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ATROPINE-RESISTANT EFFECTS OF THE MUSCARINIC AGONISTS McN-A-343 AND AHR 602 ON CARDIAC PERFORMANCE AND THE RELEASE OF NORADRENALINE FROM SYMPATHETIC NERVES OF THE PERFUSED RABBIT HEART

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1 The effects of 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) and *N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602) on cardiac performance and noradrenaline release from terminal sympathetic fibres were measured in isolated perfused hearts of rabbits.

2 In the presence of sufficient atropine to block muscarinic receptors, high concentrations of McN-A-343 and AHR 602 caused no cardiac stimulation and there was no increase in the resting output of noradrenaline into the perfusates.

3 McN-A-343 and AHR 602 increased both the mechanical responses and the transmitter overflow evoked by electrical stimulation of the sympathetic nerves (SNS) but inhibited both parameters during perfusion with 1,1-dimethyl-4-phenylpiperazinium (DMPP). The effects were atropine-resistant and qualitatively similar to those seen with cocaine. Hexamethonium inhibited DMPP, but affected neither SNS *per se* nor the facilitatory effects of McN-A-343 and AHR 602 on SNS.

4 McN-A-343, cocaine and desipramine (but not AHR 602 or hexamethonium) blocked the net cardiac noradrenaline uptake and increased the positive chronotropic effect of noradrenaline.

5 Prior perfusion with concentrations of cocaine and desipramine sufficient to block uptake reduced or abolished the facilitatory effects of both McN-A-343 and AHR 602 on SNS.

6 Cocaine, McN-A-343 and AHR 602 displayed local anaesthetic properties on the guinea-pig wheal and frog nerve plexus tests, and their relative potencies in this respect were similar to those for inhibition of DMPP-evoked transmitter overflow. Hexamethonium did not produce local anaesthesia.

7 The results indicate that the facilitated release of noradrenaline after SNS and the inhibition of release after DMPP produced by McN-A-343 and AHR 602 are the result of their combined local anaesthetic action and inhibition of amine uptake.

Introduction

4-(*m*-Chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) and *N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602) are powerful stimulants of the muscarinic receptors present in mammalian sympathetic ganglia which mediate depolarization and ganglionic stimulation when activated (Roszkowski, 1961; Franko, Ward & Alphin, 1963; Jones, Gomez Alonso de la Sierra & Trendelenburg, 1963; Murayama & Unna, 1963; Smith, 1966; Jaramillo & Volle, 1967a and b; Aiken & Reit, 1969; Green, 1969; Hancock & Volle, 1970; Watson, 1970). They are selective in that they activate classical peripheral muscarinic receptor sites only at relatively high concentrations (Roszkowski, 1961; Franko *et al.*, 1963; Levy &

Ahlquist, 1962; Smith, 1966; Hobbiger, Mitchelson & Rand, 1969; Pappano & Rembish, 1971; Fozard & Muscholl, 1972a).

In recent experiments to determine whether the selectivity for the ganglionic sites extended to the muscarinic inhibitory receptors of the terminal sympathetic fibres of the rabbit heart (Fozard & Muscholl, 1972a), McN-A-343 and AHR 602 were found to increase the transmitter release evoked by electrical nerve stimulation and to decrease the release evoked by perfusion of 1,1-dimethyl-4-phenylpiperazinium. These effects were often seen at concentrations below those necessary to elicit myocardial muscarinic responses, and were unaffected by atropine at a concentration which fully abolished the inhibitory responses to acetylcholine.

The object of the present paper is to describe in detail the effects of McN-A-343 and AHR 602 on transmitter release from cardiac sympathetic nerves, and to present the results from experiments to clarify the mechanisms of their actions.

A preliminary account of some of these findings has already appeared (Fozard & Muscholl, 1972b).

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 then bled. Hearts were rapidly removed, some with the right sympathetic nerves attached (Huković & Muscholl, 1962), and perfused according to the Langendorff technique as previously described (Fozard & Muscholl, 1972a). Right ventricular tension and rate, and right atrial tension, were recorded as described by Beckett (1970) and Fozard & Muscholl (1971).

Experimental procedures

Noradrenaline release from the heart was evoked either by electrical nerve 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 (DMPP, 9.6×10^{-5} M). The experimental design adopted to measure the effects of modifying drugs on transmitter release is illustrated in Figure 2. In control experiments, three periods of SNS (SNS 1 to 3) separated by intervals of 10 min were followed after 30 min by two periods of DMPP (DMPP 1 and 2) separated by 15 min (Figure 2a). The procedure in the test experiments was similar except that modifying drugs were perfused 1 min before and during SNS 2 and DMPP 2 (Figure 2b). In some experiments the effects of combinations of drugs were measured. For SNS, one drug was perfused from 8 min before SNS 1 until the end of SNS 3, with a second drug being added 1 min before and during SNS 2. For DMPP, one drug was perfused from 8 min before, and a second drug from 1 min before, DMPP 2, until the end of the experiment. The increases in myocardial tension and rate, and noradrenaline overflow, arising from the second periods of either SNS or DMPP, were expressed as proportions of the equivalent first period responses, and compared with the corresponding changes occurring in the control experiments. Three minute samples of the perfusates were collected for assay of noradrenaline, starting with the onset of SNS or perfusion with DMPP.

In separate experiments the effects of drugs on the removal of noradrenaline from the perfusion medium were studied. The hearts were perfused with 5.9×10^{-8} M (−)-noradrenaline, and four consecutive 4 min samples of the venous effluent were collected starting 4 min after the onset of noradrenaline perfusion. Arterial samples were taken from the inflow cannula just before and immediately after the perfusion period and the chemical recoveries of noradrenaline measured. The amounts of noradrenaline estimated in the four perfusates of the same experiment were adjusted using the mean of the two recovery figures. Since approximately equal amounts of noradrenaline were found in the four samples, they were averaged to calculate the percentage of noradrenaline removed during passage through the heart. Modifying drugs were perfused 1 min before and during the noradrenaline perfusion. In these experiments heart rate was counted by inspection immediately before and repeatedly during perfusion with noradrenaline.

Local anaesthesia was measured by the frog nerve plexus and guinea-pig wheal methods described by Bülbring & Wajda (1945).

Estimation of noradrenaline

All perfusion samples were immediately acidified with 1N H_2SO_4 to pH 3. Noradrenaline was measured 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 μg 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 in the perfusates were not corrected for this recovery. None of the drugs either individually or in combination interfered with the estimation of noradrenaline.

Statistical analysis

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

Drugs used

These were acetylcholine chloride (Deutsche Hoffmann-La Roche A.G. Grenzach); atropine sulphate (Boehringer Sohn, Ingelheim); (−)-cocaine hydrochloride (E. Merck, Darmstadt); 1,1-dimethyl-4-phenylpiperazinium iodide (Fluka, Buchs, Switzerland); hexamethonium iodide (Cassella, Frankfurt); 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McNeil-A-343, McNeil Labs. Inc., Pittsburg, Pa., U.S.A.); *N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602, A.H. Robins, Co., Richmond, Va., U.S.A.).

Concentrations are expressed as molarities.

Results

Effects of McN-A-343 and AHR 602 alone and in the presence of atropine on the perfused rabbit heart

Infusions of McN-A-343 (1.25×10^{-6} to 1.5×10^{-4} M) and AHR 602 (8.0×10^{-7} to 5.0×10^{-4} M) produce concentration-dependent inhibition of atrial tension and ventricular rate with little effect on ventricular tension (Fozard & Muscholl, 1972a; Figure 1). In the presence of atropine (1.4×10^{-6} M) a further perfusion with the highest concentration of either McN-A-343 or AHR 602 failed to evoke a response (Figure 1).

The resting release of noradrenaline from the untreated rabbit heart averaged 1.9 ± 1.2 ng/min ($n = 6$). This level was not significantly changed during perfusion with McN-A-343 (2.0×10^{-5} M) or AHR 602 (2.0×10^{-4} M) either alone (in both cases, $n = 2$) or in the presence of atropine (1.4×10^{-6} M) ($n = 3$ and $n = 2$ respectively).

Effects of McN-A-343 and AHR 602 on the mechanical responses and noradrenaline output evoked by stimulation of the cardiac sympathetic nerves

The results are summarized in Fig. 2 and Table 1. In control experiments the increase in right ventricular tension and rate, and atrial tension, in response to repeated periods of SNS and DMPP stimulation were reproducible (Fig. 2a), although

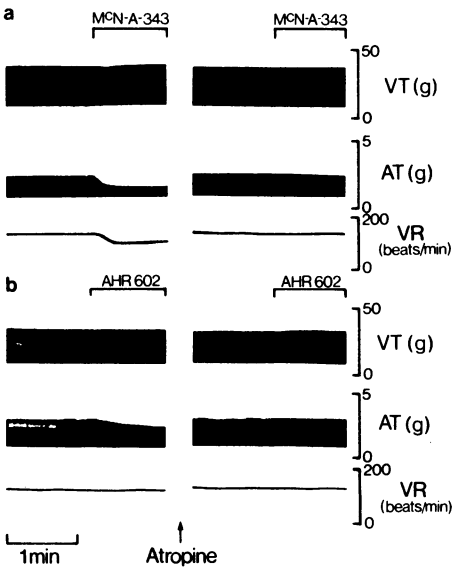

Fig. 1 Effects of McN-A-343 and AHR 602 on the rabbit isolated heart. VT, ventricular tension in g; AT, atrial tension in g; VR, ventricular rate in beats per minute. Time, 1 minute. After a 1 min control period, hearts were perfused for a further 1 min with (a) McN-A-343 (4.9×10^{-4} M) or (b) AHR 602 (5.0×10^{-4} M). Left panels before, and right panels 10 min after, perfusion with atropine (1.4×10^{-6} M).

in each case the noradrenaline output declined with repeated stimulation (Table 1). Atropine (1.4×10^{-6} M) or hexamethonium (2.7×10^{-6} M) perfused from 8 min before SNS 1 until the end of SNS 3 affected neither the output of noradrenaline arising from SNS 1, nor the decline in output from the first to each subsequent stimulation period (Table 1). Atropine (1.4×10^{-6} M), perfused in the present experiments from 8 min before until the end of DMPP 2, has previously been shown not to affect the output of noradrenaline evoked by perfusion with DMPP (Lindmar, Löffelholz & Muscholl, 1968).

McN-A-343 (2.0×10^{-5} M) or AHR 602 (2.0×10^{-4} M) added to the perfusion fluid 1 min before and for the duration of SNS 2 caused a small inhibition of atrial tension and ventricular rate. However, the mechanical responses evoked by SNS were usually slightly greater (Fig. 2b), and the outputs of noradrenaline were consistently increased (Table 1) compared to the equivalent observations during control experiments. In the presence of atropine (1.4×10^{-6} M) or hexamethonium (2.7×10^{-6} M), McN-A-343 and AHR 602 could still facilitate both the output of noradrena-

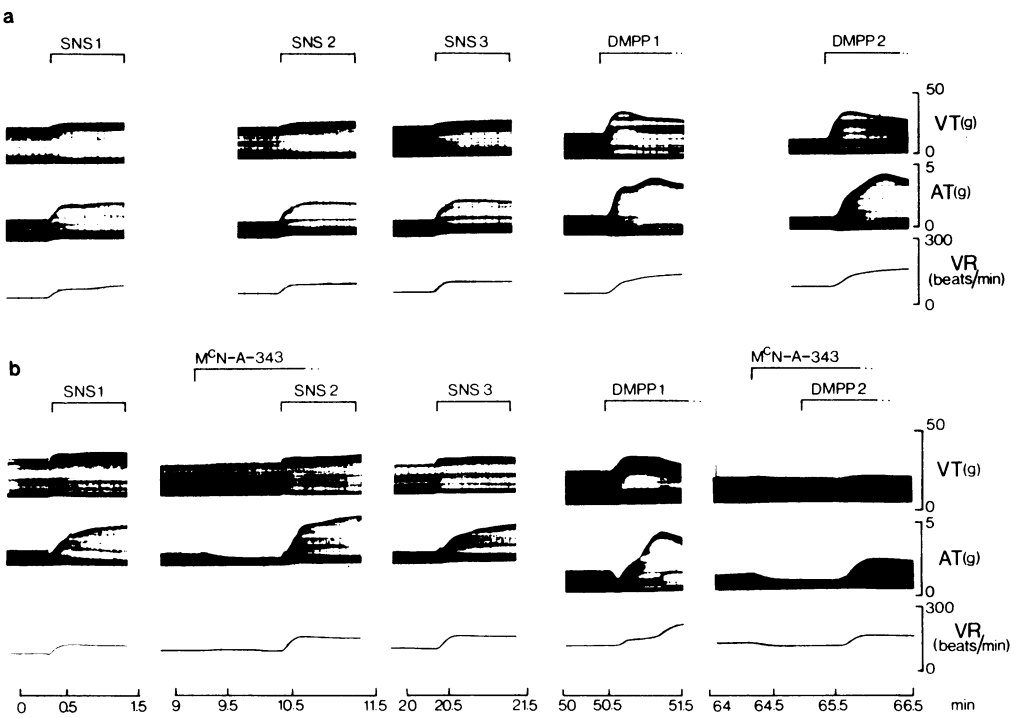

Fig. 2 Effects of McN-A-343 on the mechanical responses evoked by stimulation of the sympathetic nerves of the rabbit isolated heart. SNS = electrical stimulation of the fibres leaving the stellate ganglion (600 pulses, 1 ms, 10 Hz, supramaximal voltage). DMPP, 3 min perfusion with 1,1-dimethyl-4-phenylpiperazinium (9.6×10^{-5} M). The scale along the base shows the time course of the experiments in minutes. (a) Control experiments; (b) test experiments in which McN-A-343 (2.0×10^{-5} 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. Other details as Figure 1.

line (Table 1) and the mechanical responses evoked by SNS.

In contrast to their effects on SNS, both McN-A-343 (2.0×10^{-5} M) and AHR 602 (2.0×10^{-4} M) inhibited the mechanical response of the heart (Fig. 2b), and the output of noradrenaline (Table 1) evoked by perfusion with DMPP. Perfusion with atropine (1.4×10^{-6} M) did not prevent these effects.

Comparison of the effects of McN-A-343 and AHR 602 with those of cocaine, acetylcholine and hexamethonium

All drugs were perfused from 1 min before, and during SNS 2 or DMPP 2. The results are presented in Figure 3. With concentrations of McN-A-343 from 0.39 to 2.0×10^{-5} M, both the increases in noradrenaline output after SNS and the decreases after DMPP, were concentration-dependent. AHR 602 (0.2 to 2.0×10^{-4} M) also inhibited the release of noradrenaline by DMPP. At the higher doses (1.0 to 2.0×10^{-4} M) the release of noradrenaline after SNS was facilitated, although the maximum output observed was less than that attained with McN-A-343. These observations were similar to those obtained with cocaine, but differed from those observed with classical muscarinic receptor agonist drugs (Fozard & Muscholl, 1972a), exemplified here by acetylcholine. Thus, cocaine at a concentration of 1.8×10^{-5} M, increased both the cardiac stimulant effects and the output of noradrenaline as a result of SNS, yet inhibited both during perfusion with DMPP. Acetylcholine (0.28 to 6.9×10^{-6} M), in contrast, caused a concentration-dependent inhibition of transmitter output evoked either by SNS or DMPP. Hexamethonium (2.7×10^{-6} M) caused inhibition of the noradrenaline output after perfusion with DMPP, yet had no significant effect on either the noradrenaline output or the mechanical response to SNS.

Table 1 Effects of McN-A-343 and AHR 602 both alone and in the presence of atropine and hexamethonium on noradrenaline release from the sympathetic nerves of the rabbit heart

Drug	Concentration (M)	Noradrenaline release by SNS					Noradrenaline release by DMPP		
		S_1 (ng)	S_2/S_1	n	S_3/S_1	n	S_1 (ng)	S_2/S_1	n
None		170 ± 46	0.62 ± 0.04	10	0.36 ± 0.05	9	1051 ± 107	0.60 ± 0.02	19
Atropine*	1.4×10^{-6}	203 ± 27	0.59 ± 0.08	4	0.23 ± 0.08	4			
Hexamethonium*	2.7×10^{-6}	124	0.67	1	0.44	1			
Combined (control)		176 ± 31	0.62 ± 0.03	15	0.33 ± 0.04	14	1051 ± 107	0.60 ± 0.02	19
McN-A-343**	2.0×10^{-5}	112 ± 6	1.28 ± 0.12	3	0.65 (0.85, 0.45)	2	786 (938, 634)	0.04 (0.01, 0.08)	2
Atropine* + McN-A-343**	1.4×10^{-6} + 2.0×10^{-5}	136 ± 87	1.28 ± 0.20	4	0.44 ± 0.13	3	500 (631, 369)	0.05 (0.03, 0.06)	2
Hexamethonium* + McN-A-343	2.7×10^{-6} + 2.0×10^{-5}	196 ± 74	1.36 ± 0.22	3	0.39 ± 0.10	3			
Combined (McN-A-343)		147 ± 39	1.30 ± 0.10††	10	0.47 ± 0.08	8	643 ± 116	0.04 ± 0.02††	4
AHR 602**	2.0×10^{-4}	201 ± 43	0.85 ± 0.16	3	0.29 ± 0.11	3	1352 (1850, 855)	0.03 (0.02, 0.03)	2
Atropine* + AHR 602**	1.4×10^{-6} + 2.0×10^{-4}	273 (247, 300)	0.81 (1.19, 0.44)	2	0.19 (0.28, 0.10)	2	1148 (1715, 582)	0.02 (0.04, 0.01)	2
Hexamethonium* + AHR 602**	2.7×10^{-6} + 2.0×10^{-4}	94 (84, 103)	0.98 (1.17, 0.80)	2	0.39 (0.38, 0.41)	2			
Combined (AHR 602)		191 ± 33	0.88 ± 0.11†	7	0.29 ± 0.06	7	1250 ± 313	0.03 ± 0.01††	4

For SNS, drugs marked * were perfused from 8 min before SNS 1 until the end of SNS 3. Those marked ** were perfused from 1 min before SNS 2 until the end of the collection period. For DMPP, drugs marked * were perfused from 8 min before and those marked ** from 1 min before until the end of SNS 2. † and †† indicates a significant difference from the relevant combined (control) mean value ($P < 0.05$ and $P < 0.001$ respectively).

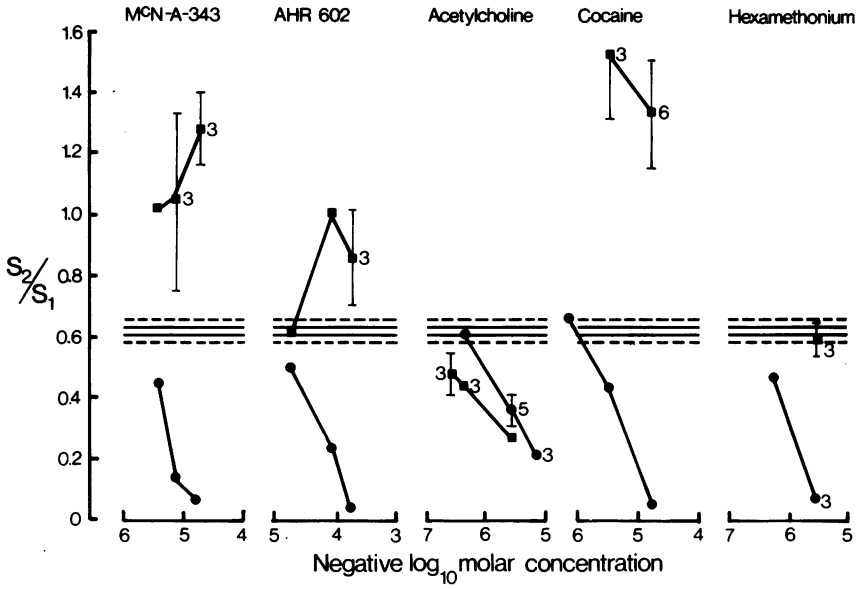

Fig. 3 Comparison of the concentration-dependent effects of McN-A-343, AHR 602, acetylcholine, cocaine and hexamethonium on noradrenaline output evoked by stimulation of the sympathetic nerves of the isolated rabbit heart. S_2/S_1, ratio of outputs between second and first stimulation periods for SNS (▪) and DMPP (•). The points represent the means of from two (unnumbered) to six experimental determinations. The vertical bars represent the standard errors of the mean values. The horizontal lines represent the mean S_2/S_1 ratios of noradrenaline output (with standard errors) in 15 SNS (upper two lines) and 19 DMPP (lower two lines) control experiments in which no modifying drugs were present.

Effects of McN-A-343, AHR 602, cocaine, desipramine and hexamethonium on the removal of noradrenaline from the perfusion medium of rabbit isolated perfused hearts

The results are given in Table 2. In control experiments, 51.3 ± 3.0% of the noradrenaline was removed during perfusion with a 5.9 x 10^{-8} M solution. There was a slight positive chronotropic effect of 12.4 ± 5.5 beats per minute. After cocaine (1.8 x 10^{-5} M), desipramine (5.2 x 10^{-6} M) and McN-A-343 (2.0 x 10^{-5} M) the removal was significantly reduced to 8.8, 6.5 and 28.2% respectively. The positive chronotropic effects from the infusion were increased in each case, and the increases were proportional to the

Table 2 The effects of drugs on the removal of noradrenaline (59 nM) from the perfusion medium by rabbit isolated hearts

Drug	*Concentration (M)*	*Number of hearts**	*% Noradrenaline eliminated*	*Mean pre-infusion heart rate (beats/min)*	*Mean increase in heart rate due to noradrenaline (beats/min)*
Drug free	–	8	51.3 ± 3.0	122.5 ± 5.2	12.4 ± 5.5
Cocaine	1.8 x 10^{-5}	3	8.8 ± 3.3††	116.0 ± 2.3	73.0 ± 21.4†
Desipramine	5.2 x 10^{-6}	4	6.5 ± 5.2††	137.5 ± 18.5	61.9 ± 5.6††
McN-A-343	2.0 x 10^{-5}	3	28.2 ± 1.8††	128.0 ± 16.2	19.7 ± 4.9
AHR 602	2.0 x 10^{-4}	4	50.9 ± 2.6	130.0 ± 8.7	3.0 ± 4.0
Hexamethonium	2.7 x 10^{-6}	3	51.3 ± 2.0	106.7 ± 2.7†	12.2 ± 6.7

* Four separate noradrenaline determinations were made for each heart and the results averaged.
† and †† indicates a significant difference from the equivalent drug-free mean value ($P < 0.05$ and $P < 0.001$ respectively).

degree of uptake inhibition. After AHR 602 (2.0 x 10^{-4} M) and hexamethonium (2.7 x 10^{-6} M), neither the elimination of noradrenaline nor the chronotropic effects were significantly different from the drug-free control values (Table 2).

Effects of McN-A-343, AHR 602, cocaine and desipramine on noradrenaline output evoked by electrical stimulation of the sympathetic nerves

Cocaine (1.8 x 10^{-5} M) and McN-A-343 (2.0 x 10^{-5} M) or AHR 602 (2.0 x 10^{-4} M) were perfused together from 1 min before and during SNS 2, and the output of noradrenaline obtained was compared with that obtained during perfusion with each drug separately. The results are presented in Figure 4. The output in the presence of both cocaine and McN-A-343 although increased, was not significantly different from that seen after either drug used alone, and less than the output which would have arisen had summation of the two effects occurred. When cocaine and AHR 602 were combined, the resultant output almost exactly equalled the sum of the increases arising from each drug used alone.

In separate experiments, cocaine (1.8 x 10^{-5} M) or desipramine (5.2 x 10^{-6} M) was perfused from 8 min before SNS 1 until the end of SNS 3. McN-A-343 (2.0 x 10^{-5} M) or AHR 602 (2.0 x 10^{-4} M) was perfused concurrently from 1 min before and during SNS 2. The results are presented in Table 3. After pretreatment with cocaine or desipramine, the overflow of noradrenaline after SNS 1 was greater than that obtained in untreated

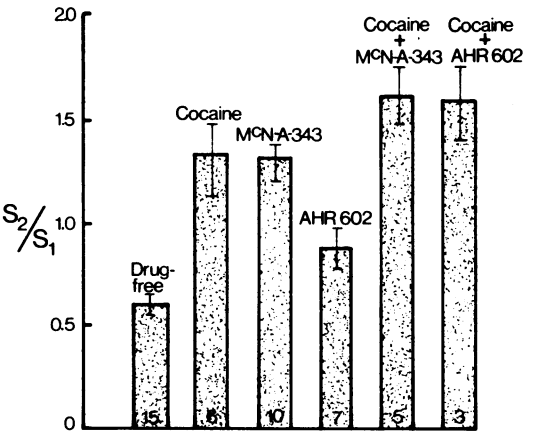

Fig. 4 Effects of McN-A-343 and AHR 602 both alone and in combination with cocaine on noradrenaline output evoked by electrical stimulation of the sympathetic nerves of the rabbit isolated heart. S_2/S_1, ratio of outputs between second and first SNS stimulation periods. Cocaine (1.8 x 10^{-5} M), McN-A-343 (2.0 x 10^{-5} M) and AHR 602 (2.0 x 10^{-4} M) were perfused either alone, or in combination, from 1 min before and during SNS 2. The number of individual experimental observations comprising each mean value is shown within the histograms. The vertical lines indicate the standard errors of the mean values. Details of significance can be inferred from the text.

hearts. Thus, the mean output of noradrenaline from SNS 1 obtained by combining values from the control groups given in Table 3 was

Table 3 Effects of McN-A-343 and AHR 602 both alone and in combination with cocaine or desipramine on noradrenaline output evoked by electrical stimulation of the sympathetic nerves of the rabbit isolated heart

Drug	*Concentration (M)*	S_1 *(ng)*	S_2/S_1	n	S_3/S_1	n
None	–	176 ± 31	0.62 ± 0.03	15	0.33 ± 0.04	14
McN-A-343**	2.0 x 10^{-5}	147 ± 39	1.30 ± 0.10††	10	0.47 ± 0.08	8
AHR 602**	2.0 x 10^{-4}	191 ± 33	0.88 ± 0.11†	7	0.29 ± 0.06	7
Cocaine*	1.8 x 10^{-5}	290 ± 87	0.66 ± 0.04	4	0.41 ± 0.10	3
Cocaine* McN-A-343**	1.8 x 10^{-5} 2.0 x 10^{-5}	478 ± 171	0.96 ± 0.03††	4	0.56 ± 0.04	4
Cocaine* AHR 602**	1.8 x 10^{-5} 2.0 x 10^{-4}	378 ± 142	0.66 ± 0.05	4	0.36 ± 0.08	4
Desipramine*	5.2 x 10^{-6}	450 ± 74	0.67 ± 0.04	4	0.48 ± 0.05	4
Desipramine* McN-A-343**	5.2 x 10^{-6} 2.0 x 10^{-5}	202 ± 52	0.62 ± 0.08	4	0.44 ± 0.10	4

Drugs marked * were perfused from 8 min before SNS 1 until the end of SNS 3. Those marked ** were perfused from 1 min before SNS 2 until the end of the collection period. † and †† indicate a significant difference from the relevant control mean value ($P < 0.05$ and $P < 0.001$ respectively). Details of the significances of the effects of cocaine and desipramine on the overflow of noradrenaline after SNS 1 are given in the text.

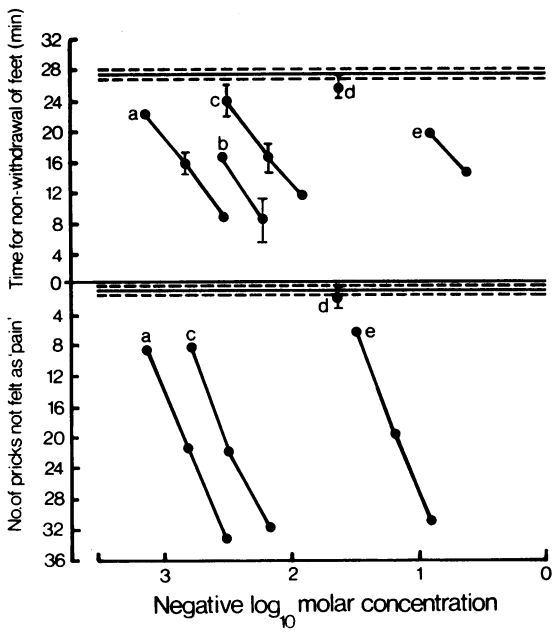

Fig. 5 Effects of cocaine (a and b); McN-A-343 (c); hexamethonium (d); and AHR 602 (e) on the frog nerve plexus and guinea-pig wheal tests for local anaesthesia. Regression lines relating reduction in time for non-withdrawal of the feet (frog test, upper graph) and increase in pricks producing no reaction (guinea-pig test, lower graph) to concentration were calculated by the method of least squares. The horizontal lines represent for each test, the mean values obtained (with standard errors) when the vehicle solution (0.7% sodium chloride) was used alone. Each point represents the mean of from three to six (frog test) or six to 12 (guinea-pig test) individual determinations. The vertical lines indicate the standard errors of the mean values. In the frog test, the cocaine (a) curve was obtained with *Rana temporaria* and relates only to the results with McN-A-343. Cocaine (b) was obtained five months later, with *Rana pipiens* and relates to AHR 602.

170 ± 19 ng ($n = 32$). After cocaine or desipramine, the equivalent values were significantly higher than this at 382 ± 76 ng ($n = 12, P < 0.01$) and 330 ± 63 ng ($n = 8$, $P < 0.05$) respectively. The decline in output from SNS 1 to SNS 3 was in each case, however, similar to that observed in control experiments.

In the presence of cocaine, AHR 602 failed to increase the output of noradrenaline evoked by SNS 2. In contrast, McN-A-343 retained significant facilitatory activity ($P < 0.001$) but its effects were reduced to about half those seen in untreated hearts. In the presence of desipramine, McN-A-343 no longer increased the output of noradrenaline as a result of SNS (Table 3).

Effects of McN-A-343, AHR 602, cocaine and hexamethonium on the guinea-pig wheal and frog nerve plexus tests for local anaesthesia

The results are presented in Figure 5. Cocaine, McN-A-343 and AHR 602 displayed significant local anaesthetic activity on each of the tests, and the concentration-effect curves were parallel. McN-A-343 was 2.2 and 5.2 times, and AHR 602 48.4 and 69.0 times less potent than cocaine on guinea-pig wheal and frog nerve plexus respectively. Hexamethonium, in concentrations up to 15 times the ED_{50} concentration of cocaine, displayed no activity on either test.

Discussion

Although classed as muscarinic agonists, both McN-A-343 and AHR 602 show selectivity for the depolarizing receptors of sympathetic ganglia and have little action at classical peripheral muscarinic receptor sites (see Introduction). The present results with atropine (Fig. 1 and Table 1) clearly indicate that with the exception of inhibition of cardiac performance, the effects of McN-A-343 and AHR 602 described in this study do not involve activation of muscarinic receptor sites. It is also unlikely that nicotinic receptor sites are playing any significant role in the facilitation of noradrenaline output from SNS observed after McN-A-343 and AHR 602, since perfusion with hexamethonium, which strongly inhibited noradrenaline overflow due to DMPP (Fig. 3), not only failed to modify noradrenaline output after SNS *per se*, but also left unaltered the facilitatory effects of McN-A-343 and AHR 602 (Table 1).

There are a number of theoretical means by which compounds added to the fluid perfusing the rabbit heart might increase the overflow of noradrenaline arising from stimulation of the sympathetic nerves. The possibility that facilitation could arise from an increased resting release can be dismissed since neither McN-A-343 nor AHR 602 caused any increase in the resting release of noradrenaline into the perfusates at concentrations producing facilitation of SNS. Furthermore, when the perfusion concentration of each compound was increased 25-fold and the myocardial inhibitory effects eliminated by atropine, no indication was seen of the sympathomimetic activity which would have been expected had significant release of noradrenaline occurred (Figure 1).

If the compounds possessed α-adrenoceptor antagonist activity, then they might increase the

overflow of noradrenaline by interrupting the feedback inhibitory effects of the stimulation-evoked release of noradrenaline (Starke, Montel & Schümann, 1971a; Wennmalm, 1971; Starke, 1972). However, there is no evidence in the literature for McN-A-343 or AHR 602 having α-adrenoceptor antagonist activity (Roszkowski, 1961; Franko *et al.*, 1963; Rand & Varma, 1971). Further, whereas the facilitatory effects of α-adrenoceptor antagonist drugs are additive with those produced by cocaine or desipramine (Starke *et al.*, 1971a; Starke, Montel & Wagner, 1971b; Wennmalm, 1971; Starke, 1972), those of McN-A-343 and AHR 602 were greatly reduced or abolished (Fig. 4, Table 3) under similar experimental circumstances. It therefore seems unlikely that blockade of α-adrenoceptor can explain the facilitated response to SNS after McN-A-343 and AHR 602.

The most likely explanation for the increased noradrenaline overflow is that these compounds inhibit transmitter re-uptake into the terminal sympathetic neurones. Certainly, typical blockers of noradrenaline uptake, such as cocaine and desipramine, not only inhibit net neuronal uptake of exogenous noradrenaline (Lindmar & Muscholl, 1964; Schümann, Starke, Werner & Hellerforth, 1970b; Schümann, Starke & Werner, 1970a) but also, in similar concentrations, enhance the overflow of noradrenaline (Huković & Muscholl, 1962; Löffelholz & Muscholl, 1970; Schümann *et al.*, 1970a; Wennmalm, 1971) arising from stimulation of the sympathetic nerves to the rabbit heart. The results from Fig. 3 and Tables 2 and 3 of the present study confirm these observations. In addition, they show that McN-A-343 displays a similar spectrum of activity in reducing the net neuronal uptake of noradrenaline (Table 2) at a concentration which enhances both the mechanical effects (Fig. 2) and the output of noradrenaline (Fig. 3 and Table 1) during SNS. AHR 602 on the other hand, although capable of enhancing the overflow of noradrenaline resulting from SNS (Table 1), could not be shown to inhibit the removal of noradrenaline from the perfusion fluid (Table 2). A similar effect of McN-A-343 on noradrenaline tissue uptake was reported recently by Allen, Rand & Story (1972), who demonstrated inhibition of the accumulation of tritium from a solution of (±)-[^{3}H]-noradrenaline into guinea-pig atria. The effects of AHR 602 on noradrenaline uptake appear not to have been investigated previously.

Any drug which has as the basis of its SNS facilitatory actions interference with transmitter re-uptake, should be less effective in the presence of partial uptake inhibition by another drug, and devoid of activity if uptake is completely abolished. Figure 4 shows that when McN-A-343 and AHR 602 were administered together with cocaine (at a concentration giving 83% inhibition of noradrenaline uptake, Table 2), the net facilitation caused by McN-A-343 was markedly reduced, although the much weaker effects of AHR 602 were probably additive with those of cocaine. Using a different experimental approach (cocaine or desipramine [87% uptake inhibition] present throughout the experiment, McN-A-343 or AHR 602 added 1 min before and during SNS 2), the facilitatory effects of AHR 602 were abolished, whilst those of McN-A-343 were reduced by half in the presence of cocaine and fully inhibited after desipramine (Table 3). These results would support the suggestion that both McN-A-343 and AHR 602 produce facilitation of SNS by inhibiting transmitter re-uptake.

Although the mechanism whereby DMPP releases noradrenaline from its storage sites within the sympathetic nerves is not established, it is assumed that combination with specific receptor sites—nicotinic—induces membrane depolarization to which transmitter release is coupled. There are a number of mechanisms by which McN-A-343 and AHR 602 might produce inhibition of the DMPP-evoked release of noradrenaline from the sympathetic nerves.

They might, like hexamethonium, prevent depolarization by blocking the nicotinic receptor sites. However, in experiments on the frog rectus abdominis preparation, no effects attributable to nicotinic receptor blockade could be demonstrated with either McN-A-343 (Roszkowski, 1961) or AHR 602 (Franko *et al.*, 1963). Further, although both compounds were shown to block nervous transmission through isolated cat superior cervical ganglia, the nicotinic receptor was specifically excluded as the site of the blockade (Jaramillo & Volle, 1967a and b). Finally, cocaine, which is not an antagonist at nicotinic receptors (Jones *et al.*, 1963; Palmer, 1972), is an effective inhibitor of the stimulant effects of DMPP, which implies the existence of an alternative mechanism of action.

This is most likely to be a local anaesthetic action, which would oppose the depolarizing effects of nicotinic receptor stimulation by non-specific stabilization of the neuronal cell membrane. Experimental evidence supporting such a mechanism has been provided by Lindmar & Muscholl (1961) and Westfall & Brasted (1972), who demonstrated inhibitory effects of local anaesthetic agents on the nicotine-evoked release of noradrenaline from cardiac sympathetic nerves. In the present study, cocaine, McN-A-343 and AHR 602 have all been shown to have local anaesthetic properties (Fig. 5), and significantly, there was good agreement with respect to their

relative potencies in producing local anaesthesia and in inhibiting DMPP-evoked transmitter release.

In addition to being a local anaesthetic, cocaine is a potent inhibitor of neuronal noradrenaline uptake. This is a potentially important site of blockade, in view of the recent suggestion that nicotinic drugs require to be transported intraneuronally by the noradrenaline uptake mechanism, as a prerequisite to effecting transmitter release (Su & Bevan, 1970; Bevan & Su, 1972; Allen *et al.*, 1972). If such a mechanism were operating for DMPP in the rabbit heart, then it could be argued that cocaine, McN-A-343 and AHR 602 were inhibiting its effect by blocking its intraneuronal access. However, in concentrations giving similar and marked inhibition of DMPP-evoked transmitter release (Fig. 3), blockade of exogenous noradrenaline uptake varied from 83% (cocaine), through 45% (McN-A-343), to no detectable activity (AHR 602) (Table 2). This suggests that in the rabbit heart (as in guinea-pig heart, Westfall & Brasted, 1972) the sympathomimetic effects of DMPP are not dependent on an intact noradrenaline uptake pathway. Consequently, it seeems likely that non-specific membrane stabilization is the mechanism whereby cocaine, McN-A-343 and AHR 602 exert their inhibitory effects, and that uptake inhibition represents merely an incidental non-causal event.

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References

AIKEN, J.W. & REIT, E. (1969). A comparison of the sensitivity to chemical stimuli of adrenergic and cholinergic neurones in the cat stellate ganglion. *J. Pharmac. exp. Ther.*, **169**, 211-223.

ALLEN, G.S., RAND, M.J. & STORY, D.F. (1972). Effects of McN-A-343 and DMPP on the uptake and release of [^{3}H]-noradrenaline by guinea-pig atria. *Br. J. Pharmac.*, **45**, 480-489.

BECKETT, P.R. (1970). The isolated perfused heart preparation: two suggested improvements. *J. Pharm. Pharmac.*, **22**, 818-822.

BEVAN, J.A. & SU, C. (1972). Uptake of nicotine by the sympathetic nerve terminals in the blood vessel. *J. Pharmac. exp. Ther.*, **182**, 419-426.

BÜLBRING, E. & WAJDA, I. (1945). Biological comparison of local anaesthetics. *J. Pharmac. exp. Ther.*, **85**, 78-84.

FOZARD, J.R. & MUSCHOLL, E. (1971). A useful muscarinic parameter and the differential recording of atrial and ventricular tension in the perfused rabbit heart. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **270**, 319-325.

FOZARD, J.R. & MUSCHOLL, E. (1972a). Effects of several muscarinic agonists on cardiac performance and the release of noradrenaline from sympathetic nerves of the perfused rabbit heart. *Br. J. Pharmac.*, **45**, 616-629.

FOZARD, J.R. & MUSCHOLL, E. (1972b). Atropine resistant effects of 4-(m-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) on norepinephrine release from sympathetic nerves. Abstract 424 of *Fifth International Congress on Pharmacology*, San Francisco, July 23-28, 1972.

FRANKO, B.V., WARD, J.W. & ALPHIN, R.S. (1963). Pharmacological studies of N-benzyl-3-pyrrolidyl acetate methobromide (AHR 602), a ganglion stimulating agent. *J. Pharmac. exp. Ther.*, **139**, 25-30.

GREEN, R. (1969). The effect of denervation on the sensitivity of the superior cervical ganglion of the pithed cat. *J. Pharmac. exp. Ther.*, **167**, 143-150.

HANCOCK, J.C. & VOLLE, R.L. (1970). Cholinoceptive sites in rat sympathetic ganglia. *Archs int. Pharmacodyn. Thér.*, **184**, 111-120.

HOBBIGER, F., MITCHELSON, F. & RAND, M.J. (1969). The actions of some cholinomimetic drugs on the isolated taenia of the guinea-pig caecum. *Br. J. Pharmac.*, **36**, 53-69.

HUKOVIĆ, S. & MUSCHOLL, E. (1962). Die Noradrenalin-Abgabe aus dem isolierten Kaninchenherzen bei sympathischer Nervenreizung und ihre pharmakologische Beeinflussung. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **244**, 81-96.

JARAMILLO, J. & VOLLE, R.L. (1967a). Non-muscarinic stimulation and block of a sympathetic ganglion by 4-(m-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride (McNeil-A-343). *J. Pharmac. exp. Ther.*, **157**, 337-345.

JARAMILLO, J. & VOLLE, R.L. (1967b). Ganglion blockade by muscarine, oxotremorine and AHR 602. *J. Pharmac. exp. Ther.*, **158**, 80-88.

JONES, A., GOMEZ ALONSO DE LA SIERRA, B. & TRENDELENBURG, U. (1963). The pressor response of the spinal cat to different groups of ganglion-stimulating agents. *J. Pharmac. exp. Ther.*, **139**, 312-320.

LEVY, B. & AHLQUIST, R.P. (1962). A study of sympathetic ganglionic stimulants. *J. Pharmac. exp. Ther.*, **137**, 219-228.

LINDMAR, R., LÖFFELHOLZ, K. & MUSCHOLL, E. (1968). A muscarinic mechanism inhibiting the release of noradrenaline from peripheral adrenergic nerve fibres by nicotinic agents. *Br. J. Pharmac.*, **32**, 280-294.

LINDMAR, R. & MUSCHOLL, E. (1961). Die Wirkung von Cocain, Guanethidin, Reserpin, Hexamethonium, Tetracain und Psicain auf die Noradrenalin-Freisetzung aus dem Herzen. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **242**, 214-227.

LINDMAR, R. & MUSCHOLL, E. (1964). Die Wirkung

von Pharmaka auf die Elimination von Noradrenalin aus der Perfusionsflüssigkeit und die Noradrenalinaufnahme in das isolierte Herz. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 247, 469-492.

LÖFFELHOLZ, K. & MUSCHOLL, E. (1970). Der Einfluss von d-Amphetamin auf die Noradrenalinabgabe aus dem isolierten Kaninchenherzen. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **266**, 393.

MURAYAMA, S. & UNNA, K.R. (1963). Stimulant action of 4-(m-chloro-phenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343) on sympathetic ganglia. *J. Pharmac. exp. Ther.*, **140**, 183-192.

PALMER, W.A. (1972). Unpublished observation reported by Allen, G.S., Rand, M.J. and Story, D.F. (1972). Effects of McN-A-343 and DMPP on the uptake and release of ^{3}H-noradrenaline by guinea-pig atria. *Br. J. Pharmac.*, **45**, 480-489.

PAPPANO, A.J. & REMBISH, R.A. (1971). Effects of the muscarinic agonist McNeil-A-343 and nicotine in isolated guinea-pig atria: Insensitivity to blockade by tetrodotoxin. *J. Pharmac. exp. Ther.*, **177**, 40-47.

RAND, M.J. & VARMA, B. (1971). Effect of the muscarinic agonist McNeil-A-343 on responses to sympathetic nerve stimulation in the rabbit ear artery. *Br. J. Pharmac.*, **43**, 536-542.

ROSZKOWSKI, A.P. (1961). An unusual type of ganglionic stimulant. *J. Pharmac. exp. Ther.*, **169**, 109-119.

SCHÜMANN, H.J., STARKE, K. & WERNER, U. (1970a). Interactions of inhibitors of noradrenaline uptake and antiotensin on the sympathetic nerves of the isolated rabbit heart. *Br. J. Pharmac.*, **39**, 390-397.

SCHÜMANN, H.J., STARKE, K., WERNER, U. & HELLERFORTH, R. (1970b). The influence of angiotensin on the uptake of noradrenaline by the isolated heart of the rabbit. *J. Pharm. Pharmac.*, **22**, 441-446.

SMITH, J.C. (1966). Pharmacologic interactions with 4 - (m - chlorophenyl - carbamoyloxy) - 2 - butynyltrimethylammonium chloride, a sympathetic ganglion stimulant. *J. Pharmac. exp. Ther.*, **153**, 276-284.

STARKE, K. (1972). Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **274**, 18-45.

STARKE, K., MONTEL, H. & SCHÜMANN, H.J. (1971a). Influence of cocaine and phenoxybenzamine on noradrenaline uptake and release. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **270**, 210-214.

STARKE, K., MONTEL, H. & WAGNER, J. (1971b). Effect of phentolamine on noradrenaline uptake and release. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **271**, 181-192.

SU, C. & BEVAN, J.A. (1970). Blockade of the nicotine-induced norepinephrine release by cocaine, phenoxybenzamine and desipramine. *J. Pharmac. exp. Ther.*, **175**, 533-540.

WATSON, P.J. (1970). Drug receptor sites in the isolated superior cervical ganglion of the rat. *Eur. J. Pharmac.*, **12**, 183-193.

WENNMALM, A. (1971). Studies on mechanisms controlling the secretion of neurotransmitters in rabbit heart. *Acta physiol. scand.*, Suppl. **365**.

WESTFALL, T.C. & BRASTED, M. (1972). The mechanism of action of nicotine on adrenergic neurons in the perfused guinea-pig heart. *J. Pharmac. exp. Ther.*, **182**, 409-418.

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