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EFFECTS OF 5-HYDROXYTRYPTAMINE ON THE DORSAL MUSCLE OF THE LEECH (HIRUDO MEDICINALIS)

BY

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5-Hydroxytryptamine has an inhibiting effect on the leech muscle. It reduces the contractions produced by acetylcholine or nicotine and accelerates the relaxation of the muscle when these substances are washed out. This acceleration of relaxation allows a more rapid assay of acetylcholine in this preparation.

It has been found by Poloni that 5-hydroxytryptamine causes relaxation of the isolated leech muscle and that this effect can be utilized for its assay in concentrations as low as 10^{-12} g/ml. (Poloni, 1955b). Poloni had reported earlier that samples of cerebrospinal fluid from schizophrenics relax the leech muscle (Poloni, 1951). He suggested that 5-hydroxytryptamine was the active principle in these fluids (Poloni, 1955a).

The present experiments confirm the relaxing effect of 5-hydroxytryptamine on the leech muscle and describe some further actions of this substance on this preparation. However, the relaxing effect has not been found suitable as a basis for the assay or identification of 5-hydroxytryptamine.

METHODS

The experiments were carried out on twin strip preparations of the dorsal muscle of the leech *Hirudo medicinalis*. The muscle strips were suspended in 5 ml. mammalian Locke solution diluted 1 to 1.4 with distilled water. Eserine sulphate was added to the bath fluid at concentrations of 5 to 10 μg/ml. The bath temperature was kept at about 20° C. The method is described in detail by MacIntosh & Perry (1950).

The compounds tested on the leech muscle were acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, nicotine hydrogen tartrate, potassium chloride and (+)-lysergic acid diethylamide. All values refer to the salts. The acetylcholine, nicotine and potassium were left in contact with the muscle for 60 to 90 sec. When the actions of 5-hydroxytryptamine or (+)-lysergic acid diethylamide were being studied, their concentrations were often maintained by adding them to the bath after each washing.

An attempt was made to determine if 5-hydroxytryptamine could be normally found in leech tissue. Extracts were made by mincing whole leeches and extracting them with 80% acetone, 25 ml. per leech, for 24 hr at 4° C. After filtration, the acetone was evaporated off under a vacuum pump and the residue re-suspended in 3 ml. distilled water.

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RESULTS

Fig. 1 shows the contractions produced by 3 ng/ml. of acetylcholine and their reduction on the addition of 5-hydroxytryptamine (200 ng/ml.) to the bath. After washing out the 5-hydroxytryptamine together with the acetylcholine, the sensitivity of the muscle to acetylcholine gradually returns. Besides diminishing the size of the acetylcholine contraction, the inhibitory effect of 5-hydroxytryptamine is evident

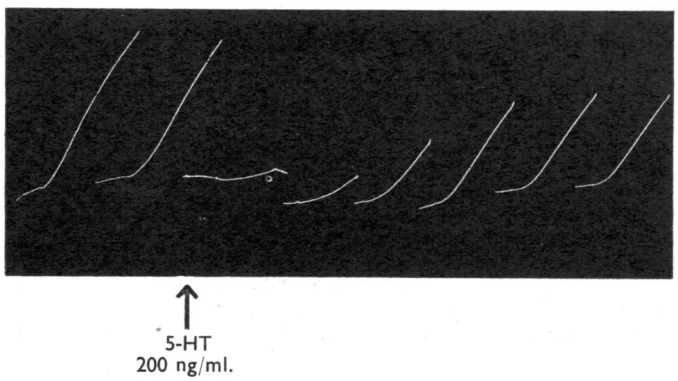

Fig. 1. Contraction of the eserinized leech muscle to 3 ng/ml. acetylcholine given at 5 min intervals and left in the bath for 90 sec. Three minutes before the third addition of acetylcholine (at arrow), 200 ng/ml. of 5-hydroxytryptamine was added to the bath and washed out 4.5 min later together with the acetylcholine.

by the fact that it accelerates relaxation of the muscle after washing out the acetylcholine. The relaxing effect is also seen in a muscle not contracted by acetylcholine, but the effect is less pronounced. This small relaxation has not been found suitable for the assay of 5-hydroxytryptamine because of the difficulty in establishing a stable base line for comparison of relaxations.

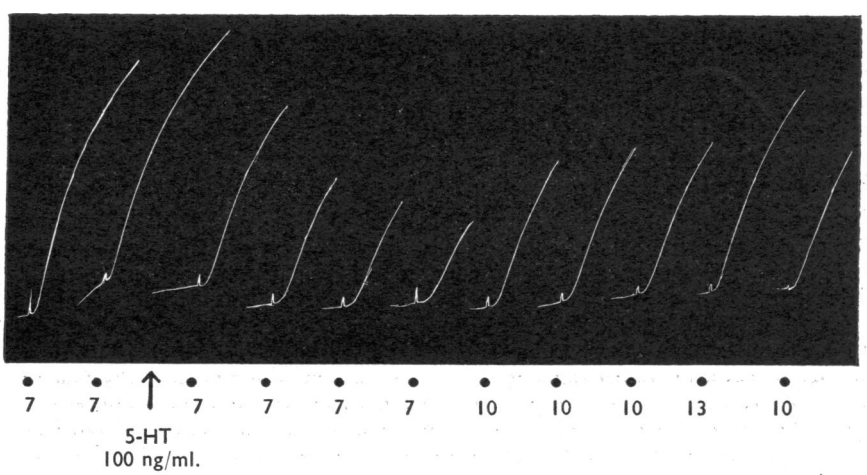

Fig. 2. Contraction of the eserinized leech muscle to varying amounts of acetylcholine given at 5 min intervals. At the arrow, 100 ng/ml. 5-hydroxytryptamine is added to the bath and again after each renewal of the bath fluid. The figures below the contractions refer to the amounts of acetylcholine in ng/ml. added to the bath.

The threshold concentration of 5-hydroxytryptamine sufficient to reduce the size of contractions produced by 3 to 5 ng/ml. of acetylcholine is between 25 and 50 ng/ml. When the 5-hydroxytryptamine is kept in the bath fluid, the degree of inhibition increases during the first 20 to 30 min and then becomes stable as shown in Fig. 2. The time required for the recovery from the effect of 5-hydroxytryptamine is dependent on the time the 5-hydroxytryptamine has been in contact with the muscle. At least 1 hr is required for full recovery of the acetylcholine sensitivity of the tissue following contact with 5-hydroxytryptamine for half an hour.

The relaxing effect of 5-hydroxytryptamine can be utilized to perform the acetylcholine assay more rapidly on the leech muscle. The presence of 100 to 300 ng/ml. of 5-hydroxytryptamine in the bath fluid renders it possible to elicit acetylcholine contractions at about 5 min intervals without undue changes in the base line. In

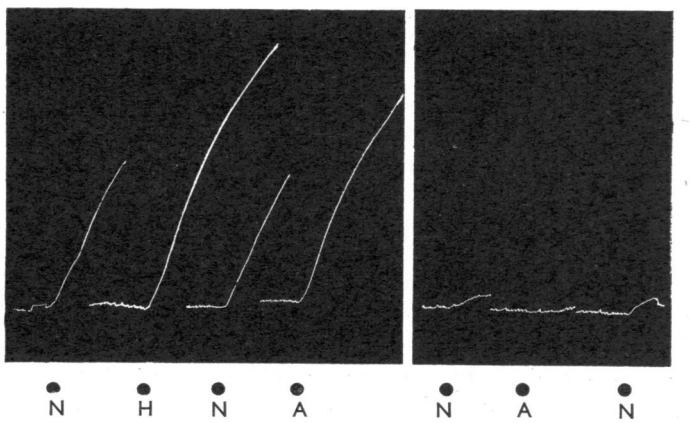

Fig. 3. Contractions of the eserinized leech muscle to 400 ng/ml. nicotine (N) and to 2 ng/ml. acetylcholine (A). Between the two tracings, there is an interval of 30 min during which 400 ng/ml. of 5-hydroxytryptamine was kept in the bath.

the experiment of Fig. 2, the acetylcholine is given for 90 sec at 5 min intervals. It can be seen that in the presence of 100 ng/ml. 5-hydroxytryptamine, the base line remains relatively constant and that there is good discrimination between 7, 10 and 13 ng/ml. of acetylcholine.

In contrast to 5-hydroxytryptamine, lysergic acid diethylamide does not reduce acetylcholine contractions even if added to the bath in amounts up to 1 μg/ml. With this concentration of lysergic acid diethylamide, a slight relaxation of the muscle is usually obtained.

The nicotine contractions of the leech muscle are inhibited by 5-hydroxytryptamine in the same way as are acetylcholine contractions (Fig. 3), but potassium contractions are affected to a much lesser degree (Fig. 4).

Acetone extracts of the leech contracted the rat uterus and the guinea-pig ileum preparations. The effects were obtained by the addition to the 5 ml. bath of 0.05 ml. extract corresponding to about 35 mg of fresh tissue. The contractions were not due to acetylcholine, histamine or 5-hydroxytryptamine, since they were not abolished by atropine, mepyramine or lysergic acid diethylamide. They were not

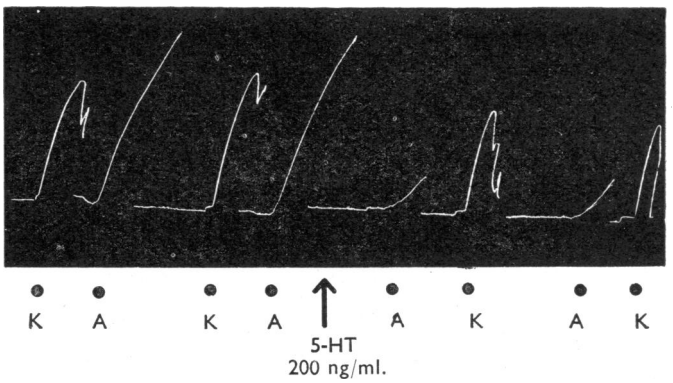

Fig. 4. Contractions of the eserinized leech muscle to 0.3 mg/ml. of potassium chloride (K) and to 2 ng/ml. of acetylcholine (A). From the arrow until the end of the record, the bath contained 200 ng/ml. of 5-hydroxytryptamine.

due to potassium, since the extracts became inactive upon ashing; nor due to bradykinin, since they remained active after incubation with chymotrypsin. No further attempt was made to identify the active principle, or principles, in the extracts.

DISCUSSION

Relaxing effects of 5-hydroxytryptamine on smooth muscle preparations have been reported by Brecht & Jeschke (1960) on the isolated frog lung and by Twarog (1954, 1960) on the molluscan retractor muscle. According to Twarog, 5-hydroxytryptamine prevents the maintenance of tension which develops in response to acetylcholine, but the initial tension developed is potentiated. This author therefore suggests that 5-hydroxytryptamine may be involved in a tension-maintaining system of molluscan smooth muscle that is independent of the tension-developing system. In the leech muscle, both development and maintenance of tension were found to be inhibited by 5-hydroxytryptamine.

In an earlier report, Erspamer (1955) failed to find appreciable quantities of 5-hydroxytryptamine in the ventral ganglionic chain of the leech using paper chromatography and bioassay techniques. Our finding, using less refined dissection methods, was the same. However, recently Welsh & Muirhead (1960) made an extensive survey of the distribution of 5-hydroxytryptamine in invertebrates. They measured the 5-hydroxytryptamine content of pooled nerve cords dissected out from leeches, and found 6.9 μg of 5-hydroxytryptamine per gramme of fresh tissue. This was a relatively large amount of 5-hydroxytryptamine as compared to the content in neural tissues of other invertebrate species examined.

On the basis of the sensitivity of muscles of crustaceans and molluscans to 5-hydroxytryptamine and the presence of this substance in neural tissue of these classes, Welsh (1957) has proposed that 5-hydroxytryptamine has a neurohumoral role in the physiology of invertebrates. The relaxing effect of 5-hydroxytryptamine upon the annelid *Hirudo medicinalis* is another example of the responsiveness of invertebrate organs to this substance.

At present, it seems to be not justifiable to attribute the relaxation of leech muscle produced by samples of cerebrospinal fluid from schizophrenic patients to an action of 5-hydroxytryptamine. Other substances also cause relaxation of the leech muscle such as guanidine (Fühner, 1918), adenylic acid (Minz, 1932), and potassium chloride in threshold concentrations (Vartiainen & Kostia, 1937). Furthermore, the presence of 5-hydroxytryptamine in the cerebrospinal fluid of schizophrenics has not been confirmed by other workers using different methods of assay (Schain, 1960).

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