh $4{\cdot}5 \times 10^{-7}$M) was perfused 1 min before and during the second periods of SNS and DMPP. Noradrenaline outputs into the perfusates during each period of SNS and DMPP were assayed and compared as described in the text.

The output of noradrenaline during the second period of stimulation was expressed as the proportion of the output detected in the respective first period and compared with the equivalent change occurring in the control experiments. The third period of nerve stimulation served to indicate the continuing effectiveness of electrical stimulation throughout each experiment. Regression lines relating percentage reduction in noradrenaline output to drug concentration were calculated by the method of least squares.

From the concentration-effect curves obtained on each parameter the affinity of each compound was expressed as the negative $\log_{10}$ of the molar concentration giving 50% of a maximum response, the $pD_2$ value of Ariëns (1964). The affinity is also given relative to acetylcholine (taken as 100). Affinity ratios, which are the ratios of the molar concentrations giving 50% of a maximum response, were calculated after Van Rossum (1965). The geometrical means of the affinity ratios are presented.

*Estimation of noradrenaline*

To determine the output of noradrenaline, 3 min samples of the perfusate were collected starting with the onset of SNS or DMPP infusion. The perfusates were immediately acidified with 1 N $H_2SO_4$ adjusting the pH to 3. Noradrenaline was estimated fluorimetrically by a modification of the trihydroxyindole method after absorption on and elution from alumina (Lindmar & Muscholl, 1964). The recovery of 0·2 and 0·5 $\mu$g of noradrenaline added to 50 ml Tyrode solution was repeatedly tested. It ranged from 60% to 84% with a mean value of 72·2% obtained in 39 experiments. The amounts of endogenous noradrenaline released into the perfusates were not corrected for this recovery. None of the drugs either individually or in combination interfered with the recovery or estimation of noradrenaline.

*Statistical analysis*

All measures of variation of means quoted are standard errors. Student's *t* test was used to assess the significance of a difference between mean values. *n* is the number of observations.

*Drugs used*

These were acetylcholine chloride (Deutsche Hoffmann–La Roche A.G. Grenzach); atropine sulphate (Boehringer Sohn, Ingelheim); carbachol chloride (E. Merck, Darmstadt); 1,1-dimethyl-4-phenylpiperazinium iodide (Fluka, Buchs, Switzerland); furtrethonium iodide (Professor E. Ariëns, Nijmegen); hexamethonium iodide (Cassella, Frankfurt); 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343, McNeil Labs. Inc., Pittsburg, Pa., U.S.A.); methacholine chloride (Schuchard, Munich); *N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602, A. H. Robins, Co., Richmond, Va., U.S.A.); oxotremorine (E. G. A. Chemie, Steinheim); pilocarpine hydrochloride (Boehringer Sohn, Ingelheim); *N*-methyl-1,2,5,6, tetrahydro-nicotinic acid prop-2-yne ester (MH-1; Mutschler & Hultzsch, 1972; made available by Professor E. Mutschler, Pharmacy Department, University of Mainz).

Concentrations are expressed as molarities.

## Results

### *Effects of muscarinic agonists on atrial tension development and ventricular rate in the perfused rabbit heart*

All the agonists produced concentration-dependent depression of atrial tension development (Fig. 2A) and ventricular rate (Fig. 2B). The maximum responses obtained with each compound were fully antagonized by perfusion of the heart with atropine ($1{\cdot}4 \times 10^{-6}$M) thus confirming the muscarinic character of the responses. The negative $\log_{10}$ of the EC50 molar concentration, the potency relative to acetylcholine (taken as 100) and the atrial tension/ventricular rate affinity ratio for each compound are presented in Table 1. The compounds are ranked in descending order of potency for depression of atrial tension development. With

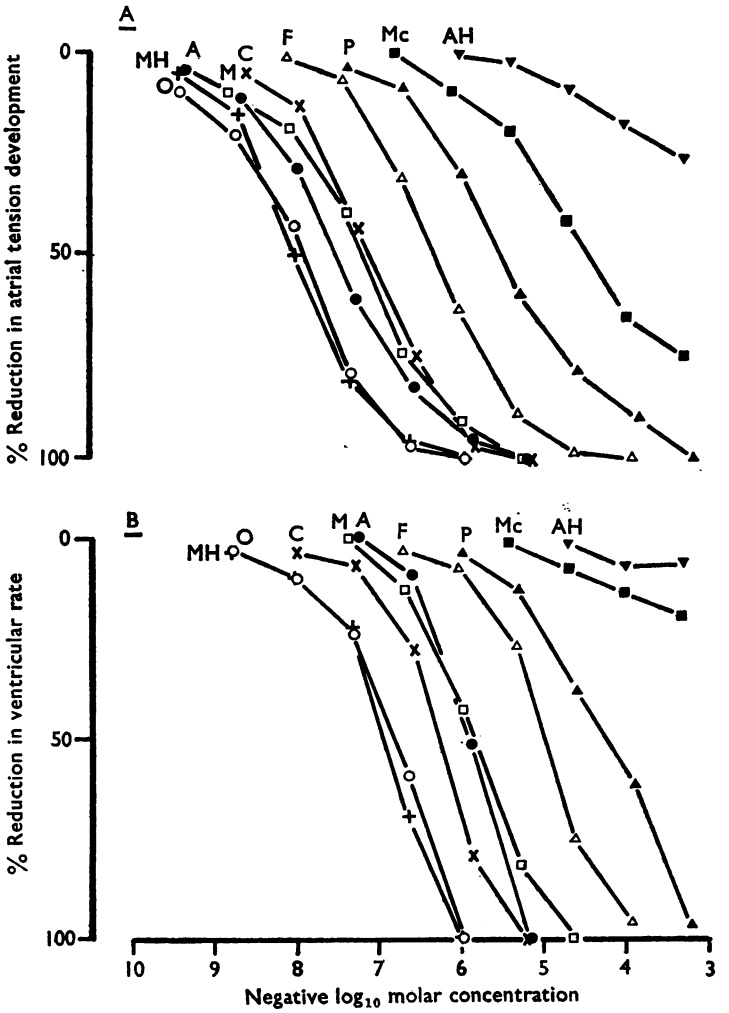


FIG. 2. Effects of various muscarinic agonists on atrial tension development (A) and ventricular rate (B) of the isolated perfused rabbit heart. Each point represents the mean of 4 to 6 experimental observations. ●, A=acetylcholine; ▼, AH=AHR 602; ×, C=carbachol; △, F=furtrethonium; □, M=methacholine; ■, Mc=McN-A-343; +, MH=MH-1; ○, O=oxotremorine; ▲, P=pilocarpine.

TABLE 1. *The negative $log_{10}$ of the EC50 molar concentrations ($pD_2$), the potencies relative to acetylcholine (Rel. Pot.) and the affinity ratios (Aff. Rat.) for the effects of muscarinic compounds on the isolated perfused rabbit heart*

| | Effects on | | | | | | | Effects on | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Atrial Tension | | | Ventricular rate | | | Aff. Rat. tension / rate | SNS noradrenaline release | | | DMPP noradrenaline release | | | Aff. Rat. SNS / DMPP |
| Compound | $pD_2$ | Rel. Pot. | $n_1$ | $pD_2$ | Rel. Pot. | $n_1$ | | $pD_2$ | Rel. Pot. | $n_2$ | $pD_2$ | Rel. Pot. | $n_2$ | |
| MH-1 | 8·02±0·13 | 339 | 5 | 6·89±0·16 | 1047 | 5 | 14 | 6·27 | 479 | 7 | 5·64 | 166 | 9 | 4·3 |
| Oxotremorine | 7·95±0·15 | 289 | 5 | 6·80±0·21 | 849 | 5 | 14 | 5·94 | 224 | 8 | 5·38 | 91 | 7 | 3·6 |
| Acetylcholine | 7·49±0·15 | 100 | 6 | 5·87±0·10 | 100 | 6 | 42 | 5·59 | 100 | 10 | 5·42 | 100 | 10 | 1·5 |
| Methacholine | 7·21±0·14 | 52 | 6 | 5·79±0·18 | 83 | 6 | 26 | 5·18 | 39 | 9 | 4·91 | 31 | 7 | 1·9 |
| Carbachol | 7·15±0·08 | 46 | 6 | 6·26±0·09 | 245 | 6 | 8 | 4·83 | 17 | 6 | 4·82 | 25 | 5 | 1·0 |
| Furtrethonium | 6·34±0·15 | 7·1 | 5 | 5·02±0·10 | 14 | 5 | 21 | 4·12 | 3·4 | 11 | 4·14 | 5·2 | 6 | 0·95 |
| Pilocarpine | 5·52±0·14 | 1·1 | 4 | 4·23±0·25 | 2·3 | 4 | 20 | 3·09 | 0·32 | 6 | 3·45 | 1·1 | 8 | 0·44 |
| McNeil-A-343 | 4·48±0·11 | 0·10 | 4 | <3·31 | <0·28 | 4 | — | *f** | — | 8 | 5·25* | 68 | 6 | — |
| AHR 602 | <3·30 | <0·006 | 4 | <3·30 | <0·27 | 4 | — | *f** | — | 7 | 4·25* | 6·8 | 6 | — |

$n_1$=Number of individual concentration–effect curves
* =Effect not antagonized by atropine ($1·4 \times 10^{-6}$M)

$n_2$=Number of individual estimations used to calculate regression lines
*f* =Facilitation

the exception of carbachol the order of potency on ventricular rate was in good agreement with this. However, for all the compounds, the potencies relative to acetylcholine were consistently higher for depression of ventricular rate than for depression of atrial tension. The mean atrial tension/ventricular rate affinity ratio which indicates the relative sensitivity of the two receptors to muscarinic stimulants was 28·9. The two compounds suggested to be selective ganglion stimulants AHR 602 and McN-A-343, were markedly less potent on both parameters than the other muscarinic agonists.

*Effects of hexamethonium on the release of noradrenaline from terminal sympathetic nerve fibres of the perfused rabbit heart*

Hexamethonium ($2{\cdot}7 \times 10^{-6}$M) was perfused either 1 min before and during the second periods of SNS or DMPP, or 8 min before and during the first period of SNS. The results are shown in Figure 3. In control experiments there was a decline in noradrenaline output of 38·2% and 40·2% from the first to the second stimulation periods for SNS and DMPP respectively. Hexamethonium had no significant effects on the noradrenaline release induced by SNS under either condition of perfusion (Figs. 3A and 3B). In contrast, the noradrenaline output induced by DMPP was almost completely abolished after only 1 min perfusion with hexamethonium (Fig. 3A).

*Effects of the muscarinic compounds alone and in the presence of atropine on the release of noradrenaline from terminal sympathetic fibres of the perfused rabbit heart*

The effects of each agonist on noradrenaline release by SNS and DMPP are shown in Figure 4. The negative $\log_{10}$ of the EC50 molar concentration, the

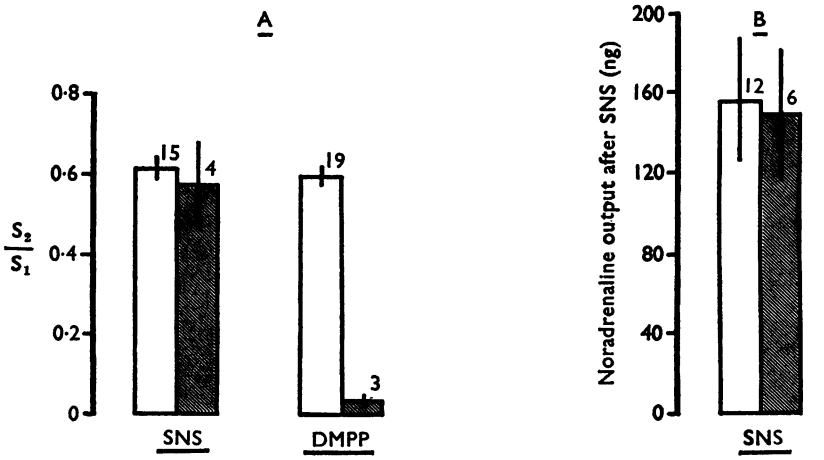


FIG. 3. The effects of hexamethonium on the output of noradrenaline from the sympathetic nerves of the isolated perfused rabbit heart. SNS=Electrical stimulation of the sympathetic nerves (600 pulses, 1 ms, 10 Hz, supramaximal voltage). DMPP=3 min perfusion with 1,1-dimethyl-4-phenylpiperazinium ($9{\cdot}6 \times 10^{-5}$M). $s_2/s_1$=ratios of outputs between second and first stimulation periods. Open columns, control experiments. Hatched columns, hexamethonium ($2{\cdot}7 \times 10^{-6}$M) one minute before and during second period of SNS or DMPP (A), or eight minutes before and during the first period of SNS (B). Number of experiments above each column; vertical bars indicate standard errors of the mean values.

potency relative to acetylcholine and the noradrenaline release after SNS/noradrenaline release after DMPP affinity ratio for each compound are given in Table 1. In Fig. 5 the effects of atropine ($1{\cdot}4 \times 10^{-6}$M) on the responses evoked by each muscarinic compound are presented.

The overflow of noradrenaline after SNS or DMPP was not corrected for the resting release of noradrenaline into the perfusates which in six experiments averaged $1{\cdot}9 \pm 1{\cdot}2$ ng/minute. None of the muscarinic compounds in the maximum concentrations used caused an appreciable change in this level during their first minute of perfusion, either alone or in the presence of atropine. Atropine ($1{\cdot}4 \times 10^{-6}$M) alone had no effect on the release of noradrenaline by SNS ($n=23$), or on the normal decline in output from the first to the second stimulation periods ($n=4$). Concentrations of atropine below $2{\cdot}9 \times 10^{-6}$M do not alter the noradrenaline output caused by DMPP (Lindmar *et al.*, 1968). The implications of the

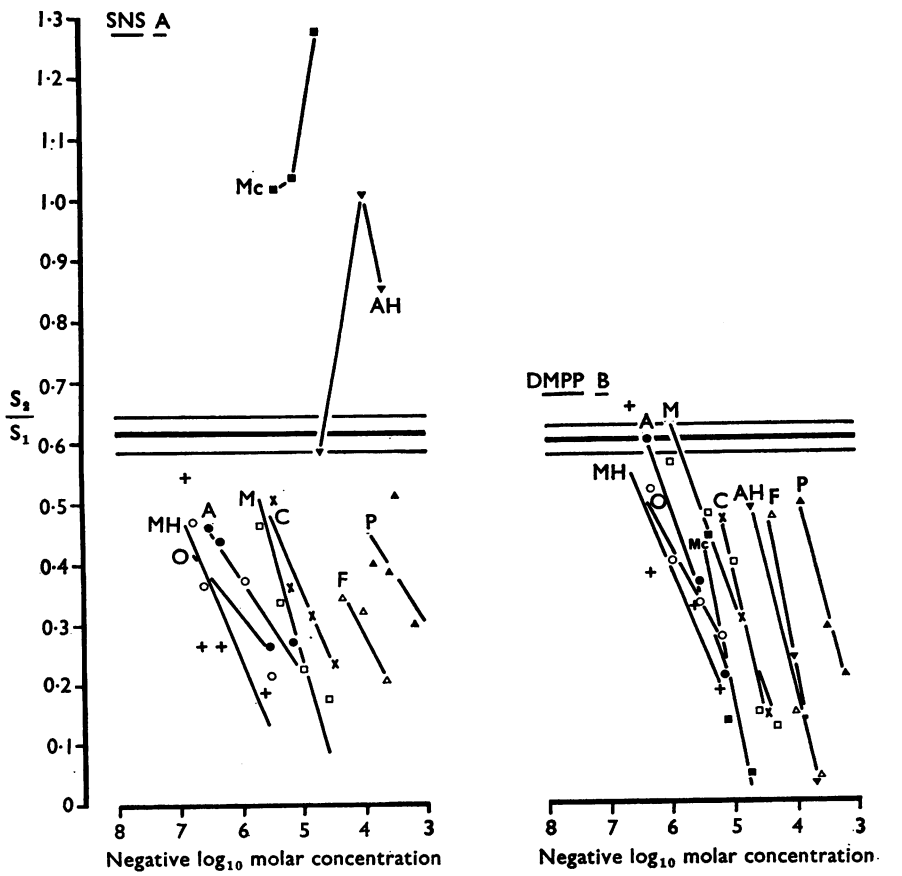


FIG. 4. Effects of various muscarinic agonists on the output of noradrenaline from the sympathetic nerves of the isolated perfused rabbit heart. $S_2/S_1$=Ratio of outputs between second and first stimulation periods for SNS (A) and DMPP (B). Regression lines relating percentage reduction in noradrenaline output to drug concentration were calculated by the method of least squares. The number of individual values from which each line was constructed are given in Table 1. The points represent the values from 1 to 5 individual determinations. The horizontal lines represent the mean $S_2/S_1$ ratios of noradrenaline output (with standard errors) in 15 (SNS) and 19 (DMPP) control experiments in which no modifying drugs were present. Other details as Figs. 2 and 3.

antagonism of DMPP noradrenaline output by concentrations of atropine greater than $2{\cdot}9 \times 10^{-6}$M (Lindmar *et al.*, 1968 ; Löffelholz, 1970) is considered fully in the **Discussion**.

Acetylcholine, carbachol, furtrethonium, methacholine, MH-1, pilocarpine and oxotremorine inhibited the noradrenaline release evoked by SNS in a concentration-

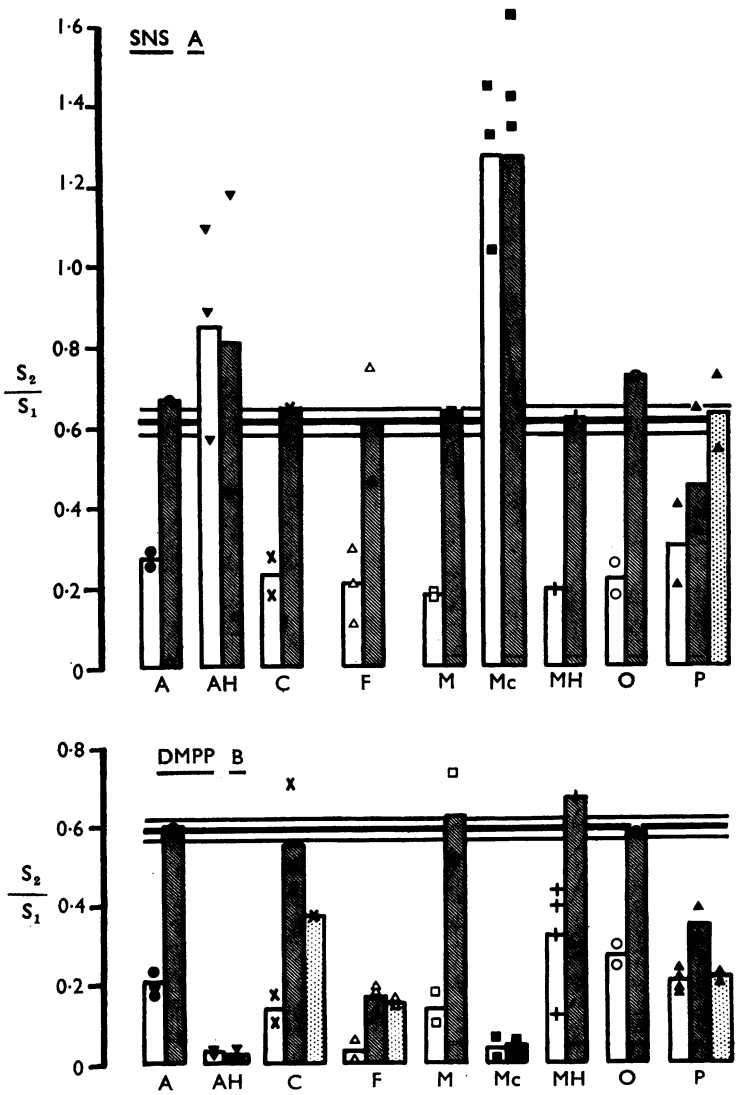


FIG. 5. Modification by atropine of the drug-induced changes in noradrenaline output evoked by SNS (A) and DMPP (B) from the isolated perfused rabbit heart. $s_2/s_1$=Ratio of outputs between second and first stimulation periods for SNS and DMPP. The open columns represent for each compound the mean output of noradrenaline during perfusion with the highest (or in the case of MH-1 and methacholine in part B, the second highest) concentrations shown in Fig. 4. The hatched and stippled columns represent the same concentrations repeated in the presence of atropine, $1{\cdot}4 \times 10^{-6}$ and $5{\cdot}8 \times 10^{-6}$M respectively. Each point represents a single experimental result. The horizontal lines represent the mean $s_2/s_1$ ratios of noradrenaline output (with standard errors) in 15 (SNS) and 19 (DMPP) control experiments in which no modifying drugs were present. Other details as Figs. 2 and 3.

dependent fashion (Fig. 4A). With the exception of pilocarpine the maximum responses obtained in each case were fully antagonized by atropine ($1{\cdot}4 \times 10^{-6}$M) present throughout the experiment (Fig. 5A). Pilocarpine responses were fully reversed by increasing the atropine concentration to $5{\cdot}8 \times 10^{-6}$M. After AHR 602 ($1{\cdot}0 \times 10^{-4}$ to $2{\cdot}0 \times 10^{-4}$M) and McN-A-343 ($3{\cdot}9 \times 10^{-6}$ to $2{\cdot}0 \times 10^{-5}$M) there was facilitation of the output of noradrenaline after SNS (Fig. 4A) which was unaffected by perfusion with atropine ($1{\cdot}4 \times 10^{-6}$M) (Fig. 5A).

The noradrenaline output produced by DMPP was inhibited in a concentration-dependent fashion by each of the compounds under investigation (Fig. 4B). The inhibitory responses obtained with acetylcholine, carbachol, methacholine, MH-1 and oxotremorine were fully antagonized, those to pilocarpine and furtrethonium partially antagonized, and those to AHR 602 and McN-A-343 unaffected by atropine ($1{\cdot}4 \times 10^{-6}$M) (Fig. 5B). No further reversal of the effects of furtrethonium and pilocarpine was obtained by increasing the atropine concentration to $5{\cdot}8 \times 10^{-6}$M. Indeed, the output of noradrenaline in the pilocarpine experiments was less than that measured after the smaller concentration of atropine (Fig. 5B).

For those compounds whose effects were antagonized wholly or partially by atropine, the orders of potency and the potencies relative to acetylcholine for inhibition of noradrenaline output after SNS and DMPP were in good agreement and similar to the equivalent values for inhibition of atrial tension and ventricular rate (Table 1). The mean noradrenaline release after SNS/noradrenaline release after DMPP affinity ratio was 1·52.

## Discussion

Misu & Kubo (1969) suggested that electrical nerve stimulation of the heart by the method of Huković & Muscholl (1962) involved transmission via a ganglionic pathway. Since inhibitory muscarinic receptors are present in sympathetic ganglia (see **Introduction**), it was important to establish that the stimulation performed in the present experiments was postganglionic.

The observations with hexamethonium (Fig. 3) supplement the earlier results of Huković & Muscholl (1962), Löffelholz & Muscholl (1969) and Wennmalm (1971), and confirm that electrical stimulation of the sympathetic nerves by the method of Huković & Muscholl (1962) is postganglionic. Furthermore, hexamethonium failed to inhibit noradrenaline release after SNS (Fig. 3A) under identical experimental conditions to those under which the muscarinic compounds were optimally effective (Fig. 4A). Taken together these observations preclude a ganglionic locus for the muscarinic inhibitory activity and suggest that in these experiments the results with SNS can truly be referred to events at the terminal sympathetic fibres. The discrepancy between these results and those of Misu & Kubo (1969) may simply reflect differences in the preparation of the nerve fibres for stimulation. In the present experiments, the electrode was positioned as close as possible to the heart thus ensuring that the postganglionic fibres were stimulated.

The inhibitory effects of each compound on cardiac performance (i.e. atrial tension and ventricular rate) were muscarinic since they were fully antagonized by atropine ($1{\cdot}4 \times 10^{-6}$M). Although, except for carbachol, there was reasonable agreement with respect to order of potency, all the compounds were more potent relative to acetylcholine in depressing ventricular rate than atrial force. The difference may reflect the type and/or distribution of cardiac cholinesterase

enzymes which control the quantitative response of the myocardium to acetylcholine (Webb, 1950). If, as is suggested, there is a greater concentration of cholinesterase activity in the region of the pacemaker than in contractile muscle cells (Webb, 1950 ; Roberts & Konjovic, 1969), then not only would a larger concentration of acetylcholine be needed to inhibit rate than force, but also differences in relative potencies would be expected between acetylcholine and those compounds not hydrolyzed by cholinesterases. In support of this interpretation the atrial tension/ventricular rate affinity ratios for acetylcholine and methacholine were the highest of all the compounds, and both are substrates of rabbit atrial cholinesterases (Briscoe, 1954).

In line with their reported specificity for the depolarizing receptors of the ganglion, AHR 602 and McN-A-343 displayed muscarinic inhibitory activity only at relatively high concentrations. Although earlier reports had described no activity of these compounds on the whole heart (Roszkowski, 1961 ; Franko *et al.*, 1963), more recently Pappano & Rembish (1971) have described atropine-sensitive slowing of guinea-pig atria after McN-A-343 ($2{\cdot}0 \times 10^{-5}$ to $10^{-4}$M). Clearly detection of muscarinic activity depends on the sensitivity of the test system, and atrial tension is at least a 28 times more sensitive indicator of muscarinic inhibitory activity than either ventricular rate or force (Fig. 2 ; Table 1 ; Lindmar *et al.*, 1968 ; Fozard & Muscholl, 1971a).

In contrast to their effects on cardiac performance, certain of the compounds altered the SNS- and DMPP-evoked noradrenaline release by mechanisms which were not entirely muscarinic. In particular, facilitation of noradrenaline release by SNS and inhibition of that by DMPP after AHR 602 and McN-A-343 were atropine-resistant (Fig. 5A), and hence non-muscarinic. Similarly the inhibitory effects of furtrethonium and pilocarpine on the response to DMPP (but not SNS) appeared to be in part non-muscarinic since they were not fully reversed by atropine (Fig. 5B). An increase in the atropine concentration from $1{\cdot}4 \times 10^{-6}$ to $5{\cdot}8 \times 10^{-6}$M not only produced no further antagonism of the effects of furtrethonium and pilocarpine, but reduced the noradrenaline output by DMPP in the presence of pilocarpine or carbachol (Fig. 5B). Since concentrations of atropine greater than $2{\cdot}9 \times 10^{-6}$M have nicotinic receptor blocking activity on this preparation (Lindmar *et al.*, 1968 ; Löffelholz, 1970) it is possible that the effects of further muscarinic blockade were obscured.

Nevertheless, the slopes of the furtrethonium and pilocarpine regression lines for inhibition of noradrenaline output by DMPP ($-0{\cdot}61$ and $-0{\cdot}40$ respectively) were somewhat steeper than those for the five compounds whose effects were fully atropine sensitive ($-0{\cdot}30 \pm 0{\cdot}04$), and more similar to those of AHR 602 and McN-A-343 ($-0{\cdot}55$ and $-0{\cdot}45$ respectively) whose effects were non-muscarinic. Furthermore, whereas the five compounds were more potent in inhibiting noradrenaline output after SNS than after DMPP (mean noradrenaline release by SNS/noradrenaline release by DMPP affinity ratio, 2·1) furtrethonium and pilocarpine were more potent in inhibiting the output after DMPP than after SNS (mean noradrenaline release by DMPP/noradrenaline release by SNS affinity ratio, 1·6). The evidence is thus consistent with furtrethonium and pilocarpine having additional, atropine-resistant inhibitory activity on the release of noradrenaline by DMPP. Blockade of ganglionic transmission by an unexplained, but definitely non-muscarinic mechanism has been described for AHR 602,

McN-A-343 and furtrethonium (Jaramillo & Volle, 1967a and b). The atropine-resistant inhibition of noradrenaline release by DMPP seen in the present experiments may be a manifestation of the same property on the terminal fibres.

The atropine-resistant facilitation of noradrenaline output after SNS by AHR 602 and McN-A-343 is currently the subject of further investigation. Preliminary results to be reported (Fozard & Muscholl, 1972) show not only that McN-A-343 can block the net uptake of noradrenaline by the heart, but also that its facilitatory effects on noradrenaline output after SNS are reduced by prior perfusion with cocaine. From this evidence the ability to inhibit noradrenaline uptake would seem to be an important factor contributing to the observed facilitation. These observations may also be relevant to the results of Rand & Varma (1971) who demonstrated atropine-resistant potentiation of sympathetic nerve stimulation by McN-A-343 on the rabbit ear artery for which they had no conclusive explanation.

If a series of compounds exert their pharmacological effects through activation of the same receptors then their order of potency and relative potencies should be identical on all tissues containing those receptors. In the present experiments, when allowance is made for the atropine-resistant inhibitory effects of furtrethonium and pilocarpine and the relevance of cholinesterase activity, there was good agreement between the compounds with respect to their muscarinic relative potencies on each parameter tested. This is evidence that the muscarinic receptors mediating inhibition of atrial tension development, ventricular rate and release of neuronal noradrenaline by SNS and DMPP are similar.

It is significant that neither AHR 602 nor McN-A-343 showed selectivity for the muscarinic receptors mediating inhibition of noradrenaline release. The possibility of muscarinic inhibition or indeed excitation being obscured by the atropine-resistant effects of AHR 602 and McN-A-343 is unlikely since the output of noradrenaline after both SNS and DMPP was similarly affected before and after atropine (Fig. 5). On the superior cervical ganglion McN-A-343 was 20 to 30 times more potent and AHR 602 was only slightly less potent than acetylcholine in stimulating the depolarizing muscarinic receptor sites (Jones, 1963 ; Takeshige, Pappano, De Groat & Volle, 1963 ; Trendelenburg, 1966 ; Jaramillo & Volle, 1967a and b). Neither drug stimulated the ganglionic receptors mediating hyperpolarization (Jaramillo & Volle, 1967a and b), although the relative potencies of acetylcholine, furtrethonium, methacholine, oxotremorine and pilocarpine on these receptors were in good agreement with those shown in Table 1 (Takeshige *et al.*, 1963 ; Takeshige & Volle, 1964 ; Jaramillo & Volle, 1967a and b ; Volle, 1967). The evidence is thus compatible with there being only one type of muscarinic receptor present on the terminal sympathetic fibres of the rabbit heart. Further, this receptor is more likely to correspond to the receptor of the ganglion causing hyperpolarization than to that mediating depolarization.

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