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Mechanism of the vasoconstrictor action of isoprenaline on an isolated artery preparation

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It was not possible to demonstrate a vasodilator action of isoprenaline using the isolated artery segment from the rabbit ear. However, in high doses isoprenaline produced vasoconstriction. This vasoconstrictor response was blocked by α-receptor antagonists but not by β-receptor antagonists, except in high, non-specific doses. The response was not reduced by pretreatment of the rabbits with reserpine or by cocaine. The vasoconstriction produced by isoprenaline in this preparation therefore appears to be due to a direct action on the α-adrenotropic receptors.

An isolated perfused segment of the central artery from the rabbit ear has been shown to constrict when its sympathetic periarterial nerves are stimulated and when noradrenaline, histamine or 5-hydroxytryptamine (5-HT) is injected into the perfusion fluid (de la Lande & Rand, 1965). Furthermore, these amines enhance the responses to sympathetic nerve stimulation. It was also shown that acetylcholine produces vasodilatation in the preparation, but only when the tone is raised by continuous sympathetic stimulation. Starr & West (1966) showed that bradykinin was also vasodilator when the tone was raised.

The main effect of isoprenaline on blood vessels is dilatation, as was first shown by Konzett in 1940. More recently, Furchgott (1959) showed that isoprenaline may produce a contraction of arterial smooth muscle, although it is about 100 times less potent than noradrenaline. Eckstein & Hamilton (1959) showed that isoprenaline produces vasoconstriction in the venous system and the work of Kaiser, Ross & Braunwald (1964) suggests that this is due to the stimulation of β-adrenotropic receptors.

In the present study, the actions of isoprenaline were investigated on the isolated artery from the rabbit ear. In this preparation, isoprenaline produces vasoconstriction which, on analysis, appears to be due to a direct action on α-receptors.

Experimental

METHODS

A segment of the artery from the rabbit ear was set up as described by de la Lande & Rand (1965). The preparation was perfused with McEwen solution (1956), which was gassed with carbon dioxide 5% in oxygen. The temperature of the perfusion fluid was maintained at 37° and the preparation was perfused at a rate of 6 ml/min with a constant volume flow inducer. Changes in perfusion pressure, which arose from changes in the resistance to flow through the arterial segment, were recorded with a mercury manometer and kymograph or a Statham pressure transducer and an Offner recorder. Injections and infusions of drugs were given via a rubber connection close to the cannula. Drugs were freshly prepared in McEwen solution and the volume injected was 0·05 to 0·15 ml. Infusions were given by means of a Palmer slow injection apparatus at a rate

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of 0·05 to 0·2 ml/min through a polythene catheter inserted into the rubber connecting tube. The periarterial sympathetic nerves were stimulated by means of bipolar platinum ring electrodes.

DRUGS

Drugs used were: isoprenaline hydrochloride (racemic isoprenaline), (−)-isoprenaline bitartrate, (−)-noradrenaline bitartrate, (−)-adrenaline tartrate, histamine acid phosphate, phentolamine hydrochloride, phenoxybenzamine hydrochloride, tolazoline hydrochloride, pronethalol hydrochloride, propranolol hydrochloride and cocaine hydrochloride. All doses are expressed in terms of these salts.

Results

ACTIONS OF CATECHOLAMINES ON THE ARTERIAL SEGMENT

Very low concentrations of noradrenaline and adrenaline produced constriction of the artery, the threshold doses being 0·1 to 1 ng; moderate vasoconstriction was produced by 1 to 5 ng. Noradrenaline and adrenaline were found to be approximately equipotent, confirming the results of de la Lande & Harvey (1965). Isoprenaline was without action in the dose range in which noradrenaline was effective, but in doses of 1 to 10 μg it too produced constriction. The vasoconstrictor potency of racemic isoprenaline was approximately 5,000 times less than that of noradrenaline. The vasoconstrictor potency of (−)-isoprenaline was about twice that of racemic isoprenaline.

EFFECT OF ISOPRENALINE ON SYMPATHETICALLY INDUCED VASOCONSTRICTION

In an attempt to demonstrate a vasodilator action of isoprenaline in the artery, injections were given during an arterial spasm produced by continuous stimulation of the sympathetic nerves. Doses of isoprenaline ranging from 1 ng to 20 μg were given during the periods of sustained vasoconstriction. Low doses had no effect: in no case was there any hint of vasodilatation. Higher doses produced only a further increase in vasoconstriction. The same findings were obtained when infusions of

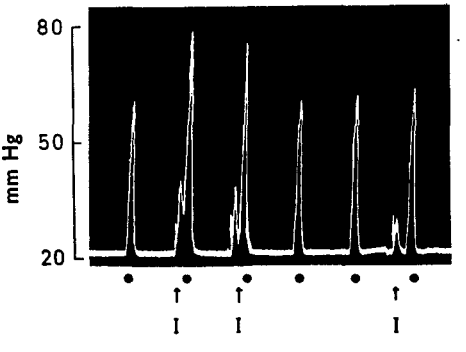

FIG. 1. Isolated artery segment. Periarterial stimulation (●) for 10 sec every 2 min using 1 msec pulses at 10/sec and supramaximal voltage. At I, isoprenaline (20 μg) was injected.

noradrenaline (2 to 20 ng/ml) or ergotamine (2 to 20 ng/ml) were given to produce a prolonged spasm.

Injections of isoprenaline before a short burst of sympathetic stimulation resulted in an increase in the size of the vasoconstrictor response. Fig. 1 shows that the increase in size depended on the interval between the isoprenaline injection and nerve stimulation, and did not persist beyond the duration of the vasoconstrictor effect of the isoprenaline. The increase is therefore merely due to summation of the two vasoconstrictor stimuli and thereby differs from the facilitation of sympathetic vasoconstriction produced by 5-HT as reported by de la Lande & Rand (1965).

Continuous infusions of isoprenaline were ineffective in diminishing vasoconstrictor responses to short bursts of nerve stimulation, and, if the concentration of isoprenaline was raised, the sympathetic vasoconstrictor responses were increased (Fig. 2).

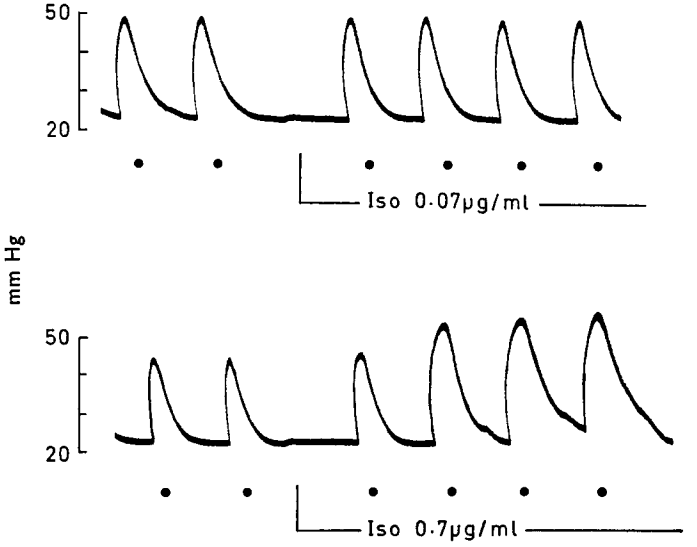

FIG. 2. Responses of the isolated artery segment to periarterial stimulation (●) for 10 sec every 2 min using 1 msec pulses at 10/sec and supramaximal voltage. Infusions of isoprenaline (Iso) are marked by the horizontal bars.

EFFECTS OF THE β-RECEPTOR ANTAGONIST PROPRANOLOL ON RESPONSES TO CATECHOLAMINES

Fig. 3 (upper panel) shows that propranolol (80 ng/ml) increased the vasoconstrictor responses to both isoprenaline and noradrenaline. When the concentration of propranolol was increased to 7 μg/ml, the response to isoprenaline was reduced, but there was a similar reduction in the response to noradrenaline (Fig. 3, lower panel).

EFFECTS OF α-RECEPTOR ANTAGONISTS ON RESPONSES TO CATECHOLAMINES

Phentolamine (16 ng/ml) blocked the vasoconstrictor responses to noradrenaline and isoprenaline. The recovery of the responses to the

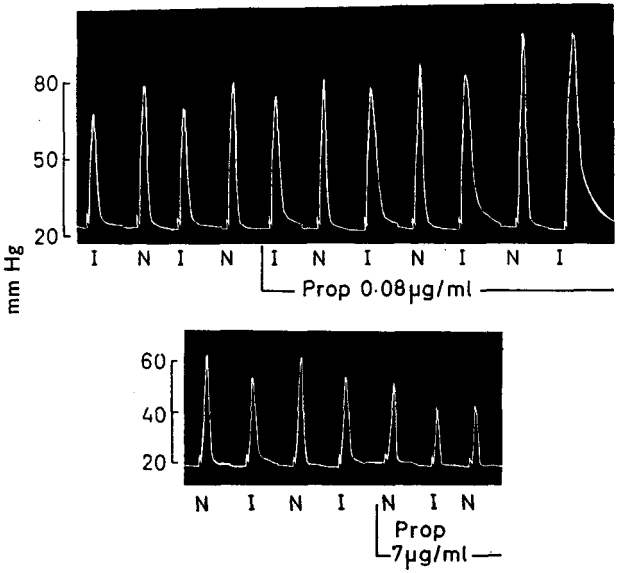

FIG. 3. Responses of isolated artery segments to injections of isoprenaline, 5 μg (I) and noradrenaline, 0·5 ng (N). Infusions of propranolol (Prop), 0·08 μg/ml and 7 μg/ml, are marked by the horizontal bars.

two drugs after blockade by phentolamine followed a similar time-course (Fig. 4). Similar results were obtained with phenoxybenzamine. In a concentration of 25 ng/ml, phenoxybenzamine blocked the responses to noradrenaline and isoprenaline without affecting the vasoconstrictor response to histamine (1 μg).

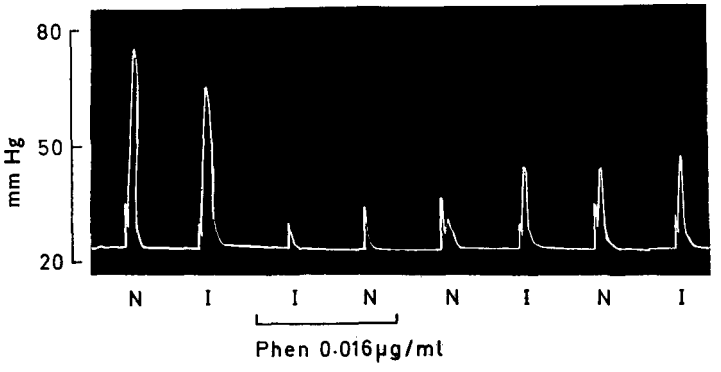

FIG. 4. Responses of the isolated artery segment to noradrenaline 0·002 μg (N) and isoprenaline 5 μg (I). An infusion of phentolamine (Phen), 0·016 μg/ml, is marked by the horizontal bar.

EFFECTS OF COCAINE AND RESERPINE

Observations were made with these drugs to determine whether isoprenaline may have been acting by releasing noradrenaline from stores within the adrenergic axons innervating the vascular smooth muscle.

Cocaine (0·2 to 0·5 μg/ml) enhanced the vascoconstrictor responses to nerve stimulation and potentiated the vasoconstrictor activity of both noradrenaline and isoprenaline as shown in Fig. 5. The vasoconstrictor action of histamine (1 μg) was also potentiated by cocaine.

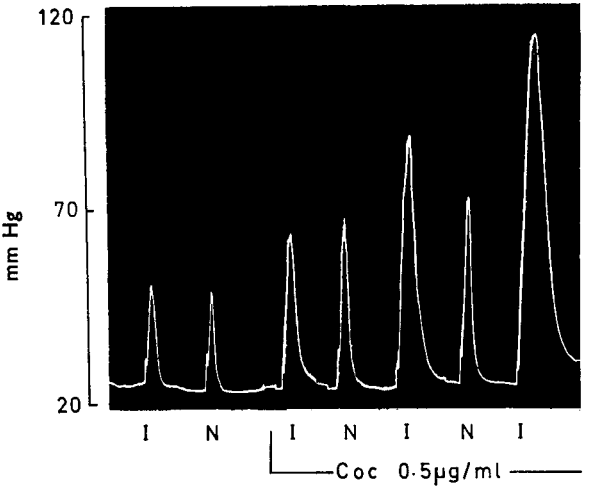

FIG. 5. Responses of the isolated artery segment to isoprenaline 7 μg (I), and to noradrenaline 0·002 μg (N). An infusion of cocaine (Coc), 0·5 μg/ml is marked by the horizontal bar.

Artery segments taken from rabbits treated with reserpine (2 mg/kg) 20 hr before setting up the isolated preparations showed slightly reduced vasoconstrictor responses to isoprenaline and noradrenaline. However, the relative potency of the two amines was unaffected. These observations indicate that isoprenaline acts directly on the receptors, since otherwise it would have been expected that cocaine or reserpine pre-treatment would decrease its vasoconstrictor activity.

Discussion

Vasodilator responses to acetylcholine and bradykinin have been demonstrated in the isolated artery preparation in which tone was maintained by vasoconstrictor stimulation (de la Lande & Rand, 1965; Starr & West, 1966). Thus the presence of drug receptors which mediate vasodilatation when combined with the appropriate agonist may be deduced. However, no vasodilator action of isoprenaline could be observed, which suggests either that β-receptors are absent in the preparation or that their stimulation does not lead to vasodilatation.

The only effect of isoprenaline on the arterial segment was to cause vasoconstriction, and this was additive with the vasoconstriction produced by nerve stimulation. Constriction of veins produced by isoprenaline

has been attributed by Kaiser & others (1964) to an action on β-receptors, but it seems unlikely that the vasoconstrictor action of isoprenaline observed in the arterial segment in our experiments is due to β-receptor stimulation since the action was not specifically depressed by propranolol. In fact, propranolol caused an equal degree of antagonism of isoprenaline and noradrenaline, as did phentolamine and phenoxybenzamine. These findings suggest that the vasoconstrictor action of isoprenaline is due to its combination with α-receptors. In accord with this is the finding that isoprenaline is several thousand-fold weaker than noradrenaline. The effects of propranolol on the actions of the two agonists indicate that it may combine with α-receptors as an antagonist, but it is weak in this respect.

The potentiation of the vasoconstrictor action of isoprenaline by low concentrations of propranolol might suggest at first sight that a vasodilator component of the response was being masked by blockade of β-receptors. However, there was an equal degree of potentiation of noradrenaline, and it is most unlikely that it too could be acting on β-receptors to produce a masked vasodilator component of response equivalent to that of isoprenaline.

The potentiation of the action of noradrenaline by propranolol and by cocaine may be explained by the blocking of its uptake into the adrenergic neurons in the tissue, since both drugs interfere with noradrenaline uptake mechanisms (Euler, 1967). However, this explanation cannot apply to isoprenaline because it is a poor substrate for catecholamine uptake mechanisms (Hertting, 1964).

From experiments with cocaine and reserpine it appears that the action of isoprenaline is directly on the receptors. Therefore blockade of reuptake of released noradrenaline cannot explain the potentiation of isoprenaline. The vasoconstrictor action of histamine was also potentiated by cocaine. The only explanation for the findings that we are able to offer is that cocaine sensitizes the arterial smooth muscle to vasoconstrictor drugs. Nevertheless, it should be pointed out that de la Lande (submitted for publication) has good evidence that cocaine does in fact potentiate the action of noradrenaline on the isolated artery segment by blocking its uptake.

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