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THE EFFECT OF BUFOTENINE, MELATONIN, PSILOCYBIN, AND RELATED COMPOUNDS ON THE 5-HYDROXYTRYPTAMINE EXCITATORY RECEPTORS OF *HELIX ASPERSA* NEURONS

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ABSTRACT

1. Intracellular recordings were made from identifiable neurons in the suboesophageal ganglionic mass of the snail, *Helix aspersa*. All the cells used were excited by 5-hydroxytryptamine (5-HT) and inhibited by dopamine.

2. The equipotent molar ratios were determined for a number of tryptamine analogues. The following compounds closely resembled 5-HT in their mode of action on 5-HT excitatory receptors: *N*-methyl-5-hydroxytryptamine; α-methyl-5-hydroxytryptamine; bufotenine; 5-methoxytryptamine.

3. A comparison is made between the structural requirements for the snail 5-HT excitatory receptor and for 5-HT receptors present in 8 other animal tissues.

4. The requirements for potent 5-HT-like activity on snail neurons include the presence of an indole nucleus with a hydroxyl group in the 5 position and for a side chain with no greater substitution than a methyl group on either the terminal nitrogen or the α carbon.

5-HYDROXYTRYPTAMINE (5-HT) has been shown to have a potent effect on a wide range of animal tissues (Garattini and Valzelli, 1965; Eichler and Farah, 1966; Garattini and Shore, 1968). Gaddum (1953) suggested that 5-HT, whose actions qualitatively resembled those of tryptamine (Laidlaw, 1912), was acting on tryptamine receptors of mammalian gut. He observed that high doses of tryptamine would block the responses of the gut to both tryptamine and 5-HT. By the use of blocking agents Gaddum and Picarelli (1957) presented evidence for the presence of two types of tryptamine receptor in the gut. They found evidence for D-receptors, which were blocked by phenoxybenzamine and lysergic acid diethylamide, and M-receptors, which were blocked by morphine, atropine, and cocaine. The D-receptors are thought to be on the smooth muscle while the M-receptors are probably located on nervous tissue. The 5-HT receptors on the rat uterus and rabbit ear are also considered to be D-receptors (Robson and Stacey, 1962). Since morphine antagonizes the 5-HT receptors on autonomic ganglion cells, it is likely that these are M-receptors (Trendelenburg, 1957).

The presence of tryptamine receptors in the cerebral cortex has been reviewed by Vogt (1968). Structure activity studies involving the actions of indolealkylamines on neurons have been undertaken by Curtis and Davis (1962) and Krnjevic and Phillis (1963). 5-HT receptors have been shown to be present on molluscan neurons (Gerschenfeld and Tauc, 1961; Kerkut and Walker, 1961, 1962; Gerschenfeld and Stefani, 1966, 1968). The present study presents evidence concerning the structure activity requirements for the 5-HT receptor of neurons in the snail, *Helix aspersa*, which are excited by 5-HT.

METHODS

All experiments were performed on the isolated suboesophageal ganglionic mass of snails, *Helix aspersa*, which were collected locally. The brain was prepared for experimentation according to the method of Walker (1968). The preparation was immersed in a 20-ml. bath containing snail Ringer solution of the following composition:

NaCl, 80 m*M*: KCl, 4 m*M*: $MgCl_2$, 5 m*M*: $CaCl_2$, 5 m*M*: Tris-HCl buffer, 5 m*M*. The *p*H of the Ringer was 8·0.

The micro-electrodes were pulled from Pyrex, using a vertical puller, and filled with *M* potassium acetate. The recording electrodes had a resistance of from 5 to 30 MΩ and a tip diameter of less than 0·5 μm. The activity of the neurons was recorded using a Medistor negative capacity electrometer amplifier and Tektronix 502A and 564 oscilloscopes, and permanent traces were obtained using either an A.E.I. or a Watanabe pen oscillograph.

Drugs were dissolved and applied in snail Ringer directly to the bath. Certain drugs had first to be dissolved in acid and then neutralized with alkali. The following compounds were used: 5-hydroxytryptamine creatinine sulphate, tryptamine-HCl, tyramine hydrochloride (B.D.H.); *N*-methyltryptamine, α-methyltryptamine, 5-methoxygramine, tryptophol (R. Emanuel); *N*-methyl-5-hydroxytryptamine oxalate (Aldrich); *N*-acetyl-5-hydroxytryptamine (Sigma); 6-hydroxytryptamine (Hassle); 5-methoxytryptamine, melatonin, *N*,*N*-dimethyltryptamine bioxalate, 5-hydroxyindole, 5-hydroxyindolylacetic acid Koch-Light; bufotenine bioxalate, psilocin substance, psilocybin Sandoz; 5,6-dihydroxytryptamine-HCl Texas Research Institute of Mental Sciences; lyseric acid diethylamide tartrate United Pharmaceutical Works; α-methyl-5-hydroxytryptamine creatinine sulphate Upjohn. The dose of 5-HT was added to the bath in the same volume (0·1–0·5 ml.) as the analogue under investigation. The preparation was washed with 50 ml. of Ringer between additions of compounds. The potency of all compounds was measured by finding the amount of the compound that would produce the same degree of excitation/depolarization as a standard dose of 5-HT. Potency is expressed as the equipotent molar ratio, i.e., the ratio:—

$$\frac{\text{Amount of compound (nmoles)}}{\text{Amount of 5-HT (nmoles)}}$$

producing the same degree of excitation/depolarization.

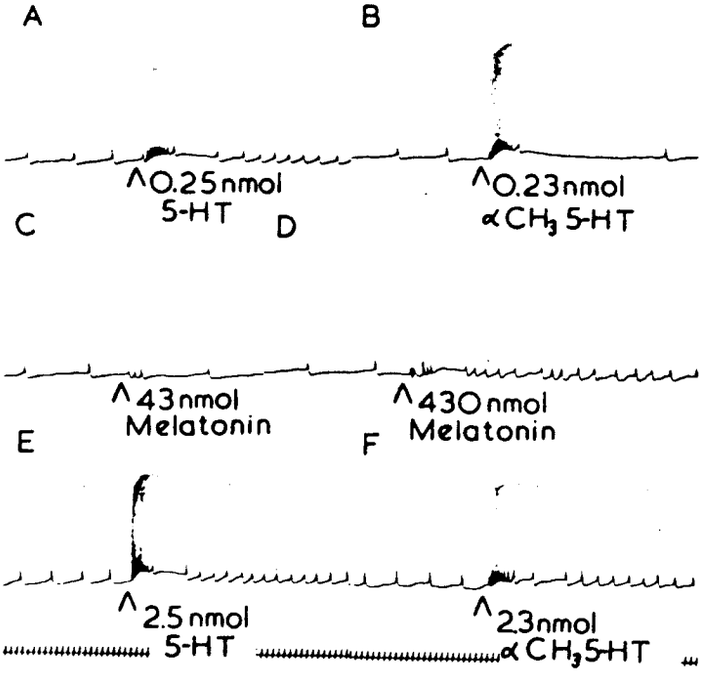

FIG. 1. The effects of 5-HT, α-methyl-5-HT, and melatonin on the electrical activity of a cell in the brain of *Helix aspersa*. A, 6 mV. depolarization in response to 0·25 nmole 5-HT. B, 8 mV. depolarization in response to 0·23 nmoles α-methyl-5-HT. C, Slight excitation in response to 43 nmoles melatonin. D, 4 mV. depolarization in response to 430 nmoles melatonin. E, 12 mV depolarization in response to 2·5 nmole 5-HT. F, 7 mV. depolarization in response to 2·3 nmoles α-methyl-5-HT. All traces are from the same cell. The time trace is in intervals of 1 second. The action potentials were 80 mV. in amplitude.

RESULTS

Two groups of cells were used in carrying out this study, both groups were excited by

Table I.—Structure and Excitatory Activities of Some Tryptamine Analogues tested on Specific Neurons in the Central Nervous System of *Helix aspersa*

Compound	Equipotent Molar Ratio Range	Mean	Number of Observations	R_1*	R_2	R_3	R_4
5-Hydroxytryptamine	1	1		$-CH_2-NH_2$	H	OH	H
N-Methyl-5-hydroxytryptamine	0·3–7	4·5	10	$-CH_2-N(H)(CH_3)$	H	OH	H
α-Methyl-5-hydroxytryptamine	1–10	6·7	8	$-CH(CH_3)-NH_2$	H	OH	H
Bufotenine	13–130	45·6	14	$-CH_2-N(CH_3)_2$	H	OH	H
5-Methoxytryptamine	42–210	120	10	$-CH_2-NH_2$	H	OCH_3	H
Melatonin	172–8600	3088	8	$-CH_2-N(H)(COCH_3)$	H	OCH_3	H
Psilocin	2000–10,000	3500	5	$-CH_2-N(CH_3)_2$	OH	H	H
Psilocybin	2000–10,000	4500	7	$-CH_2-N(CH_3)_2$	OP	H	H
N-Acetyl-5-hydroxytryptamine	370–18,400	7130	6	$-CH_2-N(H)(COCH_3)$	H	OH	H
N,*N*-dimethyltryptamine	2500–>40,000	>10,000	6	$-CH_2-N(CH_3)_2$	H	H	H
6-Hydroxytryptamine	Inhibition of activity		6	$-CH_2-NH_2$	H	H	OH

*

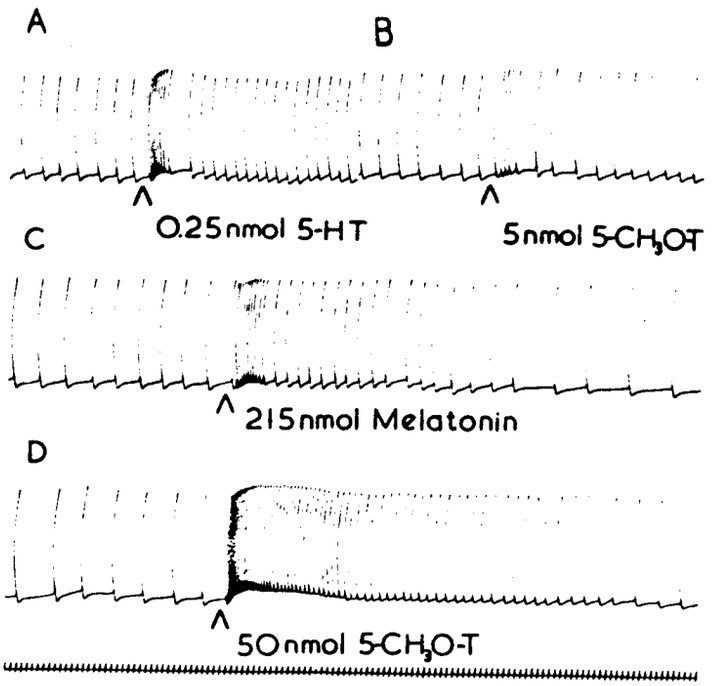

FIG. 2. The effects of 5-HT, 5-methoxytryptamine, and melatonin on the electrical activity of a cell in the brain of *Helix aspersa*. A, 5 mV. depolarization in response to 0·25 nmole 5-HT. B, 3 mV. depolarization in response to 5 nmoles 5-methoxytryptamine. C, 4 mV. depolarization in response to 215 nmoles melatonin. D, 8 mV. depolarization in response to 50 nmoles 5-methoxytryptamine. All traces are from the same cell. The time trace is in intervals of 1 second. The action potentials were 85 mV. in amplitude.

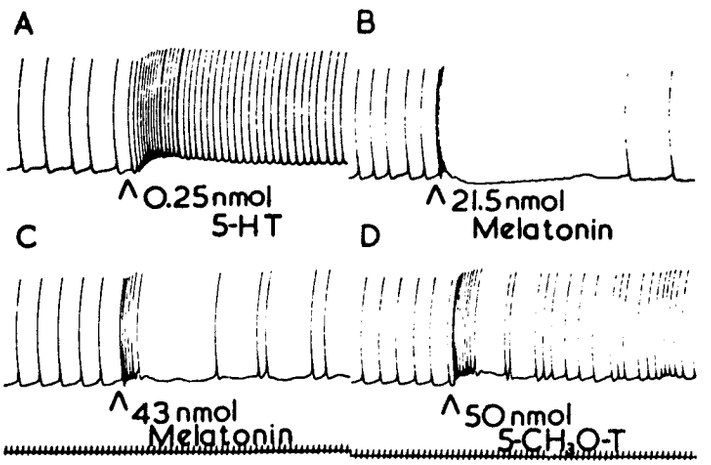

FIG. 3. - The effects of 5-HT, melatonin, and 5-methoxytryptamine on the electrical activity of a cell from the brain of *Helix aspersa*. A, 10 mV. depolarization in response to 0·25 nmole 5-HT. B, Slight excitation followed by inhibition in response to 21·5 nmoles melatonin. C, 5 mV. depolarization followed by inhibition in response to 43 nmoles melatonin. D, 8 mV. depolarization in response to 50 nmoles 5-methoxytryptamine. All traces are from the same cell. The time trace is in intervals of 1 second. The action potentials were 75 mV. in amplitude.

5-hydroxytryptamine and acetylcholine and inhibited by dopamine. One group of cells was in the posterior region of the right parietal ganglion, close to the boundary with the visceral ganglion. These cells include cell 12, shown in *Fig.* 1 of the paper of Walker, Lambert, Woodruff, and Kerkut (1970). The second group of cells was in the posterior visceral ganglion, close to the boundary with the right parietal ganglion. These cells include cell 16 of the same figure. The threshold concentration for 5-hydroxytryptamine excitation varied somewhat from cell to cell but was generally around 2·5–25 pmoles. In general, a dose of ten times this value was used as the 5-HT standard dose. A dose of 250 pmoles gave a rapid depolarization of between 5 and 10 mV. (*Fig.* 1A).

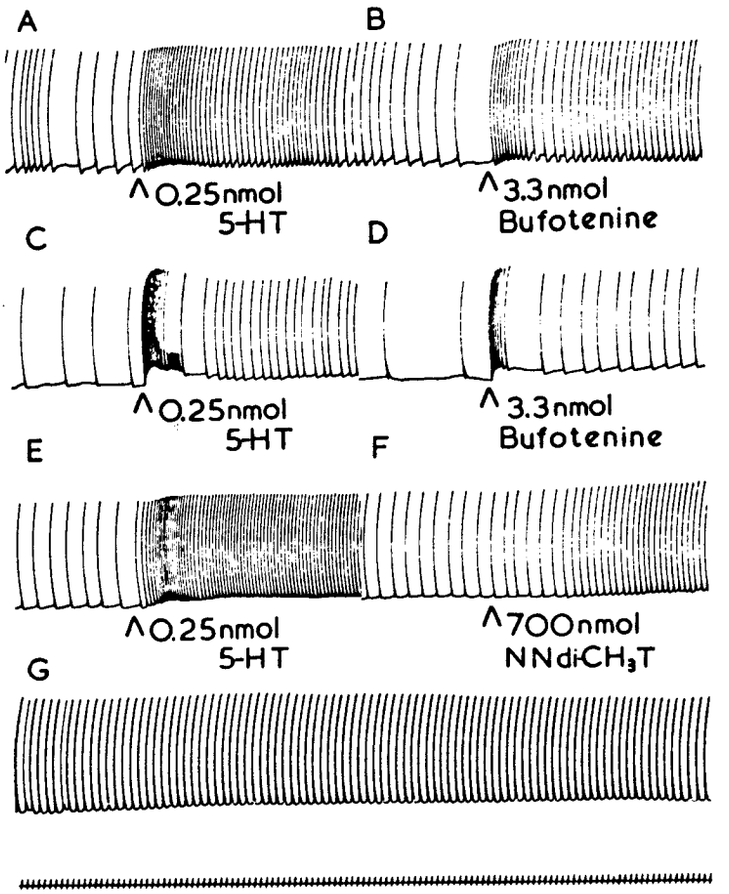

FIG. 4.—The effects of 5-HT, bufotenine, and *N*,*N*-dimethyltryptamine on the electrical activity of 3 cells in the brain of *Helix aspersa*. A, 4 mV. depolarization in response to 0·25 nmole 5-HT. B, 4 mV. depolarization in response to 3·3 nmoles bufotenine. C, 12 mV. depolarization in response to 0·25 nmole 5-HT. D, 9 mV. depolarization in response to 3·3 nmoles bufotenine. E, 4 mV. depolarization in response to 0·25 nmole 5-HT. F and G, Gradual 6 mV. depolarization in response to 700 nmoles *N*,*N*-dimethyltryptamine. Traces A, B, C, D, E, F, and G are from the same cells. The time trace is in intervals of 1 second for traces A, B, E, F, and G, and in intervals of 2 seconds for traces C and D. The action potentials were 80 mV. in amplitude for traces A and B, and 75 mV. for the other traces.

The equipotent molar ratios for each compound tested were determined as described in the Methods section and are shown in *Table I*. As can be seen from *Table I*, any substitution on the basic 5-HT molecule decreased the potency of the compound.

Substitution of a methyl group on the α carbon of the side chain or on the terminal nitrogen had the least effect on activity; α-methyl-5-hydroxytryptamine in some cases was equipotent with 5-HT (*Fig.* 1B) but was generally slightly less potent (*Fig.* 1F). The form of the excitation and the duration was the same for both compounds. On a few cells *N*-methyl-5-hydroxytryptamine was slightly more potent than 5-HT.

The replacement of the hydroxyl group on the indole nucleus by a methoxy group reduced the potency of the compound by over one hundredfold (*Table I*). In *Fig.* 2 traces B and D show the response of a cell to 5 nmoles and 50 nmoles respectively of 5-methoxytryptamine. The addition of an acetyl group to the terminal nitrogen of the side chain of 5-methoxytryptamine further reduced the potency of the compound (*Fig.* 1, traces C and D; *Fig.* 2, trace C). The equipotent molar ratio of melatonin compared to 5-HT is over 3000. *N*-Acetyl-5-hydroxytryptamine had a similar effect to melatonin on snail neurons, with an equipotent molar ratio of 7130. In a few experiments the cells failed to respond to either melatonin or to *N*-acetyl-5-hydroxytryptamine. These experiments have not been included in the present studies. In some cells the excitatory response to melatonin was followed by a period of inhibition (*Fig.* 3, traces B and C). This inhibitory effect was also shown to a lesser extent in this cell by 5-methoxytryptamine (trace D). In this cell the addition of 5-HT produced only excitation (trace A). On some occasions the excitatory response to 5-HT was followed by an inhibitory phase. The removal of the hydroxyl group from 5-HT rendered the compound an

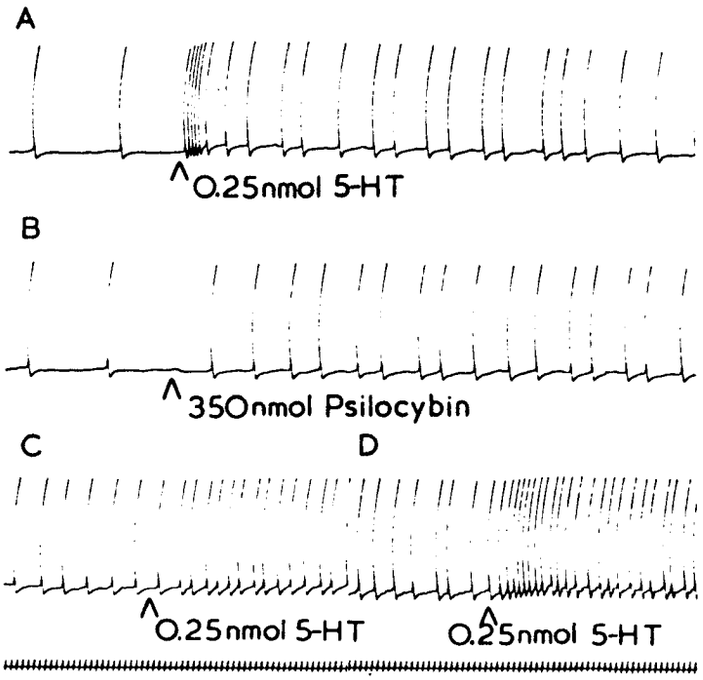

FIG. 5. The effects of 5-HT and psilocybin on the electrical activity of a cell from the brain of *Helix aspersa*. A, 4 mV. depolarization in response to 0·25 nmole 5-HT. B, Gradual 2 mV. depolarization in response to 350 nmoles psilocybin. C, Partial block of the 5-HT response in the presence of psilocybin. D, 4 mV. depolarization in response to 0·25 nmole 5-HT after prolonged washing with Ringer to remove the psilocybin. The presence of psilocybin reduces the response to 5-HT. All traces are from the same cell. The time trace is in intervals of 1 second. The action potentials were 90 mV. in amplitude.

antagonist of 5-HT. At high concentrations tryptamine inhibited cell activity.

Addition of 2 methyl groups to the terminal nitrogen of the side chain also reduced the potency of the compound. For example, bufotenine (*Fig.* 4, traces B and D) was less potent than 5-HT, the equipotent molar ratio for this compound being 45·6. However, the addition of methyl groups to the terminal nitrogen of the side chain of tryptamine did result in a compound with some agonist activity (*Fig.* 4, traces F and G).

N,*N*-Dimethyltryptamine is, however, more than ten thousand times less potent than 5-HT. At the doses required for agonist activity the action of this compound was very difficult to reverse, the cell remaining depolarized. Moving the hydroxyl group to a different position on the indole nucleus also resulted in a striking loss of 5-HT-like activity. Both psilocin and psilcybin were more potent than *N*,*N*-dimethyltryptamine (*Table 1*) but are still over a thousand times less potent than 5-HT. The onset of the actions of both compounds was slow (*Fig.* 5, trace B; *Fig.* 6, trace B) and their actions very long lasting. In the presence of psilocybin the response to 5-HT was reduced (*Fig.* 5, trace C). Similarly, psilocin reduced the response to 5-HT (*Fig.* 6, trace C). On washing off the psilocybin the 5-HT response fully returned (*Fig.* 5, trace D). Addition of an extra hydroxyl group on the 6 position of the indole nucleus reduced the potency and sometimes resulted in a biphasic response: excitation followed by inhibition (*Fig.* 7, traces B, D, and E). The presence of a hydroxyl group on position 6 in the absence of a hydroxyl group on position 5 resulted in the reversal of action of the compound. 6-HT hyperpolarized the cell membrane potential and inhibited cell activity (*Fig.* 8,

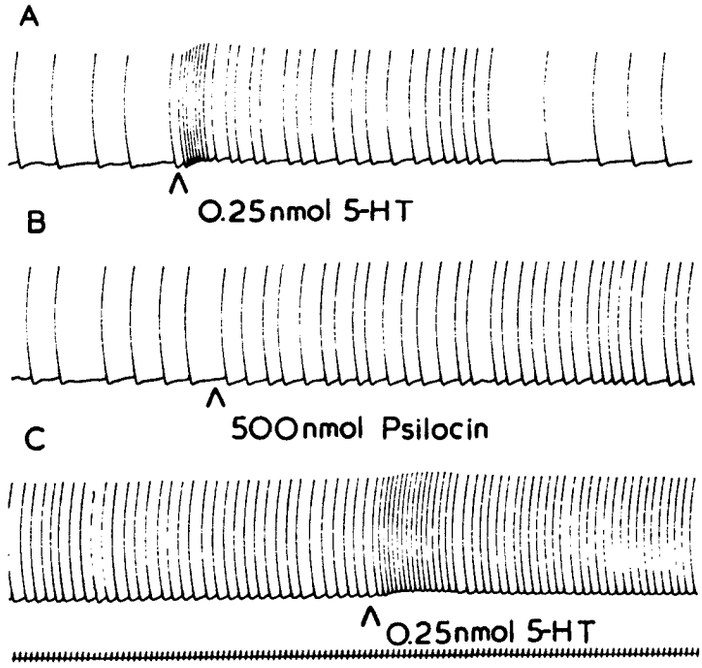

FIG. 6.—The effects of 5-HT and psilocin on the electrical activity of a cell from the brain of *Helix aspersa*. A, 6 mV. depolarization in response to 0·25 nmole 5-HT. B, Gradual 3 mV. depolarization in response to 500 nmoles psilocin. C, 3 mV. depolarization in response to 0·25 nmole 5-HT in the presence of 500 nmoles psilocin. Psilocin reduces the response to 5-HT. All traces are from the same cell. The time trace is in intervals of 1 second. The action potentials were 85 mV. in amplitude.

traces C, D, and E. There was no indication of an excitatory phase with this compound.

A number of other compounds related to 5-HT were also tested but were almost entirely devoid of excitatory agonist activity. These compounds included: *N*-methyltryptamine, tryptamine, 5-hydroxyindole, 5-hydroxyindolylacetic acid, 5-methoxygramine, lyseric acid diethylamide, α-methyltryptamine, 5-methyltryptamine, tryptophol. In a few preparations single large doses of some of these compounds sometimes resulted in a slight excitation of the cell.

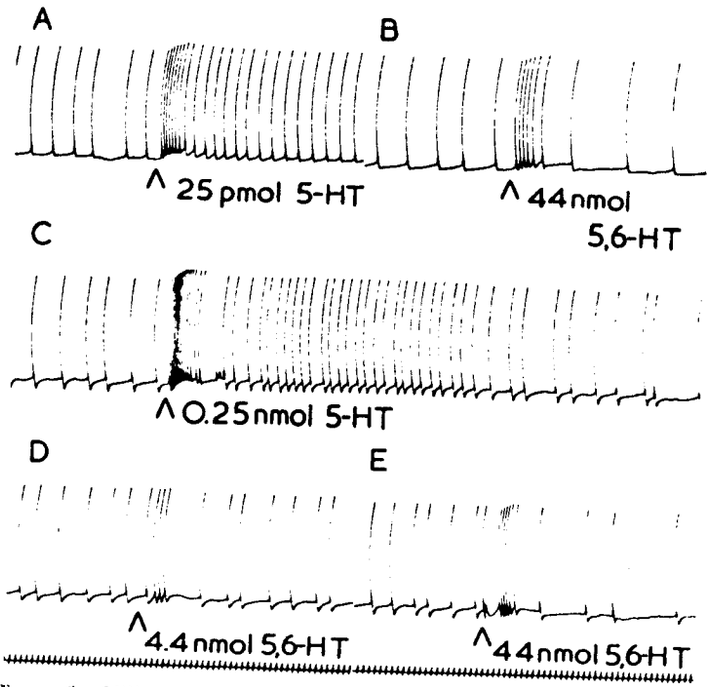

FIG. 7. The effects of 5-HT and 5,6-dihydroxytryptamine on the electrical activity of 2 cells from the brain of *Helix aspersa*. A, 6 mV. depolarization in response to 25 pmoles 5-HT. B, 4 mV. depolarization in response to 44 nmoles 5,6-dihydroxytryptamine. C, 8 mV. depolarization in response to 0·25 nmoles 5-HT. D, Threshold excitatory response to 4·4 nmoles 5,6-dihydroxytryptamine. E, 2 mV. depolarization followed by slight inhibition in response to 44 nmoles 5,6-dihydroxytryptamine. Traces A, B, C, D, and E are from the same cell. The time trace is in intervals of 1 second. The action potentials were 80 mV. in amplitude.

DISCUSSION

Structure–activity studies for 5-HT and tryptamine receptors have been carried out by a number of investigators; for example, rat uterus (Barlow and Khan, 1959a, b; Bertaccini and Zamboni, 1961), rat stomach strip (Barlow and Khan, 1959a, b; Vane, 1959), guinea-pig ileum (Bertaccini and Zamboni, 1961), rabbit ear (Bertaccini and Zamboni, 1961), *Helix* heart (Bertaccini and Zamboni, 1961), *Mercenaria* (*Venus*) heart (Greenberg, 1960), *Tapes* heart (Chong and Phillis, 1965), and the cat inferior mesenteric ganglion (Gyermek and Bindler, 1962). The structural requirements for the 5-HT receptors of 8 tissues compiled from the above work, together with the results from the present study, are summarized in *Table II*. In general only compounds that have been tested in the present study have been included in this table.

TRYPTAMINE

It can be seen from *Table II* that in several tissues (for example, rabbit ear, *Mercenaria* heart, *Tapes* heart, and *Helix* heart)

tryptamine is a potent agonist of 5-HT, being about ten times less potent than 5-HT. Tryptamine would also appear to be about ten times less potent than 5-HT in decreasing the tone of the catch muscle of *Anodonta* (Salanki and Labos, 1969). On *Helix* neurons tryptamine is devoid of any excitatory activity and is, in fact, a fairly potent and rapidly acting antagonist of 5-HT (Gerschenfeld and Stefani, 1966). At high concentrations tryptamine in fact inhibits cell activity (Walker and Woodruff, 1971). The only other tissue in *Table II* in which tryptamine has little if any activity is the cat inferior mesenteric ganglion (Gyermek and Bindler, 1962); although Curtis and Davis (1962), using the technique of iontophoretic injection, found that tryptamine was much less active compared to 5-HT in depressing orthodromic excitation of lateral geniculate neurons by volleys in the optic nerve of the cat. In their studies on the relative potencies of amines on cerebral cortical cells Krnjevic and Phillis (1963), also applying compounds by iontophoretic injection, found that tryptamine was equipotent with 5-HT as an inhibitory agent. However, these experiments were performed on animals under barbiturate anaesthesia and this anaesthetic has been shown to block the excitatory action of 5-HT (Roberts and Straughan, 1967). Krnjevic and Phillis found only inhibitory effects to analogues of tryptamine. It would be interesting to repeat a structure-activity study of mammalian neurons, where recently 5-HT has been shown to be excitatory (Roberts and Straughan, 1967; Boakes, Bradley, Briggs, and Dray, 1970).

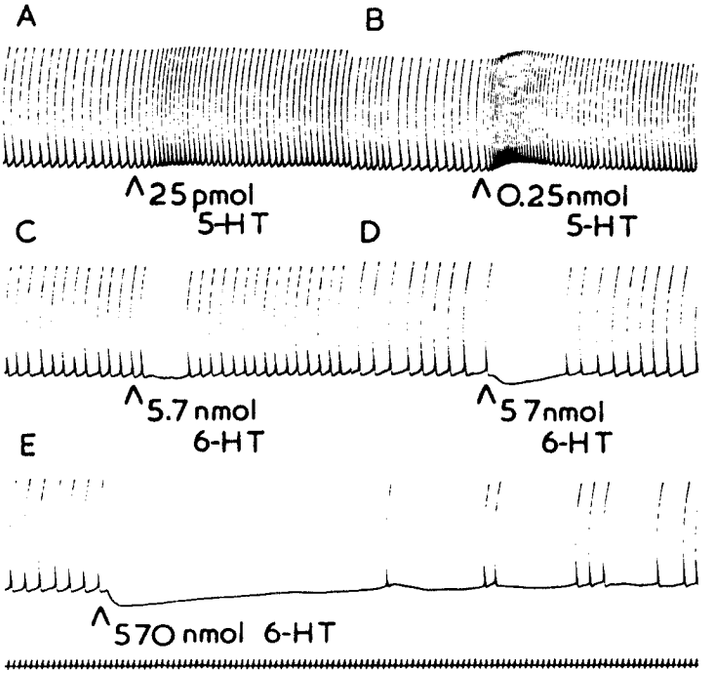

FIG. 8. The effects of 5-HT and 6-HT on the electrical activity of a cell from the brain of *Helix aspersa*. A, Threshold response to 25 pmoles 5-HT. B, 6 mV. depolarization in response to 0·25 nmole 5-HT. C, Threshold 2 mV. hyperpolarization in response to 5·7 nmoles 6-HT. D, 8 mV. hyperpolarization in response to 57 nmoles 6-HT. E, 12 mV. hyperpolarization in response to 570 nmoles 6-HT. All traces are from the same cell. The time trace is in intervals of 1 second. The action potentials were 80 mV. in amplitude.

α-METHYL-5-HYDROXYTRYPTAMINE

The addition of a methyl group on the α carbon of the side chain produced only a

Table II.—A Comparison of the Equipotent Molar Ratios of a Series of Tryptamine Analogues on 9 Tissues

Compound	Tissue								
	Rat Uterus	Guinea-pig Ileum	Rabbit Ear	Rat Stomach	Cat Ganglion	*Mercenaria* Heart	*Lytechinus* Heart	*Helix* Heart	*Helix* Neurons
5-Hydroxytryptamine	1	1	1	1	1	1	1	1	1
Tryptamine	176	280	20	480	3000	10	13	22	Inhibition
α-Methyl-5-hydroxytryptamine	1·3	8	3·3	1·4	500	—	—	8·2	6·7
α-Methyltryptamine	0	0	0	31	—	8·6	—	0	0
N-Methyl-5-hydroxytryptamine	1	7	1·7	6·3	2	—	—	—	4·5
N-Methyltryptamine	128	48	17	1120	—	3·7	—	—	0
Bufotenine	4	4	11	10	1·2	0·028	1	0·3	45·6
N,*N*-Dimethyltryptamine	200	38	35	196	80	10·7	—	10·7	10,000
5-Methoxytryptamine	9·6	9	3·4	20	320	—	7	0·5	120
Melatonin	0	0	345	—	—	—	—	—	3088
4-Hydroxytryptamine	11	26	22	1·8	0	—	100	15	—
Psilocin	—	—	—	—	0	—	—	—	3500
Psilocybin	330	760	0	—	0	—	—	18	4500
6-Hydroxytryptamine	0	0	133	460	0	—	35	47	Inhibition

slight reduction in potency on snail neurons. This finding is in agreement with results from many other tissues where α-methyl-5-hydroxytryptamine is approximately equipotent with 5-HT. In fact, Greenberg (1960) found α-methyl-5-hydroxytryptamine to be six times less potent on *Mercenaria* heart compared with 5-HT. This value agrees closely with the value of 6·7 for *Helix* neurons. It is difficult to account for the relative inactivity of this compound in stimulating the cat inferior mesenteric ganglion where it is five hundred times less potent than 5-HT. Further addition or substitution does seriously reduce potency on snail neurons.

α-Methyltryptamine

The important role of the hydroxyl group on the 5 position of the indole nucleus for activity can clearly be seen by comparing the potencies of α-methyltryptamine with α-methyl-5-hydroxytryptamine (*Table II*). The loss of the hydroxyl group reduces the activity in 5 tissues: rat uterus, guinea-pig ileum, rabbit ear, and *Helix* heart and neurons; from one to ten times less active than 5-HT to a complete loss of activity. Only on the rat stomach and *Mercenaria* heart has α-methyltryptamine any 5-HT-like activity.

N-Methyl-5-hydroxytryptamine

Generally the addition of a methyl group to the terminal nitrogen of 5-HT has a similar effect to that of adding a methyl group to the α carbon of the side chain (*Table II*). There is one noticeable exception: the cat inferior mesenteric ganglion, where *N*-methyl-5-hydroxytryptamine is approximately equipotent with 5-HT while α-methyl-5-hydroxytryptamine is five hundred times less potent.

N-Methyltryptamine

The importance of the hydroxyl group is again emphasized by comparing the potencies of *N*-methyltryptamine and *N*-methyl-5-hydroxytryptamine. Removal of the hydroxyl group reduces the potency by a factor ranging from ten to a hundred. When added in high doses to snail neurons this compound produces a slight excitation but the potency

ratio would be more than 100,000 compared with 5-HT.

BUFOTENINE (*N,N*-DIMETHYL-5-HYDROXYTRYPTAMINE)

Bufotenine is forty-five times less potent than 5-HT on snail neurons. From *Table II* it can be seen that the snail neuron 5-HT receptor is the least reactive to bufotenine. On some tissues (for example, cat ganglion and *Tapes* heart) it is equipotent with 5-HT, while on the *Mercenaria* heart bufotenine is about fifty times more potent than 5-HT. Phillis and Tebecis (1967) found that bufotenine was more powerful than 5-HT as a depressant of the firing of thalamic neurons, though it is less potent than 5-HT as a depressor agent in the lateral geniculate (Curtis and Davis, 1962). It is equipotent with 5-HT as an excitant of the heart of the lamellibranch *Tapes* (Chong and Phillis, 1965). Bufotenine is of interest since it occurs naturally in a number of animals; in, for example, the skin of several amphibians (Erspamer, 1961). However, there is no evidence that it occurs in the nervous system or that it might act as a neurotransmitter agent.

N,N-DIMETHYLTRYPTAMINE

The loss of the hydroxyl group on the 5 position of bufotenine reduces the potency from 45 to less than 10,000 compared with 5-HT on snail neurons. It is difficult to estimate an accurate potency value for this compound as when it is given at high doses it slowly causes depolarization of the membrane potential with an increase in cell firing-rate. This acceleration is difficult to reverse. From *Table II* it can be seen that in most other tissues this compound is considerably less potent than bufotenine; for example, five hundred times less potent on *Mercenaria* heart. This compound is a very weak agonist of 5-HT in the lateral geniculate (Curtis and Davis, 1962). However, *N,N*-dimethyltryptamine is only three times less potent than bufotenine on the rabbit ear.

5-METHOXYTRYPTAMINE

Substitution of the hydroxyl group on the 5 position of the indole nucleus by a methoxy group results in a one-hundred-and-twenty-fold drop in potency on snail neurons. This is similar to the situation in the cat inferior mesenteric ganglion, where 5-methoxytryptamine is three-hundred-and-twenty times less potent than 5-HT. On many other tissues it is approximately ten times less potent than 5-HT. In one tissue, the snail heart, 5-methoxytryptamine is twice as potent as 5-HT. This compound is equipotent with bufotenine as a depressor of activity in the lateral geniculate (Curtis and Davis, 1962). 5-Methoxytryptamine has been shown to be present in the nervous system (Maickel and Miller, 1968).

MELATONIN (*N*-ACETYL-5-METHOXYTRYPTAMINE)

This compound occurs naturally in the vertebrate pineal gland (Lerner, Case, Takahashi, Lee, and Mori, 1958) and is a potent agent for lightening frog skin by acting on the melanocytes. It has little or no 5-HT-like activity on most of the tissues tested (*Table II*). Bertaccini and Zamboni (1961) found that melatonin had no effect on the rat uterus or guinea-pig ileum. It does have slight activity on the rabbit ear, in the lateral geniculate (Curtis and Davis, 1962), and on snail neurons. Even at high doses melatonin rarely produced a rapid depolarization of the snail neuron membrane potential but only increased the firing rate, which resulted in a slight depolarization. *N*-Acetyl-5-hydroxytryptamine had a similar effect to melatonin on snail neurons. This indicates the importance of an unsubstituted terminal nitrogen on the side chain.

PSILOCIN AND PSILOCYBIN (*N,N*-DIMETHYL-4-HYDROXYTRYPTAMINE AND THE PHOSPHATE DERIVATIVE)

Changing the position of the hydroxyl group from the 5 to the 4 position generally results in only a slight loss of potency (*Table II*). However, on the cat inferior mesenteric ganglion 4-hydroxytryptamine is inactive. In contrast, it is more potent than 5-HT in the lateral geniculate (Curtis and Davis, 1962). Since there are certain similarities between the 5-HT receptor on the cat

inferior mesenteric ganglion and the snail neuron 5-HT receptor, it would be interesting to test the effect of this compound on snail neurons. Both psilocin and psilocybin are relatively inactive on snail neurons and never produce a rapid depolarization of the membrane potential but only a slow depolarization which is very difficult to reverse. However, both compounds appear to act on 5-HT receptors, since in the presence of either compound the 5-HT response is reduced. Structurally both psilocin and psilocybin are similar to *N*,*N*-dimethyltryptamine, except for the addition of the hydroxyl group on the 4 position. If the potencies of psilocybin and *N*,*N*-dimethyltryptamine are compared (*Table II*), considerable variation can be seen between tissues. For example, while *N*,*N*-dimethyltryptamine is active on both the rabbit ear and cat ganglion, psilocybin is inactive. In one case—the rabbit ear—4-hydroxytryptamine is fairly potent, while it is inactive on the cat ganglion. Conversely, on the snail heart and neurons psilocybin is slightly more potent compared with *N*,*N*-dimethyltryptamine. Both psilocin and psilocybin are more potent than *N*,*N*-dimethyltryptamine on the lateral geniculate (Curtis and Davis, 1962).

5,6-Dihydroxytryptamine and 6-Hydroxytryptamine

Both these compounds cause inhibition of the activity of snail neurons. The dihydroxytryptamine analogue causes either excitation or inhibition, or both, depending on the preparation and on the dose of the compound. On some preparations it was devoid of activity. 6-Hydroxytryptamine always inhibited cell activity and higher doses hyperpolarized the membrane potential. These cells are also inhibited by dopamine and it is possible that 6-hydroxytryptamine is interacting with the dopamine receptor. Further experiments are required to determine the site of action of this compound, and of tryptamine. There have been few observations on the potency of 5,6-dihydroxytryptamine on 5-HT receptors, though Carlisle (1964) found that it was ten to one hundred times more potent than 5-HT on certain crustacean hearts. In general, 6-hydroxytryptamine mimics 5-HT but is less potent. On the rat uterus and the guinea-pig ileum it is inactive, which is of interest as tryptamine has some 5-HT-like activity on these tissues. On both the *Tapes* and *Helix* hearts 6-hydroxytryptamine is only slightly less potent than tryptamine. 6-Hydroxytryptamine has been shown to be present in heart extracts of the crab *Carcinus maenas* and is about ten times more potent than 5-HT as an excitant of crab heart activity (Kerkut and Price, 1964). In the mammalian central nervous system Krnjevic and Phillis (1963) found 4-hydroxytryptamine, 5-HT, and 6-hydroxytryptamine to be equipotent, but neither 4-hydroxytryptamine nor 6-hydroxytryptamine had any excitatory action on the cat ganglion (Gyermek and Bindler, 1962). Curtis and Davis (1962) found that 6-hydroxytryptamine was less potent while 7-hydroxytryptamine was more potent than 5-HT on cells in the cat lateral geniculate nucleus.

From the present study certain general points concerning 5-HT excitatory receptors on snail neurons can be made. The action of tryptamine analogues on snail neurons can be divided into four groups:

1. Those which are inactive as excitatory agonists, and at high concentrations may antagonize the action of 5-HT; for example, tryptamine.

2. Those which mimic 5-HT in their mode of action; for example, α-methyl-5-hydroxytryptamine, *N*-methyl-5-hydroxytryptamine, bufotenine, 5-methoxytryptamine.

3. Those which produce a slow depolarization when applied at high doses and increase the action potential frequency; for example, psilocin, psilocybin, *N*,*N*-dimethyltryptamine, *N*-methyltryptamine, α-methyltryptamine. These also desensitize the membrane to 5-HT.

4. Those which inhibit cell activity and hyperpolarize the membrane potential; for example, 6-hydroxytryptamine, tryptamine.

From the present study it would appear that for potent 5-HT-like activity agonists should contain the following groups:—

1. An indole nucleus.

2. A hydroxyl group on the 5 position.

3. A substituent on either the terminal nitrogen or the α carbon of the side chain no greater than one methyl group.

NOTE (added at proof stage)

Recently a sample of 4-HT has been tested on *Helix aspersa* neurons. This compound was found to be eight hundred times less active compared with 5-HT.

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