Short communications

Rat superfused aortic strip for the bioassay of noradrenaline and adrenaline

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The use of spiral strips of rat aorta superfused with Krebs solution is described for the assay of noradrenaline and adrenaline. The preparation responds to threshold doses of 0·1–1 ng of amine, is easy to set up and maintain and offers an inexpensive alternative to other available assay systems.

Bioassay remains the only readily available technique by which nanogramme quantities of catecholamines can be detected. The best known assay is the pressor response of the pithed rat (Shipley & Tilden, 1947; Muscholl & Vogt, 1958). With this system quantities of amines around 0·5–1·0 ng can be estimated. However, the pithed rat assay has those disadvantages inherent in any whole animal preparation, and in some hands is notoriously difficult to set up and maintain. Several other assays have been used. The rat superfused stomach strip (Armitage & Vane, 1964) is useful for monitoring changes in concentration of blood borne amine (Vane, 1966); however, it is incapable of reliably estimating small quantities of amines in extracts. The rabbit perfused ear artery (de la Lande & Harvey, 1965) and the rabbit superfused portal vein (Hughes, 1970) appear to have the requisite sensitivity. Nevertheless, in view of the frequent need to reduce laboratory running costs, it would be preferable to have an alternative assay system using a less expensive animal. This report examines the rat superfused aortic strip for such potential.

Methods.—Male Wistar rats of 200–300 g weight were killed by a blow on the head and the dorsal aorta was removed from the level of the aortic arch to that of the renal arteries. The vessel was cut into a spiral strip having a width of about 1·5 mm and a period of about 4 mm. Strips from large rats were sometimes divided into two lengths. The strips were suspended in a superfusion cascade (Vane, 1964) of Krebs solution (NaCl, 6·9; KCl, 0·35; $CaCl_2.6H_2O$, 0·55; $MgSO_4.7H_2O$, 0·29; $NaHCO_3$, 2·1; KH_2PO_4, 0·16; dextrose, 1·0 g/litre gassed with 95% oxygen and 5% carbon dioxide) which was usually maintained at 38° C, and which flowed at 10 ml/minute. Contractor activity was recorded with isometric transducers exerting a resting tension of 1 g on the tissues and displayed using an ink-writing Beckman Dynograph. Noradrenaline and adrenaline bitartrate, 5-hydroxytryptamine creatinine phosphate and bradykinin were dissolved in normal saline and injected or infused into the superfusion stream proximal to the roller pump.

Results.—Most strips exhibited some spontaneous activity. In general those strips taken from larger rats were more satisfactory in that they showed less activity. This might reflect an intrinsic difference in the muscles or might be due merely to the greater trauma involved in preparing the smaller vessels.

Strips regularly responded with measurable contractions to injections of 1 ng or more of noradrenaline. In about half the preparations, measurable responses to 0·1–0·2 ng were obtained. Reproducible responses were obtained when successive injections were given at any time after relaxation was complete. This meant that an assay cycle time of 2–3·5 min was possible, depending on the doses of noradrenaline (Fig. 1).

Adrenaline was approximately equipotent with noradrenaline.

Certain isolated smooth muscle tissues are more sensitive to agonist drugs at lower than body temperature (Armitage & Vane, 1964). The rat superfused aortic strip, however, was about 10 times less sensitive to noradrenaline at 34° C than at 38° C.

The presence in the superfusion fluid of 5-hydroxytryptamine (5-HT) (5–10 ng/ml) or bradykinin (50 ng/ml) did not affect the sensitivity of the strips to noradrenaline. Higher concentrations of 5-HT (50–100 ng/ml) caused sustained contraction of the strips and a reduction in noradrenaline sensitivity. This situation

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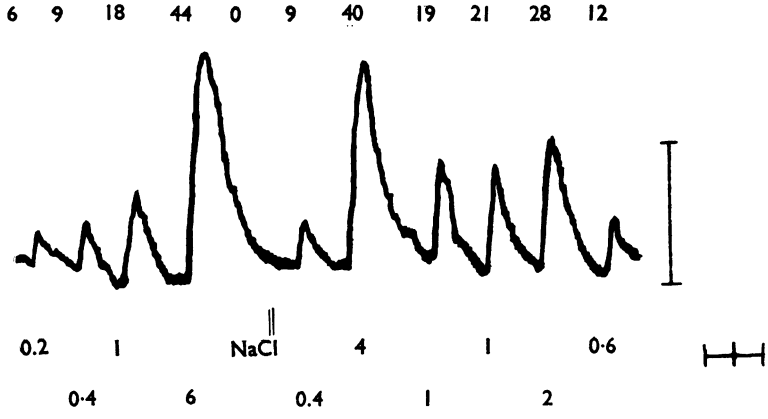

FIG. 1. Contractor responses of a rat superfused aortic strip at 38° C to injections of 0·2–6 ng noradrenaline. The numbers above the responses represent mm recorded on the original trace. Calibrations: 10 mg increase in tension and 1 minute.

contrasts with the increased sensitivity of the rabbit perfused ear artery produced by 5-HT (de la Lande, Cannell & Waterson, 1966).

Discussion.—Valette & Ngun-Ba-Muoi (1962) reported that the rat aortic strip immersed in Krebs solution exhibited a high sensitivity to adrenaline. However, the long time course of responses of this preparation (7 min or more) made it impractical as a routine assay system. In this study use of superfusion rather than immersion has greatly increased the rapidity of responses, while maintaining a sensitivity to adrenaline and noradrenaline at least equal to those of established assay systems. Although the technique has been developed for the specific purpose of assaying extracts of biological media, the fact that appreciable concentrations of 5-HT and bradykinin do not affect the responses indicate that the rat aortic strip could also be used in the blood bathed cascade system for detection of blood borne catecholamines.

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