THE OCCURRENCE OF 5-HYDROXYTRYPTAMINE IN SCORPION VENOM

BY K. R. ADAM AND C. WEISS

Department of Physiology, University of Khartoum, Sudan

(With Plate i)

(Received 1 July 1957)

The constituents of scorpion venom have not yet been fully identified. During preliminary investigation of the venom of *Leiurus quinquestriatus* by paper chromatography, a spot corresponding in position to that due to 5-hydroxytryptamine (HT) was seen. This paper is concerned with the methods of identification and assay of this substance in venom, and also considers the histochemistry of the venom gland in relation to this finding.

METHODS

Venom was obtained exclusively from *Leiurus quinquestriatus* (Hemprich & Ehrenberg, 1829). It was collected by stimulating the telson, using an induction coil. The pooled venom was dried and kept in a vacuum desiccator. Its toxic potency did not appear to vary over a period of several weeks. It was redissolved in water as required.

Ascending paper chromatography was carried out using Whatman papers nos. 1 and 4, and the following solvent mixtures: n-butanol, acetic acid, water (4:1:5); amyl alcohol, pyridine, water (2:2:1); n-butanol saturated with N-HCl. Various reagents were used to demonstrate the HT spots, including β-dimethylamino-benzaldehyde, diazotized β-nitroaniline, and α-nitroso-β-naphthol. The green-blue colour in ultra-violet light after treatment with an acetic acid-ninhydrin mixture (Jepson & Stevens, 1953) was probably the most useful and sensitive method. It also demonstrated other venom constituents by virtue of the ninhydrin reaction. Paper electrophoresis was also used, and good resolution of the constituents was obtained in normal acetic acid (pH 2.3).

The assay of HT was mainly carried out on the isolated rat uterus (by the method of Amin, Crawford & Gaddum, 1954), but estimations were also made on the isolated guinea-pig ileum. For fluorometric estimations, the venom was extracted with 90% (v/v) acetone. This extract was dried, redissolved in 3N-HCl, and the HT estimated in a Farrand spectrofluorometer, using an activating wave-length of 300 mÅ and measuring the fluorescence at 540 mÅ (cf. Bogdanski, Pletscher, Brodie & Udenfriend, 1956). In all cases, the HT used as the standard was the creatinine sulphate complex, but all quantities are expressed as the base.
For histological examination, venom glands were dissected free of chitin, fixed in buffered 10% formalin (with the addition, for the chromaffin reaction, of 3% potassium dichromate), and embedded in paraffin. The argentaffin reaction was obtained with Fontana's ammoniacal silver solution as detailed by Gomori (1952). The diazo reagents for demonstrating enterochromaffin granules were: diazotized o-safranin (Lillie, Burtner & Henson, 1953), fast red salt B, and diazotized p-nitroaniline (Pearse, 1953). Ehrlich's reaction with p-dimethylaminobenzaldehyde was also carried out by the method of Pearse (1953).

RESULTS

Paper chromatography. 0.5 mg. quantities of venom were run simultaneously with standard HT, and, in all three solvent mixtures, distinct spots with the same flow rates and colour reactions as the standard HT were obtained. The sizes of the spots, compared with known quantities of pure HT, indicated concentrations of about 1–5 µg. HT/mg. dry weight of venom. No other spots appeared after treatment with the above colour reagents, except in the case of ninhydrin which showed other components with lower rates of flow and poorly separated. Better resolution of these components was obtained by paper electrophoresis, and here too a line was found having the same mobility as pure HT.

Assay of HT in venom. Various batches of crude venom were assayed on the isolated rat uterus, and were found to contain 2–4 µg. HT/mg. dry venom. This oxytocic activity could be abolished completely by dihydroergotamine, a potent antagonist of HT (Gaddum & Hameed, 1954). A similar result was obtained with assays on isolated guinea-pig ileum, though this method is less precise. One batch of venom was extracted with acetone, and the extract assayed fluorometrically. In this case the concentration of HT was found to be 2.9 µg./mg. venom. The fluorescence spectrum of the extract appeared identical with that of pure HT.

Histology of the venom gland. The gland has two lobes, the lumen of each communicating with the sting. Each lobe consists of folded epithelium with rather sparse supporting cells surrounded by a coat of smooth muscle. After formalin fixation, the epithelium is highly granular, and a considerable proportion of these granules are capable of reducing ammoniacal silver nitrate (Pl. 1, fig. 1). They also show to some extent a chromaffin reaction after fixation in a formalin-dichromate mixture. However, the diazo methods, which demonstrate mammalian enterochromaffin granules clearly, gave negative results, and no fluorescence was seen when sections were viewed in ultra-violet light. Some cells in the basal parts of the gland gave a clearly positive indole reaction with p-dimethylaminobenzaldehyde (Pl. 1, fig. 2). Usually two or three of such cells could be seen in each section.

DISCUSSION

The ubiquity of HT has been noted before (Erspamer, 1954), and it had already been found in wasp venom (Jaques & Schachter, 1954) when the present results were obtained. Its concentration in the venom of Leiurus quinquestriatus is
The occurrence of 5-hydroxytryptamine in scorpion venom

apparently the highest yet reported in any biological medium, but there is no
indication as yet of any function it may perform. The considerable local pain
resulting from a sting may be due to the HT content of the venom (cf. Armstrong,
Dry, Keele & Markham, 1952). This point may be settled by experiments using
venom from which the HT has been extracted. It is perhaps relevant that Moham-
med & El Karemi (1953) have claimed that atropine and dihydroergotamine, both
of which can antagonize HT, can protect rats to some extent against injected
scorpion venom.

The high concentration of HT in the venom adds interest to the histochemistry
of the gland. From the results given above, it would appear that there are no cells
present which closely resemble the mammalian enterochromaffin cell. Although
some cells show argentaffin and chromaffin granules, they do not give the diazo
reactions or fluorescence in ultra-violet light, and the granules themselves are generally
much coarser than those seen in the enterochromaffin cell.

The indole reaction given by some basal cells may mean that these are the
producers of HT. Enterochromaffin cells do not give the indole reaction, and
Barter & Pearse (1955) have suggested that, after formalin fixation, HT is converted
by ring closure into a β-carboline derivative. In the present case, however, it does
not seem likely that this reaction occurred since no fluorescence in ultra-violet light
was seen, and it remains possible that the indole reaction does indicate the site
of HT.

SUMMARY

5-hydroxytryptamine has been identified as one of the constituents of the venom
of *Leiurus quinquestriatus* and its concentration estimated to be 2–4 µg/mg.
dry weight of venom. The relevant histochemistry of the venom gland is
discussed.

Our thanks are due to Prof. D. A. Smith for his advice and encouragement; to
Dr T. B. B. Crawford, Pharmacology Department, University of Edinburgh, for
the spectrofluorometric estimation; and to Abbott Laboratories, Chicago, for a gift
of 5-hydroxytryptamine creatinine sulphate.

REFERENCES

and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol.* 126,
596-618.


Pharmacol.* 9, 240-8.


Fig. 1. Epithelium of venom gland. Formalin fixation. Argentaffin reaction. $\times 200$.

Fig. 2. Cell from basal part of gland showing Ehrlich (indole) reaction. $\times 760$.

ADAM AND WEISS—THE OCCURRENCE OF 5-HYDROXYTRYPTAMINE IN SCORPION VENOM

(Pacing p. 42)