LETTERS TO THE EDITOR

A Sensitive Method for the Assay of Acetylcholine

SIR,—Several methods have previously been described for the detection or assay of minute amounts of acetylcholine. Unfortunately most of these methods are either very laborious or the preparations used are unstable and vary in sensitivity throughout the assay. We have developed a method, based on that described by Paton (1957), with which we were able to assay amounts of acetylcholine of about 1 picogram (pg.) or less.

The terminal ileum of large guinea-pigs, weighing between 750 g. and 1 kg., was removed, washed in saline solution and cut into segments of approximately 3 cm. in length. The ileum from smaller guinea-pigs proved to be insensitive.

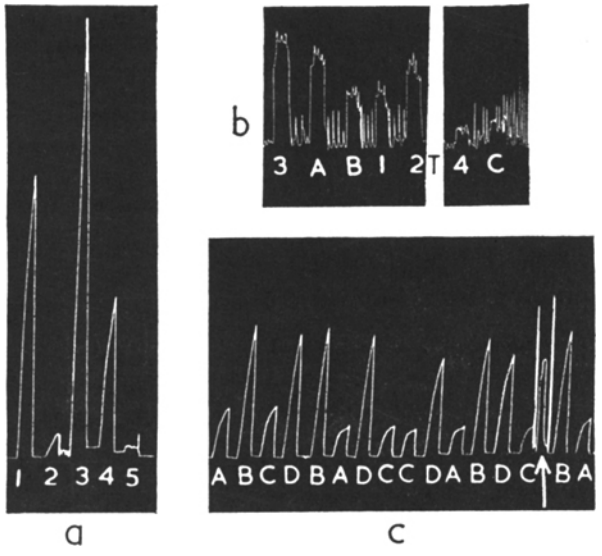

FIG. 1a. Guinea-pig ileum suspended in double-glucose Tyrode solution, sensitised to acetylcholine. 1, 20 ng. histamine; 2, 0·5 μg. 5-hydroxytryptamine; 3, 0·05 pg. acetylcholine chloride; 4, 0·025 pg. acetylcholine chloride; 5, 0·2 ml. double glucose Tyrode solution.

b. Guinea-pig ileum suspended in Hanks' balanced salt solution sensitised to acetylcholine. The numbers denote the amount of standard acetylcholine chloride solution in pg. Doses of test solution A, 0·2 ml.; B, 0·1 ml. and C, 0·4 ml. In period T, atropine sulphate (10^{-8} g./ml.) was added to the bath for 5 min.

c. Guinea-pig ileum suspended in double-glucose Tyrode solution, sensitised to acetylcholine. Four point Latin square assay of two acetylcholine chloride solutions. A = C = 0·1 pg.; B = D = 0·15 pg. Dose interval 90 sec., contact time 30 sec. At the arrow the tissue went into spasm.

The segments of ileum were placed in about 1 litre of a suitable aerated physiological salt solution which contained 10^{-5}M di-isopropylphosphorofluoridate and allowed to stand at 37° for 1 hr. A segment of this ileum was then cleared of mesentery and mounted in a 2·0 ml. organ bath at 28–32°; the sensitivity of the tissue to acetylcholine did not appear to be affected by slight changes in temperature but reduced temperature minimized the spontaneous movements. The physiological salt solution in which the ileum was mounted varied in composition according to the tissue from which extracts containing acetylcholine were collected but always contained 5 mg./l. of morphine sulphate. Paton (1957 has shown that this concentration of morphine sulphate reduces the violent intermittent spasms of the ileum which usually occur after cholinesterase inhibition. In different experiments the ileums were suspended in Tyrode solution containing twice the usual quantity of glucose and in Hanks' balanced salt Solution. Similar sensitivities to acetylcholine were obtained in each solution. Standard acetylcholine solution and test solutions were diluted with the same solution as that in which the ileum was suspended; the dose volume did not exceed 0·2 ml.

The threshold amount of acetylcholine varied with the preparation and was occasionally as low as 0·0125 pg. which represents a bath concentration of $6{\cdot}25 \times 10^{-15}$ g./ml; more usually the threshold amount fell between 0·1 and 1 pg. The sensitivity to other common spasmogens such as histamine and 5-HT was similar to that in untreated ileum (Fig. 1a); Atropine 10^{-8} was effective in blocking the action of acetylcholine and a typical response is shown in Fig. 1b.

Fig. 1c shows a four point Latin square assay. The dose response curve was steep and a dose ratio of 1 : 1·5 was used. The index of precision (λ) calculated for this assay was 0·024 and the fiducial limits (P = 0·05) of the potency ratio were 94·5 to 105·8 per cent.

The difficulties encountered with the preparation were (i) that when mounted in double-glucose Tyrode solution the tissue often goes into spasm at 20–30 min. intervals making it almost impossible to perform a complete latin square assay—see Fig. 1c; and (ii) that in Hanks' solution the spontaneous movements were troublesome. A small proportion of the guinea-pigs proved to be completely insensitive to all concentrations of acetylcholine after the sensitising treatment, some showing a slow rhythmical contraction which could be abolished by atropine.

We hope that this method may prove suitable for assaying minute amounts of acetylcholine liberated from tissues. The method is suitable for use with the two physiological salt solutions described, and possibly a variety of other mammalian physiological salt solutions can be used.

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