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The effects of cholinomimetic drugs on responses to sympathetic nerve stimulation and noradrenaline in the rabbit ear artery

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Summary

1. The effects of infusions of the cholinomimetic drugs acetylcholine, methacholine, muscarine, carbachol, arecoline and pilocarpine were examined on vasoconstrictor responses of the perfused rabbit ear artery to sympathetic nerve stimulation and to injections of noradrenaline.

2. The first effect of very low concentrations of acetylcholine or muscarine was a slight enhancement of responses to sympathetic nerve stimulation, but when higher concentrations of acetylcholine, methacholine, muscarine, carbachol and arecoline were infused, these vasoconstrictor responses were decreased. With still higher concentrations the responses tended to increase in size during the infusion. After stopping an infusion, the depressed vasoconstrictor responses rapidly recovered and became enhanced.

3. Infusions of pilocarpine in a wide range of concentrations generally caused enhancement of responses.

4. The depressant effects of cholinomimetic drugs on the responses to sympathetic nerve stimulation were antagonized by atropine. Larger concentrations of the drugs overcame the blockade by atropine.

5. The effects of acetylcholine, methacholine and muscarine on the responses to sympathetic nerve stimulation were more pronounced at low than at high frequencies of stimulation.

6. Vasoconstrictor responses to injected noradrenaline were enhanced by acetylcholine or methacholine, whereas responses to sympathetic nerve stimulation were decreased by the same concentrations of these choline esters.

7. It is suggested that cholinomimetic drugs may act on receptor sites associated with the adrenergic terminal axon and that they may facilitate or impair the release of noradrenaline and impair noradrenaline uptake.

Introduction

Acetylcholine may cause effects resembling those of sympathetic nerve stimulation, but in some circumstances it may also block the effects of sympathetic nerve stimulation. Thus Brücke (1935) and Burn & Rand (1960) showed that small doses of acetylcholine produced piloerection in the cat's tail, whereas large doses caused a transient response and then abolished the piloerector response to sympathetic

nerve stimulation. Furthermore, acetylcholine caused vasoconstriction in the perfused rabbit ear, whereas the addition of acetylcholine to the perfusion fluid led to loss of the vasoconstrictor response to sympathetic nerve stimulation (Burn & Rand, 1960). Acetylcholine in a high concentration in the presence of atropine abolished the effects of sympathetic nerve stimulation in rabbit isolated atria, although lower concentrations produced an increase in the rate and force of beating (Huković, 1960).

The effects of acetylcholine which mimicked the responses to sympathetic nerve stimulation were due to release of noradrenaline from the terminal sympathetic axons in these tissues, and the depressant effects of acetylcholine on the responses to sympathetic nerve stimulation were presumably due to blockade of release of noradrenaline. Since nicotine acted like acetylcholine in the experiments cited, and since the actions of acetylcholine were exhibited in the presence of atropine, these excitatory and inhibitory effects of acetylcholine were taken to be nicotinic. However, a muscarinic action of acetylcholine on noradrenergic axons has been demonstrated by Lindmar, Löffelholz & Muscholl (1968) and by Haeusler, Thoenen, Haefely & Huerlimann (1968). They showed, first, that acetylcholine, methacholine and pilocarpine caused a reduction in the amount of noradrenaline released from the perfused rabbit or cat heart by nicotine and dimethylphenylpiperazinium, and, second, that the inhibitory effects of these drugs on noradrenaline release were abolished by atropine. Furthermore, Malik & Ling (1969) showed that acetylcholine usually reduced or abolished the vasoconstrictor responses to sympathetic nerve stimulation in the perfused mesenteric artery of the rat and that this effect was blocked by atropine; however, low concentrations of acetylcholine caused an increase in the responses to sympathetic nerve stimulation.

This paper deals with experiments on the perfused rabbit ear artery which were designed to study the effects of a number of cholinomimetic drugs with mainly muscarinic actions, both in the presence and absence of atropine, on the vasoconstrictor responses to sympathetic nerve stimulation. Observations were also made on the effects of acetylcholine and methacholine on the responses of the artery to injections of noradrenaline.

Methods

Segments of the central artery of the rabbit ear were set up as described by de la Lande & Rand (1965). The preparations were perfused with McEwen's solution gassed with 5% carbon dioxide in oxygen, at a temperature of 37° C. The rate of perfusion was maintained at 6 ml/min with a Watson-Marlow flow inducer. Changes in perfusion pressure, which arose from the changes in the resistance to flow through the arterial segment, were recorded in mm Hg (1 mm Hg≡1·333 mbar) with a Statham pressure transducer on an Offner Dynograph recorder.

Infusions of drug solutions at a rate of 0·03 to 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. The concentrations of the infused drugs were calculated as μg/ml in the perfusion fluid reaching the artery.

Injections of noradrenaline were made directly into the rubber connecting tube by means of an insulin syringe or by an automatic device. The volume of the solution injected was 0·05 to 0·1 ml.

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. Generally the frequency was 20 Hz, but in some experiments lower frequencies of 3 to 10 Hz were used. The pulses were given for 10 s periods at 3 min intervals.

Drugs used were: acetylcholine chloride (Hopkins & Williams Ltd.), arecoline hydrobromide (B.D.H.), atropine sulphate (Macfarlan Smith Ltd.), carbachol (carbamylcholine chloride, B.D.H.), methacholine (±)-acetyl-β-methylcholine chloride, Sigma Chemical Co.), natural (±)-muscarine hydrochloride (Professor C. H. Eugster), neostigmine bromide (Prostigmine, Hoffman-La Roche), noradrenaline bitartrate (Levophed, Winthrop), and pilocarpine nitrate (Macfarlan Smith Ltd.). All solutions of drugs were freshly prepared and the amounts referred to in the text are expressed in terms of salts except for noradrenaline, which is expressed as the base.

Results

Responses to sympathetic nerve stimulation

Effects of acetylcholine

In most experiments the frequency of sympathetic nerve stimulation was 20 Hz. Infusions of acetylcholine caused a reduction in vasoconstrictor responses to sympathetic nerve stimulation, the threshold for this effect being about 10 ng/ml of perfusion fluid. The maximal reduction of response varied from 30 to 85% in different experiments, and the concentrations of acetylcholine producing maximal effects ranged from 1 to 3 μg/ml. With higher concentrations of acetylcholine, the responses increased in size again and approached the height of the control responses. The results of experiments with stepwise increases in concentration of acetylcholine from 0·01 to 100 μg/ml are summarized in Fig. 1.

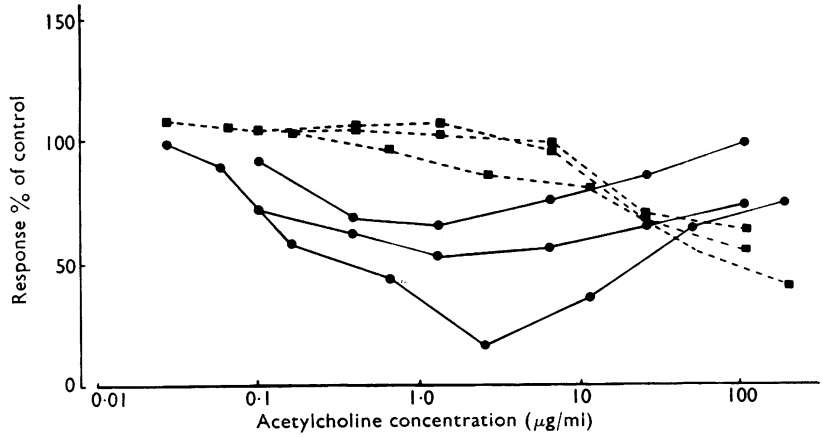

FIG. 1. Perfused rabbit ear artery. Effects of acetylcholine infusions with stepwise increases in concentration on responses to periarterial nerve stimulation (20 Hz, 10 s). Each point is the mean of five responses and is expressed as the percentage of the mean initial responses. No atropine, ●——●; in the presence of atropine (0·1 μg/ml), ■- - -■.

The results of one experiment are illustrated in Fig. 2. Low concentrations of acetylcholine (0·01 and 0·04 μg/ml) reduced the responses to nerve stimulation, but the effect diminished with time; higher concentrations (up to 2·56 μg/ml) had a greater inhibitory effect, and there was no diminution with time. With still higher concentrations (10·24 μg/ml), the inhibitory effect on responses to nerve stimulation gradually wore off, and with concentrations above 40·96 μg/ml the responses tended to return to control levels. Acetylcholine had no effect on the base line perfusion pressure. On terminating an infusion during which responses were depressed, they returned rapidly to their control level and often exceeded it.

Atropine, in a concentration of 0·1 μg/ml, produced a slight but significant increase in vasoconstrictor responses to sympathetic nerve stimulation in six out of ten experiments (Table 1). In the presence of atropine (0·1 μg/ml), acetylcholine did not cause a reduction in the responses to sympathetic nerve stimulation unless its concentration exceeded 10 μg/ml (Fig. 1).

In a few experiments, brief infusions of low concentrations of acetylcholine (1 to 2 ng/ml) caused a slight increase in vasoconstrictor responses to sympathetic nerve stimulation at 20 Hz, and the increase persisted or became greater after termination of the infusions. In experiments with lower frequencies of nerve stimulation (10 Hz

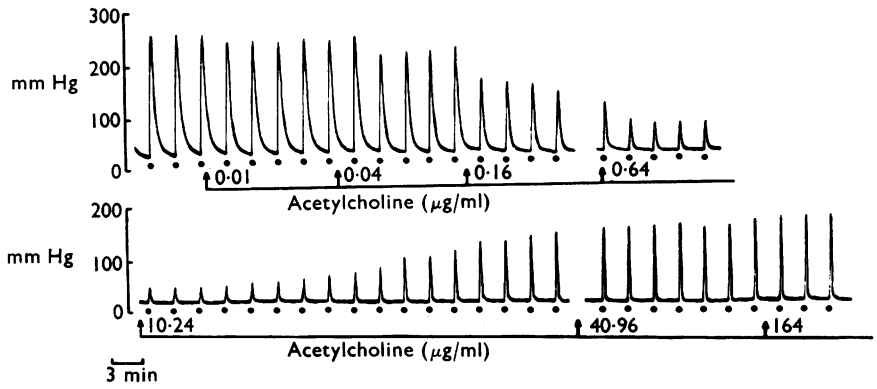

FIG. 2. Effects of increasing concentrations of acetylcholine on responses to periarterial nerve stimulation (●, 20 Hz, 10 s). Horizontal bars indicate infusion of acetylcholine with concentrations (μg/ml of perfusion fluid) next to arrows.

TABLE 1. *Effects of atropine (0·1 μg/ml) on responses to sympathetic nerve stimulation*

	Control		Atropine		
Exp. No.	Mean ± S.E.		Mean ± S.E.		% of control
1	42·7 ± 0·27		42·2 ± 0·12		98·8NS
2	30·2 ± 0·20		29·9 ± 0·33		99·0NS
3	41·8 ± 0·58		41·4 ± 0·24		99·0NS
4	34·8 ± 0·58		35·5 ± 0·66		101·4NS
5	46·0 ± 0·00		48·4 ± 0·60		105·2**
6	39·8 ± 0·37		42·3 ± 0·83		106·3*
7	52·5 ± 0·35		57·3 ± 0·53		109·1***
8	52·9 ± 0·33		60·1 ± 1·27		113·6***
9	44·4 ± 0·40		51·1 ± 1·05		115·1***
10	39·6 ± 0·67		51·8 ± 0·25		130·8***

NS, Not significant; * $P<0·05$; ** $P<0·01$; *** $P<0·001$.
Pen deflection in mm on record; means and standard errors of five responses before and after administration of atropine.

or less), the facilitation of vasoconstrictor responses after a brief infusion or single injection of small amounts of acetylcholine was more marked ; the inhibitory effects of acetylcholine were also more marked. In the experiment shown in Fig. 3, in which the response to stimulation at 3 Hz was small, a brief infusion of a low concentration of acetylcholine (0·005 μg/ml for 3 min) reduced this response considerably, but after stopping the infusion, there was an immediate increase in the response which eventually exceeded the pre-infusion height by 50%. This brief infusion was repeated a number of times until the responses were increased about threefold in height. Now, a single injection of 10 ng of acetylcholine, 2·5 min before a stimulation, caused only facilitation. Thereafter, during a longer infusion of acetylcholine (0·005 to 0·01 μg/ml) the responses were affected in a way which may be interpreted as being due to a mixture of facilitatory and inhibitory effects. During infusion with 0·04 μg/ml of acetylcholine, the vasoconstrictor responses were completely abolished, but were restored immediately on cessation of the infusion and were greater than before. The facilitatory effect of low concentrations of acetylcholine was not affected by atropine (0·1 μg/ml).

Effects of methacholine

Infusions of methacholine with stepwise increases in concentration from 0·01 to 10 μg/ml produced decreases in responses to nerve stimulation at 20 Hz (Fig. 4). In two experiments the effect was slight, but in three others the responses were decreased to 50% or less of the control value. Concentrations above 10 μg/ml had less inhibitory effects (Fig. 5, middle tracing) ; this finding is similar to that observed with acetylcholine. The reduction of responses by methacholine was most marked

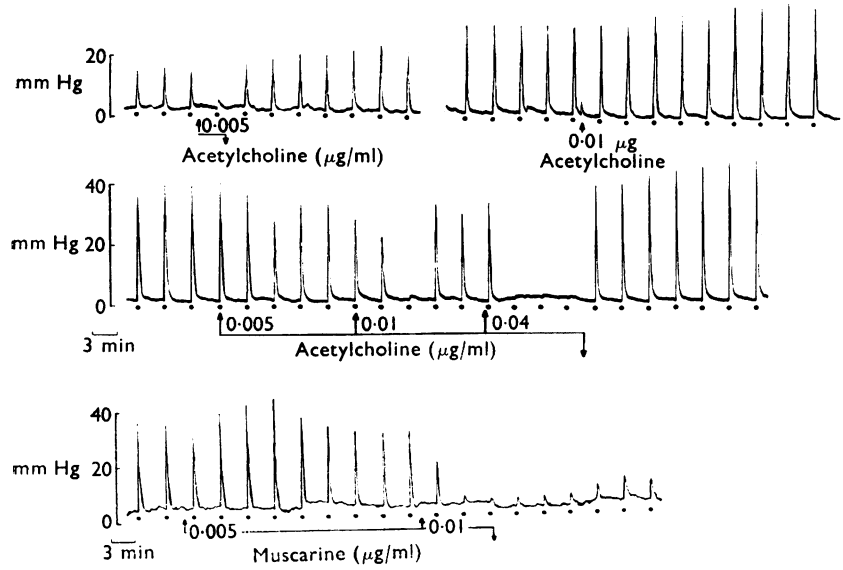

FIG. 3. Effects of acetylcholine and muscarine on responses to low frequency stimulation of the periarterial nerves. Upper and middle tracings continuous record: horizontal bars with arrows, infusion of acetylcholine (μg/ml perfusion fluid); arrow without bar, injection of acetylcholine into cannula. During the gap in the upper tracings, three infusions of acetylcholine (0·005 μg/ml). Stimulation (●), 3 Hz, 10 s. Lower tracing of another preparation ; horizontal bars with arrows, infusion of muscarine (μg/ml perfusion fluid). Stimulation (●), 5 Hz, 10 s.

immediately after the start of infusion ; thereafter, the responses tended to return to the control level (Fig. 5, upper tracing). The reduction in responses produced by methacholine was rapidly reversed after termination of an infusion, and the responses were then increased above the control level (Fig. 5, upper and middle tracings). Lower concentrations of methacholine were required for the inhibition of the responses to low (<10 Hz) than to high frequencies (20 Hz) of stimulation.

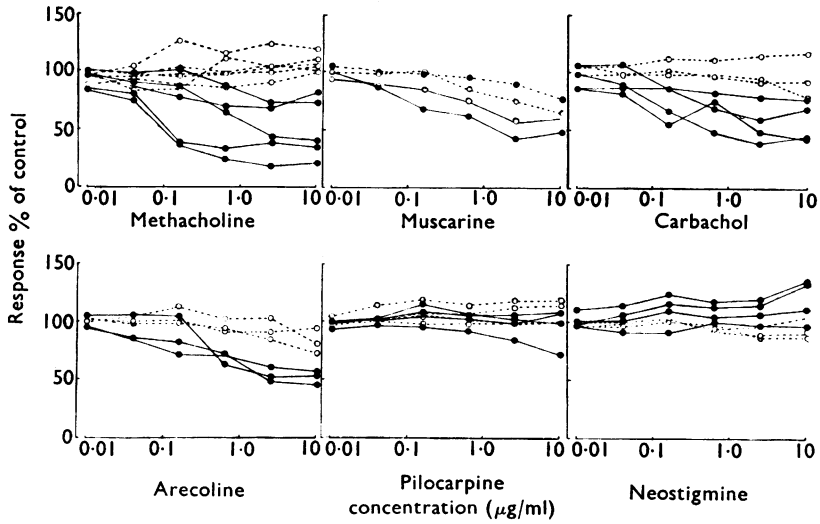

FIG. 4. Effects of cholinomimetics and neostigmine on responses to periarterial nerve stimulation (20 Hz, 10 s). Each point is the mean of five responses and is expressed as the percentage of the mean initial responses. No atropine, ——— ; in the presence of atropine (0·1 μg/ml), - - - - - -. With muscarine, the closed circles are results from observations in one preparation, the open circles are from another preparation ; with all the other drugs, separate preparations were used for each experiment.

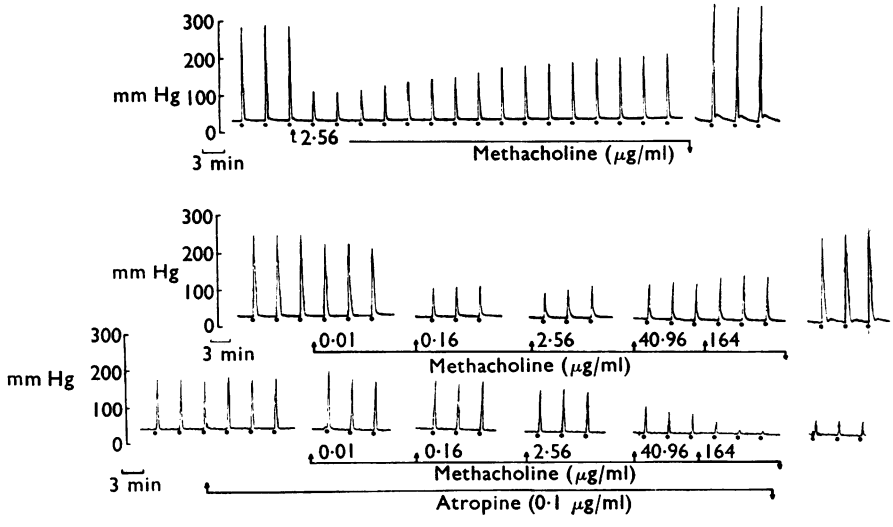

FIG. 5. Effects of methacholine on responses to periarterial nerve stimulation (●, 20 Hz, 10 s) ; three preparations. Horizontal bars with arrows indicate infusions of methacholine or atropine (μg/ml perfusion fluid).

Atropine (0·1 μg/ml) reduced or abolished the inhibitory effect of methacholine on the responses to sympathetic nerve stimulation; when the concentration of methacholine was 10 μg/ml, the responses were slightly above the control level (Fig. 4). In one experiment, all concentrations of methacholine produced increases in the responses in the presence of atropine, but in this experiment methacholine raised the resting perfusion pressure. High concentrations of methacholine (up to 100 μg/ml) were required to cause a reduction in the responses in the presence of atropine, a finding comparable to that obtained with acetylcholine (Fig. 5, lower tracing).

Effects of carbachol

The results with carbachol (0·01 to 10 μg/ml) were similar to those with methacholine. Figure 4 shows that responses to sympathetic nerve stimulation were reduced by carbachol and that this effect was abolished by atropine. After terminating an infusion, the responses rapidly recovered and eventually exceeded the control level. In one of three experiments in which atropine was present, carbachol caused an increase in the responses.

Effects of muscarine

In two experiments, muscarine, in concentrations of 0·04 to 10 μg/ml, reduced responses to sympathetic nerve stimulation at 20 Hz; this action was antagonized by atropine (0·1 μg/ml) (Fig. 4). In low concentrations (0·005 to 0·01 μg/ml), muscarine sometimes caused increases in the vasoconstrictor responses (Fig. 6). After the termination of an infusion that had decreased the responses, they eventually increased to above the control level.

When the frequency of nerve stimulation was low (<10 Hz), muscarine had a greater facilitatory action in low concentrations, and a greater inhibitory action in higher concentrations (Fig. 3, lower tracing). At low frequencies of stimulation, the responses recovered only gradually after termination of the infusion.

Effects of arecoline

Arecoline had, in general, effects similar to those of the choline esters and muscarine; responses to stimulation at 20 Hz were reduced by high concentrations (0·64 to 10 μg/ml) and the effect was antagonized by atropine (Fig. 4). In one experiment (Fig. 6), lower concentrations (0·01 to 0·16 μg/ml) increased the responses slightly and higher concentrations reduced them; then, after the termination of the infusion, the responses recovered to above the control level.

Effects of pilocarpine

Pilocarpine differed from the other cholinomimetic drugs in that it generally caused a slight increase in the responses to nerve stimulation over a wide range of concentrations (0·01 to 164 μg/ml), both in the absence and in the presence of atropine (Figs. 4 and 6).

Effects of neostigmine

Infusions of neostigmine in gradually increasing concentrations caused increases in vasoconstrictor responses to sympathetic nerve stimulation, the effect being abolished by atropine (Figs. 4 and 6).

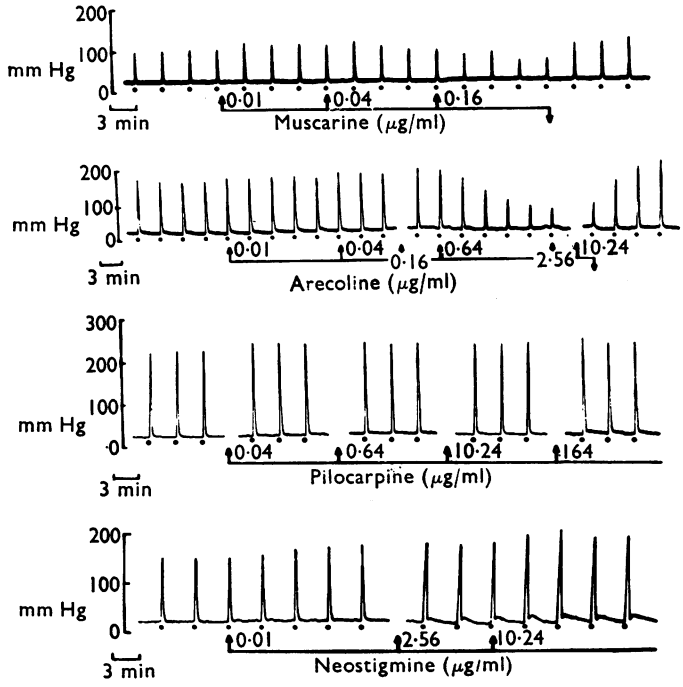

FIG. 6. Effects of muscarine, arecoline, pilocarpine and neostigmine on responses to peri-arterial nerve stimulation (●, 20 Hz, 10 s). Horizontal bars with arrows indicate infusions of drugs (μg/ml perfusion fluid).

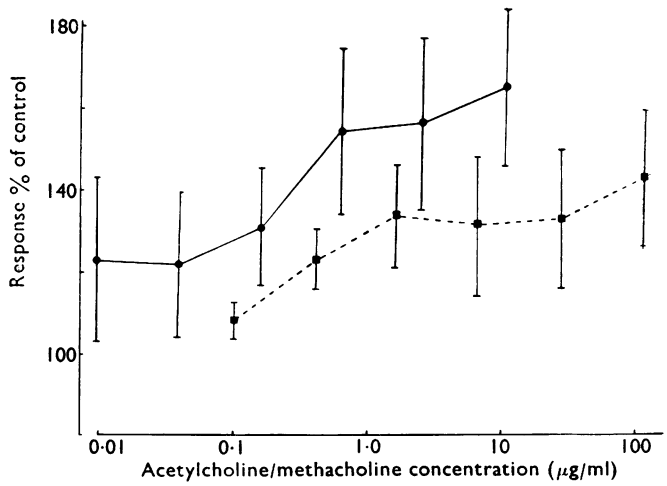

FIG. 7. Facilitatory effects of acetylcholine and methacholine on the vasoconstrictor responses to injections of noradrenaline (2·5 to 10 ng). Abscissa, concentration of choline esters (μg/ml); ordinate, mean increases in vasoconstrictor response (% of control). Acetylcholine (six experiments), ●——●; methacholine (seven experiments), ■- - -■; vertical bars, S.E. of the means (five responses to noradrenaline were elicited at each concentration of the choline esters).

Responses to noradrenaline injections

Effects of acetylcholine and methacholine

Acetylcholine and methacholine caused increases in the vasoconstrictor responses to injected noradrenaline (Fig. 7). These results are in marked contrast to those obtained with sympathetic nerve stimulation, in which the main effects of acetylcholine and methacholine, in the same ranges of concentrations, were reduction in vasoconstriction. Figure 8 illustrates experiments in which the facilitatory effects of acetylcholine and methacholine on the responses to noradrenaline were compared, in the same preparation, with their inhibitory effects on responses to sympathetic nerve stimulation. When similar experiments were carried out with atropine present, acetylcholine still enhanced the vasoconstrictor responses to noradrenaline but no longer reduced those to sympathetic nerve stimulation.

Discussion

Acetylcholine had two effects on the vasoconstrictor responses of the rabbit isolated ear artery preparation to sympathetic nerve stimulation. With high frequencies of stimulation (20 Hz) and concentrations of acetylcholine in the range of 0·01 to 10 μg/ml, the responses were decreased. However, very low concentrations of acetylcholine (less than 10 ng/ml) produced increases in the responses, particularly

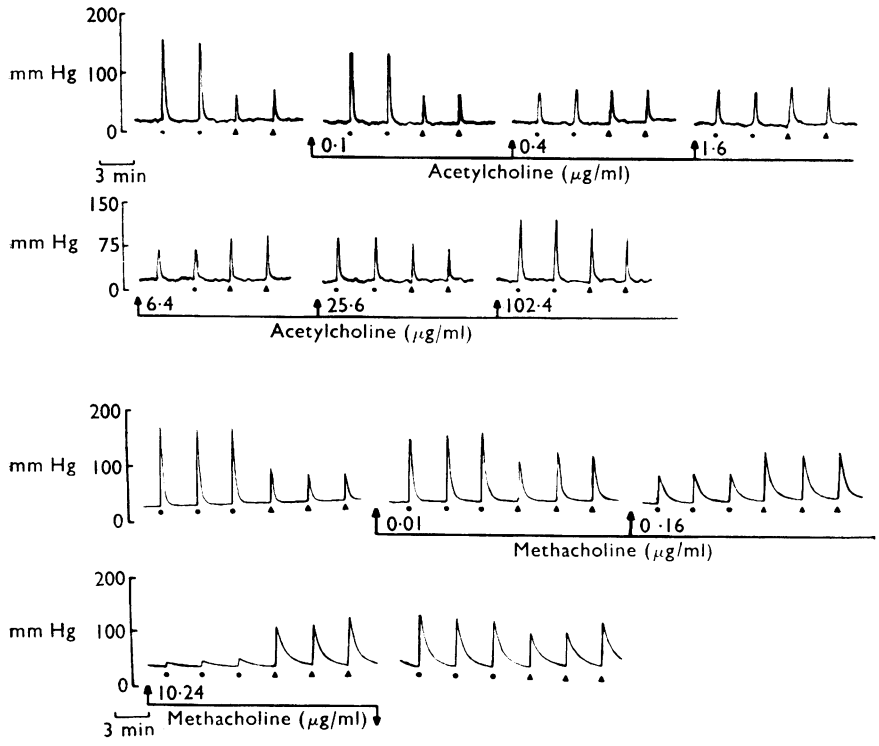

FIG. 8. Effects of acetylcholine or methacholine on the responses to periarterial nerve stimulation (●, 20 Hz, 10 s) and to injections of noradrenaline (▲, 2·5 ng). Horizontal bars with arrows indicate infusions of acetylcholine or methacholine (μg/ml perfusion fluid).

when the frequency of stimulation was low (<10 Hz); furthermore, responses were increased after termination of an infusion of acetylcholine that had decreased responses. These findings are essentially in accord with those of Malik & Ling (1969), who used the rat isolated mesenteric artery preparation of McGregor (1965).

The inhibitory effect of acetylcholine on responses to sympathetic nerve stimulation was shared by methacholine, muscarine, carbachol and arecoline. The decrease in response may be due (1) to the vasodilator action of these drugs, or (2) to reduction in noradrenaline release, possibly by an action they may have on the sympathetic terminal axon. The first possibility is unlikely, for concentrations of acetylcholine and methacholine similar to those which reduced the vasoconstrictor responses to sympathetic nerve stimulation did not cause reduction of vasoconstriction induced by injections of noradrenaline. Vasodilator drugs, such as glyceryl trinitrate and papaverine, reduced responses to noradrenaline more readily than those to sympathetic nerve stimulation (Gay, Rand & Ross, 1969). The evidence suggests, therefore, that acetylcholine and methacholine reduced the responses to sympathetic nerve stimulation by impairing noradrenaline release.

In view of the possibility that acetylcholine may be concerned in mediating the release of noradrenaline by the nerve impulse (Burn & Rand, 1959), a reduction in noradrenaline release by acetylcholine could be due to a blockade by an excess of acetylcholine of its own actions, as suggested by Burn & Rand (1962). Closely related to this interpretation is the possibility that acetylcholine, in a persistently high concentration, exerts an adrenergic neurone blocking action (Rand & Wilson, 1967). However, it is thought that the inhibitory action of acetylcholine on noradrenaline release is nicotinic, since it is demonstrable in the presence of atropine (for references, see **Introduction**), and is also exhibited by the nicotinic stimulants nicotine and dimethylphenylpiperazinium (Bentley, 1962; Wilson, 1962; Birmingham & Wilson, 1965; Rand & Wilson, 1967). Atropine increased the concentrations of acetylcholine or methacholine which were required to reduce responses to sympathetic nerve stimulation by a factor of about 100. Since methacholine is virtually devoid of nicotinic activity, and muscarine reduced the responses to sympathetic nerve stimulation, it is suggested that impairment of noradrenaline release may result from an action of cholinomimetic drugs on muscarinic receptors of terminal adrenergic neurones. Other evidence for such receptors has been advanced by Lindmar *et al.* (1968), who found that acetylcholine reduced the amount of noradrenaline released by dimethylphenylpiperazinium from the isolated perfused rabbit heart, and by Haeusler *et al.* (1968), who observed that atropine increased about 80-fold the amount of noradrenaline liberated from the isolated perfused cat heart by acetylcholine.

The increase in vasoconstrictor responses to sympathetic nerve stimulation produced by very low concentrations of acetylcholine may be due to facilitation of release of noradrenaline by nerve impulses. If acetylcholine is concerned in mediating the release of noradrenaline, as suggested by Burn & Rand (1959), added acetylcholine may have a facilitatory action in low concentrations although excess acetylcholine may block release as suggested above. The enhancement of responses to sympathetic nerve stimulation by low concentrations of muscarine or by a wide range of concentrations of pilocarpine may also be due to facilitation of noradrenaline release. The increases in responses which were observed after termination of infusions of acetylcholine, methacholine, muscarine, carbachol or arecoline that had

caused decreases in responses may be due to persistence of a facilitatory effect on noradrenaline release when the inhibitory effect of these drugs on the release had worn off.

The facilitatory effect of acetylcholine on responses to nerve stimulation could be due to excitation of sympathetic terminal axons, as has been demonstrated in the cat spleen (Ferry, 1963) and the perfused cat heart (Cabrera, Torrance & Viveros, 1966 ; Haeusler *et al.*, 1968). Acetylcholine sets up antidromic action potentials in the sympathetic nerves of these organs. Haeusler *et al.* (1968) showed that atropine reduced the antidromic excitation caused by acetylcholine although it increased the output of noradrenaline from the perfused cat heart, an observation which indicates that noradrenaline release by acetylcholine may be independent of the production of action potentials. Furthermore, they found that the muscarinic drugs, pilocarpine and methacholine, did not cause antidromic excitation.

The enhancement of responses to sympathetic nerve stimulation by small amounts of muscarine suggests that a muscarinic receptor is involved. However, the facilitatory effect of small amounts of acetylcholine was not abolished by atropine. The inhibitory effects of higher concentrations of both drugs were readily blocked by atropine. It is possible that muscarinic receptors concerned with excitatory effects are less susceptible to blockade by atropine than are those concerned with inhibitory effects.

It appears that muscarine may either facilitate or depress sympathetic transmission. There are other examples of such dual muscarinic actions. For example, muscarine and acetylcholine have excitatory actions on isolated atria in which the beat is suppressed by cooling, although they have inhibitory actions in a normally beating atria (Burn & Rand, 1958). Furthermore, there is considerable evidence that acetylcholine and a number of other cholinomimetics have excitatory and inhibitory actions on muscarinic receptors in ganglion cells (reviewed by Volle, 1966a, b). Thus, the ganglion cell and the adrenergic terminal axon show certain similarities in their pharmacological reactivities to muscarinic as well as to nicotinic cholinomimetic drugs.

Pilocarpine increased responses to sympathetic nerve stimulation in the artery. However, Lindmar *et al.* (1968) found that pilocarpine, like acetylcholine, reduced noradrenaline release. Pilocarpine stimulates chromaffin cells (Douglas, Kanno & Sampson, 1967), but not sympathetic nerve endings in the heart (Haeusler *et al.*, 1968). The finding that pilocarpine differed from other muscarinic cholinomimetics in that it enhanced but did not depress responses to sympathetic nerve stimulation may be related to the difference between its effects and those of other muscarinic substances on ganglion cells (Takeshige & Volle, 1964).

The potentiating effect of neostigmine on responses to stimulation may be due to protection from hydrolysis of acetylcholine concerned in mediating the release of noradrenaline by the nerve impulse ; this observation may be added to the findings of a number of workers (reviewed by Burn, 1968), who demonstrated that anticholinesterases caused increases in responses to sympathetic nerve stimulation. In most cases the effects of the anticholinesterases were observed in the presence of atropine: in the rabbit ear artery, however, the effect was blocked by atropine and was therefore mediated by muscarinic receptors. Atropine alone often caused a slight increase in responses to stimulation, an observation which suggests that if

acetylcholine is concerned with the mediation of noradrenaline release, it also acts on muscarinic receptors which inhibit release. The apparent paradox would be resolved if either neostigmine had an action other than inhibition of cholinesterase, or atropine has an action other than blockade of muscarinic receptors.

The potentiation of the vasoconstrictor action of noradrenaline by acetylcholine and methacholine might be explained by impairment of uptake of noradrenaline, but in the rabbit heart acetylcholine does not inhibit the removal of noradrenaline from the perfusion fluid (Muscholl, 1968). There is no reason to suppose that the potentiation in the rabbit ear is due to synergism of vasoconstrictor effects, since acetylcholine and methacholine have no vasoconstrictor activity, except when they are given together with noradrenaline.

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