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THE ACTION OF 5-HYDROXYTRYPTAMINE AND RELATED COMPOUNDS ON THE ACTIVITY OF RETZIUS CELLS OF THE LEECH *Hirudo medicinalis*

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1 The equipotent molar ratios of a range of tryptamine analogues, as compared with 5-hydroxytryptamine (5-HT), have been determined on the basis of their ability to hyperpolarize the membrane potential of the Retzius cell of the leech, *Hirudo medicinalis.*

2 The substitution of methyl, fluoro, chloro, methoxy or acetyl groups onto the 5-HT molecule progressively reduced the potency.

3 5-Methoxylation or terminal *N*-methylation of tryptamine considerably increased the potency of tryptamine but these compounds tended to depolarize cells rather than cause hyperpolarization. In some experiments they were ineffective on preparations pretreated with 5-HT.

4 It is suggested that these compounds may act by a different mechanism from the 5-hydroxylated indoles, perhaps involving a different receptor.

Introduction

Structure-activity studies have been performed on a wide range of vertebrate and invertebrate tissues in an attempt to gain information concerning the nature of the tryptamine receptor site (Barlow & Khan, 1959a, b; Vane, 1959; Greenberg, 1960; Bertaccini & Zamboni, 1961; Curtis & Davis, 1962; Gyermek & Bindler, 1962; Chong & Phillis, 1965; Berridge, 1972; Walker & Woodruff, 1972). Vane (1959) has shown that such studies may be complicated by the presence of monoamine oxidase.

Intracellular electrical recordings from leech Retzius cells were first made by Eckert (1963) and Hagiwara & Morita (1962). Using similar methods, Kerkut & Walker (1967) have shown that these cells are hyperpolarized by 5-hydroxytryptamine (5-HT) and this inhibition is mediated through an increase in permeability to chloride ions (Walker & Smith, 1973). 5-HT has been shown to be present in leech segmental ganglion cells, including the Retzius cells, and is a possible transmitter agent in this nervous system (Kerkut, Sedden & Walker, 1967; Rude, Coggeshall & Van Orden, 1969). Recently, the 5-HT level and aromatic acid decarboxylase activity has been determined in Retzius cells (Coggeshall, Dewhurst, Weinreich & McCaman, 1972; McCaman, Weinreich & McCaman, 1973); a level of 2.5 pmol/cell of 5-HT was found. However, as yet there is no evidence for monoamine oxidase activity in leech neurones (Marsden, 1970).

This paper presents evidence concerning the structure-activity requirements for the 5-HT receptor of the leech Retzius cell. A preliminary report of some of this work has been given to the Pharmacological Society (Smith & Walker, 1973).

Methods

All experiments were performed on the isolated segmental ganglia of the medicinal leech *Hirudo medicinalis.* The animals were obtained from a local dealer and kept in distilled water in an aquarium at a temperature of around 10°C. Leeches were pinned ventral side down to a wax block and dissected by a dorsal incision. The viscera were cleared aside to expose the ventral nerve cord enclosed in its blood sinus. The nerve cord was carefully removed from the animal and stored in leech Ringer. Fresh leeches were used each day. A section of cord containing three ganglia was mounted on 'silastic' on a glass slide and viewed with a binocular dissecting microscope, magnification 24x. The blood sinus was carefully slit to expose the ventral surface of the ganglion. The Retzius cells were then clearly visible. The preparation was placed in the experimental bath (volume 20 ml) and viewed with an 'Olympus' binocular microscope, magnification 80x. Nicholls & Kuffler (1964) have shown that compounds added to the bathing

medium easily reach the neurones of the leech ganglia without further dissection.

Retzius cells were identified by their position on the anterior ventral side of the ganglion, by their size (60-80 μm diameter) and by the form of their bioelectric potentials. The resting potential was 40-50 mV and the action potentials with their characteristic after potentials were generally between 30 and 40 mV and did not have an overshoot. Later, Retzius cells could be further identified by their hyperpolarization when 5-HT was added to the bath. The Ringer used had the following composition (mM) (Kuffler & Potter, 1964): NaCl 115; KCl 4; $CaCl_2$ 2; glucose 10; tris HCl buffer pH 7.4 10.

Intracellular recordings were made from Retzius cells with glass microelectrodes, 20-60 MΩ resistance, filled with molar potassium acetate, pH 7. The potentials were amplified with a Medistor negative capacity electrometer amplifier and displayed on a Tektronix 502A oscilloscope or a Solartron digital voltmeter. Permanent traces were obtained with an AEI or a Watanabe pen oscillograph.

To determine the potency of analogues, a standard dose of 5-HT (2.6-10.4 nmol), corresponding to a concentration of 10^{-7} -4×10^{-7} M, was administered and the resulting hyperpolarization noted. After washing with leech Ringer, doses of analogue were given in a 5 min cycle until a response the same size as that to the standard 5-HT was obtained. A second standard dose of 5-HT was then administered to ensure that no desensitization had occurred. In other preparations a dose of 5-HT was bracketed between two standard doses of analogue in an attempt to eliminate any desensitization.

Such a sequence was not possible for all the analogues as some of them were blocked by 5-HT and one blocked the response to 5-HT. The equipotent molar ratio (epmr) for each analogue was calculated for at least six experiments from the ratio of the number of nmol producing comparable responses. All the analogues were less potent than 5-HT so more was required to produce the response and the epmr was greater than one. For some compounds the magnitude of the response was examined both before and after the preparation had been exposed to iproniazid (50 μg; bath concentration 0.9×10^{-5} M) for three periods of 1 minute.

All drugs were dissolved in leech Ringer wherever possible but some had to be dissolved in distilled water or dilute HCl and then neutralized with NaOH. A similar amount of acid and alkali had no effect on cell activity when administered without the analogue.

The dose-response curves represent the average of five experiments. With 5-HT and α-methyl-5-hydroxytryptamine a 4 min cycle was used but with 5-methoxy *N,N*-dimethyltryptamine it had to be lengthened to 10 minutes. High doses of α-methyl-5-hydroxytryptamine desensitized the preparation but when a single large dose was applied to a fresh preparation a maximum response similar in size to a 5-HT response was obtained.

Drugs used

5-Hydroxytryptamine creatinine sulphate complex (Sigma); *N*-methyl-5-hydroxytryptamine oxalate (Aldrich); α-methyl-5-hydroxytryptamine creatinine sulphate (Upjohn); 5-chlorotryptamine hydrochloride (gift from Dr M.J. Berridge); Bufotenine (*N,N*-dimethyl 5-hydroxytryptamine) (Sandoz); tryptamine hydrochloride (B.D.H.); 5-methoxytryptamine (Koch-Light); 5-methyltryptamine hydrochloride (Ralph N. Emanuel); α-methyltryptamine (Ralph N. Emanuel); *N*-methyltryptamine (Ralph N. Emanuel); *N,N*-dimethyltryptamine bioxalate (Koch-Light); *N,N*-dimethyl 5-methoxytryptamine (Aldrich); 6-hydroxytryptamine creatinine sulphate complex (Regis Chemical Co.); Psilocin (*N,N*-dimethyl-4-hydroxytryptamine) (Sandoz); 4-hydroxytryptamine bioxalate (gift from Dr S. Maddrell); 5-hydroxytryptophol (Regis Chemical Co.); DL-5-hydroxytryptophan (Koch-Light); *N*-acetyl-5-hydroxytryptamine (Sigma); 3(2-aminoethyl)-5-hydroxyindene oxalate (gift from Dr R. Pinder); 5-fluorotryptamine hydrochloride (Koch-Light); Melatonin (*N*-acetyl-5-methoxytryptamine) (Koch-Light); iproniazid phosphate (Sigma).

Results

These are summarized in Table 1. *N*-methyl-5-hydroxytryptamine, α-methyl-5-hydroxytryptamine, bufotenine, 4-hydroxytryptamine, 6-hydroxytryptamine, 3(2-aminoethyl)-5-hydroxyindene, 5-chlorotryptamine and 5-fluorotryptamine produced hyperpolarizations which were very similar to those produced by 5-HT, although the responses were longer in the case of the two halogenated analogues. In some preparations bufotenine was almost as potent as 5-HT (Figure 1 a and b).

Certain compounds without an hydroxyl group, that is, 5-methoxytryptamine, α-methyltryptamine, *N*-methyltryptamine, *N,N*-dimethyltryptamine and 5-methoxy *N,N*-dimethyltryptamine as well as *N*-acetyl-5-hydroxytryptamine, tended to depolarize and excite cells. This was in general at doses greater than 30 nmol (1.5 x

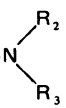
Table 1 Equipotent molar ratios (epmr) of tryptamine-like substances as compared with 5-hydroxytryptamine (5-HT) on the leech Retzius cell preparation

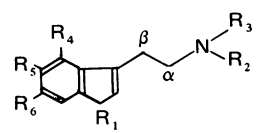
Drug	R_1	R_2	R_3	R_4	R_5	R_6	α	*No. of expt.*	*Equipotent molar ratio Range*	*Mean*
5-Hydroxytryptamine	N	H	H	H	OH	H	H	–	–	1.0
3(2-Aminoethyl)5-hydroxyindene	C	H	H	H	OH	H	H	6	1.4-4.6	2.7
α-Methyl-5-HT	N	H	H	H	OH	H	CH_3	6	1.9-4.8	3.1
N-methyl-5-HT	N	CH_3	H	H	OH	H	H	6	3.9-8.7	6.5
N,N-dimethyl 5-HT*	N	CH_3	CH_3	H	OH	H	H	7	1.7-26.4	10.0
N-methyltryptamine	N	CH_3	H	H	H	H	H	6	2.3-22.9	15.3
N,N-dimethyltryptamine	N	CH_3	CH_3	H	H	H	H	7	2.8-55.0	16.7
5-Fluorotryptamine	N	H	H	H	F	H	H	6	14.2-37.8	26.8
N,N-dimethyl 5-methoxytryptamine	N	CH_3	CH_3	H	CH_3O	H	H	6	9.6-96.0	31.9
5-Chlorotryptamine	N	H	H	H	Cl	H	H	7	8.7-139.2	54.7
5-Methoxytryptamine	N	H	H	H	CH_3O	H	H	7	21.3-284.5	77.1
Tryptamine	N	H	H	H	H	H	H	7	38.3-204.0	118.5
5-Methyltryptamine	N	H	H	H	CH_3	H	H	6	63.4-380.0	205.5
6-Hydroxytryptamine	N	H	H	H	H	OH	H	6	100.0-750.0	295.8
4-Hydroxytryptamine	N	H	H	OH	H	H	H	5	152.0-570.0	326.5
N,N-dimethyl-4-HT**	N	CH_3	CH_3	OH	H	H	H	6	263.9-2639	1031
α-Methyltryptamine	N	H	H	H	H	H	CH_3	7	91.6-3435	1289
N-acetyl-5-HT	N	CH_3CO	H	H	OH	H	H	6	1843-7371	3104
Melatonin	N	CH_3CO	H	H	CH_3O	H	H	–	inactive	–
5-Hydroxytryptophol	N	OH†	–	H	OH	H	H	–	inactive	–
5-Hydroxytryptophan	N	H	H	H	OH	H	COOH	–	inactive	–

As the numerical value for the epmr increases so the potency of the compound compared with 5-HT decreases.

* *N,N*-dimethyl 5-HT = Bufotenine

** *N,N*-dimethyl 4-HT = Psilocin

† 5-Hydroxytryptophol,  replaced by OH.



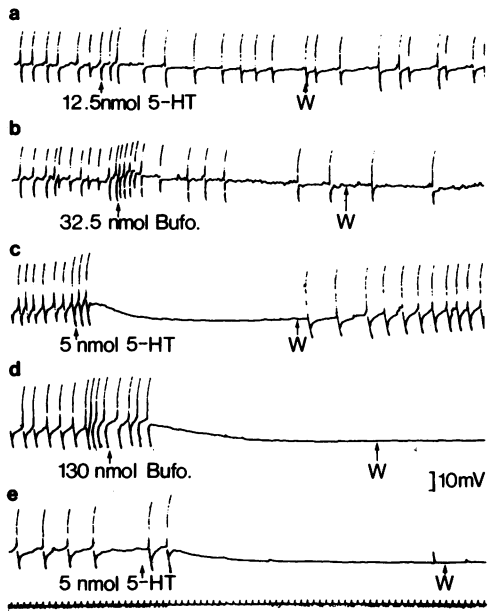


Fig. 1 A comparison of the effect of bufotenine on two different leech preparations: (a and b; c, d and e). (a) 12.5 nmol 5-hydroxytryptamine (5-HT) produced a 3 mV hyperpolarization; (b) 32.5 nmol bufotenine (Bufo) was required to produce the same response (epmr = 2.6). Preparation, 130 nmol bufotenine produced a 6 mV hyperpolarization (c), the same as that produced by 5 nmol 5-HT (epmr = 26) (c) and (e). Time trace in intervals of 1 second.

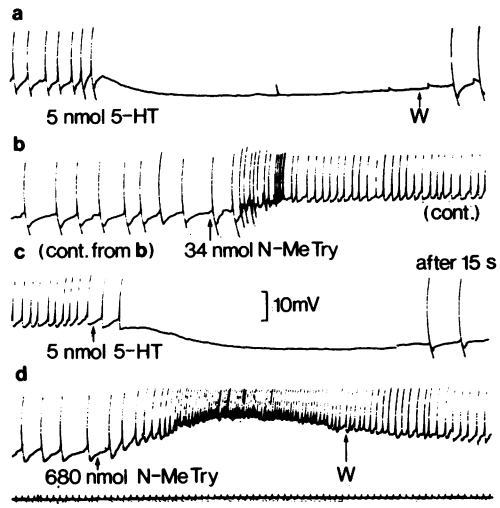


Fig. 2 Traces show that certain tryptamine analogues produced depolarization rather than hyperpolarization of Retzius cell membrane potential. (a) The preparation hyperpolarized by 6.5 mV when treated with 5 nmol 5-hydroxytryptamine (5-HT) but depolarized by 5 mV when treated with 34 nmol *N*-methyltryptamine (N-Me Try) (b). Treatment of the depolarized cell with 5 nmol 5-HT resulted in an 11 mV hyperpolarization, i.e. the cell was hyperpolarized to the same level as before (c). Larger doses (680 nmol), of *N*-methyltryptamine produced larger depolarizations (11 mV in d). Time trace in intervals of 1 second.

10^{-6} M). The response to *N*-methyltryptamine, the most potent excitatory compound tested, is illustrated in Figure 2 b and d. At low doses this compound produced a permanent hyperpolarization. When the response to this compound was tested in a Ringer with high magnesium content (20 mM), the excitatory response still occurred. High magnesium Ringer is known to block synaptic transmission and this experiment suggests that this excitatory effect is a direct one and not mediated via an interneurone.

In some experiments the responses to analogues could be blocked by pretreatment with 5-HT. This occurred in three cells out of 11 with tryptamine, in all cells tested with 5-methoxytryptamine, in three out of six with 5-methyltryptamine, in one cell out of five with α-methyltryptamine, in three cells out of six with *N*-methyltryptamine, in three cells out of seven with *N,N*-dimethyltryptamine and in four cells out of six with 5-methoxy *N,N*-dimethyltryptamine. It is possible that the above compounds may have been acting by the release of 5-HT or some other inhibitory transmitter. However, the response to tryptamine persisted in the presence of high magnesium (20 mM), suggesting that the release of 5-HT or some other inhibitory transmitter is not involved (Baylor & Nicholls, 1969).

Both α-methyltryptamine and 6-hydroxytryptamine sensitized cells to 5-HT. The only substance examined which blocked the action of 5-HT was 3(2-aminoethyl)-5-hydroxyindene which is a potent 5-HT agonist (Table 1: Figure 3a, b and c).

5-Hydroxytryptophol, 5-hydroxytryptophan and melatonin were all inactive on Retzius cells in the concentrations tested (which were partly limited by their solubilities). The maximum final bath concentrations administered were 6.25×10^{-4} M, 2.37×10^{-4} M and 1.73×10^{-4} M respectively.

The slightly different character of the responses of the 5-hydroxylated tryptamines as compared with those without a 5-hydroxyl group is also illustrated by their log dose-response

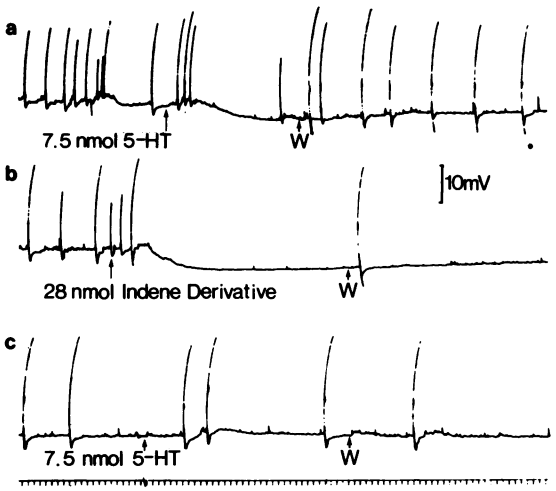


Fig. 3 Comparison of the effects of 3(2-aminoethyl)-5-hydroxyindene and of 5-hydroxytryptamine (5-HT) on Retzius cell activity. (a) 7.5 nmol 5-HT causes a 4 mV hyperpolarization; 28 nmol of the indene derivative produced a 6 mV hyperpolarization (b). After treatment with this substance, the response to a 7.5 nmol dose of 5-HT is blocked (c). Time traces in intervals of 1 second.

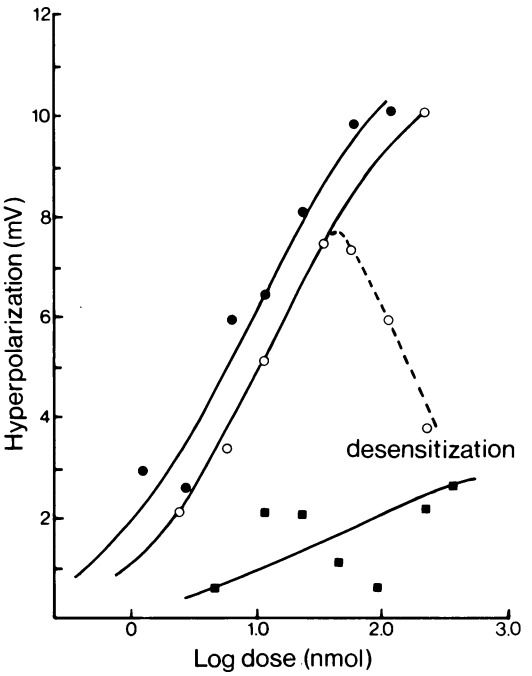


Fig. 4 A plot of $\log_{10}$ dose-response curve for 5-hydroxytryptamine (5-HT) (●), α-methyl-5-hydroxytryptamine (○) and 5-methoxy *N,N*-dimethyltryptamine (■). All plots are the average of five experiments. Note the different slope and maxima observed with the latter compound. Dotted curve, desensitization.

curves (Figure 4). The 5-hydroxylated analogue tested, α-methyl-5-hydroxytryptamine, gives the same maximal response as 5-HT and the log dose-response curve has a similar gradient. However, 5-methoxy *N,N*-dimethyl-tryptamine cannot produce the same maximal response and the log dose-response curve has a much shallower slope.

Discussion

The relative activities of tryptamine analogues on the leech Retzius cell are similar in some respects to results on other tissues. When the position of the hydroxyl group of the indole ring is altered, decreases in potency, similar to those observed in the leech, have been observed in the cat mesenteric ganglion (Gyermek & Bindler, 1962), in *Tapes* heart (Chong & Phillis, 1965), in *Calliphora* salivary gland (Berridge, 1972) and in *Helix* neurones (Walker & Woodruff, 1972). α-Methylation of both 5-HT and tryptamine decreases potency in agreement with the observations of Bertaccini & Zamboni (1961). *N*-methylation of tryptamine increases its activity in the leech and in the cat mesenteric ganglion (Gyermek & Bindler, 1962), in the *Mercenaria* heart (Greenberg, 1960) and in the guinea-pig ileum (Bertaccini & Zamboni, 1961) but not in the rat stomach (Vane, 1959).

Differences in relative activity on various preparations might be due to different rates of inactivation. Results with the leech Retzius cell are of particular importance because of its apparent lack of monoamine oxidase, and it was found that the magnitude of response was not increased by exposing the preparation to iproniazid. The results might also be affected by differences in inactivation by presynaptic uptake. Inactivation of 5-HT by presynaptic uptake has been demonstrated in the snail *Helix* (Cottrell, 1971).

The compounds appear to be of two types, the 5-hydroxylated and 5-halogenated compounds, which produce hyperpolarization at all doses and were not blocked by pretreatment with 5-HT, and the *N*-methylated, 5-methoxy-lated, and other compounds which tended to depolarize cells in high doses and whose hyperpolarizing action in low doses was blocked by pretreatment with 5-HT. The differences

between the two might be due to differences in inactivation, though not in inactivation by amine oxidase, but there is the strong suggestion that the two types are acting in different ways and possibly even at two different receptors. The log dose-response curves could be taken to indicate that some of the compounds are partial agonists or that they are acting at different receptors but the different effects of pretreatment with 5-HT suggest that the two types act in different ways. Experiments with combinations of the two types of compound might establish whether or not they were acting at the same receptors. The difference between the two types is of particular interest because the second type includes *N*-methyl and 5-methoxytryptamine, which are hallucinogenic, and it is possible that the two properties might be associated.

High activity on the leech Retzius cell is limited to molecules closely related to 5-HT; all the analogues were less active. The adverse effects on activity of 4-, 5-, and 6- substituents are not dissimilar from those observed on other preparations but in the present work it has been possible to note the relative unimportance of the indole NH group. In contrast to the marked effects of altering the side-chain amino group, the change from indole to indene in the 5-HT molecule produced a compound with half to one-third of its activity. It seems therefore that this part of the molecule is not particularly important in the interaction between 5-HT and the receptor (or receptors).

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