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# EFFECTS OF THE MUSCARINIC AGONIST McN-A-343 ON THE RELEASE BY SYMPATHETIC NERVE STIMULATION OF [$^3$H]-NORADRENALINE FROM RABBIT ISOLATED EAR ARTERIES AND GUINEA-PIG ATRIA

G.S. ALLEN, M.J. RAND & D.F. STORY
Department of Pharmacology, University of Melbourne, Parkville, Victoria 3052, Australia

1 McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride) in concentrations of $10^{-5}$ and $10^{-4}$ M inhibits the stimulation-induced efflux of radioactivity from segments of rabbit ear artery that have previously been incubated with (−)-[$^3$H]-noradrenaline, and also decreases the contractile response.

2 The inhibitory effects of McN-A-343 ($10^{-5}$ M) on the efflux of radioactivity and the contractions induced by low frequencies of stimulation (2 and 5 Hz) are reversed by atropine, but atropine does not modify these effects with high frequencies of stimulation (20 and 50 Hz).

3 McN-A-343 ($10^{-4}$ M) enhances the stimulation-induced efflux of radioactivity from guinea-pig atria that have previously been incubated with (−)-[$^3$H]-noradrenaline, and prolongs the positive inotropic response. These effects are not modified by atropine.

4 It is concluded that McN-A-343 has different effects on adrenergic transmitter release in the two tissues. In the artery, it acts as an agonist on muscarinic receptors of adrenergic terminals to inhibit transmitter release at low frequencies of stimulation. In the atria it enhances transmitter efflux from the tissue, largely by inhibiting re-uptake.

## Introduction

Roszkowski (1961) reported that McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride) was a selective stimulant of muscarinic receptors of sympathetic ganglion cells. McN-A-343 also acts as an agonist on muscarinic receptors in other tissues. Hobbiger, Mitchelson & Rand (1969) reported that McN-A-343 acts on muscarinic receptors of the guinea-pig taenia caeci, Chiba, Hashimoto, Kubota & Satoh (1971) suggested that the bradycardia caused by McN-A-343 in cats was due to stimulation of muscarinic receptors at the sinoatrial node, and Taira, Matsumura & Hashimoto (1971) presented evidence suggesting that McN-A-343 stimulates parasympathetic ganglion cells in the dog urinary bladder by an action on muscarinic receptors.

McN-A-343 has been shown to have a dual effect on the response of the isolated artery of the rabbit ear to sympathetic nerve stimulation (Rand & Varma, 1971). With low frequencies of stimulation (2-5 Hz), McN-A-343 caused a decrease in responses to nerve stimulation. This effect was also attributed to a muscarinic action since in the presence of atropine it was reversed to a marked enhancement. However, responses to injected noradrenaline were not decreased by McN-A-343, which suggested that the decrease in responses to sympathetic nerve stimulation was due to an effect on transmitter release. With high frequencies of stimulation (10-20 Hz), McN-A-343 caused an increase in the responses of the artery to periarterial nerve stimulation and this effect was not changed qualitatively in the presence of atropine.

Recently it was shown that McN-A-343 is a potent inhibitor of [$^3$H]-noradrenaline uptake by guinea-pig atria, and that this action was atropine-insensitive (Allen, Rand & Story, 1972). This finding suggested that inhibition of re-uptake of released noradrenaline may be the mechanism by which McN-A-343 enhances responses to sympathetic nerve stimulation. Alternatively, McN-A-343 may facilitate noradrenaline release.

The present experiments were designed to investigate the effect of McN-A-343 on the efflux of radioactivity produced by stimulation of sympathetic nerves of guinea-pig isolated atria and the central artery of the rabbit ear following incubation with [$^3$H]-noradrenaline. Some of

these findings were communicated to the Fifth International Congress of Pharmacology, San Francisco, July, 1972.

## Methods

*Experiments with isolated arteries*

Preparations were set up as described by Allen, Rand & Story (1973). Rabbits of either sex (weighing 2-3 kg) were killed by cervical dislocation. The proximal 25-30 mm segment of each central ear artery was cannulated at each end with polythene tubing, dissected free from surrounding tissue and mounted in an air-filled jacketed organ bath. The arteries were perfused-superfused at 37° C, with Krebs-Henseleit solution (composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25.0, $MgSO_4$ 0.45, $KH_2PO_4$ 1.03, $CaCl_2$ 2.5, dextrose 11.1 and disodium ethylenediamine tetra-acetic acid (EDTA) 0.067), which had been equilibrated with a mixture of 5% carbon dioxide in oxygen. The perfusion-superfusion flow rate was maintained at 4 ml/min with a Watson-Marlow flow inducer and the perfusion pressure was monitored with a Statham P23Db transducer coupled to a Rikadenki chart recorder. The adventitial sympathetic nerves were stimulated by means of bipolar platinum ring electrodes, through which monophasic square wave pulses of 1 ms duration and supramaximal voltage were applied. Arteries were set up as described and tested to ensure responsiveness to stimulation. They were then temporarily removed and incubated at 37° C with (−)-[$^3$H]-noradrenaline (0.29 μg/ml; 10 μCi/ml) in Krebs-Henseleit solution (1 ml) for 60 minutes. After incubation the artery segments were perfused-superfused for 60 min, by which time the efflux of radioactivity had approached a steady state (Allen *et al.*, 1973).

The efflux of radioactivity was determined by counting the total radioactivity of aliquots of the perfusate-superfusate collected in 30 s periods. Stimulation of the adventitial sympathetic nerves elicited vasoconstriction as described by de la Lande & Rand (1965), as well as an increased efflux of radioactivity. Stimulation was applied for 30 s periods at various frequencies. Each preparation was given three periods of stimulation at 30 min intervals. The 'resting' efflux of radioactivity was taken as the efflux in the 30 s period immediately preceding each 30 s period of stimulation, and the 'stimulation-induced' efflux was calculated by subtracting the resting efflux from the efflux during the stimulation period.

Drug solutions were infused into the perfusion fluid by means of a Braun slow injection apparatus; concentrations are expressed in terms of that in the perfusion fluid reaching the artery. Drugs were added only for the second period of stimulation: infusions were commenced 20 min beforehand and were discontinued immediately after the second period of stimulation. The stimulation-induced effluxes of radioactivity during the second and third periods of stimulation were calculated as percentages of the first stimulation-induced efflux. Thus each artery served as its own control and time-dependent changes were assessed from separate experiments in which no drugs were used.

*Experiments with isolated atria*

Guinea-pigs (weighing 300-500 g) were killed by cervical dislocation, exsanguinated and the hearts rapidly removed. The atria were dissected free and suspended between two platinum electrodes about 1 cm apart in an organ bath containing 2 ml Krebs-Henseleit solution. The solutions in the organ bath, and in the reservoir supplying the organ bath, were aerated with a mixture of 5% carbon dioxide in oxygen and maintained at a temperature of 37° C. The force of spontaneous contractions of the atria was recorded on a Brush Mark 240 pen recorder using a high compliance strain gauge transducer; the initial tension was adjusted to about 1 gf (≃9.8 mN). The atria were allowed to equilibrate under these conditions for about 45-60 min, the Krebs-Henseleit solution in the organ bath being replaced every 5 minutes.

After equilibration the atria were incubated with (−)-[$^3$H]-noradrenaline (0.21 μg/ml; 5 μCi/ml) for 20 min and then washed repeatedly with noradrenaline-free solution for 50 min, after which time the efflux of radioactivity from the atria had approached a steady level.

The intramural nerves of the atria were stimulated with monophasic square wave impulses of 1 ms duration and about 10 V amplitude applied across the platinum electrodes at a frequency of 5 Hz for 30 s periods.

The efflux of radioactivity into the surrounding solution was measured after 3 min periods of contact with the atria. The 'resting' efflux was taken as the mean efflux during the 3 min periods immediately preceding and following the 3 min period during which the 30 s stimulation was applied. The stimulation-induced component of efflux was calculated by subtracting the resting efflux from the efflux during the stimulation period.

The effects of drugs on the release of radioactivity were determined by replacing the solutions in the organ bath with drug-containing solution which had been pre-warmed and gassed in

a separate reservoir. The atria were equilibrated with the drugs for 15 minutes. In order that each atrial preparation could serve as its own control, the resting and stimulation-induced effluxes were determined first in the absence and then in the presence of the drug under investigation. To assess time-dependent changes in efflux, control experiments were performed in the absence of drugs.

*Counting the radioactivity*

Samples of Krebs-Henseleit solution collected from the artery (1 ml) and atria (0.5 ml) preparations were added to counting vials containing approximately 0.2 ml 6 M HCl and 10 ml scintillation solution. The composition of the scintillation solution was 5.5 g 2,5-diphenyloxazole (PPO), 0.1 g 1,4-*bis*-2-(5-phenoxazolyl)-benzene (POPOP) and 333 ml Triton-X, per litre of toluene. Radioactivity (counts per minute) in the vials was measured in a Packard Tri-carb liquid scintillation counter and was calculated as disintegrations per minute (d/min), corrections being made for counting efficiency by use of an internal reference standard of [$^3$H]-*n*-hexadecane.

*Radiochemicals and drugs*

Tritiated *laevo*-noradrenaline, (−)-[7-$^3$H]-noradrenaline acetate, was obtained from the Radiochemical Centre, Amersham. The specific activity of the [$^3$H]-noradrenaline used in the experiments with atria was 4.1 Ci/mmol, and the specific activity of that used in experiments with arteries was 5.8 Ci/mmol. The solutions were stored at −30°C and used without further dilution. The concentrations of [$^3$H]-noradrenaline referred to in the text are expressed in terms of noradrenaline base.

The following drugs were used: atropine sulphate (David G. Bull Laboratories, Melbourne); 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343, McNeil Laboratories); hexamethonium bromide (May & Baker). All solutions of drugs were freshly prepared in distilled water.

## Results

*Rabbit ear arteries*

Infusions of McN-A-343 in concentrations of $10^{-5}$ and $10^{-4}$ M (3.17 and 31.7 μg/ml) had no significant effect ($P > 0.05$) on the resting efflux of radioactivity from artery segments in which the transmitter stores had been labelled with

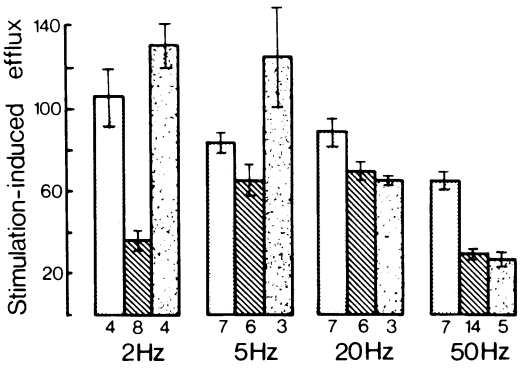


**Fig. 1** Effects of McN-A-343 ($10^{-5}$ M) in the absence (hatched columns) and in the presence (stippled columns) of atropine ($3.5 \times 10^{-7}$ M) on the stimulation-induced efflux of radioactivity from arteries stimulated at frequencies of 2, 5, 20 and 50 Hz. Dotted columns represent control (drug-free) experiments. Stimulation-induced effluxes shown were in the second period of stimulation and are expressed as a percentage of the efflux in the first period of stimulation. Columns represent means and vertical lines their standard errors. The number of experiments is shown under each column.

[$^3$H]-noradrenaline. However, the vasoconstrictor responses to sympathetic nerve stimulation and the accompanying release of radioactivity were reduced by McN-A-343 in both the concentrations used and at all frequencies of stimulation tested. The magnitudes of these effects depended on the concentration of McN-A-343 and on the frequency of stimulation.

The effects of McN-A-343 ($10^{-5}$ M) on the stimulation induced efflux of radioactivity are summarized in Figure 1. The reduction of stimulation-induced efflux was greater with low (2 Hz) and high (50 Hz) frequencies of stimulation than with stimulation at intermediate frequencies (5 and 20 Hz). With the higher concentration of McN-A-343 ($10^{-4}$ M), there was a greater decrease in efflux of radioactivity with stimulation at 5, 20 and 50 Hz.

The effects of McN-A-343 in a concentration of $10^{-5}$ M on the vasoconstriction response and on the stimulation-induced efflux of radioactivity in an experiment with stimulation at 2 Hz are shown in Figure 2.

The simultaneous infusion of atropine ($3.5 \times 10^{-7}$ M) prevented the effect of McN-A-343 ($10^{-5}$ M) in reducing the efflux of radioactivity and vasoconstrictor responses to low frequencies of stimulation, as shown for one experiment with stimulation at 2 Hz in Figure 2b. In the experiment illustrated, there was a slight increase

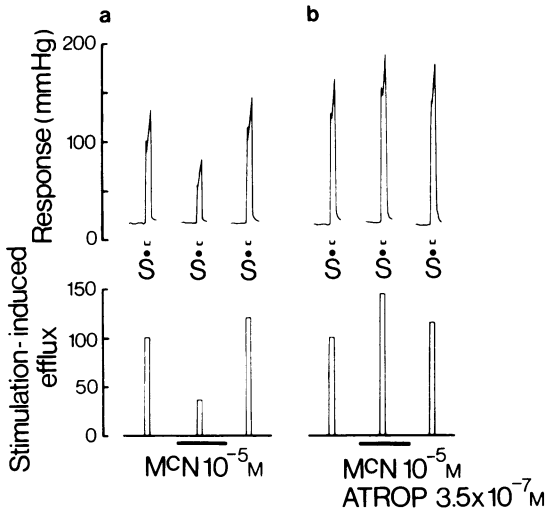


**Fig. 2** Effects of McN-A-343 (McN $10^{-5}$ M) in (a) the absence and (b) presence of atropine (ATROP 3.5 x $10^{-7}$ M) on the vasoconstrictor response of an artery segment to periarterial stimulation (S) at 2 Hz and on the accompanying stimulation-induced efflux of radioactivity. Upper records: vasoconstrictor responses, in mmHg (1 mmHg ≃ 133.3 $Nm^{-2}$), before, during and after infusion of McN-A-343 or McN-A-343 plus atropine. Lower records: stimulation-induced effluxes of radioactivity, expressed as percentages of that during the first period of stimulation.

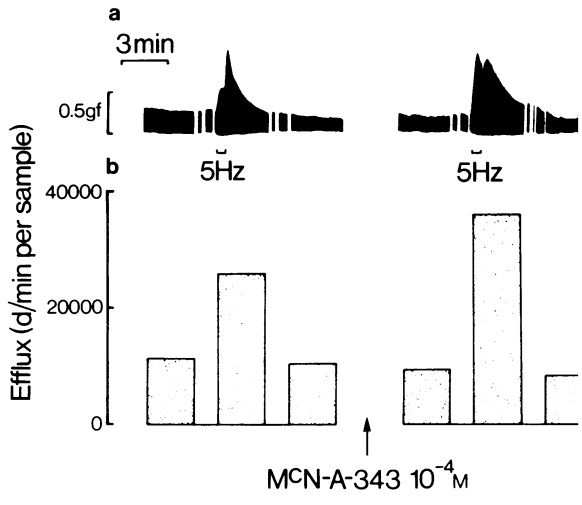


**Fig. 3** Effects of McN-A-343 ($10^{-4}$ M) on (a) the response of guinea-pig atria to field stimulation at 5 Hz and (b) the efflux of radioactivity determined simultaneously. (a) Force of contraction; (1 gf ≃ 9.8 mN); the tracing is interrupted at intervals when bath fluid was removed and replaced with fresh fluid. (b) Efflux of radioactivity during 3 min periods.

in the stimulation-induced release of radioactivity, but in four such experiments with stimulation at 2 Hz the mean increase in efflux was not significantly greater than in control experiments. However, with stimulation at 5 Hz the stimulation-induced efflux was significantly enhanced ($P < 0.05$) by McN-A-343 ($10^{-5}$ M) in presence of atropine. There were corresponding increases in the vasoconstrictor responses. With higher frequencies of stimulation (20 and 50 Hz), atropine had no effect on the depression of stimulation-induced efflux by McN-A-343 ($10^{-5}$ M). These results are summarized in Figure 1.

The effects of the lower concentration of McN-A-343 ($10^{-5}$ M) in reducing the responses and the efflux of radioactivity were reversed after 30 min perfusion of the arteries with drug-free Krebs-Henseleit solution. However, after terminating infusion of the higher concentration ($10^{-4}$ M) there was only partial recovery of the vasoconstrictor responses and of the stimulation-induced efflux.

*Guinea-pig atria*

McN-A-343 in concentrations up to $10^{-4}$ M had no effect on the resting efflux of radioactivity from atria labelled with [$^3$H]-noradrenaline (Allen *et al.*, 1972). However, in a concentration of $10^{-4}$ M, McN-A-343 significantly enhanced the stimulation-induced release of radioactivity, and there was also an increase in the duration of the inotropic response to stimulation, as illustrated for one experiment in Figure 3. The mean increase in efflux of radioactivity is shown in Figure 4.

Atropine, in a concentration of 3.5 x $10^{-7}$ M, did not significantly modify the effect of McN-A-343 ($10^{-4}$ M) on the stimulation-induced efflux of radioactivity (Figure 4) or the responses of atria to field stimulation.

## Discussion

McN-A-343 had opposite effects on the stimulation-induced efflux of radioactivity from arteries and atria which had been previously incubated with tritiated noradrenaline: that from arteries being decreased and that from atria being increased.

The stimulation-induced efflux of radioactivity from artery segments which had been incubated

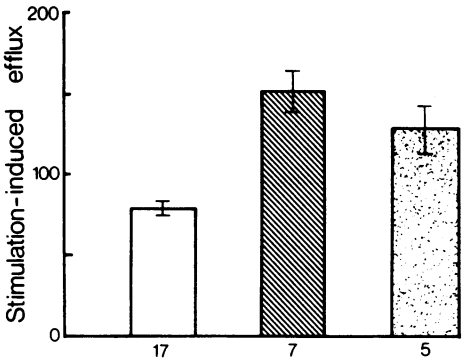


**Fig. 4** Effects of McN-A-343 ($10^{-4}$ M) in the absence (hatched column) and presence (stippled column) of atropine ($3.5 \times 10^{-7}$ M), on the stimulation-induced efflux of radioactivity from guinea-pig isolated atria. Open column represents control (drug-free) experiments. Effluxes in the second period of stimulation are expressed as percentages of the efflux in the first period. Columns represent means and vertical lines their standard errors. The number of experiments is shown under each column.

with (−)-[$^3$H]-noradrenaline is due to release of tritiated noradrenaline that has entered the transmitter pool in the adrenergic neurones of the artery. After such incubation, stimulation-induced release of radioactivity can be elicited for at least 5 h and is abolished by tetrodotoxin (Allen *et al.*, 1973). At least 50% of the radioactivity is attributed to unchanged noradrenaline, and most of the remainder consists of non-catechol (presumably *O*-methylated) metabolites (unpublished observations). Reduction of stimulation-induced efflux of radioactivity may therefore be taken as evidence for reduction of transmitter release. An increase of stimulation-induced efflux, however, could be due to either an increase in transmitter release or a decrease in transmitter re-uptake.

Rand & Varma (1971) reported that McN-A-343 reduced the vasoconstrictor response of the isolated artery to low frequencies of sympathetic nerve stimulation (2 and 5 Hz) but increased the responses to stimulation at higher frequencies. We have confirmed the reduction of responses to low frequency stimulation and have shown that it is due to a reduction of transmitter release. However, we also found that there was a reduction of transmitter release and of vasoconstriction in response to higher frequencies of stimulation. The discrepancy between the findings of Rand & Varma (1971) and these reported here may be due to the differences in the methods of setting up the artery preparation and in the regimes of stimulation.

The effects of atropine on the inhibition of transmitter release produced by McN-A-343 depend on the frequency of stimulation. With low frequencies (2 and 5 Hz) the inhibition was abolished and reversed to an enhancement which was statistically significant with stimulation at 5 Hz. There was a corresponding increase in vasoconstrictor responses, as had been shown by Rand & Varma (1971). On the other hand, atropine had no effect on the inhibitory action of McN-A-343 on transmitter release or the vasoconstrictor responses with stimulation at high frequencies (20 and 50 Hz).

The atropine-sensitive inhibition of transmitter release produced by McN-A-343 probably involves muscarinic receptors of terminal adrenergic axons (see reviews: Muscholl, 1970; Kosterlitz & Lees, 1972), but our results suggest that these receptors are only atropine-sensitive at low frequencies of stimulation. An alternative explanation is that the mechanism by which McN-A-343 inhibits transmitter release at high frequencies of stimulation differs from that at low frequencies: it may, for example, be acting as a guanethidine-like adrenergic neurone blocking drug as suggested by Rand & Varma (1971), who demonstrated reversal of the inhibitory action of McN-A-343 by dexamphetamine.

The facilitatory effects of McN-A-343 in the presence of atropine on the efflux of radioactivity and on the vasoconstrictor response to stimulation at low frequencies are probably due to increased transmitter release, rather than to inhibition of transmitter uptake. Although McN-A-343 inhibits noradrenaline uptake in the atria, $10^{-5}$ M producing about 50% inhibition (Allen *et al.*, 1972), this concentration had no effect on the responses of the artery to injections of noradrenaline (Rand & Varma, 1971).

McN-A-343 was less active on the adrenergic nerves in the guinea-pig atria than on those of the rabbit ear artery and in a concentration of $10^{-4}$ M produced the opposite effects to those observed in the artery, increasing the efflux of radioactivity and prolonging the response. These effects of McN-A-343 were not significantly altered by atropine. Allen *et al.* (1972) showed that McN-A-343 in a concentration of $10^{-4}$ M produced 80% inhibition of noradrenaline uptake by atria and this effect also was not altered by atropine.

Atropine converts the inhibitory effect of McN-A-343 on the force of contraction of spontaneously beating atria to a stimulant effect (Bhagat, 1966), although there is no corresponding increase in the efflux of radioactivity (Allen *et al.*, 1972). It is possible that the enhanced release of

radioactivity during stimulation stems from inhibition by McN-A-343 of neuronal re-uptake of released noradrenaline. However, two other inhibitors of noradrenaline uptake, cocaine and desipramine, produce only small increases in stimulation-induced efflux of radioactivity from atria (unpublished observations). The difference may be attributable to partial inhibition of the release of noradrenaline by cocaine and desipramine; such an effect would partly counteract the increased efflux resulting from blockade of re-uptake of the transmitter.

On balance, it seems that the main effect of McN-A-343 on responses to adrenergic nerve stimulation in guinea-pig atria is to inhibit re-uptake of transmitter noradrenaline, giving a prolongation of response and an increased efflux of transmitter, although facilitation of transmitter release may also contribute.

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