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Modification by choline of adrenergic transmission in rat mesenteric arteries

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Summary

1. The action of choline on the vasoconstrictor responses of the perfused mesenteric arteries of the rat to sympathetic nerve stimulation and to injected noradrenaline has been investigated.

2. The infusion of choline (500 μg/ml), for periods of 15 s, increased the response to sympathetic nerve stimulation, whereas the infusion of the same concentration for 20 min greatly reduced the response to nerve stimulation. Choline (up to 500 μg/ml), infused either for short or long periods, did not alter the response to injected noradrenaline.

3. The inhibitory action of choline on the response to nerve stimulation was abolished either by an increase in the calcium concentration from 1·8 to 5·4 mM or by simultaneous infusion of (+)-amphetamine or atropine.

4. The results suggest that choline in concentrations of 500 μg/ml has the same effect on adrenergic transmission in mesenteric arteries as acetylcholine at concentrations of 5 ng/ml.

Introduction

Evidence for the view that the release of noradrenaline (NA) from the sympathetic postganglionic fibres is mediated by acetylcholine (ACh) (Burn & Rand, 1959) has recently been greatly strengthened by the histochemical findings of Eränkö, Rechardt, Eränkö & Cunningham (1970) in the rat pineal body which is innervated by postganglionic sympathetic fibres from the superior cervical ganglion. These workers have shown that the terminal of sympathetic fibres in the rat pineal is invested by acetylcholinesterase. In contrast, Graham, Lever & Spriggs (1968) have shown in the cat that the nerve bundles innervating pancreatic arterioles consist exclusively of adrenergic or cholinergic axons. In addition, they observed some nerve bundles which contained a mixture of discrete adrenergic and cholinergic axons juxtaposed, without any Schwann cell cytoplasm intervening. Cholinesterase staining elements have also been demonstrated in the artery of the rabbit ear at the medial-adventitial border (Waterson, Hume & de la Lande, 1970), a region containing a network of noradrenergic nerve terminals (de la Lande & Waterson, 1967). The

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staining associated with cholinesterase enzymes together with noradrenaline disappeared 21 days after the removal of the superior cervical ganglion. After pretreatment with reserpine, noradrenaline disappeared but the staining due to the enzymes remained unaffected.

We have reported that anticholinesterase agents potentiate the constrictor response of mesenteric arteries to postganglionic sympathetic nerve stimulation at frequencies of less than 6 Hz, the effect being greatest at 1 Hz, the lowest frequency tested (Malik, 1970). It was also demonstrated that ACh, applied in very low concentrations (2 ng/ml) for periods of 15 s, augmented the response to postganglionic sympathetic nerve stimulation (Malik & Ling, 1969a). Choline has a variety of actions dependent in part on its structural similarity to ACh. Thus, it has weak cholinergic stimulatory effects, some of which are blocked by atropine (Dale, 1914 ; Le Heux, 1921 ; Fatt, 1950 ; Gebber & Volle, 1965), an anticurare effect (Hutter, 1952), an anticholinesterase action (del Castillo & Katz, 1957) and a capacity to release ACh from preganglionic nerve endings (Brown & Feldberg, 1936 ; Matthews, 1963 ; Desiraju, 1966). We have examined the effects of choline on adrenergic transmission in mesenteric arteries in an attempt to show similarities to ACh at this neuroeffector junction.

Methods

Female albino rats, 250–300 g, were anasethetized with ether and the abdomen opened by a midline incision. The superior mesenteric artery was cannulated and isolated with its small resistance vessels according to the method of McGregor (1965). The vessels were perfused with Tyrode solution at a constant flow of 25 ml/min using a Harvard peristaltic pump (Model 1210) as described earlier (Malik & Ling, 1969a). Tyrode solution of the following composition (mM) was used: NaCl, 136 ; KCl, 2·7 ; $CaCl_2$, 1·8 ; $MgCl_2$, 1·1 ; $NaHCO_3$, 12 ; $NaHPO_4$, 0·42 and dextrose, 5·6. The temperature of the perfusion fluid was maintained at 22° C and the solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Changes of perfusion pressure were recorded manometrically from a cannula tied in the artery using a frontal writing lever on a kymograph. Before the cannulation of the superior mesenteric artery, the pressure in the cannula was 60 mmHg (1 mmHg≡1.333 mbar) at a flow rate of 25 ml/minute. During perfusion of the arteries, the pressure was 85 mmHg.

The periarterial nerves were stimulated for 20–22 s at intervals of 4 min with a Grass stimulator (Model S4) using biphasic rectangular pulses of 1 ms duration, frequency of 6 Hz and supramaximal voltage (20 V). The responses to noradrenaline (1–3 μg) were obtained by injecting it directly into the cannula leading to the superior mesenteric artery. All other drugs were added to Tyrode solution.

Drugs

Choline chloride (Fischer Scientific), (−) noradrenaline bitartrate monohydrate (NA), atropine sulphate (K & K Laboratories) and (+)-amphetamine sulphate (Smith, Kline & French). The drugs were dissolved in small volumes of Tyrode solution and diluted with the perfusion solution to obtain the final concentration which was expressed as that of the salt.

Results

Effect of choline on the responses to sympathetic nerve stimulation and to injected noradrenaline

The infusion of choline at concentrations of 500 μg/ml for periods of 15 s usually potentiated the response to sympathetic nerve stimulation as shown in Fig. 1. This

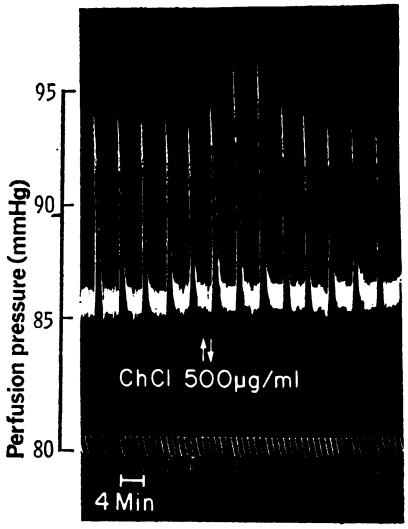

FIG. 1. Effect of choline chloride (ChCl) on the pressor responses of mesenteric arteries to sympathetic nerve stimulation. The mesenteric arteries were perfused with Tyrode solution at a rate of 25 ml/min at 22° C. The periarterial nerves were stimulated with biphasic rectangular pulses (20 V ; 1 ms ; 6 Hz) every 4 min for 22 seconds. The infusion of choline (500 μg/ml) for 15 s increased the responses to sympathetic nerve stimulation.

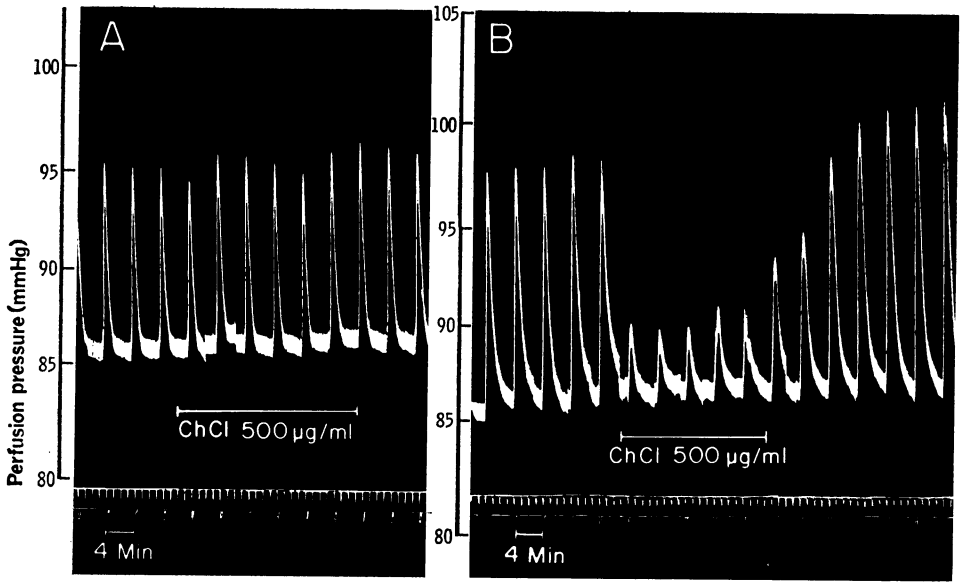

FIG. 2. (A) The action of prolonged infusion of choline on the responses to injected noradrenaline, (2 μg) and to sympathetic nerve stimulation (B). Recordings as in Fig. 1. The infusion of choline chloride (ChCl 500 μg ml), did not alter the response to injected NA, but reduced the responses to sympathetic nerve stimulation.

effect of choline was observed in six of nine experiments. In contrast, the continued infusion of choline (500 μg/ml) for 20 min markedly diminished the response to stimulation (Fig. 2B). When perfusion with choline-free Tyrode solution was resumed, the response to nerve stimulation was sometimes restored to the control value, but was often higher than the initial control response. The blocking effect of choline was observed in fifteen experiments and could be repeated as often as 4 times in the same preparation. However, in five of these experiments, infusion of choline inhibited the initial two responses to nerve stimulation ; this depression was

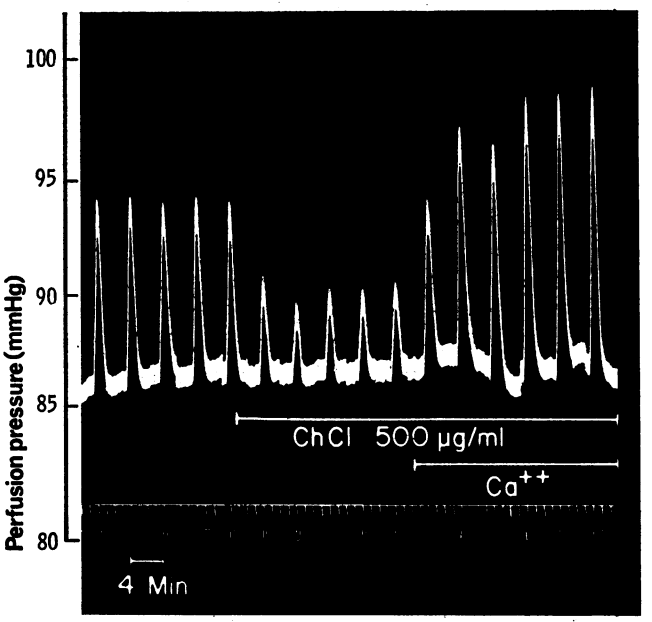

FIG. 3. Effect of increasing the calcium concentration on the inhibitory action of choline. Recordings as in Fig. 1. Responses to nerve stimulation were inhibited by the infusion of choline chloride (ChCl 500 μg/ml). When the calcium concentration in the perfusion fluid was raised from 1·8 to 5·4 mM, the inhibition due to choline was reversed and the amplitude of the responses potentiated.

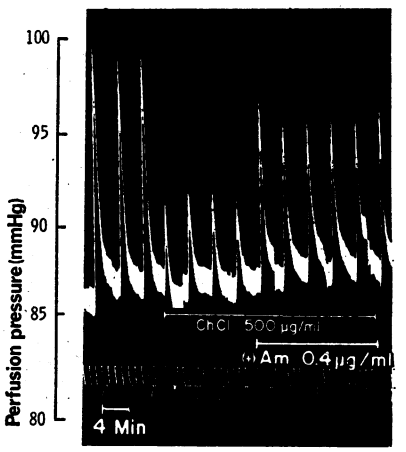

FIG. 4. Effect of (+)-amphetamine on the inhibitory action of choline. Recordings as in Fig. 1. The inhibitory effect of choline chloride (ChCl 500 μg/ml), on the responses to nerve stimulation was diminished by the simultaneous infusion of (+)-amphetamine (0·4 μg/ml).

followed by potentiation to above control levels while the choline was being infused. Choline, in concentrations lower than 100 μg/ml, either increased or did not alter the responses to nerve stimulation. The infusion of choline of up to 500 μg/ml, for either shorter or longer periods did not affect the responses to injected NA (2 μg) (Fig. 2A); this was so whether the responses to NA were recorded before or after the responses to nerve stimulation.

Effect of increased calcium concentration, (+)-amphetamine or atropine on the inhibitory action of choline on the response to sympathetic nerve stimulation

Effect of increased calcium concentration

Since blockade of responses to sympathetic nerve stimulation produced by guanethidine, ACh or dimethylphenylpiperazinium (DMPP) is reversed by raising the concentration of calcium in the medium (Burn & Welsh, 1967; Kirpekar, Wakade, Dixon & Prat, 1969; Malik & Ling, 1969a, b), experiments were carried out to determine if increases in the calcium concentration of the perfusion fluid would alter the inhibitory action of choline on the response to sympathetic nerve stimulation in mesenteric arteries. We have reported (Malik & Ling, 1969a) that increases in calcium concentration potentiate the responses to sympathetic nerve stimulation without altering the response to injected NA. Choline (500 μg/ml) inhibited the response to nerve stimulation in the presence of calcium concentrations of 1·8, 3·6 and 5·4 mM. In four experiments, the inhibitory effect of infusion of choline (500 μg/ml) on the response to nerve stimulation was abolished by raising the calcium concentration from 1·8 to 5·4 mM (Fig. 3).

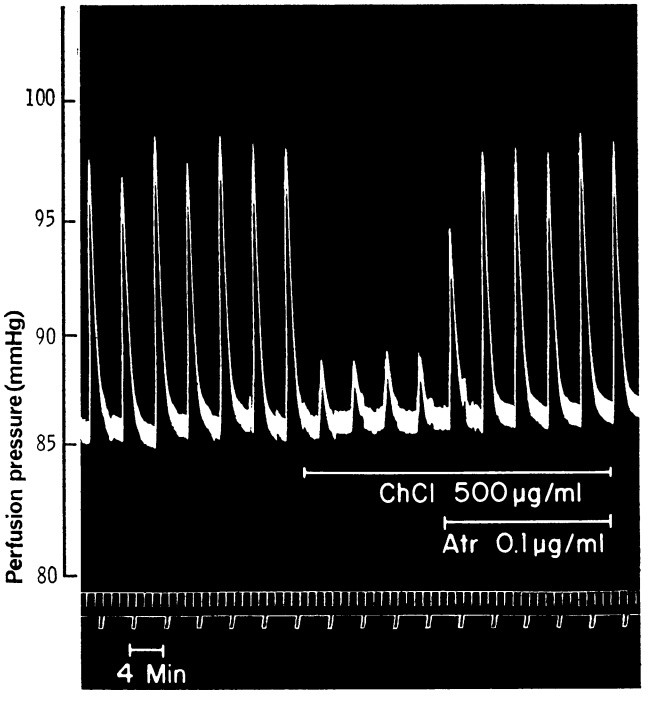

FIG. 5. Effect of atropine on the inhibitory action of choline on the response to nerve stimulation. The inhibitory effect of choline chloride (ChCl 500 μg/ml), on the responses to nerve stimulation was reversed by atropine (Atr, 0·1 μg ml).

Effect of +-amphetamine

(+)-Amphetamine increases the responses to sympathetic nerve stimulation (Rogers, Atkinson & Long, 1966 ; Malik & Ling, 1969a ; Löffelholz & Muscholl, 1970) and reverses the blockade of sympathetic nerves due to adrenergic neurone blocking agents (Day, 1962), ACh and DMPP (Malik & Ling, 1969a, b). In five experiments, (+)-amphetamine (0·4 μg/ml) invariably reversed the inhibitory effect of choline on the responses to sympathetic nerve stimulation (Fig. 4).

Effect of atropine

Atropine (0·1 μg/ml) had either no effect or produced a small reduction in the responses to sympathetic nerve stimulation in twelve experiments. This concentration of atropine reversed completely the inhibitory action of ACh on the response to nerve stimulation (Malik & Ling, 1969a). The inhibiting effect of choline on the responses to nerve stimulation was abolished by simultaneous infusion of atropine (0·1 μg/ml) (Fig. 5). This effect of atropine was observed in each of five experiments.

Discussion

Choline modified the responses of rat mesenteric arteries to sympathetic nerve stimulation in different ways depending upon the duration of its infusion. Infusion of choline (500 μg/ml) for periods of 15 s, potentiated the response to sympathetic nerve stimulation. However, the infusion of the same concentrations for periods as long as 20 min greatly reduced the responses to nerve stimulation. Since the infusion of choline for either shorter or longer periods did not alter the responses to injected NA and did not change basal perfusion pressure, these effects of choline on the vasoconstrictor responses to sympathetic nerve stimulation are not due to its direct action on vascular smooth muscle but rather to its effect on the release of NA from the sympathetic fibres. The adrenergic nerve terminals contain receptors which are sensitive to ACh and DMPP (Malik & Ling, 1969a, b). The infusion of ACh and DMPP may stimulate or block these receptors depending on the time course and concentration of the agents used. The receptors for ACh can be blocked by atropine, whereas receptors for DMPP are unaffected by atropine. However, the receptors for DMPP are blocked by hexamethonium (Lindmar & Muscholl, 1961).

If one accepts the cholinergic link hypothesis (Burn & Rand, 1959) the effect of infusion of choline for short periods presumably either summates with ACh, which is released from adrenergic nerve fibres or increases the synthesis or release of ACh from these fibres as has been shown for choline in preganglionic fibres (Brown & Feldberg, 1936 ; Matthews, 1963 ; Desiraju, 1966). This, in turn, may increase the release of NA from adrenergic nerve terminals and result in a greater constrictor response.

The infusion of choline for longer periods presumably would cause accumulation of ACh or interfere with the action of ACh at receptors on the adrenergic nerve terminal. Therefore, the response to nerve stimulation would be greatly reduced or abolished. Choline potentiates the contractor response of the vas deferens to postganglionic sympathetic nerve stimulation (Bell, 1967). Since this effect was

abolished by hyoscine, it was suggested that it was due to an action at muscarinic receptors on the muscle. However, in perfused mesenteric arteries the inhibitory effect of choline on the response to postganglionic sympathetic nerve stimulation was reversed by the simultaneous infusion of atropine. Thus, it appears that the adrenergic nerve terminals, like the superior cervical ganglion (Ambache, Perry & Robertson, 1956) contain muscarinic receptors in addition to nicotinic receptors, which may be stimulated or inhibited depending upon the time course and concentration of cholinomimetic agents (Malik & Ling, 1969a). Inhibitory muscarinic receptors have been proposed for the adrenergic nerve terminal in the heart of rabbit and cat (Lindmar, Löffelholz & Muscholl, 1968 ; Haeusler, Thoenen, Haefely & Hürliman, 1968) and rabbit ear artery (Rand & Varma, 1970). The blockade of receptors at the adrenergic nerve terminal, whether muscarinic or nicotinic, presumably prevents the entry of calcium into the nerve terminal and thus causes a decrease in the release of the neurotransmitter. An increase in calcium concentration or simultaneous infusion of (+)-amphetamine would abolish this inhibition (Malik & Ling, 1969a, b). Since the inhibitory action of choline on the response to sympathetic nerve stimulation is abolished by increasing the calcium concentration of the perfusion fluid or by the infusion of (+)-amphetamine it appears that choline acts on adrenergic transmission in mesenteric arteries in a manner similar to that of ACh.

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