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MODULATION BY ACETYLCHOLINE OF ADRENERGIC TRANSMISSION IN THE RABBIT EAR ARTERY

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1 Low concentrations of acetylcholine (4×10^{-11} and 1×10^{-10} M) increase the vasoconstrictor response of the isolated ear artery of the rabbit to stimulation of the periarterial sympathetic nerves. Higher concentrations (4×10^{-8} M and greater) decrease the response.

2 Low concentrations of acetylcholine (1×10^{-11} and 1×10^{-10} M) increase the stimulation-induced efflux of radioactivity from artery segments previously incubated with [^{3}H]-noradrenaline. Higher concentrations (3×10^{-8} M and greater) decrease the efflux.

3 Neither atropine nor hexamethonium affects the facilitatory action of low concentrations of acetylcholine on adrenergic transmission in the rabbit ear artery.

4 Atropine antagonizes the inhibitory effect of higher concentrations of acetylcholine on adrenergic transmission.

Introduction

Acetylcholine and other cholinomimetic drugs modify the responses to sympathetic nerve stimulation in isolated artery preparations; depending upon the drug used, the concentration and the frequency of stimulation, the responses may be enhanced or reduced (Malik & Ling, 1969; Rand & Varma, 1970). High concentrations of acetylcholine reduce or abolish the vasoconstrictor responses to sympathetic nerve stimulation in the perfused mesenteric artery of the rat (Malik & Ling, 1969) and the perfused ear artery of the rabbit (Rand & Varma, 1970; Steinsland, Furchgott & Kirkepar, 1973), and this effect is blocked by atropine. However, low concentrations of acetylcholine increase the responses to sympathetic nerve stimulation in these two preparations. It was suggested that the changes in response to sympathetic nerve stimulation produced by acetylcholine were due to changes in release of transmitter. Having developed a method of studying transmitter release in the isolated perfused ear artery of the rabbit (Allen, Rand & Story, 1973a), we set out to test this postulate directly. A preliminary account of some of the findings has been given to the British Pharmacological Society (Allen, Glover, Rand & Story, 1972).

Methods

Vasoconstrictor responses

Rabbits of either sex weighing 2-4 kg were killed by cervical dislocation. The central artery of each ear was cannulated and set up as described by de la Lande & Rand (1965). The preparations were perfused at 6 ml/min with Krebs-Henseleit solution. Perfusion pressure was measured with a Statham P23Db pressure transducer and recorded on a Rikadenki potentiometric recorder, calibrated from 0 to 200 mmHg. Periarterial nerve stimulation was delivered through concentric platinum bipolar electrodes using square wave pulses of 1 ms duration and supramaximal voltage (60-80 V) at a frequency of 2 Hz in trains of 10 s every 2 minutes. Solutions of acetylcholine were infused, by means of a Palmer slow infusion pump at rates of 0.05-0.2 ml/min; infusion of drug-free Krebs-Henseleit solution had no effect on basal pressure or responsiveness of the artery as determined in control experiments.

Experiments with tracer noradrenaline

Segments of the central artery of the rabbit ear were incubated with [^{3}H]-(−)-noradrenaline (1.72 μM; 10 μCi/ml) and then set up for perfusion-superfusion with Krebs-Henseleit solution as described by Allen *et al.* (1973a): the segment was perfused through the lumen and the effluent then superfused the adventitial surface. The flow rate was maintained at 4 ml/min and the perfusion pressure was monitored. Samples of the perfusate-superfusate were collected at 3 min periods for measurement of the efflux of radioactivity from the artery segment. The adventitial sympathetic nerves were stimulated

with trains of pulses at 5 Hz for 30 s periods. The first period of stimulation was given 50 min after the period of incubation with [^{3}H]-noradrenaline. The second period was given 30 min after the first. The resting efflux of radioactivity was taken as the efflux in the 3 min period immediately preceding each period in which stimulation was applied. The stimulation-induced efflux was calculated by subtracting the resting efflux from the efflux during the stimulation period. In some experiments the effect of acetylcholine on the resting efflux was monitored by collecting samples of the perfusate-superfusate immediately before, during and after the infusion of acetylcholine. The total radioactivity of samples was calculated in disintegrations per minute (d/min) per collection sample. Corrections for counting efficiency were made using an internal reference standard ([^{3}H]-*n*-hexadecane). Drug solutions were perfused into the perfusion fluid, using a Braun slow injection apparatus.

The Krebs-Henseleit solution was of the following composition (mM): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; $MgSO_4$, 0.45; KH_2PO_4, 1.03; $CaCl_2$, 2.5; and D-glucose, 11.1. Disodium ethylenediamine tetraacetic acid (EDTA) was added to the Krebs-Henseleit solution to retard oxidation of noradrenaline.

Radiochemicals and drugs

Tritiated *laevo*-noradrenaline ([7-^{3}H]-(−)-noradrenaline acetate) with a specific activity of 5.8 Ci/mmol was obtained from the Radiochemical Centre, Amersham, and was stored at −30° C.

The following drugs were used: acetylcholine perchlorate (B.D.H.); atropine sulphate (David G. Bull Laboratories, Melbourne); hexamethonium bromide (May & Baker), neostigmine methylsulphate (Prostigmin, Roche). All solutions of drugs were freshly prepared in distilled water.

Results

Effects of acetylcholine on vasoconstrictor responses

After the artery segments were set up and basal pressure was steady (usually around 15 mmHg), stimulation at 2 min intervals was begun. The rise in perfusion pressure with stimulation at 2 Hz for 10 s was about 80 mmHg. At least 6 constant responses were obtained to provide the mean control value, then acetylcholine was infused with step-wise increases in concentrations from 1×10^{-11} to 1×10^{-6} M; in this range of concentrations acetylcholine did not alter the basal perfusion pressure. With each rate of infusion, the responses were allowed to stabilize at the new level and the mean of at least the last 4 constant responses was taken. For each artery preparation, the mean responses during infusions of acetylcholine were expressed as percentages of the mean control response. The results are shown in Figure 1. The vasoconstrictor responses were significantly increased by acetylcholine in concentrations of 4×10^{-11} M ($P < 0.01$) and 1×10^{-10} M ($P < 0.05$), and were significantly decreased by concentrations of 4×10^{-8} M ($P < 0.05$) and greater ($P < 0.01$).

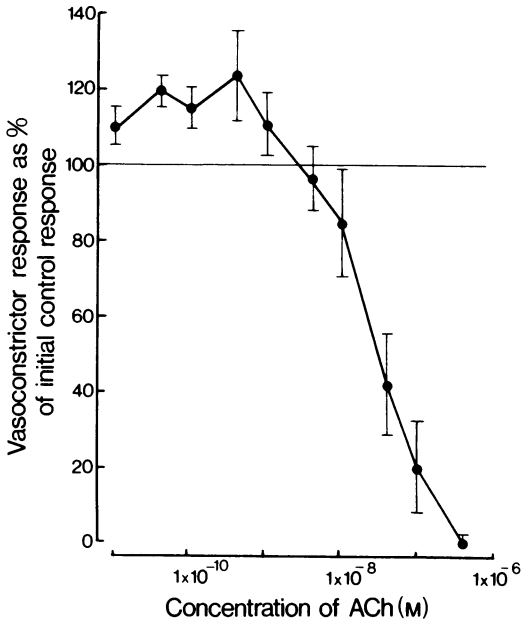

Figure 1 Effects of acetylcholine (ACh) on vasoconstrictor responses to sympathetic nerve stimulation (2 Hz, 10 s) of rabbit isolated ear artery. The rise in perfusion pressure with stimulation in the presence of each concentration of acetylcholine was expressed as a percentage of the initial control value. The points represent means and the vertical bars the standard errors of means of 5-7 experiments.

In similar experiments, it was found that the increases in vasoconstrictor responses produced by low concentrations of acetylcholine (4×10^{-11} and 1×10^{-10} M) were not affected by the addition of atropine (2.9×10^{-7} M) or hexamethonium (5.5×10^{-4} M). However, the decrease in vasoconstrictor responses produced by a higher concentration of acetylcholine (1×10^{-7} M), to a mean of 25% (s.e.mean = 7.6; $n = 5$) of control, was abolished by atropine (2.9×10^{-7} M), the

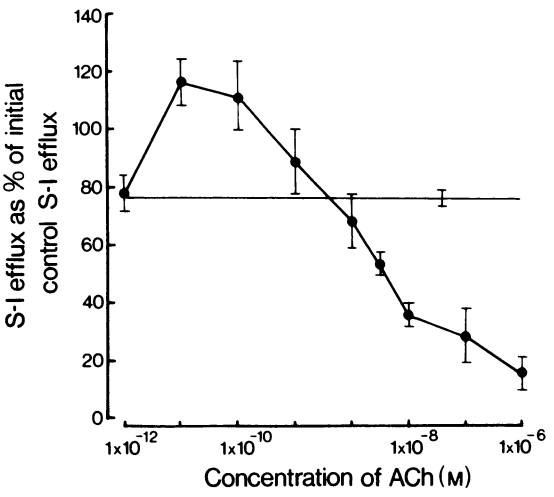

Figure 2 Effects of acetylcholine on the stimulation-induced (S-I) efflux of radioactivity from artery segments previously incubated with [^{3}H]-(—)-noradrenaline. The S-I efflux occurring in the presence of acetylcholine in the second period of stimulation was expressed as a percentage of the S-I efflux in the first period of stimulation. Symbols represent the means of 3-6 experiments and the vertical bars the standard errors of the means. The horizontal line represents the mean efflux in the second period in 7 control experiments in the absence of acetylcholine; standard error of that mean is indicated at the right hand end.

mean responses being 112% (s.e.mean = 7.6) of control. With atropine alone, the responses were 114% (s.e.mean = 9.1) of control.

Effects of acetylcholine on stimulation-induced efflux of tritium from arteries labelled with [^{3}H]-noradrenaline

Acetylcholine infusions were started 20 min before the second period of stimulation. The stimulation-induced efflux of radioactivity during the second period of stimulation was calculated as a percentage 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. Figure 2 summarizes the effects of acetylcholine in concentrations between 1×10^{-12} and 1×10^{-6} M on the stimulation-induced efflux of radioactivity from arteries with stimulation at 5 Hz. In concentrations of 1×10^{-11} and 1×10^{-10} M the stimulation-induced effluxes were significantly enhanced ($P < 0.05$). In concentrations of 3×10^{-8} M and greater, acetylcholine caused significant concentration-dependent reductions in the efflux. The resting efflux of radioactivity was transiently increased by the highest concentration of acetylcholine (1×10^{-5} M), but after 20 min exposure, the resting efflux was not affected by acetylcholine in the range of concentrations used.

The observations made on the vasoconstrictor responses during the course of the experiments with [^{3}H]-noradrenaline were in accord with those made in the experiments with stimulation at 2 min intervals. Furthermore, the changes in efflux corresponded with the changes in vasoconstrictor response within each experiment.

The facilitatory effects of low concentrations of acetylcholine persisted after termination of the infusions; the responses and the efflux were still enhanced after 60 min of perfusion of the arteries with drug-free Krebs-Henseleit solution. On the other hand, the inhibition of responses and stimulation-induced efflux which occurred with higher concentrations of actylcholine were restored to control levels after 30 min perfusion with drug-free Krebs-Henseleit solution. In some experiments the reduction in efflux produced by acetylcholine in a high concentration was reversed to an enhancement after washout of the drug.

The simultaneous infusion of atropine (2.9×10^{-7} M) did not significantly alter the effect of a low concentration of acetylcholine (1×10^{-10} M) in enhancing the radioactive efflux and constrictor responses in 7 experiments. However, atropine prevented the reductions caused by a higher concentration of acetylcholine (1×10^{-5} M) in the constrictor responses and release of radioactivity: the mean efflux in the presence of acetylcholine was 16.7% (s.e.mean = 4.2; $n = 6$) of the initial control; in the presence of acetylcholine plus atropine it was 128.6% (s.e.mean = 43.8; $n = 5$); in the absence of drugs the corresponding efflux was 75.9% (s.e.mean = 2.8; $n = 7$). Infusions of atropine (2.9×10^{-7} M) alone did not significantly alter the stimulation-induced efflux.

In seven experiments the simultaneous infusion of hexamethonium (5.5×10^{-4} M) did not modify the facilitatory effect of a low concentration of acetylcholine (1×10^{-10} M) on the responses and accompanying efflux of radioactivity. Similarly the inhibitory effect of a higher concentration of acetylcholine (1×10^{-5} M) was not altered in the presence of hexamethonium. Infusions of hexamethonium (5.5×10^{-4} M) alone did not alter the stimulation-induced efflux or the vasoconstrictor responses.

The anticholinesterase drug, neostigmine (1×10^{-4} M) was without effect on the enhancement of radioactive efflux in the presence of a low

concentration of acetylcholine (1×10^{-10} M). Neostigmine alone did not alter the pattern of efflux or constrictor responses in control experiments.

Discussion

The findings of Malik & Ling (1969) and Rand & Varma (1970) that low concentrations of acetylcholine increased vasoconstrictor responses to sympathetic nerve stimulation in isolated artery preparations whereas high concentrations decreased the response were attributed by these authors to increases and decreases, respectively, in the amounts of transmitter noradrenaline released since the vasoconstrictor responses to exogenous noradrenaline were not affected by the low concentrations of acetylcholine and were increased by the high concentrations. The findings have been confirmed, and it has been shown that the changes in the vasoconstrictor responses to sympathetic nerve stimulation are associated with corresponding changes in efflux of radioactivity from arteries in which transmitter stores have been labelled with [^{3}H]-noradrenaline.

In arteries previously incubated with [^{3}H]-noradrenaline, the increased stimulation-induced efflux of radioactivity in the presence of low concentrations of acetylcholine cannot be explained by block of reuptake of released noradrenaline, since acetylcholine has only a slight inhibitory effect on noradrenaline uptake in a million-fold greater concentration (Allen, Rand & Story, 1973b). The increased stimulation-induced efflux must, therefore, be attributed, to increased release of adrenergic transmitter.

The facilitatory effect of low concentrations of acetylcholine on adrenergic transmission in the rabbit ear artery was not affected by either hexamethonium or atropine. Thus the receptors involved in this action of acetylcholine are different from the nicotinic receptors on ganglion cells and the muscarinic receptors in various tissues. It is possible that the receptor site is identical with one or other of the classical nicotinic or muscarinic types, but its location is such that blocking drugs do not penetrate to it; for example, it may be intracellular and acetylcholine may have access to it through a specific transport mechanism which is not available to the blocking drug.

The inhibitory effect of high concentrations of acetylcholine on adrenergic transmission is a better known phenomenon. Brücke (1935) first demonstrated that large doses of acetylcholine can abolish the effects of sympathetic stimulation of the pilomotor muscles of the tail of the cat. These observations were confirmed by Coon & Rothman (1940) and by Burn & Rand (1960). More recently, acetylcholine has been shown to inhibit stimulation-induced release of noradrenaline, or responses to adrenergic nerve stimulation, or both, in the isolated perfused heart of the rabbit (for review, see Muscholl, 1970) and cat heart (Haeusler, Thoenen, Haefely & Huerlimann, 1968), mesenteric artery of the rat (Malik & Ling, 1969), rabbit ear artery (Rand & Varma, 1970; Hume, de la Lande & Waterson, 1972; Steinsland *et al.*, 1973), dog saphenous vein (Vanhoutte & Shepherd, 1973; Vanhoutte, Lorenz & Tyce, 1973) and cat spleen (Kirkepar, Prat, Puig & Wakade, 1972).

The inhibitory effect of acetylcholine on adrenergic transmission is antagonized by atropine and is not affected by hexamethonium, and presumably involves muscarinic receptors.

The finding that acetylcholine reduces transmitter efflux from adrenergic vasomotor nerves raises the possibility that part of the vasodilator action of acetylcholine may be attributed to this effect, but the extent to which acetylcholine-induced vasodilatation is due to this action, rather than to an action on muscarinic receptors in vascular smooth muscle, can only be resolved by further experiments directed at this question.

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