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Effects of the muscarinic agonist McN-A-343 on responses to sympathetic nerve stimulation in the rabbit ear artery

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Summary

1. Observations were made on the effects of 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) on responses of isolated segments of the central artery of the rabbit's ear to sympathetic nerve stimulation and noradrenaline.

2. With low frequencies of nerve stimulation (2–5 Hz), McN-A-343 caused a decrease in responses to sympathetic nerve stimulation. This effect of McN-A-343 was abolished by dexamphetamine or atropine. In the presence of atropine, McN-A-343 caused an increase in responses to sympathetic nerve stimulation.

3. With high frequencies of nerve stimulation (10–20 Hz), McN-A-343 caused an increase in responses. This effect was not qualitatively changed in the presence of atropine.

4. When McN-A-343 had an inhibitory effect on responses to sympathetic nerve stimulation, responses to noradrenaline were unaffected.

5. It is suggested that McN-A-343 acts on muscarinic receptors through which noradrenaline release may be inhibited; it may also act on the cholinergic stage in adrenergic transmission postulated by Burn & Rand (1959).

Introduction

Acetylcholine and other cholinomimetic drugs affect the responses to sympathetic nerve stimulation in some isolated preparations: depending on the frequency of stimulation and the concentration of drug, the responses may be enhanced or decreased (Malik & Ling, 1969a; Rand & Varma, 1970a). Both these effects are produced by a drug which acts solely on nicotinic receptors, DMPP (Malik & Ling, 1969b). However, there is also evidence for the presence of muscarinic receptors at adrenergic nerve terminals through which noradrenaline release may be inhibited (Lindmar, Löffelholz & Muscholl, 1968; Haeusler, Thoenen, Haefely & Huerlimann, 1968; Löffelholz & Muscholl, 1969; Malik & Ling, 1969a; Varma & Rand, 1969; Rand & Varma, 1970a, b) or facilitated (Rand & Varma, 1970a).

The type of receptor involved in the action of cholinomimetic drugs on responses to sympathetic nerve stimulation is deduced from the spectrum of action of the drugs on muscarinic and nicotinic receptors and from the use of receptor antagonists. The drug McN-A-343 [4(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride:

$$m\text{-}Cl\cdot C_6H_4\cdot NH\cdot CO\cdot O\cdot CH_2\cdot C:C\cdot CH_2\cdot N^+(CH_3)_3\cdot Cl^-]$$

stimulates muscarinic receptors and is selective for those of sympathetic ganglion cells (Roszkowski, 1961; Smith, 1966), but it does act on muscarinic receptors of the guinea-pig taenia caeci (Hobbiger, Mitchelson & Rand, 1969). The effects of McN-A-343 on ganglion cells and smooth muscles are abolished by atropine. However, McN-A-343 may have an atropine-insensitive action on adrenergic neurones; thus, it had a sympathomimetic effect due to release of noradrenaline in atropinized guinea-pig atria (Bhagat, 1966) and caused relaxation of atropinized taenia caeci (Hobbiger *et al.*, 1969).

We decided to investigate the effects of McN-A-343 on responses to sympathetic nerve stimulation and noradrenaline in the perfused isolated central artery of the rabbit's ear to compare them with the effects of other cholinomimetic drugs (Rand & Varma, 1970a). A preliminary account of this work was reported to a meeting of the British Pharmacological Society (Rand & Varma, 1970b).

Methods

Segments of the central artery of the rabbit ear were set up as described by de la Lande & Rand (1965). They were maintained at 37° C and perfused at the rate of 6 ml/min, using a Watson-Marlow flow inducer, with McEwen's (1956) solution bubbled with 5% carbon dioxide in oxygen. Changes in perfusion pressure, which arose from the changes in the resistance to flow through the arterial segment, were recorded with a Statham pressure transducer on an Offner Dynograph recorder. Infusions of drug solutions (0·03–0·5 ml/min) were given through a polythene catheter inserted into a rubber connecting tube close to the perfusion cannula by means of a Palmer slow injection apparatus. When drugs were infused into the cannula, the concentrations were expressed in terms of that in the perfusion fluid reaching the artery. Injections of noradrenaline (0·05–0·1 ml) were made directly into the rubber connecting tube by means of an insulin syringe. The periarterial sympathetic nerves were stimulated by means of bipolar platinum ring electrodes, through which pulses of 1 ms duration and supramaximal voltage were applied. The frequencies of nerve stimulation used ranged from 2 to 20 Hz. Trains of pulses were given for 10 s at 3 min intervals. In some of the experiments, arteries denervated as described by de la Lande & Rand (1965) were used.

Drugs

The following drugs were used: acetylcholine chloride (Hopkins and Williams Ltd.), atropine sulphate (Macfarlan Smith Ltd.), dexamphetamine sulphate (Smith Kline and French Laboratories), 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343, McNeil Laboratories), and (−)-noradrenaline bitartrate (Winthrop). Doses in the text refer to the salts except for noradrenaline, which is expressed as the base.

Results

Responses to sympathetic nerve stimulation

The effects of infusions of McN-A-343 on responses of the artery to sympathetic nerve stimulation depended on the concentration and the frequency of stimulation. Figure 1 illustrates the results obtained with stimulation rates of less than 5 Hz and

a rate of 20 Hz. With the lower frequency range, infusion of increasing concentrations of McN-A-343 caused a progressive reduction in responses to sympathetic nerve stimulation. After termination of McN-A-343 infusion, the impairment of responses to sympathetic stimulation persisted with only slight recovery after 60 minutes. With a frequency of 20 Hz, in five experiments, responses to nerve stimulation were increased by McN-A-343. The persistence of effects after terminating infusions were variable; in some experiments the responses returned to control levels within 30 min, in others there was only partial return by 60 minutes. The difference in effect of McN-A-343 on responses to low and high frequencies of nerve stimulation is shown more sharply in Fig. 2 in which a concentration of 2·5 μg/ml was infused without previous administration of lower concentrations.

Effects of atropine

The reduction of responses to nerve stimulation at low frequencies produced by McN-A-343 was reversed by atropine (0·1 μg/ml) to a marked enhancement (Fig. 2). In the presence of atropine (0·1 μg/ml), infusions of McN-A-343 in increasing concentrations caused an enhancement of responses to low frequencies of nerve stimulation, but responses to high frequencies of nerve stimulation were not qualitatively different from those in the absence of atropine (Fig. 1).

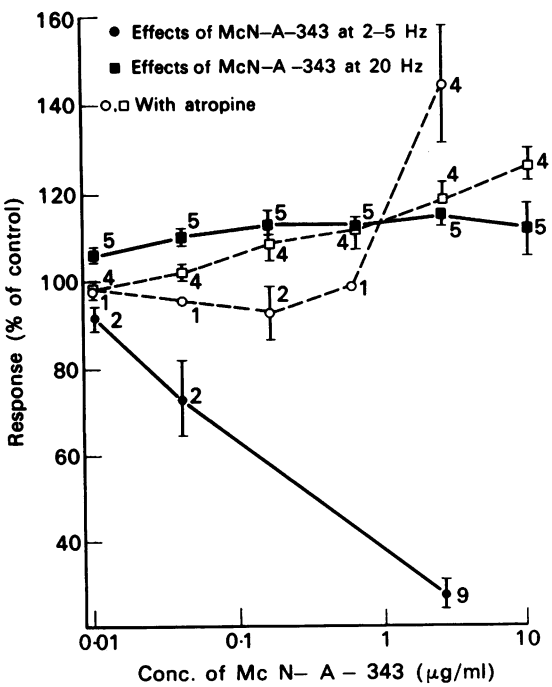

FIG. 1. Effects of increasing concentrations of McN-A-343 (μg/ml) on responses to nerve stimulation at low frequencies (2–5 Hz) and at high frequency (20 Hz). Observations were also made in the presence of atropine (0·1 μg/ml.). The number of experiments for each point is shown by the adjacent figure. The vertical lines are standard errors. In each experiment, the means of five responses at each concentration were expressed as a percentage of the control values for that experiment.

Effects of dexamphetamine

The reduction in responses to low frequencies of nerve stimulation by McN-A-343 were almost completely reversed by dexamphetamine (0·25 to 1 μg/ml) in three experiments, one of which is illustrated in Fig. 3.

Responses to noradrenaline

In some experiments, vasoconstrictor responses were obtained by injections of noradrenaline (2·5–10 ng) as well as to low frequencies of nerve stimulation.

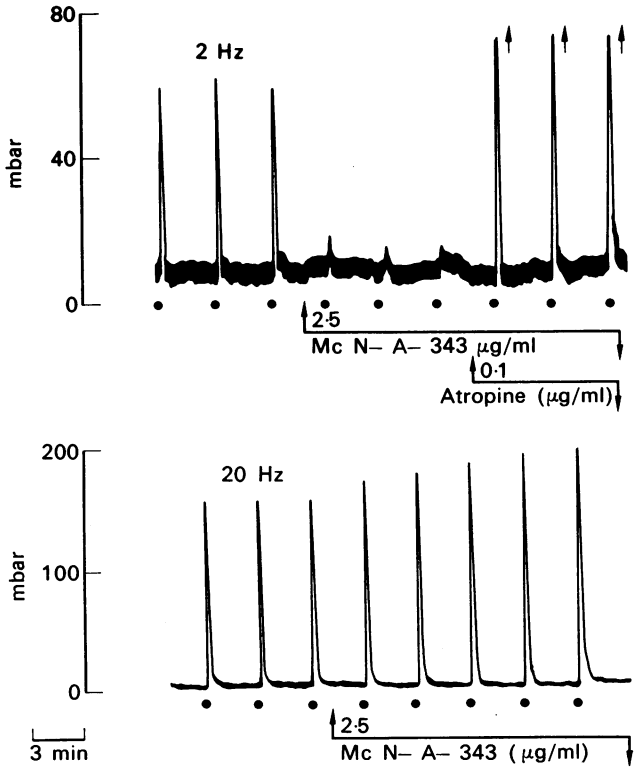

FIG. 2. Effects of infusions of McN-A-343 at the same concentration (2·5 μg/ml) in two separate experiments at different frequencies of nerve stimulation. (Upper tracing, 2 Hz, 10 s; lower tracing 20 Hz, 10 s). Horizontal bars with arrows indicate infusions of McN-A-343 or atropine (μg/ml perfusion fluid).

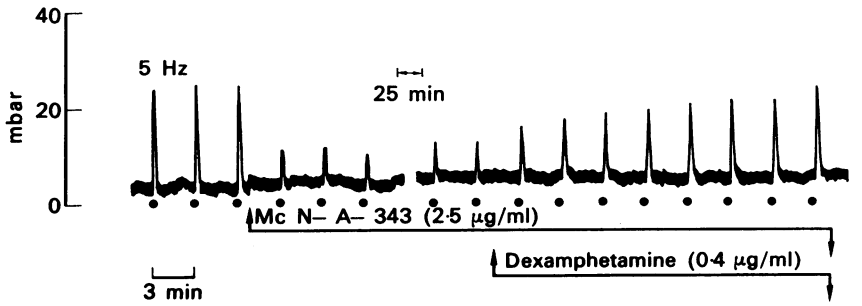

FIG. 3. Antagonism by dexamphetamine of the action of McN-A-343 on responses to a low frequency of nerve stimulation.

Responses to injected noradrenaline were not affected when responses to nerve stimulation (2 Hz) were almost completely abolished by McN-A-343, as shown in one experiment (Fig. 4). In three such experiments, after McN-A-343 (2·5 μg/ml), the mean of the first three nerve stimulations was 32 ± 5% of control and that to noradrenaline was 98 ± 1·3% and did not differ significantly from control.

In five experiments with denervated arteries, infusions of McN-A-343 (2·5–10 μg/ml) had no significant effect on responses to noradrenaline injections. The mean responses in the presence of McN-A-343 (2·5, 5 and 10 μg/ml) were, respectively, 109, 103 and 93% of control.

Discussion

The effects of McN-A-343 on responses to sympathetic nerve stimulation are similar to those of other cholinomimetic agonists, including acetylcholine (Malik & Ling, 1969a), muscarine itself and pilocarpine (Varma & Rand, 1969; Rand & Varma, 1970a).

McN-A-343 causes a decrease in vasoconstrictor responses to postganglionic sympathetic nerve stimulation at low frequencies (up to 5 Hz). It is unlikely that the decrease was due to a vasodilator action of McN-A-343 since the responses to noradrenaline were not reduced. A more likely explanation for the effect follows from the observations that there are muscarinic receptors at terminal adrenergic neurones through which noradrenaline release induced either by nicotinic drugs (Lindmar *et al.*, 1968; Haeusler, Thoenen, Haefely & Huerlimann, 1968), or by electrical stimulation of sympathetic nerve (Löffelholz & Muscholl, 1969), can be inhibited. The inhibitory effect of McN-A-343, like that of acetylcholine (Malik & Ling, 1969a) and other muscarinic agonists (Rand & Varma, 1970a) is abolished by a low concentration of atropine (0·1 μg/ml); the most likely conclusion is that McN-A-343 acts on these muscarinic inhibitory receptors to cause inhibition of noradrenaline release. It has been suggested that the muscarinic receptors at adrenergic neurone terminals can play a physiological role in inhibition of noradrenaline release (Löffelholz & Muscholl, 1969, 1970; reviewed by Muscholl, 1970).

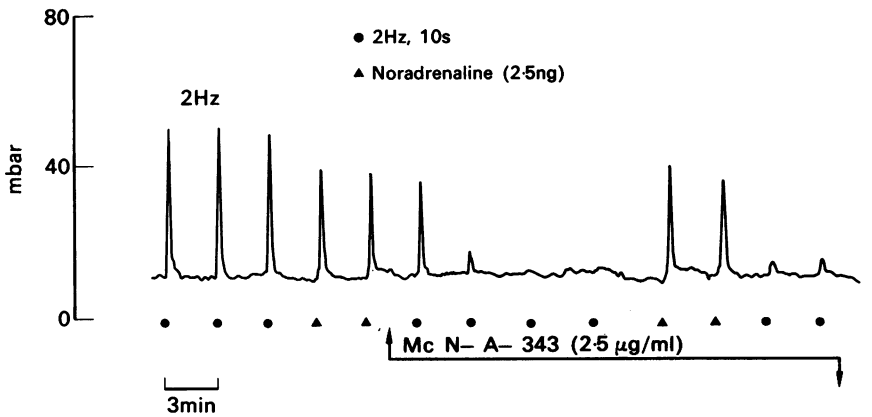

FIG. 4. Effects of McN-A-343 on responses to periarterial nerve stimulation and injections of noradrenaline. Horizontal bars with arrows indicate infusion of McN-A-343.

Increases in vasoconstrictor responses to sympathetic nerve stimulation were observed with McN-A-343 when high frequencies (10–20 Hz) were used and also in the presence of atropine at low frequencies of nerve stimulation. These effects could be due to blockade of noradrenaline uptake; however, there was no significant increase in responses to exogenous noradrenaline. The absence of evidence for blockade was not due to a balance between blockade of receptors and uptake as occurs with desipramine (Glover, McCulloch & Story, communicated to the Austr. Soc. clin, exp. Pharmac., Nov. 1970, unpublished) since McN-A-343 had no effect on responses to noradrenaline in denervated tissues in which the possibility of neuronal uptake is eliminated. On the other hand, McN-A-343 blocks the uptake of ^{3}H-noradrenaline in guinea-pig atria (Allen, Story & Varma, communicated to the Austr. Soc. clin, exp. Pharmac., Nov. 1970, unpublished). Another way in which McN-A-343 could increase responses to sympathetic nerve stimulation may be by facilitation of release of noradrenaline. In isolated guinea-pig atria, McN-A-343 releases noradrenaline (Bhagat, 1966). Further investigation is required to determine the mechanisms by which McN-A-343 causes an increase in responses to sympathetic nerve stimulation in the artery. Furthermore, it is possible that the increase in response to high frequency stimulation might be produced by a mechanism different from that responsible for the increase in response to low frequency stimulation in the presence of atropine.

Atropine reverses the effect of acetylcholine on responses to low frequencies of sympathetic nerve stimulation (Malik & Ling, 1969a). This finding suggests blockade of a muscarinic inhibitory effect and unmasking of a nicotinic facilitatory effect. However, atropine also caused reversal of the effect of McN-A-343 on responses to low frequencies of stimulation and McN-A-343 is devoid of nicotinic activity (Roszkowski, 1961; Smith, 1966). Rand & Varma (1970a) suggested that muscarinic receptors concerned with excitatory effects on adrenergic neurones are less susceptible to blockade by atropine than are those concerned with inhibitory effects; the observations with McN-A-343 support this suggestion.

Depending on the conditions of the experiment, McN-A-343 may increase or decrease responses to sympathetic nerve stimulation, as may some adrenergic neurone blocking drugs (Rand & Wilson, 1967; Lederer, Rand & Wilson, 1970). These observations strengthen the suggestion made by Burn & Rand (1965) that the differences between acetylcholine and adrenergic neurone blocking drugs are quantitative rather than qualitative, and that other cholinomimetic drugs, including McN-A-343, have intermediate actions. All these drugs may act on a cholinergic step in adrenergic transmission (Burn & Rand, 1959, 1962). The reversal of the blocking action of McN-A-343 by dexamphetamine indicates its similarity to adrenergic neurone blocking drugs.

The difference in effect of the muscarinic agonist McN-A-343 at high and low frequencies of stimulation may arise from the difference in amounts of acetylcholine liberated at the two frequencies. With low frequencies, noradrenaline release may be produced with minimal effect of the acetylcholine mediator on the inhibitory muscarinic receptors, hence addition of McN-A-343 causes inhibition. With high frequencies, the mediator acetylcholine is released in larger amounts and the inhibitor muscarinic receptors may be saturated; noradrenaline release cannot therefore be inhibited, in fact it is possible that McN-A-343, being a partial agonist, may reduce the action of acetylcholine on the inhibiting muscarinic receptors.

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