Brit. J. Pharmacol. (1956), **11**, 186.

IDENTIFICATION OF 5-HYDROXYTRYPTAMINE IN THE STING OF THE NETTLE *(URTICA DIOICA)*

BY

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(RECEIVED JANUARY 26, 1956)

Emmelin and Feldberg (1947) showed that there were three smooth-muscle stimulating substances in the sting of the nettle: acetylcholine (ACh), histamine, and a third they did not identify. They described six properties of this unidentified third substance: constriction of the blood vessels of rabbit's ear; stimulation of *in vitro* preparations of rabbit's and guinea-pig's small intestine; lowering of rabbit's blood pressure; solubility in ether; and instability to boiling in alkaline solution. Since these properties coincided with those of 5-hydroxytryptamine (5-HT) subsequently described, we explored the possibility that the third substance of Emmelin and Feldberg might be 5-HT. Demonstration that this was so has already been briefly reported (Chesher and Collier, 1955) and the present paper sets out the detailed evidence on which identification rests and describes further investigations of nettle sting.

MATERIALS AND METHODS

For ease of supply we used the dioecious nettle, *Urtica dioica*. Both sexes showed the characteristic activity; but in all except a very few experiments female plants were used. Twenty-five or more individual stings were removed with forceps from the stalk or leaf and suspended in 0.5 ml. of the Ringer solution appropriate to the preparation in use. Acetylcholine chloride, atropine sulphate, 5-hydroxytryptamine (serotonin) creatinine sulphate, lysergic acid diethylamide tartrate (LSD), mepyramine maleate, and tryptamine hydrochloride were used and weights given are those of the salts. Dried whole wasp venom apparatus was used for reference as a source of kinin (Jaques and Schachter, 1954).

Preparations of rat's colon were suspended in a 25 ml. bath at 22–24° C. in an oxygenated Ringer solution containing: NaCl, 0.9%; KCl, 0.04%; $CaCl_2$, 0.003%; $NaHCO_3$, 0.015%; glucose, 0.1%, as described by Gaddum, Peart, and Vogt (1949). For rat's uterus the temperature was adjusted to 29° C. and the concentration of calcium in the Ringer solution doubled. For guinea-pig's ileum a temperature of 35–37° C. was used and the Ringer adjusted to contain 0.024% $CaCl_2$, 0.05% $NaHCO_3$ and 0.0005% $MgCl_2$ (Feldberg, 1951). To antagonize ACh on all these preparations, 24 ng./ml. atropine, and to antagonize histamine on the guinea-pig's ileum, 80 ng./ml. mepyramine, were added to the Ringer solution. For most preparations of the uterus, young rats received on the previous day 20 μg. stilboestrol in oil by the intramuscular route. This pretreatment was omitted before experiments on a possible fourth substance in nettle sting.

In preparing extracts for chromatography, leaf and stalk of nettle were extracted with acetone in a chill room at 4° C. and the extract concentrated by evaporation at this temperature for 3 days. Unidirectional descending paper chromatograms were run on Whatman No. 1 paper at 4° C. for 18 hr., using as solvent *n*-butanol-acetic acid (10 to 1) saturated with water. 1% *p*-Dimethylaminobenzaldehyde (Ehrlich's reagent) in aqueous *n*-butanol containing 0.5% HCl was used as developer.

RESULTS

Comparison of Third Substance with 5-HT.—When nettle sting suspensions were applied to the rat's uterus in the presence of atropine, they evoked a contraction which could be attributed to third substance. Since LSD antagonized this contraction, we compared its antagonism of third substance and of 5-HT. In a typical experiment (Fig. 1) a dose of LSD reduced the response to 5-HT by 51% and the response to third substance by 50%. This antagonism was specific, since, in the absence of atropine, LSD did not reduce the response of the preparation to ACh.

Large doses of tryptamine or 5-HT desensitize the guinea-pig's ileum towards themselves, but not to drugs of other types (Gaddum, 1953). Large doses of tryptamine also desensitized the guinea-pig's ileum towards nettle stings in the presence of atropine and mepyramine. For example, at the beginning of one experiment, 30 nettle stings provoked a contraction of the same height as 0.3 μg. 5-HT. The preparation was then exposed for 15 min. to 300 μg. tryptamine and the bath

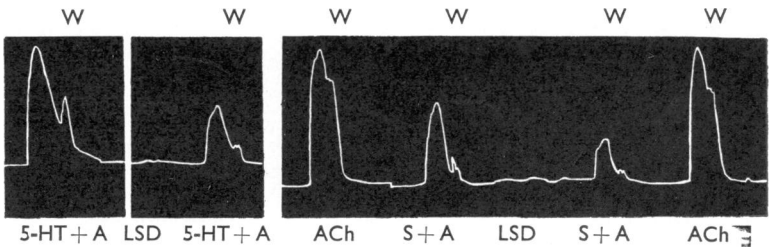

FIG. 1.—Rat's uterus in 25 ml. bath. Antagonism of 5-hydroxytryptamine and of the unidentified substance in nettle stings by lysergic acid diethylamide. A, 0·6 μg. atropine; ACh, 15 μg. acetylcholine; 5-HT, 0·2 μg. 5-hydroxytryptamine; LSD, 0·1 μg. lysergic acid diethylamide; S, 50 nettle stings; W, wash.

washed out. When doses of 5-HT and nettle stings were then given in the presence of 200 μg. tryptamine, together with atropine and mepyramine, the response to 30 nettle stings was reduced by 68% and that to 0.3 μg. 5-HT by 81%.

Experiments with the rat's uterus confirmed this. Desensitization of a preparation by 17 repeated doses of 5-HT reduced the response to 50 nettle stings in the presence of atropine by 53% and the response to 0.4 μg. 5-HT by 51% (Fig. 2). The response to ACh in the absence of atropine was unaffected by desensitization with 5-HT.

Assay of Third Substance.—Third substance was assayed against 5-HT by a matching technique in 7 independent experiments on each of 3 different test preparations (Table I). These assays gave mean values of activity equivalent to 3.43–4.86 ng. 5-HT/sting. A *t* test gave a value of $P=0.2$–0.3 for the difference between the extreme means, which shows that it is not significant.

Paper Chromatography.—In paper chromatograms, both 5-HT and acetone extract of nettle

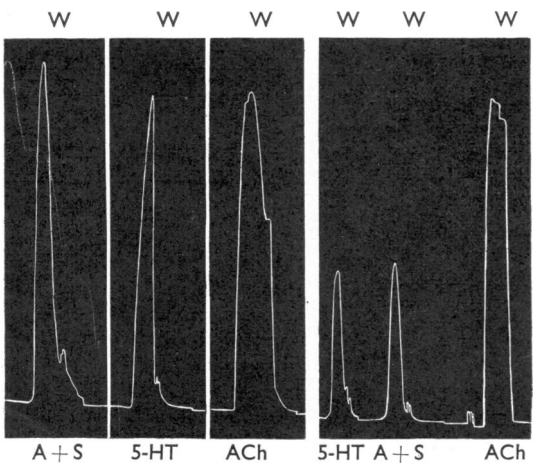

FIG. 2.—Rat's uterus in 25 ml. bath. Desensitization by 5-hydroxytryptamine towards itself and towards the unidentified substance in nettle stings. 5-HT, 0·4 μg. 5-hydroxytryptamine; other abbreviations as in Fig. 1. Between the third and fourth panels, 17 doses of 0·4 μg. 5-HT were given.

TABLE I

THE UNIDENTIFIED SMOOTH-MUSCLE STIMULATING SUBSTANCE IN NETTLE STING ESTIMATED AGAINST 5-HYDROXYTRYPTAMINE (5-HT)

Values obtained on three different preparations *in vitro*

Activity in ng. 5-HT/sting		
Rat's Uterus	Guinea-pig's Ileum	Rat's Colon
2	5	3
2	5	6
8	2	2
4	4	2
7	4	4
8	4	4
3	4	3
Mean .. 4·86	4·00	3·43
S.E. .. 1·03	0·38	0·53

gave a pink spot, turning purple and finally grey, with an R_F value of 0.34. Tryptamine gave a more stable pink spot with an R_F value of 0.61.

Instability of 5-HT in Nettle Sting Suspensions.—750 ng. quantities of 5-HT were added to a number of fresh suspensions of 50 nettle stings and the 5-HT-activity of one of them estimated immediately on the rat's colon preparation. After the remaining suspensions had stood for various times at room temperature, their activities were estimated in the same way. 5-HT-activity declined sharply on standing, reaching about 10% of its initial value within 30 min. (Fig. 3). After addition of a further 750 ng. 5-HT to suspensions that had stood for 30 min., activity again declined, but more slowly. When 5-HT was added to suspensions of stinge that had previously been boiled, it did not loss activity (Fig. 3).

In another series of experiments the stability of third substance in suspensions of nettle stings, without addition of 5-HT, was examined in the same way. On standing at room temperature, third-substance-activity declined at first sharply and then gradually (Fig. 4). When sting suspensions were boiled dry and the residue taken up in fresh Ringer solution, about 97% of the expected third-substance-activity was lost.

Search for a Fourth Substance.—For the reason discussed below, a possible fourth smooth-muscle

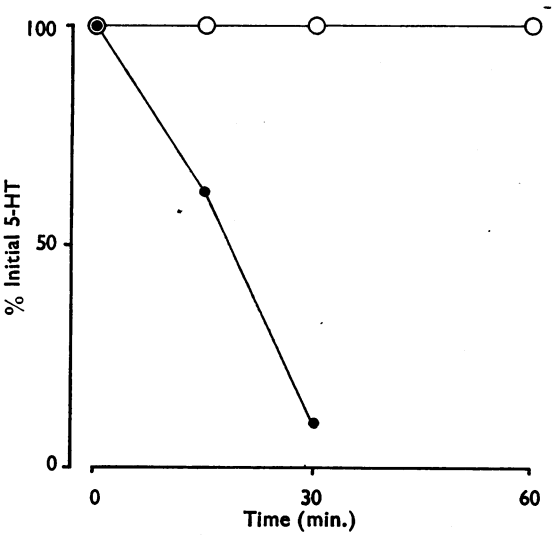

FIG. 3.—Destruction of 5-hydroxytryptamine (5-HT) by nettle stings. 750 ng. 5-HT was added to boiled and unboiled suspensions of 50 stings in Ringer solution and stood at room temperature. 5-HT estimated on rat's colon in presence of atropine. ●—●, sting suspensions unboiled. ○—○, sting suspensions boiled before adding 5-HT.

stimulating substance was sought in nettle stings. Fresh sting suspensions were applied to *in vitro* preparations pre-treated with atropine, LSD and, if necessary, mepyramine. In response to 400 nettle stings, a small contraction of rat's uterus occurred, but this was not greater than might have been

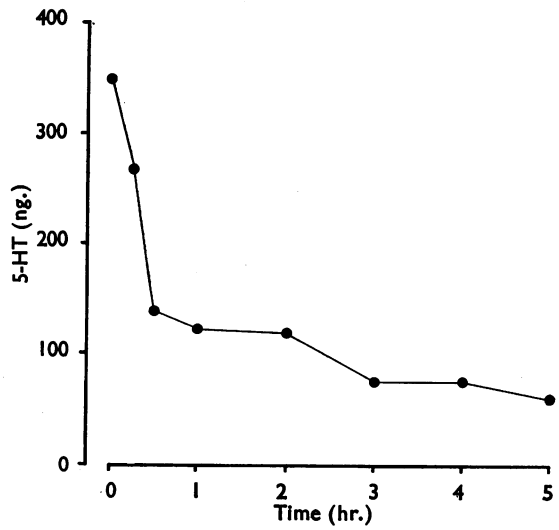

FIG. 4.—Decay of activity of unidentified substance in nettle stings, when suspensions of 50 stings in Ringer solution were stood at room temperature. Activity estimated as 5-hydroxytryptamine (5-HT) on rat's colon in the presence of atropine.

expected from a summation of the residual responses to the substances known to be present.

In the presence of antagonists, 400 nettle stings evoked from guinea-pig's ileum a scarcely detectable response, even less than that which a mixture of ACh, histamine and 5-HT, each at the dose expected in 400 stings, produced. On the other hand, a suspension containing 50 μg. dry weight whole wasp venom apparatus evoked from the same preparation, in the presence of the same antagonists, a strong slow contraction, attributable to the kinin which Jaques and Schachter (1954) have shown to be present.

DISCUSSION

The experiments described above showed that the third substance stimulated rat's colon and uterus *in vitro*, was antagonized by LSD, and was inactivated by standing in the presence of nettle stings. Large doses of 5-HT and of tryptamine desensitized *in vitro* preparations towards third substance. These facts indicate the identity of third substance and 5-HT, which quantitative results establish more firmly. On the rat's uterus preparation, LSD antagonized 5-HT and third substance to the same extent, and 5-HT desensitized the preparation towards both substances to the same degree. In quantitative estimates of third substance in terms of 5-HT in three different *in vitro* preparations, the mean values obtained did not differ significantly from one another. The identification of third substance with 5-HT is supported by the detection of 5-HT in paper chromatograms of extracts of nettle leaf and stalk. The detection of 5-HT in nettle adds a second source among plants to that already reported in cowhage by Bowden, Brown, and Batty (1954).

If the average nettle sting contains 7–9 μg. fluid (Emmelin and Feldberg, 1947; Haberlandt, 1886), then 3.4–4.9 ng. 5-HT/sting provides a concentration more than sufficient to evoke pain in human skin (Armstrong, Dry, Keele, and Markham, 1953). It is interesting that Jaques and Schachter (1954) found a very similar concentration of 5-HT (not less than 0.32 mg./g.) in wasp venom.

Although histamine, ACh and 5-HT are more than sufficiently concentrated in nettle sting to account for the triple response and pain, their presence may not explain the pricking or tingling felt even 24 hr. after being stung. This observation suggested that a fourth possible pain-producing substance might exist in nettle. If so, it either does not stimulate smooth-muscle directly or is present in very small amount, since we obtained no evidence of a fourth smooth-muscle stimulating substance in large numbers of nettle stings. The agent which

inactivates 5-HT in unboiled, but not in boiled, suspensions of nettle stings is presumably an enzyme. It is possible that enzymes in nettle sting have pain-producing properties.

Summary

1. Nettle stings contain, besides histamine and acetylcholine, a third active substance which is probably 5-hydroxytryptamine (5-HT). Its identity with 5-HT is suggested by the following tests: the third substance stimulates the rat's uterus *in vitro*; its action is antagonized by lysergic acid diethylamide to the same extent as that of 5-HT; repeated doses of 5-HT desensitize the rat's uterus to the same degree towards the third substance and 5-HT; on paper chromatograms an extract of nettle leaf and stem gave a spot which corresponded in colour and R_F value with 5-HT.

2. *In vitro* estimates gave the following mean values in ng. 5-HT/sting: rat's uterus, 4.86±1.03; guinea-pig's ileum 4.00±0.38; rat's colon, 3.43±0.53.

3. Nettle stings contain an agent, presumably an enzyme, which inactivates 5-HT and the third nettle-sting substance in Ringer solution at room temperature.

4. Evidence of the existence of a fourth substance in nettle sting that stimulates smooth muscle was sought but not found.

We wish to thank Dr. M. Schachter for the gift of dried wasp venom, Miss P. Harknett for technical assistance, and the directors of Allen and Hanburys Ltd. for permission to publish this work. The 5-hydroxytryptamine creatinine sulphate was kindly supplied by Abbott Laboratories and the lysergic acid diethylamide tartrate by Sandoz Products Ltd.

References

Armstrong, D., Dry, R. M. L., Keele, C. A., and Markham, J. W. (1953). *J. Physiol.*, **120**, 326.

Bowden, K., Brown, B. G., and Batty, J. E. (1954). *Nature, Lond.*, **174**, 925.

Chesher, G. B., and Collier, H. O. J. (1955). *J. Physiol.*, **130**, 41P.

Emmelin, N., and Feldberg, W. (1947). Ibid., **106**, 440.

Feldberg, W. (1951). Ibid., **113**, 483.

Gaddum, J. H. (1953). Ibid., **119**, 363.

—— Peart, W. S., and Vogt, M. (1949). Ibid., **108**, 467.

Haberlandt, G. (1886). *S.B. Akad. Wiss. Wien., Math. Naturw.* Cl. **93**, Abt. 1, 123.

Jaques, R., and Schachter, M. (1954). *Brit. J. Pharmacol.*, **9**, 53.