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A SENSITIVE METHOD FOR THE ASSAY OF CATECHOL AMINES

BY

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A strip of fundus from a rat's stomach was suspended in Krebs solution containing 5-hydroxytryptamine. Movements of the muscle were recorded by means of a frontal writing lever giving a magnification of sixteen-times. The strip relaxed when isoprenaline, adrenaline or noradrenaline was added to the organ-bath in concentrations of 0.2 to 2 ng/ml. The preparation was most sensitive to isoprenaline and least sensitive to noradrenaline. The components of a mixture of two catechol amines could be assayed by superfusing the rat stomach and a chick rectum in the same stream of fluid.

The commonest method for the biological assay of adrenaline makes use of the fact that contractions of the rat isolated uterus induced by acetylcholine (Jalon, Bayo & Jalon, 1945) or by carbachol (Gaddum, Peart & Vogt, 1949; Gaddum & Lembeck, 1949) are inhibited by adrenaline. In this respect adrenaline is about 100-times more effective than noradrenaline. Noradrenaline is generally assayed by its effect in raising the blood pressure of pithed rats (Muscholl & Vogt, 1957), which noradrenaline does three- to four-times more effectively than adrenaline. In our experience the method for adrenaline, in particular, is a temperamental one and an assay can be a lengthy procedure. The method to be described here arose from the observation that catechol amines reduce the size of contractions of the rat fundal strip induced by 5-hydroxytryptamine (Vane, 1957).

METHODS

The stomach strip was prepared as described by Vane (1957). About 6 cm of rectum without the rectal caeca from 7 to 14-day-old chicks was sometimes used in conjunction with the rat stomach strip. The stomach strip was set up in a 5 ml. organ-bath of Krebs solution; this contained (g/l. of distilled water): NaCl 6.9; KCl 0.35; $CaCl_2$ $6H_2O$ 0.55; KH_2PO_4 0.16; $MgSO_4$ $7H_2O$ 0.29; glucose 1; and $NaHCO_3$ 2.1. The solution was gassed with a mixture of 5% carbon dioxide and 95% oxygen. Tyrode solution was bubbled with pure oxygen and contained (g/l. of distilled water): NaCl 8.0; KCl 0.2; $CaCl_2$ $6H_2O$ 0.396; $MgCl_2$ $6H_2O$ 0.214; NaH_2PO_4 0.05; glucose 1.0; and $NaHCO_3$ 1.0. Rat uterus Ringer solution was bubbled with pure oxygen and contained (g/l. of distilled water): NaCl 9.0; KCl 0.42; $CaCl_2$ $6H_2O$ 0.06; glucose 0.5; and $NaHCO_3$ 0.5. The same gases were bubbled vigorously

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through the organ-baths in order to ensure rapid mixing when drugs were added. Between additions of drugs, fresh solution passed slowly through the bath. The upper end of the tissue was attached to a frontal writing lever giving a magnification of sixteen-times; the movable load on the lever corresponded to a load of 1 to 2 g on the tissue.

In other experiments, the organ was superfused (Gaddum, 1953) with Krebs solution. The air temperature of the container in which the tissue was suspended was 25° C. Sometimes the rat stomach strip and the chick rectum were both superfused in organ-baths arranged one above the other so that the Krebs solution cascaded down from one organ to the next.

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (±)-isoprenaline sulphate, (±)-metanephrine hydrochloride, (±)-normetanephrine hydrochloride, theophylline ethylenediamine (0.24 % w/v), acetylcholine perchlorate and dexamphetamine sulphate. Doses are expressed as bases. All stock and diluted solutions of the catechol amines contained ascorbic acid (1 mg/ml.). The amounts of ascorbic acid which reached the tissues did not affect them nor their responses to catechol amines.

RESULTS

To relax the rat stomach strip, doses of adrenaline and noradrenaline between 50 to 500 ng in a 5 ml. organ bath are needed (Vane, 1960). These doses are large compared with those required to reduce the sizes of contractions induced by 5-hydroxytryptamine (Vane, 1957). It seemed likely, therefore, that if the tone of the preparation could be raised it would become more sensitive to the relaxant actions of catechol amines.

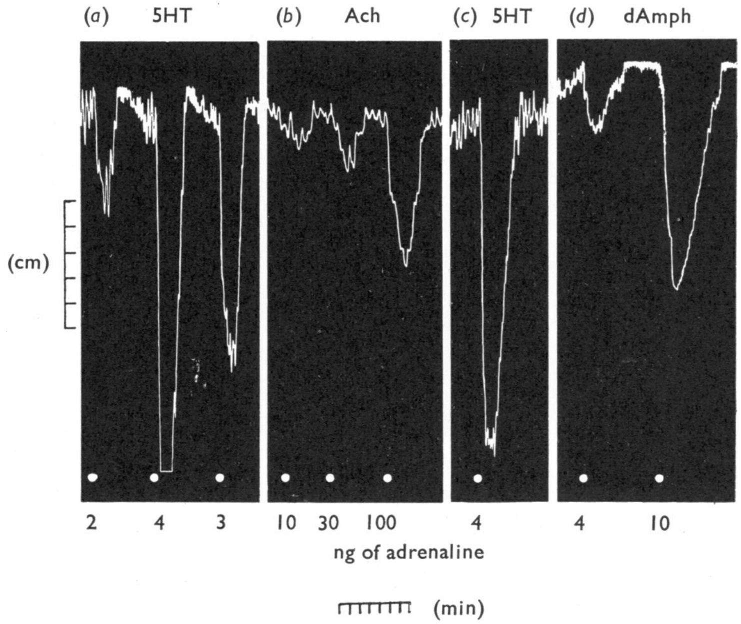

Fig. 1. Record of contractions of rat isolated stomach strip showing that the preparation is more sensitive to adrenaline when the bathing solution contains 10 μg/l. of 5-hydroxytryptamine (5HT, *a* and *c*) than when it contains 100 μg/l. of acetylcholine (Ach, *b*) or 30 mg/l. of dexamphetamine (dAmph, *d*). The figures below each white dot refer to doses of adrenaline in ng. Time in minutes: vertical scale in cm.

Comparison of compounds that increase the tone of the rat isolated stomach strip. Three drugs were used, 5-hydroxytryptamine, acetylcholine and dexamphetamine. Concentrations of each were chosen to give similar increases in the tone of the stomach strip. When one of these drugs was added to the reservoir of bathing fluid the tone increased and remained at a high plateau for several hours. The bubbling of the reservoir was stopped when it contained drugs in order to avoid oxidizing them.

When the tone increased the tissue became more sensitive to the relaxant actions of catechol amines ; it was interesting that the sensitivity was increased more by 5-hydroxytryptamine than by dexamphetamine and much more than by acetylcholine (Fig. 1). 5-Hydroxytryptamine was therefore chosen for increasing the tone.

The preparation worked satisfactorily in Tyrode solution and in Krebs solution but not in rat uterus Ringer, perhaps because the low calcium concentration in the last solution caused the strip to relax.

Effect of varying the concentration of 5-hydroxytryptamine in the bathing fluid. With no 5-hydroxytryptamine in the bathing fluid the stomach strip relaxed when

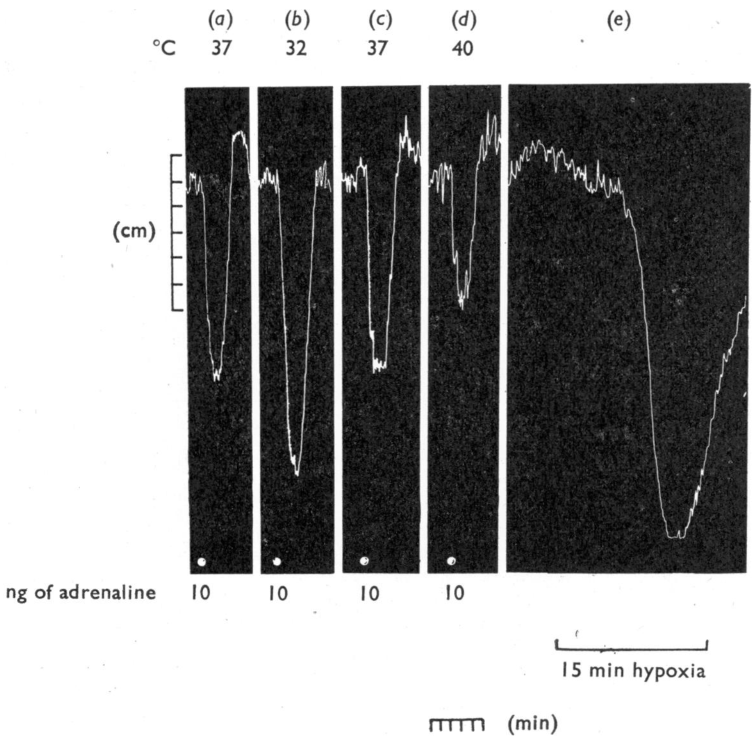

Fig. 2. Record as in Fig. 1, showing that adrenaline caused larger relaxations when the temperature of the bathing solution was 32° C (*b*) than when it was 37° C (*a* and *c*) or 40° C (*d*). The figures below each white dot refer to doses of adrenaline in ng. In (*e*), oxygenation of the bath was stopped for 15 min. The tone of the strip fell rapidly towards the end of the 15 min period but recovered when oxygenation was restarted.

adrenaline was added in doses of 50 to 200 ng. Addition of 5-hydroxytryptamine (0.5 μg/l.) increased the sensitivity so that the strip relaxed after doses of 10 or 20 ng of adrenaline. With more 5-hydroxytryptamine (10 μg/l.) in the bathing fluid adrenaline caused relaxation in doses of 5 ng or less ; higher concentrations of 5-hydroxytryptamine did not increase the sensitivity further. Therefore, in most experiments we used 5-hydroxytryptamine at a concentration of 10 μg/l. Although the concentration of 5-hydroxytryptamine in the bathing fluid affected the size of the relaxations, it did not alter the activities of the relaxant drugs relative to each other.

Effect of temperature. With 5-hydroxytryptamine (10 μg/l.) in the bathing fluid the relaxation produced by a given dose of adrenaline was greater at 32° C than at 37° C, and greater at 37° C than at 40° C (Fig. 2). Although the changes in sensitivity with temperature were not great, the lowest temperature did provide the most sensitive preparation. Fig. 2 also illustrates the effects of oxygen lack : the bubbling of oxygen through the bath was stopped for 15 min ; during this time the stomach strip relaxed, first slowly and then at a much faster rate. When oxygenation was resumed the tone recovered fairly rapidly.

Effect of p*H.* Two modifications of Krebs solution were made, containing either 0.49 or 4.9 g/l. of sodium bicarbonate instead of the usual 2.1 g/l. The *p*H's of these solutions were 6.9 and 7.8 respectively ; the *p*H of normal Krebs solution was 7.4. Adrenaline caused larger relaxations in the normal than in the more alkaline and acid solutions.

Comparison of actions of relaxant drugs. When catechol amines and other relaxant drugs were added to the bath the stomach strip relaxed rapidly. It was found convenient to leave drugs in the bath for 2 min before washing out. Recovery of tone usually took at least another 3 min, so that it was not possible to add drugs more

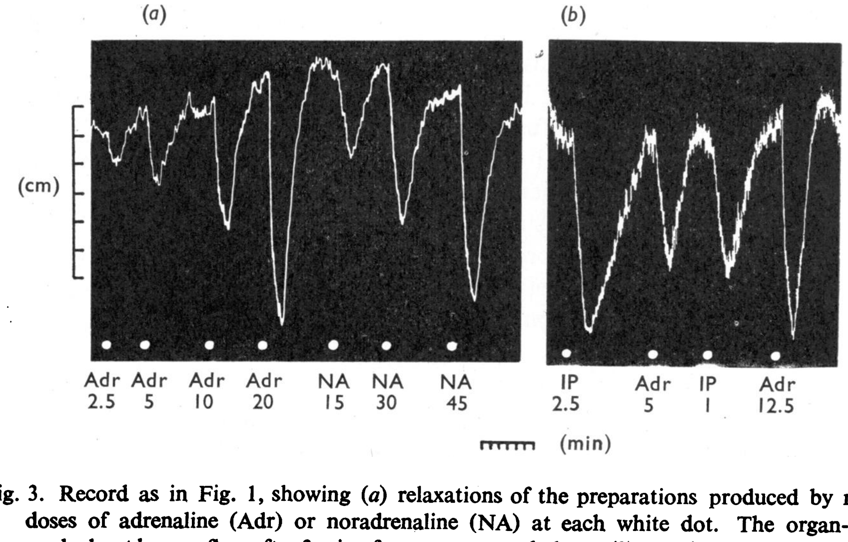

Fig. 3. Record as in Fig. 1, showing (*a*) relaxations of the preparations produced by nanogram doses of adrenaline (Adr) or noradrenaline (NA) at each white dot. The organ-bath was washed out by overflow after 3 min of exposure to each drug. (*b*), another experiment comparing the actions of adrenaline and isoprenaline (IP).

frequently than once every 5 min and sometimes it was necessary to wait 7 or 8 min between successive doses. Exact timing of the cycle was not important.

A comparison of the relaxant effects of adrenaline, noradrenaline and isoprenaline is shown in Fig. 3. In these experiments the strips were of average sensitivity to catechol amines; isoprenaline was more effective than either adrenaline or noradrenaline. Metanephrine, normetanephrine and aminophylline were also tested and their activities relative to those of the catechol amines are shown in Table 1 (the values in column 2 are equiactive doses expressed as ng of base). The preparation was extremely sensitive to isoprenaline ; sufficiently sensitive to adrenaline and noradrenaline to be suitable for the bioassay of small amounts ; and relatively insensitive to metanephrine, normetanephrine and aminophylline.

TABLE 1

APPROXIMATE EQUIACTIVE DOSES (NG OF BASE) OF COMPOUNDS RELAXING THE RAT STOMACH STRIP IN A 5 ML. ORGAN BATH

Compound	Equiactive dose
(±)-Isoprenaline	0·3
(−)-Adrenaline	1·0
(−)-Noradrenaline	2·5
(±)-Metanephrine	700
(±)-Normetanephrine	1,200
Aminophylline	14,000

Superfusion. When the strip was superfused, the responses to catechol amines were greater than when the tissue was totally immersed in Krebs solution, but the reproducibility of the effects depended critically on three factors : (1) whether the drug was applied to the tissue direct, to the thread by which the tissue was attached to the lever, or into the tube supplying the Krebs solution ; (2) whether the flow was interrupted during application of a drug and, if so, on the duration of the interruption ; and (3) on the rate of flow of the superfusing fluid. The relaxation caused by a given dose of adrenaline was usually maximal when the flow was interrupted for 1 to 2 min, but such an interruption sometimes caused the tissue to relax slightly even when no drug was added. By superfusing the tissue the sensitivity to adrenaline was increased two- to four-times. In order to avoid variability in the effects, the tissue was superfused at a constant rate of 10 ml./min and the drugs were injected into the tubing supplying the Krebs solution.

Discrimination between adrenaline and noradrenaline. This was achieved by superfusing a rat stomach strip and a chick rectum in the same stream of Krebs solution containing 10 μg/l. 5-hydroxytryptamine. The chick rectum was 20- to 100-times more sensitive to adrenaline than to noradrenaline (Mann & West, 1950 ; see also Fig. 4). The amounts of the two catechol amines in a mixture could be determined from the adrenaline and noradrenaline equivalents on the two test organs by the solution of simultaneous equations, either mathematically (Gaddum & Lembeck, 1949) or graphically (Marley & Paton, 1961).

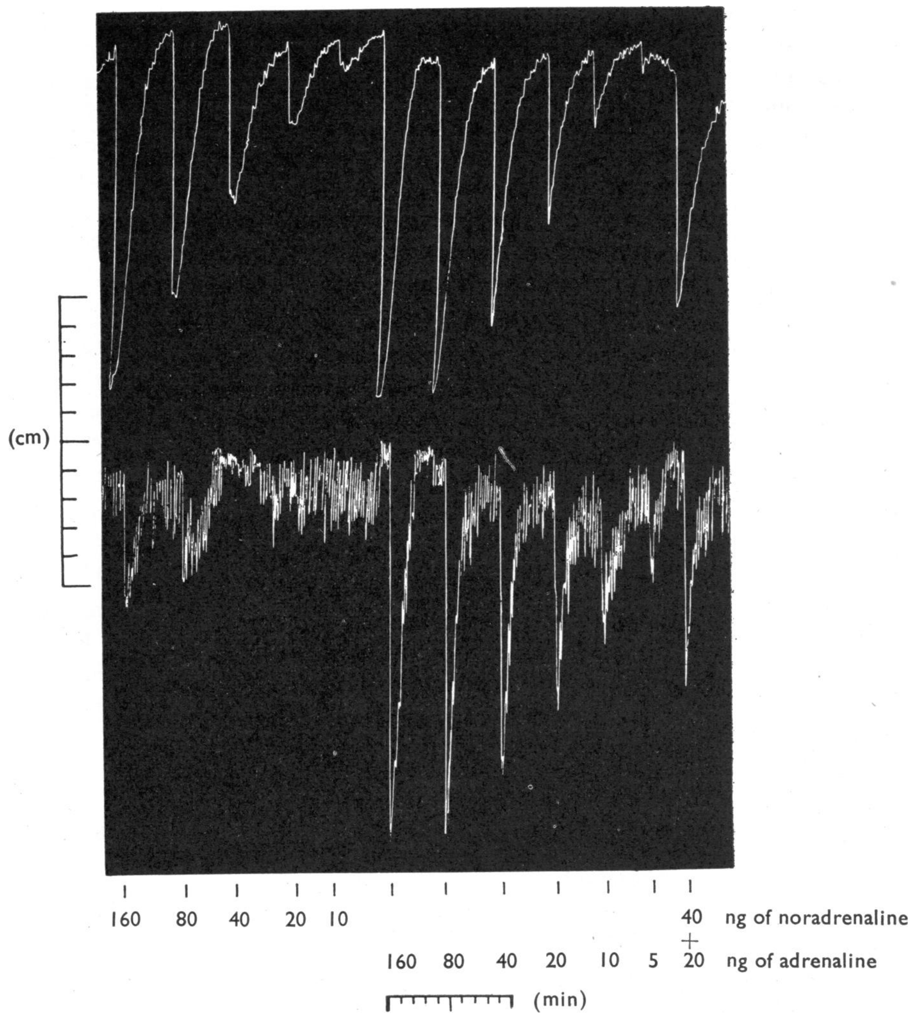

Fig. 4. Contractions of rat stomach strip (top) and chick rectum (bottom) superfused with Krebs solution containing 10 μg/l. of 5-hydroxytryptamine. The record shows the relative sensitivity of the two preparations to noradrenaline (NA, in ng) and to adrenaline (Adr, in ng). The last response was obtained with a mixture of 40 ng of noradrenaline and 20 ng of adrenaline. Solution of the simultaneous equations given by comparison of the effects with the dose/response curves for adrenaline and noradrenaline on the two preparations gave an estimate of 43 ng of noradrenaline and 20 ng of adrenaline. Time in minutes; vertical scale in cm.

DISCUSSION

With the method described, the smallest quantities of adrenaline or of noradrenaline detectable are in the same range as with the rat uterus or pithed rat preparations. The main advantages of the new method are that it requires less skill and that it is not necessary to work to a rigidly timed cycle. The method is particularly useful when assay by bracketing is sufficiently accurate.

The crucial point about the method is that the bathing solution must contain 5-hydroxytryptamine, though why 5-hydroxytryptamine should be so much more effective than acetylcholine or dexamphetamine is not clear. The concentration of 5-hydroxytryptamine in the bathing solution is not critical : the addition of as little as 0.5 μg/l. increases the sensitivity to adrenaline ten-times. The 5-hydroxytryptamine in the bathing fluid brings about not only a higher sensitivity to catechol amines but also the advantage that low concentrations of 5-hydroxytryptamine present as impurity in the extracts to be assayed have negligible effects. Moreover, because the stomach strip is already contracted, low concentrations of other contractor substances in the extracts do not interfere with the assay.

The fact that relaxations caused by a given dose of adrenaline are greater at 32° C than at 37° C suggests that at the higher temperature the tissue is slightly hypoxic : certainly it requires vigorous oxygenation, otherwise the tone is liable to fall spontaneously. Born (1956) has shown that the smooth muscle of the guinea-pig taenia coli would not maintain an increased tension when the muscle was made anoxic ; our finding may be related. Many of the experiments described in this paper were done at 37° C since the effect of temperature was one of the last factors to be studied. It is probable that the optimum temperature for the assay is 32° C or even lower.

By itself, the rat stomach strip is not suitable for assaying mixtures of catechol amines unless the components are first separated by chromatography. However, the components of a mixture of adrenaline and noradrenaline can be assayed by superfusing the rat stomach strip and the chick rectum in the same stream of fluid, as described by Vane (1958) for superfusion of several organs with blood.

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