EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON AN IDENTIFIED 5-HYDROXYTRYPTAMINE-CONTAINING NEURONE IN THE CENTRAL NERVOUS SYSTEM OF THE SNAIL *Helix pomatia*

N.N. OSBORNE

Max-Planck-Institut für experimentelle Medizin, Forschungsstelle Neurochemie, 3400 Göttingen, Germany

V.W. PENTREATH

Wellcome Laboratories of Pharmacology, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Fife, Scotland

1   The effect of 5,7-dihydroxytryptamine (5,7-DHT) on an identified 5-hydroxytryptamine (5-HT)-containing neurone in the CNS of the snail was studied by histochemical, biochemical and electro-physiological methods.

2   Low concentrations of 5,7-DHT decreased the endogenous 5-HT content of the neurone without affecting the amino acids, while relatively large amounts of the drug proportionately lowered 5-HT and in addition slightly decreased the tryptophan and methionine content of the cell.

3   5,7-DHT blocked the uptake of [^{3}H]-5-HT into the neurone; the close analogue 5,6-DHT was more potent.

4   As well as slightly influencing the accumulation of [^{3}H]-tryptophan by the neurone 5,7-DHT inhibited the metabolism of this amino acid to form 5-HT, probably by affecting the enzyme tryptophan-hydroxylase.

5   5,7-DHT produced a postsynaptic blockade of transmission from the neurone by blocking the 5-HT receptors of the follower neurones. This effect appeared to be specific for 5-HT receptors.

6   The data support the idea that 5,7-DHT is neurotoxic for indoleamine-containing neurones.

Introduction

In each cerebral ganglion of the snail *Helix pomatia*, and also a number of other gastropods, there is a large or giant neurone located in the anterior-ventral position (Kandel & Tauc, 1966; Cottrell & Osborne, 1970; Osborne & Cottrell, 1971). Conclusive evidence has shown that these giant neurones contain 5-hydroxytryptamine (5-HT or serotonin) (Osborne, 1971; Osborne & Cottrell, 1971, 1972; Osborne, 1973b) and the cells have consequently been termed 'giant serotonin cells' or 'GSCs'. The membrane properties of the GSGs in *Helix* have been studied by Kandel & Tauc (1966), who also showed each GSC to send an axon to the ipsilateral exterior lip nerve and one to each cerebral buccal connection. Further electrophysiological and autoradiographic studies on these cells (Cottrell, 1970, 1971; Pentreath & Cottrell, 1972, 1974; Cottrell & Macon, 1974) demonstrated that the two GSCs form synaptic

excitatory links with other giant neurones in the buccal ganglia. Unlike the GSCs, the buccal neurones lack 5-HT or catecholamines, and only the GSCs contain the enzymes for synthesizing 5-HT from tryptophan (Osborne, 1973c) or 5-hydroxy-tryptophan (Cottrell & Powell, 1971; Osborne, 1972).

The experiments reported here describe the effects of 5,7-dihydroxytryptamine (5,7-DHT) on the GSCs studied by histochemical and biochemical procedures, and on synaptic transmission from the GSCs. In the vertebrates, 5,7-DHT has a neurotoxic mode of action on 5-HT neurones, and to a lesser extent on noradrenaline neurones, but has apparently no effect on dopamine neurones in the CNS (Baumgarten, Björklund, Lachenmayer & Nobin, 1973). The drug is also known to influence 5-HT metabolism in brain tissue of the snail (Osborne, 1974a). The present experiments were also designed to compare the effects

of 5,7-DHT on the GSC with those known to occur on 5-HT-containing neuronal systems in the vertebrates.

Methods

Specimens of *Helix pomatia* were obtained from Gerard and Haig Ltd., East Preston, Sussex, or from Alfred Koch, 345 Holzminden, Germany and were kept at room temperature (18–22°C) in aquaria which contained a little water. All experiments were performed at room temperature.

Fluorescence microscopy

Snail nervous tissue was superfused at a constant rate with snail's saline (Meng, 1960) containing 10^{-3} M ascorbic acid and various concentrations of 5,7-DHT (10^{-6} to 5×10^{-3} M) for 15, 30, 90 or 150 minutes. Ascorbic acid was used to prevent the breakdown of 5,7-DHT. Control preparations were superfused for the same period of time in snail's saline containing ascorbic acid only. Thereafter the supraoesophageal ganglia were dissected, frozen in liquid N_2 and freeze-dried for at least two days at −35°C in a vacuum of 10^{-3} torr in an Edwards-Pearse Tissue Dryer, model EPD3. Dried tissue was exposed to formaldehyde gas for 1 h at 80°C (Falck & Owman, 1965). The vapour was obtained from paraformaldehyde (Merck) which had been kept at a relative humidity of 70% (Hamberger, Malmfors & Sachs, 1965). The nervous tissue was subsequently embedded under reduced pressure in paraffin wax and 10 μm sections were examined with a Leitz microscope fitted with a darkfield condenser, appropriate filters and a mercury vapour lamp (Corrodi & Johnson, 1967).

Amino acid and 5-hydroxytryptamine content experiments

Snails were injected through their foot musculature once a day over a period of 4 days with 0.1 ml snail's saline containing 10^{-3} M ascorbic acid and various concentrations of 5,7-DHT so that individual animals received between 10^{-5} and 10^{-3} M of the drug. Individual GSCs were then dissected from the snails as described elsewhere (Osborne, 1974b), and placed in a micro-tube containing 15 μl 50% acetone in water. After collecting 4–6 GSCs from animals injected with the same amount of 5,7-DHT, the samples were frozen in liquid nitrogen and then thawed. This 'shocking' process was repeated three times before centrifuging the tubes at 1500 g for 60 minutes. Thereafter the supernatant of each sample was analysed either fluorimetrically by the procedure of McCaman, Weinreich & McCaman (1973) for their 5-HT content, or by a micro-dansylation chroma-

tography technique (see reviews Neuhoff, 1973; Osborne, 1973a) for their amino acid content.

[³H]-Tryptophan metabolism experiments

After pinning the dissected supraoesophageal ganglia on to the plastic base of a small chamber (vol. 1 ml) which contained snail's saline, the connective tissue surrounding the GSCs was dissected with fine forceps. The saline was then replaced by a fresh solution containing 10^{-3} M ascorbic acid and [³H]-tryptophan (Radiochemical Centre, Amersham, England, specific activity 5.58 Ci/mmol; 75 μCi/ml) together with 10^{-4} M of 5,7-DHT. In controls 5,7-DHT was omitted. After an incubation time of between 30 and 150 min, the physiological solution containing the radioactivity was removed and the tissue rinsed with physiological saline for 10 minutes. The GSCs were then dissected and transferred by means of a fine pipette (see Osborne, 1974b) to microtubes containing 15 μl 50% acetone in 0.01 N HCl. After collecting 10 cells from nervous tissues treated in the same way, the sample was frozen in liquid N_2 and thawed. This 'shocking' process was repeated three times before centrifuging the tube at 1500 g for 60 minutes. Thereafter the supernatant was applied to a 5×5 cm silica gel precoated plate (Merck −60), together with small amounts (approx. 2 μg of each substance) of carrier tryptophan, 5-hydroxytryptophan (5-HTP), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). Chromatograms were then developed by ascending chromatography in covered beakers using *n*-butanol/pyridine/acetic acid/water (15:2:3:5 by vol.). Substances were subsequently identified by first spraying the chromatograms with 1% potassium ferricyanide in ammonium hydroxide solution followed by ninhydrin. 5-HTP, 5-HT and 5-HIAA appeared brown in colour and tryptophan blue. Individual substances were then eluted from the silica gel with methanol, the extraction was repeated five times, and the combined eluates from a single substance were dried in counting vials with a stream of N_2. Scintillation fluid (4 g PPO and 0.1 g POPOP/litre toluene) was added to the vials and the radioactivity counted in a Packard scintillation spectrometer.

The residual protein precipitates of the cells were dissolved in 10–20 μl of 1 N NaOH and the protein estimated by the microprocedure of Lowry, Rosebrough, Farr & Randall (1951). A series of protein standards, 0.5 μg to 6 μg of serum albumin, was carried through the procedure and used as reference standards.

Uptake experiments

GSCs (14–18) were rapidly dissected from a number of snails and preincubated for 15 min in 2 ml snail's saline at 25°C. [³H]-5-HT (Radiochemical Centre, Amersham, England, specific activity 17.3 Ci/mmol)

at a concentration of 10^{-5} M, containing 10^{-3} M ascorbic acid either alone or together with 5×10^{-5} M 5,7-DHT, or other substances were added and the incubation allowed to continue for 30 minutes. [³H]-5-HT is known to be taken up by snail nervous tissue by a high affinity process under these conditions (Osborne, Hiripi & Neuhoff, 1975). Incubations were terminated by rapid filtration of the sample through filters (0.8 μm pore size) followed by a rapid wash with 2 ml of fresh snail's saline in order to remove all traces of non-occluded radioactivity. After drying by leaving overnight at room temperature, filters were placed face up in scintillation vials and after the addition of 10 ml toluene scintillation fluid, their ³H- content was measured by scintillation counting. The efficiency of the counting was estimated by including standards with each batch of analysis. These standards consisted of filters through which non-radioactive incubated tissue samples had been filtered, and to which were then added known amounts of [³H]-5-HT or [³H]-toluene. The efficiency of counting varied between 18 and 25 per cent.

Electrophysiological experiments

The isolated cerebral and buccal ganglia were pinned to the base of a 5 ml acrylic chamber. The preparation was bathed with continuously flowing physiological solution, which was stopped when the effects of drugs were being tested. The cerebral ganglia were pinned ventral surface uppermost to expose the GSC. Most of the connective tissue layer above the GSC and the buccal ganglia neurones to be impaled was removed by dissection with fine-tipped forceps. The GSC and the postsynaptic buccal neurone being studied were each impaled with a double-barrelled electrode. Both barrels were filled with 0.6 M K_2SO_4; one barrel was used for recording, the other for stimulating.

In some experiments the cerebral buccal connection was stimulated with a plastic suction electrode connected in series with a stimulus isolation unit. Records were made on a Brush 220 series two-channel link recorder. Conventional stimulating and amplifying equipment was used.

Two types of experiments were carried out with 5,7-DHT. In the first, snails were injected each morning over a period of 3 days with 5,7-DHT (0.5 ml of snail's saline containing 2 μg/ml and 10^{-3} M ascorbic acid). The cerebral and buccal ganglia were then dissected and an electrophysiological analysis was made of the effect of the drug on the synaptic connections between the GSCs and the buccal neurones. Control animals were injected with the same volumes of saline containing only 10^{-3} M ascorbic acid. In other experiments the effect of 5,7-DHT was tested on isolated cerebral and buccal ganglia. 5,7-DHT was added to the organ bath to give a concentration of 2×10^{-4} M to 1×10^{-3} M. The nervous tissues were exposed to the drug for 10 s to 3 hours. In some studies KCl was omitted from the saline solution. 5-HT and acetylcholine (ACh) were applied to the postsynaptic neurones in the buccal ganglia from a small bore (150 μm tip diameter) syringe. Successive 5-HT applications were separated by intervals of at least 15 min because of receptor desensitization. The concentration of 5-HT was 2×10^{-5} M, that of ACh was 3×10^{-6} M.

Results

Fluorescence microscopy

Control preparations showed the GSC with its yellow fluorescing cytoplasm (indicative of 5-HT) to be situated on the ventral surface of the metacerebral part of each cerebral ganglion. Characteristically, a group of small (10–20 μm), intensely fluorescing, green cells (indicative of dopamine) was seen around the medial edge of each of the GSCs (Figure 1). As with other fluorescent cells, the nucleus of the GSC did not fluoresce. The fluorescence associated with the cytoplasm of the control GSCs was slightly mottled in appearance and not evenly distributed, as would normally be the case with the GSCs from freshly dissected ganglia (see Osborne & Cottrell, 1971). Following exposure of ganglia to 5,7-DHT, a number of changes occurred in the amine fluorescence. The fluorescence in the GSCs was slightly increased by all concentrations of 5,7-DHT perfused over a period of 15 minutes. However, over a perfusion period of 30–90 min the yellow fluorescence associated with the GSC was markedly decreased by either 10^{-6} or 5×10^{-3} M 5,7-DHT (Figure 1). Little alteration in the nature of the fluorescence of the green cells was noticed (Figure 1). Over a longer period of perfusion, viz. 150 min, 10^{-6} and 10^{-5} M 5,7-DHT produced effects similar to those observed for a perfusion period of 90 minutes. In contrast, 10^{-4} and 5×10^{-3} M 5,7-DHT (150 min perfusion) not only caused the fluorescence in the GSCs to fade, but also made the cell appear slightly swollen and apparently damaged. Moreover, a decrease in the fluorescence of the green cells and neuropile region was noted.
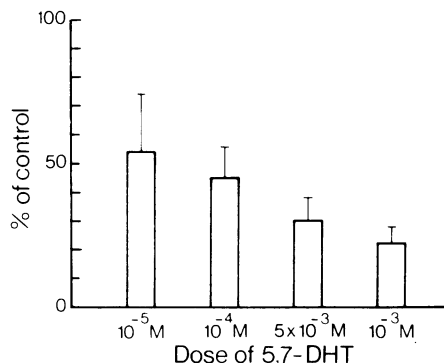
Effect of 5,7-dihydroxytryptamine on the amino acid and 5-hydroxytryptamine content of the GSC

The amino acids and related compounds which occur in the GSC and which react with [¹⁴C]-dansyl chloride have been described elsewhere (see Osborne & Cottrell, 1972; Osborne, 1973a). No significant change in the content of any substance in the GSC was produced by pretreatment of snails with 5,7-DHT at either 10^{-5} M or 10^{-4} M. Higher concentrations of the drug (10^{-3} M) produced a slight but significant decrease in the content of tryptophan and methionine.
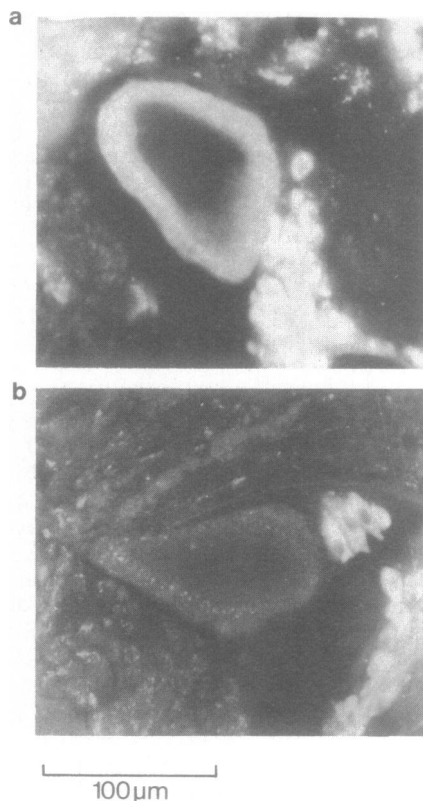
Figure 1 Sections through the cerebral ganglia of *Helix pomatia* showing the appearance of a 'giant serotonin cell' (GSC) from an untreated animal (a) and from a snail treated with 5,7-dihydroxytryptamine (5,7-DHT) (b). The tissue was processed by the formaldehyde procedure for localizing monoamines, the normal GSCs appearing yellow in colour (showing them to contain 5-hydroxytryptamine) and in close association with small green fluorescing cells (indicating the presence of dopamine). The yellow fluorescence of the GSC is decreased by 5,7-DHT.

Figure 2 Effect of four different doses of 5,7-dihydroxytryptamine (5,7-DHT) on 'giant serotonin cell' 5-hydroxytryptamine (5-HT) content. The columns give means of four determinations. Vertical lines show s.e. mean. 100% 5-HT = 700 ± 50 pg.

In our hands the sensitivity of the method described by McCaman *et al.* (1973) was of the order of 500 pg 5-HT. From a combination of 10 independent determinations (each determination required between 4 and 6 GSCs), each GSC was estimated to contain 700 ± 50 pg 5-HT. This agrees with our previous findings where other procedures were used to analyse the 5-HT content of the GSCs (Cottrell & Osborne, 1970; Osborne, 1973a). Figure 2 shows 5,7-DHT (1×10^{-5}M) to cause almost a 50% drop in the 5-HT content when injected into snails. With increasing doses of 5,7-DHT, the 5-HT content was lowered to an even greater extent. The highest dose used (10^{-3} M) produced an 85% decrease of 5-HT in the GSC.

Effect of 5,7-dihydroxytryptamine on tryptophan metabolism in the GSC

As can be seen from Figure 3, the chromatographic procedure used allows a separation of 5-HT and metabolites on plates measuring 5×5 cm. Though the procedure was sensitive, allowing less than 1 μg of substance to be detected, it was not sensitive enough to measure the endogenous content of 5-HT and metabolites in as many as 18 GSCs. Examination of chromatograms of extracts from the GSCs which had been exposed to [³H]-tryptophan showed radioactivity to be associated with three substances only: tryptophan, 5-hydroxytryptophan (5-HTP) and 5-HT. This confirmed previous data (Osborne, 1973c). From Table 1 it can be seen that only very small amounts of the accumulated [³H]-tryptophan are actually metabolized and that the quantity of [³H]-5-HT formed increases with time while the [³H]-5-HTP content stabilizes after 60 minutes. The effect of 10^{-4}M 5,7-DHT on the accumulation and metabolism of [³H]-tryptophan is also shown in Table 1. The accumulation of [³H]-tryptophan is initially unaffected by the drug but over a longer period of time a definite inhibition occurs. Moreover, the drug drastically affects the metabolism of the accumulated [³H]-tryptophan, in particular the amount of [³H]-5-HT formed. In contrast the content of [³H]-5-HTP formed from the [³H]-tryptophan taken up is not significantly affected by 5,7-DHT.

Effect of 5,7-dihydroxytryptamine on the uptake of 5-hydroxytryptamine by the GSC

Previous studies have shown that [³H]-5-HT uptake in the snail brain is inhibited by 5,7-DHT with an ID_{50} of
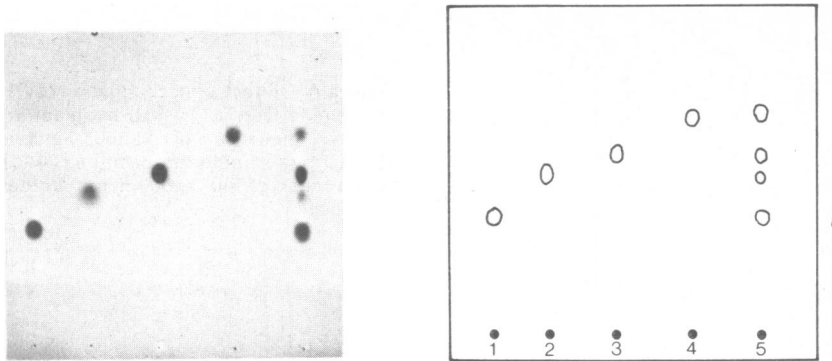
Figure 3 Chromatogram (5 × 5 cm) and map to show the separation of 5-hydroxytryptamine (5-HT) and metabolites following one dimensional chromatography. The direction of chromatography is indicated by the arrow and solution used was n-butanol/pyridine/acetic acid/water (15:2:3::5) 1 = 5-hydroxytryptophan, 2 = tryptophan, 3 = 5-HT, 4 = 5-hydroxyindoleacetic acid, 5 = mixture.

10^{-4}M (see Osborne *et al.*, 1975). It was therefore decided that in order to compare the relative potency of 5,7-DHT and other indole derivatives towards the uptake of [³H]-5-HT by the GSCs, slightly smaller concentrations of substances (5×10^{-5} M) would be tested. Figure 4 shows that at this concentration 5,7-DHT inhibited the accumulation of [³H]-5-HT by 56%. In contrast, the indole derivatives 5-hydroxyindole (5-HI), 5-HTP and 5-hydroxyindoleacetic acid (5-HIAA) only inhibited the uptake by between 7 and 15%, while 5,6-DHT was the most potent, reducing the accumulation of radioactive amine by 62%.

Electrophysiology

Stimulation of the GSC to produce a burst of action potentials resulted in excitatory responses in three large identifiable neurones (anterior, middle and posterior) on the lateral borders of each buccal ganglion (Cottrell, 1970, 1971; Cottrell & Macon, 1974). The input on to the anterior cells was different from that to the middle and posterior cells and was characterized by its long-lasting nature (Figure 5b). When the middle buccal neurone was artificially hyperpolarized, the excitatory response could be seen

Table 1   The effect of 5,7-dihydroxytryptamine (5,7-DHT) on the incorporation of radioactivity from [³H]-tryptophan into 5-hydroxytryptophan (5-HTP) and 5-hydroxytryptamine (5-HT) in 'giant serotonin cells' (GSCs).

Time (min)	Condition	Tryptophan	5-HTP	5-HT
30	Saline alone	14,000 ± 200	641 ± 34	582 ± 34
	Saline + 5,7-DHT	13,942 ± 240	632 ± 38	641 ± 32
45	Saline alone	26,000 ± 240	689 ± 38	640 ± 40
	Saline + 5,7-DHT	25,210 ± 230	700 ± 37	630 ± 33
60	Saline alone	38,120 ± 439	754 ± 39	820 ± 48
	Saline + 5,7-DHT	32,000 ± 384	714 ± 36	742 ± 68
90	Saline alone	39,800 ± 880	810 ± 40	1000 ± 68
	Saline + 5,7-DHT	34,840 ± 600	765 ± 38	800 ± 44*
120	Saline alone	47,700 ± 600	820 ± 45	1840 ± 54
	Saline +5,7-DHT	39,120 ± 594*	700 ± 50	1000 ± 50*
150	Saline alone	50,200 ± 398	829 ± 39	1840 ± 60
	Saline + 5,7-DHT	42,400 ± 520*	681 ± 48	900 ± 39*

Incorporation of radioactivity in d/min per mg protein was measured after incubation of tissue in [³H]-tryptophan for varying periods of time. Results are mean values ± s.e. mean of 4 experiments.
* $P < 0.05$ and therefore significantly different by Student's *t* test.
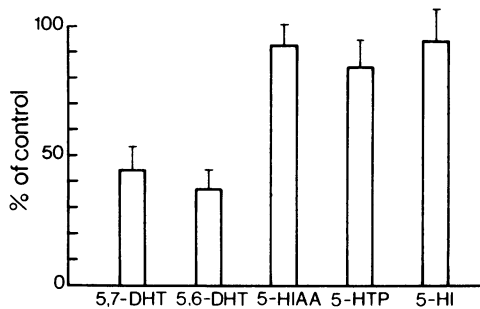
Figure 4   Effect of accumulation of $[^3H]$-5-hydroxy-tryptamine (5-HT) (10^{-5} M) by 'giant serotonin cells' in the presence of various indole derivatives (5×10^{-5}M) at room temperature (24°C). Each value is the mean of four experiments. Vertical lines show s.e. mean.
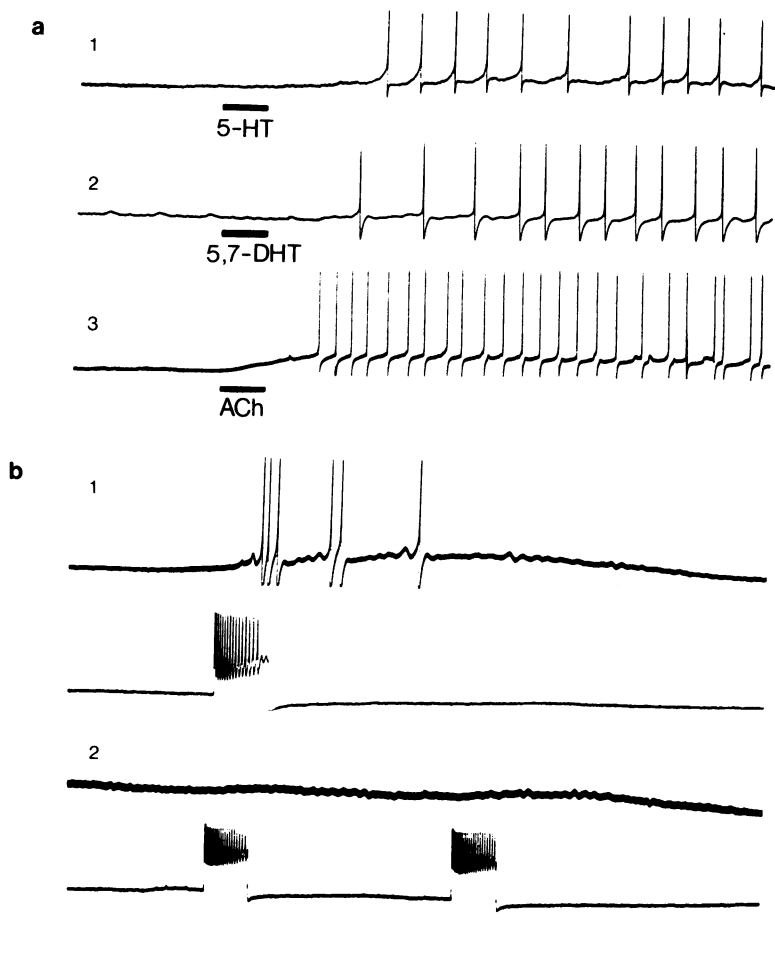
Figure 5   Effect of drugs on the middle buccal cell (a), and of 5,7-dihydroxytryptamine (5,7-DHT) on the response to 'giant serotonin cell' (GSC) stimulation of the anterior buccal cell (b). Records (a1) and (a2) are from the same preparation, (a3) and (b) are from different preparations. (a1–3) show the effect of applying 5-

to be composed of individual e.p.s.ps whose amplitude was increased at greater levels of hyperpolarization (Figure 6a). The buccal neurones were depolarized by low concentrations of 5-HT (Figure 5a).

No marked change in response to GSC stimulation was observed in either the middle or anterior cells in snails which had been injected over three days with 5,7-DHT. This finding was surprising because the drug decreased the level of 5-HT within the GSC soma. However, it is possible that any small change in e.p.s.p. size would only be apparent after analysing large numbers of drug treated animals. Such extensive analysis was not undertaken in the present study. This procedure would be necessary to rule out the natural variation in e.p.s.p. size between different animals. Cottrell & Macon (1974) found that reserpine treatment reduced, but did not abolish, the excitatory responses in the middle buccal cell.

5,7-DHT mimicked the effect of 5-HT on the middle and anterior buccal neurones; in concentrations of 2×10^{-4} to 1×10^{-3} M it caused depolarization and firing of action potentials (Figure 5a). If, after such a depolarization the preparation was briefly washed, it was found that the effect of 5-HT $(2 \times 10^{-5}$ M), but not ACh $(5 \times 10^{-6}$ M), was abolished. This suggested that the drug blocked 5-HT receptors on the somata of these neurones. The responses to 5-HT recovered gradually with washing (about 15 minutes).

The action of 5,7-DHT was tested on the buccal cell responses in freshly isolated preparations. At a concentration of 6×10^{-4} M to 2×10^{-3} M there was a rapid (approximately 2 min) abolition of input from the GSC on to the middle (Figure 6a) and anterior (Figure 5b) cells. There was an accompanying blockade of the depolarizing response to 5-HT whereas response to ACh was unaffected. After washing for approximately 15 min there was a gradual and eventually (approx. 1 h washing) complete recovery of e.p.s.ps arising from the GSC input, and concomitant recovery of the response to 5-HT. After prolonged exposure to 5,7-DHT (1–2 h), recovery of

the responses to GSC stimulation and to 5-HT required a longer period of washing (1–3 hours).

During periods in which the action of 5,7-DHT was being removed by washing, an unusual response to GSC stimulation of the middle buccal cells was observed in several preparations. Instead of excitation, a weak inhibition resulted (Figure 6b). The reason for this effect is not clear, although it appeared to be dependent upon a number of factors, such as previous concentration of 5,7-DHT employed, the time after commencement of washing, and frequency of GSC stimulation. This effect was not observed when the electrode was removed from the GSC and current passed into the bath. The effect was not mimicked by the addition of 5-HT $(2 \times 10^{-5}$ M) to the buccal cell soma, and was lost when the cell's sensitivity to 5-HT recovered.

In order to test whether the action of 5,7-DHT was specific and not the result of a reduction in membrane resistance, the effect of the drug on the membrane resistance of several middle buccal cells before and during its application was tested. This was evaluated by measuring the amplitude of potentials produced by square hyperpolarizing current impulses of constant intensity and 1 s duration given through one barrel of the double-barrelled recording electrode. No appreciable (max. 7%) reduction in membrane resistance could be recorded.

Extra evidence for the specificity of the effect of 5,7-DHT on the e.p.s.ps elicited by the GSC was obtained by stimulating the cerebro-buccal connective to produce e.p.s.ps from a different source. The results are shown in Figure 6c. Relatively large e.p.s.ps were recorded in the middle buccal cell which were probably due to the release of ACh. 5,7-DHT at a concentration of 10^{-3} M had little effect on these e.p.s.ps. 5-HT $(4 \times 10^{-5}$ M) also had little effect on these e.p.s.ps, but both drugs caused a similar but slight change in the time-course of individual e.p.s.ps (Figure 6c).

The results show that 5,7-DHT can produce an apparently specific blockade of 5-hydroxytryptaminergic transmission by blocking 5-HT receptors.

Figure 5 (cont.)

hydroxytryptamine (5-HT) $(2 \times 10^{-5}$ M), 5,7-DHT $(3 \times 10^{-4}$ M) and acetylcholine (ACh) $(3 \times 10^{-6}$ M) to the middle buccal cell. The drugs were added at time indicated by the bars. (b) Shows electrical responses in the ipsilateral anterior buccal cell (upper trace in each record) to GSC stimulation (bottom traces). (b1) Control response consisting of a relatively persistent depolarization. It was not possible to detect individual e.p.s.ps in this neurone with increased levels of hyperpolarization, probably because the cell exhibits anomalous rectification (see Cottrell & Macon, 1974). (b2) Responses 2 min after the application of 6×10^{-4} M 5,7-DHT. The excitation produced by stimulating the GSC is greatly reduced even though the GSC was made to fire at a higher frequency. Time scale 10 s. Voltage calibration, (a1–3) (middle buccal cells) 40 mV; (b1,2) top traces (anterior buccal cell) 20 mV, bottom traces (GSC) 80 mV.
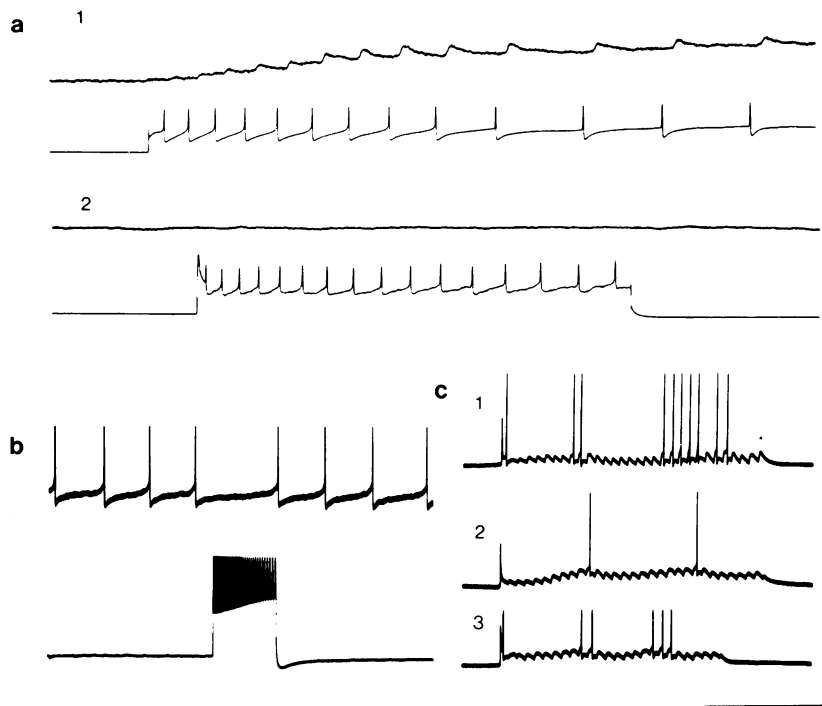
Figure 6 Effect of 5,7-dihydroxytryptamine (5,7-DHT) on the excitatory responses of the ipsilateral middle buccal cell to stimulation of the 'giant serotonin cell' (GSC) (a,b), and on the excitatory responses of the middle buccal cell to stimulation of the ipsilateral cerebro-buccal connective (c). (a) and (b) were obtained from different preparations bathed in KCl-free solution; (c) is a different preparation in normal (Meng's) saline. In (a) the middle cell (top trace in each record) has been artificially hyperpolarized from −55 mV to −100 mV. (a1) shows the control e.p.s.ps which facilitate and summate during GSC action potential firing (bottom trace). (a2) shows the complete abolition of the response 3 min after the addition of 1×10^{-3} M 5,7-DHT. (b) shows the weak inhibitory effect of GSC firing (bottom trace) on the middle buccal cell (at resting potential) 10 min after the start of washing off 6×10^{-4} M 5,7-DHT (20 min exposure) from this preparation. This effect was observed in three different preparations. (c1) Control e.p.s.ps elicited in the middle buccal neurone by stimulating the cerebro-buccal connective with 1 ms pulses at 0.6 Hz for approximately 20 seconds. Occasional e.p.s.ps elicit action potentials. (c2) and (c3) show the effect of 5,7-DHT (1×10^{-3} M) and 5-hydroxytryptamine (4×10^{-5} M) respectively on similar periods of stimulation. The nervous tissue was exposed to each drug for a period of 20 minutes. There is no apparent decrease in amplitude of the individual e.p.s.ps although in each case they possess slightly different time courses. Time scale in (a) is 2 s, in (b) and (c) is 10 seconds. Voltage calibrations, (a1,2) top traces (middle buccal cell) 9 mV, bottom traces (GDC) 120 mV; (b) top trace (middle buccal cell) 50 mV, bottom trace (GSC) 40 mV; (c1–3) (middle buccal cell) (c2) mV.

Discussion

The present fluorescent histochemical data describing the effect of 5,7-DHT on a specified 5-HT-containing cell (GSC) are similar to those of previous vertebrate studies on indoleamine neurones (Baumgarten et al., 1973) and analogous to the action of 6-hydroxy-dopamine on a characterized dopamine-containing neurone (Berry, Pentreath, Turner & Cottrell, 1974). The increase in the amine fluorescence associated with the GSC following perfusion of the CNS with 5,7-DHT for 15 min can be interpreted as an initial uptake of 5,7-DHT by the GSC. Over a longer period of perfusion (30, 90 and 150 min) the toxic effect of even small amounts of accumulated 5,7-DHT resulted in a dramatic decrease in the 5-HT fluorescence. Moreover, under the experimental conditions, longer periods of perfusion with high doses of 5,7-DHT also affected the fluorescence associated with the small green dopamine-containing cells. This is slightly surprising in that there is evidence that 5,7-DHT has little influence upon the dopamine content in vertebrate brain tissue (Baumgarten et al., 1973).

From the biochemical data obtained in this study it

is clear that 5,7-DHT causes a marked decrease in the 5-HT content of the GSCs in a similar way to that reported for vertebrate tissues (Baumgarten et al., 1973). It was also observed that the levels of a number of amino acids in the GSC including tyrosine, glutamic acid, glycine, aspartic acid, proline, GABA and alanine were unaffected by 5,7-DHT. The results obtained concerning the various suspected transmitter amino acids are further evidence for the specificity of 5,7-DHT in influencing the indoleamine content only. The reason for the very slight decrease in tryptophan and methionine as a result of high doses of 5,7-DHT is not yet clear.

These findings also showed the effect of 5,7-DHT on the synthesis and uptake of 5-HT by the GSC whereby the indoleamine content of the cell is decreased. The fact that 5,7-DHT alters the metabolism of [^{3}H]-tryptophan in the GSC in a similar way to p-chlorophenylalanine (Osborne, 1973c; Osborne & Neuhoff, 1974) suggests that the drug exerts its influence upon tryptophan-hydroxylase, as has been shown in vertebrate nervous tissue (Baumgarten, Victor & Lovenberg, 1973). Moreover, these data demonstrate that the drug also affects to a slight extent the accumulation of [^{3}H]-tryptophan by the GSC. This finding, together with the slight decrease in endogenous tryptophan following treatment with high doses of 5,7-DHT, may be of significance in relation to 5-HT turn-over. The observation that 5,7-DHT is slightly less potent than 5,6-DHT but inhibits the uptake of 5-HT into the GSC is also similar to earlier studies on vertebrate nervous tissues (Horn, Baumgarten & Schlossberger, 1973). However, unlike the studies on vertebrate tissues, much higher doses of 5,7-DHT are required to affect the uptake of 5-HT by at least 50%. This substantiates work on whole brain tissue of the snail where the ID_{50} is even higher for 5,7-DHT (Osborne et al., 1975). However, it is clear that compared to other indole derivatives tested (with the exception of 5,6-DHT), 5,7-DHT is a potent inhibitor of the uptake of 5-HT in the GSC.

The postsynaptic action of the 5,7-DHT on the GSC mediated e.p.s.ps was unexpected. The drug (concentration 10^{-4} to 10^{-3} M) rapidly abolished these e.p.s.ps and also eliminated the depolarizing response to 5-HT. On the other hand, the depolarizing response to applied ACh and to presumed cholinergic e.p.s.ps resulting from stimulation of a different input were not antagonized. Since there was a gradual and parallel recovery of the response to 5-HT and to GSC-mediated responses with washing, the blocking action of 5,7-DHT appears to be relatively specific for 5-HT receptors. In this respect the action of 5,7-DHT on 5-HT receptors is analogous to that discovered for 6-hydroxydopamine on dopamine receptors in the pond snail *Planorbis corneus* (Berry & Cottrell, 1973; Berry et al., 1974).

The experiments described in this study have shown that 5,7-DHT has a number of effects on an indoleamine-containing neurone and thus support the idea that the substance is neurotoxic. As well as altering the uptake and metabolism of 5-HT, 5,7-DHT also inhibits 5-HT transmission by blocking the receptors. However, unlike studies on vertebrate tissues (Baumgarten, Groth, Göthert & Manian, 1974), initial electron microscopical findings on the GSC could not demonstrate any pathological alterations following treatment with 5,7-DHT (Osborne & Pentreath, unpublished data).

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