The measurement of histamine in brain and its distribution

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CARLINI and Green[1] found that histamine in brain is present in highest amount in small particulate material sedimenting with microsomes; Michaelson and Dowe[2] reported that most of the histamine occurs in the nuclear fraction and in the large particulate fraction sedimenting with mitochondria. Carlini and Green used mostly rats and measured histamine by bioassay; Michaelson and Dowe[2] used guinea pigs and determined histamine by a fluorometric method.[3] The contrasting results are probably not attributable to differences between species (for the former authors found the same distribution in the brain of a guinea pig as was observed in rats) but almost certainly rest on the different methods used to measure histamine. The two methods also produce conflicting results in the regional distribution of histamine, biological assay showing a high concentration of histamine in the midbrain,[4] fluorometric assay showing a uniform distribution of histamine in brain.[3] It has been shown that the fluorometric method in its present form is not suitable for measuring histamine in brain.[1]

Using the fluorometric method, Green and Carlini found that 1 g of rat brain contains the equivalent of 246 ± 13·5 ng of histamine, a value that agrees remarkably well with that obtained by Michaelson and Dowe: 245 ± 10 ng in guinea pig brain, using the same method. Values similar to these were recorded for brains of rat, guinea pig, rabbit and dog in the original description of the fluorometric method.[3] This concentration is about fourfold that obtained by bioassay;[1] to account for the discrepancy, it was shown that the extract of brain contains at least five substances that

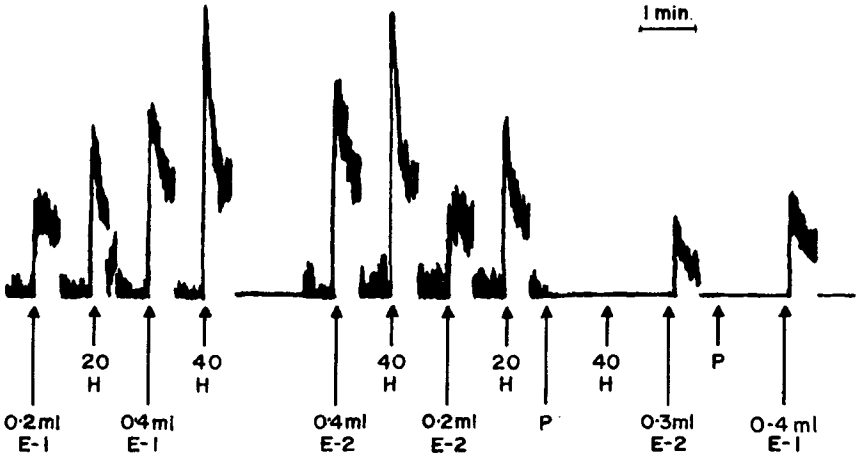

FIG. 1. The effects of extracts prepared for the estimation of histamine[3] on the guinea pig ileum. The bath contained 5 ml of Tyrode's solution. Methods have been described.[1] E-1: Extract prepared with *n*-butanol.[3] E-2: Extract prepared with $CHCl_3$-*n*-butanol.[3] H: Histamine; numbers above refer to ng. P: 0·1 μg pyrilamine.

contribute to the apparent values of histamine obtained by the fluorometric method.

What required explanation was the ostensible agreement previously reported[3] between values obtained by fluorometric and biological assays of histamine in brain extracts. It was subsequently shown that most extracts of brain on bioassay also give erroneously high values of histamine.[1] Fig. 1 shows the results of bioassay on two extracts of rat brain: one was prepared by an alkaline *n*-butanol extraction, the other by an alkaline chloroform–*n*-butanol extraction, as described.[3] Both extracts contain substances other than histamine that cause contraction of the guinea pig ileum, as shown by failure to block all the effects of the extracts with the antihistamine drug (Fig. 1). Of several extracts

of brain examined on guinea pig ileum,[1] only those prepared by the method of Adam[4] were devoid of materials interfering with the estimation of histamine.

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Mechanism of the antinatriuretic action of aldosterone

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ALDOSTERONE characteristically decreases the urinary excretion of sodium and enhances the excretion of potassium. However, little is known about the manner in which this mineralocorticoid exerts its action. Barger *et al.*[1] have infused aldosterone directly into the renal artery of the dog and noted a lag of 30 min or more before the onset of action of this hormone. This was in marked contrast to the almost immediate onset of action of the antidiuretic hormone (ADH). The marked difference in onset of action of these two hormones suggests that ADH acts directly, whereas aldosterone probably acts indirectly. A possible explanation for the lag could be that aldosterone is involved in protein synthesis. Actinomycin D was therefore used to determine whether protein synthesis *de novo* was involved in the delayed onset of action of aldosterone.

The procedure of Kagawa *et al.*[2] was used to assess the effect of the inhibitor on the action of aldosterone. Male Holtzman rats, 190–220 g, were adrenalectomized at zero time and given a 0·9% solution of sodium chloride for drinking water. Food was withdrawn at 6 hr. At 24 hr drinking water was removed and each rat given 2·5 ml of 0·9% sodium choride, subcutaneously. Animals were divided into four groups of four each and treated as follows: (1) no treatment; (2) 1 μg *d*-aldosterone-21-acetate* subcutaneously; (3) 0·3 mg actinomycin D† intraperitoneally; (4) aldosterone and actinomycin D. A 4-hr collection period was employed. Sodium and potassium outputs were determined with a Coleman flame photometer. Data were evaluated by Duncan's new multiple range test.[3] The 0·05 level of probability was the criterion of significance.

The results of this experiment are given in Table 1. Aldosterone produced a significant decrease in the excretion of sodium while significantly increasing the excretion of potassium. Actinomycin alone did not alter the excretion of either ion. However, when actinomycin D was administered to rats also receiving aldosterone, the action of the hormone on sodium excretion was completely blocked. In contrast, potassium excretion was not affected. A separation of this action of aldosterone has been reported previously.[1]

Karlson[4] believes that many hormones exert their action by promoting synthesis of enzymes. He proposes that their site of action is on DNA to somehow cause exposure of DNA receptors. Thus

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