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A new and sensitive bioassay for catecholamines

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The isolated central artery of the rabbit ear is highly sensitive to catecholamines when perfused with Krebs solution containing 5-hydroxytryptamine. The preparation responds to 1 ng of noradrenaline and is extremely long lasting. It does not discriminate between noradrenaline and adrenaline.

IN the course of experiments designed to measure the output of catecholamines from the isolated rabbit ear (de la Lande, Paton & Waud, 1964) it was observed that segments of the central artery displayed high sensitivity to nerve stimulation and to catecholamines. The effects of nerve stimulation and vasoactive drugs are discussed elsewhere (de la Lande & Rand, 1965); the present report describes a simple and highly sensitive method of assaying catecholamines on the arterial segment. It is based on the finding that 5-HT, besides causing vasoconstriction, greatly enhanced the sensitivity of the artery to noradrenaline (de la Lande & Rand, 1965).

Methods

Lop-eared rabbits are anaesthetised with urethane, 1·76 g/kg intraperitoneally. The central artery of the ear is exposed, and a portion of the artery, 5 to 7 cm in length, is excised together with the central vein and closely adhering connective tissue. The artery is cannulated at its proximal end and suspended in an organ bath (Fig. 1). The lumen is

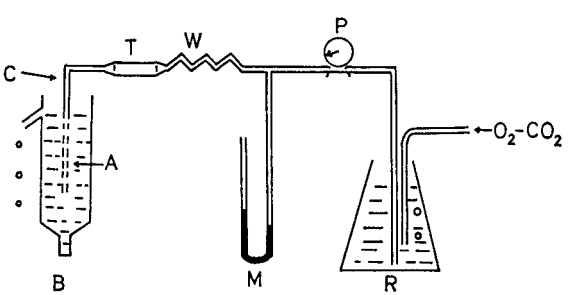

FIG. 1. Diagram of apparatus described in text. Symbols: A, isolated central artery; B, organ bath at 37°; C, cannula; M, mercury manometer; P, roller pump; R, reservoir of Krebs solution at 37°; T, rubber tubing; W, warming coil at 37°.

perfused at 37° with Krebs bicarbonate solution containing 5-hydroxytryptamine creatinine sulphate (5-HT), 0·04 μg/ml, and gassed with 95% oxygen and 5% carbon dioxide. The outflow from the artery is allowed to drain by upward displacement so that the preparation is completely immersed in the organ bath. Perfusion is by a constant-volume roller pump and the rate of perfusion is maintained at approximately 8 ml/min.

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Drugs, dissolved in normal saline, are injected into the system through rubber tubing attached to the proximal end of the cannula. Changes in the diameter of the artery cause a change in the perfusion pressure which is measured by a Condon mercury manometer. The resting perfusion pressure is usually about 30–40 mm of mercury.

Results

SENSITIVITY

The response of the preparation comprises a transient rise in perfusion pressure due to the injection volume, followed by a further rise in pressure, the magnitude of which is a function of the amount of noradrenaline injected. The relation between dose and response and the high sensitivity obtained is illustrated by the records of two preparations in Figs 2 and 3.

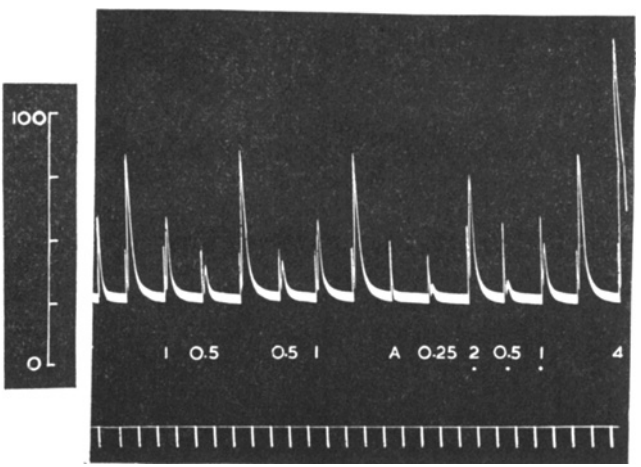

FIG. 2. The effect of graded doses of noradrenaline (ng) on the isolated perfused artery. The volumes injected are 0·2 ml except where the dose of noradrenaline is marked with a white dot, where 0·4 ml was used. Note that the response heights are depressed with the greater volumes. A is the injection of 0·2 ml of saline alone and illustrates the injection artifact. The interval between injections is 2 min. The scale on the left is perfusion pressure in mm Hg.

The sensitivity permits the detection of 0·5 ng and the precise assay of 1 ng of noradrenaline; this degree of sensitivity has been reproduced in each of eleven preparations. When expressed in terms of concentration, the limit of sensitivity is 1–2 ng/ml. The limit is imposed in part by the need to keep the volume of injection small (below 0·4 ml) to avoid interference by an excessive injection artifact (Fig. 2).

Features of the preparation are the length of time for which it can be used and the absence of major fluctuations in sensitivity over long periods. Fig. 3, upper frame, shows a portion of a record for a preparation which received a test dose of noradrenaline of 1 ng at 2 min intervals for 8 hr after commencing infusion; the lower frame shows the responses of the same preparation on the following day, after storage at 4° overnight.

SPECIFICITY

Comparison with other naturally occurring amines and polypeptides is shown in Table 1. The excitatory substances listed are those which produce a similar response to noradrenaline; of these, only adrenaline displays comparable activity. Dopamine, 5-HT, and angiotensin are less active than noradrenaline by factors of more than 30, 50 and 50 respectively. An estimate for histamine is not possible in view of the marked

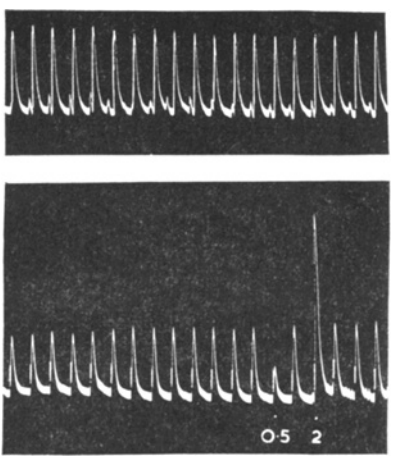

FIG. 3. Effects of cold storage. *Upper frame:* responses of a preparation to 1 ng of noradrenaline every 2 min 2 hr after commencement of infusion. *Lower frame:* responses of the same preparation after 6 hr infusion, storage at 4° for 15 hr, and infusion for a further hr. The doses are 1 ng except where otherwise shown. The vertical scale is as for Fig. 2.

TABLE 1. RELATIVE ACTIVITIES ON THE ISOLATED ARTERY

Substance	Action	Equipotent ratio (NA = 1)
Adrenaline	Excitatory	4, 1, 1, 1, 1, 0·8, 0·5
Dopamine	Excitatory	100, 66, 30, 30
Histamine	Excitatory	40, 20, 10, 10 (>100), 1·4, 1 (>1,000)
5-HT	Excitatory	250 (1,000), 250 (1,000), 100 (300), 40
Angiotensin	Excitatory	200, 125, 100, 50
Acetylcholine	Depressant	<100 (>100), >10, >20 (>150)
Bradykinin	Depressant	>100, >100, >50

With excitatory substances, a figure of 100 means that 100 ng of the substances produces a response equal to that of 1 ng of noradrenaline. The figures for the depressants refer to the amount of substance which depresses the response to noradrenaline (1 to 2 ng) by less than 10%. The figures in brackets refer to the ratios measured in the presence of the following antagonists: for histamine, mepyramine maleate 50 µg/litre, for 5-HT, methysergide bimaleate 50 µg/litre, and for acetylcholine, hyoscine hydrobromide 20 µg/litre.

variation in sensitivity between different preparations, which is some 40-fold. Acetylcholine and bradykinin depress the response to noradrenaline in concentrations of about 10 and 50 times greater, respectively, than those of noradrenaline. The vasoconstrictor effects of 5-HT, histamine and acetylcholine are substantially reduced by the addition of appropriate antagonists to the infusion fluid (Table 1). The antagonists

do not affect noradrenaline sensitivity. The absence of change to sensitivity after methysergide implies that antagonism by this drug extends only to the vasconstrictor effect of 5-HT.

PRECAUTIONS

Points of procedure which have been routinely followed in developing the method and applying it to the assay of catecholamines are as follows:

(1) 5-HT was added to the infusion medium shortly *after* commencement of infusion.

(2) The volumes of test and unknown solutions of noradrenaline, and also their concentrations, were adjusted wherever practical to provide approximately the same injection volumes (less than 0·5 ml) and response heights (less than 100 mm of mercury).

(3) A constant time interval between injections was rigorously followed. In most preparations 2 min proved a suitable compromise between the opposing requirements of speed of assay and the time required for recovery of sensitivity following the preceding response.

(4) During intervals between assays the preparation received a constant test dose of noradrenaline at 2 min intervals by automatic syringe.

Discussion

The most sensitive preparations available for the assay of noradrenaline are the pithed rat, and the superfused rat stomach strip sensitised by 5-HT (Armitage & Vane, 1964). The pithed rat is more specific for noradrenaline than the isolated perfused artery, which is also the least sensitive of the three preparations. The significant advantage of the arterial segment is that the preparation can be used for many hours. Test doses of noradrenaline can be given every 2 min by an automatic syringe and the preparation requires no further attention until required for assay. If necessary, it can be stored overnight and used the following day. The perfused artery is particularly useful when frequent assays of noradrenaline in perfusates are required, as may be the case in studies on catecholamine uptake or release from isolated tissues.

A limitation of the method is lack of specificity since it does not discriminate between adrenaline and noradrenaline. This is not likely to prove a serious drawback except with tissues such as mammalian adrenal gland which are rich in adrenaline; in those cases where the arterial segment is equally sensitive to adrenaline and noradrenaline, the lack of specificity may prove an advantage for the assay of the sum of activity of adrenaline and noradrenaline. Dopamine constricts the preparation but is unlikely to interfere significantly with a noradrenaline assay unless present in amounts ten times greater than noradrenaline. The tissues where this proportion of dopamine is exceeded are liver, intestine and lung (Schumann, 1960) and corpus striatum of the central nervous system (Carlsson, 1959). Interference by acetylcholine, 5-HT and histamine is virtually eliminated by the addition of appropriate antagonists

to the perfusion fluid. There remains, however, the possibility of interference by the vasoactive polypeptides, bradykinin and angiotensin, when these are present in high concentrations.

It must be emphasised that the success of the method depends on the presence of 5-HT in the perfusion fluid in the concentration stated in methods. The mechanism of the potentiation of catecholamine sensitivity by 5-HT is being further examined.

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References

Armitage, A. K. & Vane, J. R. (1964). *Brit. J. Pharmacol.*, **22**, 204–210.
Carlsson, A. (1959). *Pharmacol. Rev.*, **11**, 493–493.
de la Lande, I. S., Paton, W. D. M. & Waud B. (1964). *Proc. Aust. physiol. Soc.*, May, p. 39.
de la Lande, I. S. & Rand, M. J. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**. In the press.
Schűmann, H. J. (1960). In *Adrenergic Mechanisms*, Editor, Vane, J. R. Ciba Foundation Symposium, p. 11.