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A screening method for vasodilator drugs

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The use of the isolated perfused central artery of the rabbit ear for screening vasodilator drugs is described. The effects of these drugs are shown by reduction of vasoconstrictor responses to intermittent sympathetic stimulation or to injections of noradrenaline or histamine or by reduction of vascular spasms produced by continuous sympathetic stimulation or infusion of 5-hydroxytryptamine. The relative potencies of the vasodilators used against intermittent sympathetic stimulation, in order are: adenosine = sodium nitroprusside = CIBA 31,531-Ba [5-amino-1-(1-methylpiperid-4-yl)- 3-(pyrid-4-yl) pyrazole] > glyceryl trinitrate > papaverine = amino-phylline > dipyridamole ⩾ sodium nitrite = hydrallazine.

The isolated perfused central artery of the rabbit ear provides a vascular preparation with a post-ganglionic sympathetic innervation that is suitable for testing the effects of drugs directly on blood vessels. The artery segment constricts when its sympathetic periarterial nerves are stimulated or when noradrenaline, histamine or 5-hydroxytryptamine are injected into the perfusion fluid (de la Lande & Rand, 1965).

Since the smooth muscle in this preparation is already fully relaxed, vasodilator actions of drugs cannot be observed directly. However, with the tone raised by continuous sympathetic stimulation, vasodilatation has been observed with acetylcholine (de la Lande & Rand, 1965) and bradykinin (Starr & West, 1966).

An anomalous observation with this preparation was made by Gay, Rand & Wilson (1967) who showed that isoprenaline, generally a potent vasodilator drug, did not dilate the artery even when the tone was raised. Instead, it caused constriction, though its potency was several thousandfold weaker than that of noradrenaline. The explanation was that the preparation lacks β-adrenoreceptors and isoprenaline weakly stimulates α-adrenoreceptors.

This paper deals with the use of the isolated perfused rabbit ear artery preparation for screening vasodilator drugs.

EXPERIMENTAL

A segment of the central artery of the rabbit ear was set up as described by de la Lande & Rand (1965). The preparation was perfused with McEwen solution at a constant flow rate of 6 ml/min using a Watson-Marlow flow inducer. The perfusion fluid was bubbled with 5% carbon dioxide in oxygen, and was maintained at a constant temperature of 37°.

Stimulation of the periarterial sympathetic nerves or the injection of noradrenaline, histamine or 5-hydroxytryptamine (5-HT) produced constriction of the artery segment. These responses were measured as changes in perfusion pressure with a Statham pressure transducer and were recorded with an Offner Dynograph pen recorder. The sympathetic nerves were stimulated by means of bipolar platinum ring electrodes placed around the proximal end of the artery using 1 ms square wave pulses at rates of 10 to 20 pulses/s and supramaximal voltage. Injections and infusions of drugs were

given into the rubber connection near the arterial cannula. All the drugs tested were freshly prepared in McEwen solution and were injected in volumes ranging from 0·04 to 0·20 ml. Infusions were made by means of a Palmer slow injection apparatus at rates of 0·05 to 0·20 ml/min.

The drugs used were acetylcholine chloride, adenosine, aminophylline, CIBA 31,531-Ba [5-amino-2-(1-methylpiperid-4-yl)-3-(pyrid-4-yl)pyrazole hydrochloride], dipyridamole, glyceryl trinitrate, histamine hydrochloride, 5-hydroxytryptamine creatine sulphate, hydrallazine, noradrenaline hydrochloride, papaverine hydrochloride, sodium nitrite, and sodium nitroprusside. The doses and concentrations of the drugs are expressed in terms of the compounds described above.

RESULTS

Reduction of intermittent vasoconstrictor responses produced by bursts of sympathetic stimulation or injections of noradrenaline or histamine

Infusions of the vasodilator drugs tested reduced the responses of the artery to sympathetic nerve stimulaiton. Representative records are shown in Fig. 1 with papaverine, dipyridamole and CIBA 31,531-Ba.

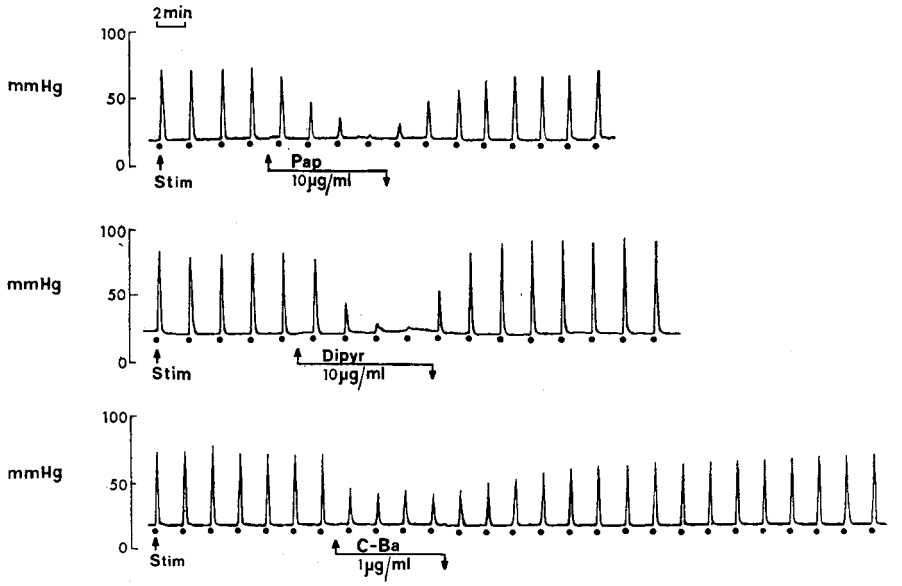

FIG. 1. Records of 3 separate experiments. In each, the periarterial sympathetic nerves were stimulated as indicated by the black dots for 10 s at 2 min intervals with 1 ms pulses at a frequency (10 to 20 pulses/s) sufficient to cause an increase in perfusion pressure of approximately 50 mm Hg. Infusions of papaverine (Pap), dipyridamole (Dipyr) and CIBA 31,531-Ba (C-Ba) were given for the periods and in the concentrations indicated beneath the records.

All of the drugs also reduced vasoconstrictor responses to injections of noradrenaline. Fig. 2 (top record) shows the effect of an infusion of sodium nitroprusside (1 μg/ml).

The potencies of the vasodilator drugs were compared by finding the minimal concentration of each drug which produced unequivocally a detectable reduction in the responses to sympathetic stimulation. The reproducibility of control responses was such that a reduction of 5 to 10% could generally be clearly recognized. Threshold concentrations were also found for the reduction of responses to injected noradrenaline

Table 1. *Threshold concentration of drugs in reducing vasoconstrictor responses to sympathetic stimulation and to injections of noradrenaline*

Vasodilator drug	Threshold concentration (μg/ml)		Relative molar potency Adenosine = 100%	
	Sympathetic stimulation	Noradrenaline injection	Sympathetic stimulation	Noradrenaline injection
Adenosine	0·5	0·01	100	100
Sodium nitroprusside	0·5	0·05	112	22
CIBA 31,531-Ba	0·5	0·05	112	22
Glyceryl trinitrate	1·0	0·20	43	4
Papaverine	2·0	0·50	36	3
Aminophylline	2·0	0·20	43	8
Dipyridamole	5·0	1·00	19	2
Sodium nitrite	50·0	1·00	0·3	0·3
Hydrallazine	50·0	0·20	0·6	3

(Table 1). In all instances, the threshold concentration to reduce the response to injected noradrenaline was lower than a concentration required to reduce the response to sympathetic stimulation. Threshold concentrations were chosen to compare potencies quantitatively because they gave more consistent results than were obtained using concentrations causing 50% depression of responses; this was probably because log dose-response lines for the substances were not always parallel. Determination of pA_2 was not used as it is not applicable to sympathetic nerve stimulation.

All of the vasodilator drugs reduced vasoconstrictor responses to injections of histamine to about the same extent as they reduced responses to noradrenaline. Examples with infusions of dipyridamole (10 μg/ml) and CIBA 31,531-Ba (1 μg/ml) are illustrated in the lower two records of Fig. 2. These are from experiments in which histamine and noradrenaline were injected alternately in doses that produced approximately equal vasoconstrictor responses.

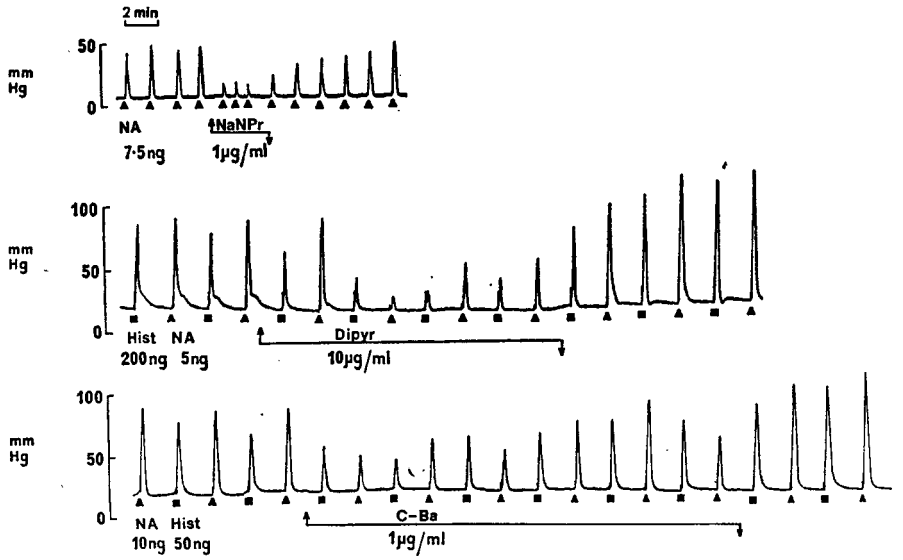

FIG. 2. Records of 3 separate experiments. Vasoconstrictor responses were induced by injections of noradrenaline (NA) as indicated by ▲ or by injections of histamine as indicated by ■ in the doses indicated beneath each record. Infusions of sodium nitroprusside (NaNPr), dipyridamole (Dipyr) and CIBA 31,531-Ba (C-Ba) were given for the periods and in the concentrations indicated beneath the records.

Reduction of vascular spasm induced by continuous sympathetic stimulation or infusion of vasoconstrictor drugs

Vasodilator actions in the perfused ear artery can also be demonstrated by a fall in perfusion pressure caused by injections or infusions of vasodilator drugs during a sustained constrictor response.

The vasodilator action of an injection of acetylcholine during sympathetically induced vascular spasm, an effect previously demonstrated by de la Lande & Rand (1965), is shown in Fig. 3. This also illustrates that an infusion of 100 μg/ml of

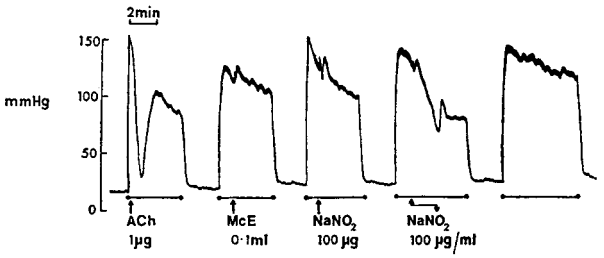

FIG. 3. Periods of sustained vasoconstriction were obtained in the preparation by stimulation with 1 ms pulses at 10 pulses/s as indicated by each pair of black dots joined by a horizontal line beneath the records. From left to right, the records show the following: The vasodilator response to 1 μg of acetylcholine (ACh) in a volume of 0·1 ml; a control injection of 0·1 ml of McEwen solution (McE); injection of 100 μg of sodium nitrite; infusion of 100 μg/ml of sodium nitrite; and a control period of stimulation.

sodium nitrite reduced the sustained vasoconstrictor response to sympathetic stimulation, but a single injection of 100 μg did not. Sodium nitrite is the weakest of the vasodilator drugs studied. With the others, vasoconstrictor spasm was reduced by injection of doses ranging from 1 to 100 μg.

Sodium nitroprusside was amongst the most potent of the vasodilator drugs studied; in a dose of 1 μg it produced marked inhibition of the sustained vasoconstriction produced either by infusion of 5-HT or by sympathetic stimulation (Fig. 4).

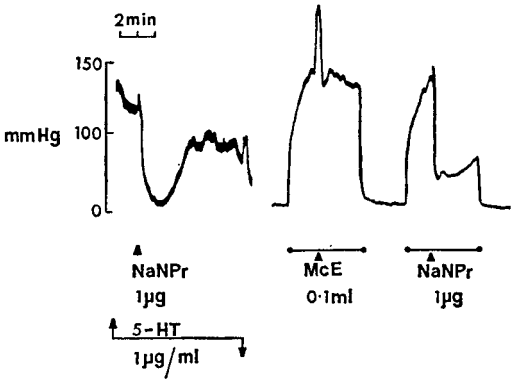

FIG. 4. Vasodilator effects of injections of 1 μg of sodium nitroprusside (NaNPr) during vascular spasms produced by infusion of 1 μg/ml of 5-hydroxytryptamine (5-HT) and sympathetic stimulation. A control injection of 0·1 ml of McEwen solution (McE) produced an artifact of an increase in perfusion pressure.

DISCUSSION

The results indicate that the isolated perfused artery from the rabbit ear provides a useful preparation for screening drugs having vasodilator activity. The preparation has the advantages that it is robust, economical and simple to set up. Furthermore, results are obtained more rapidly than with spiral strips of blood vessels and vascular spasms may be produced not only with vasoconstrictor drugs but also with sympathetic stimulation.

The preparation can be used to investigate the mode of action of vasodilator drugs. The vasodilator drugs tested reduced the vasoconstrictor responses to injected noradrenaline and histamine, to sympathetic stimulation and to infusion of 5-HT which suggests that their main effect is exerted directly on arterial smooth muscle. Relatively specific blocking drugs such as phenoxybenzamine and phentolamine produced a marked reduction in the vasoconstrictor responses to nerve stimulation and to injected noradrenaline but not to injected histamine (Gay & others, 1967). Each of the vasodilator drugs used was more effective in reducing the vasoconstrictor responses to noradrenaline than those to sympathetic stimulation. The ratios of threshold concentrations of the drugs in producing these two effects ranged as follows: papaverine, glyceryl trinitrate, dipyridamole, 1 : 5; sodium nitroprusside, aminophylline, CIBA 31,531-Ba, 1 : 10; adenosine, sodium nitrite, 1 : 50; hydrallazine, 1 : 250. The fact that these drugs were effective in lower concentrations in counteracting the effect of injected noradrenaline than the effect of noradrenaline released by nerve stimulation is in accord with general experience. Possible explanations for the wide divergence in ratios are that drugs with a low ratio may reduce the release of noradrenaline or that drugs with a high ratio may facilitate the release.

Acknowledgments

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