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RELATIVE ACTIVITIES OF SUBSTANCES RELATED TO 5-HYDROXYTRYPTAMINE AS DEPOLARIZING AGENTS OF SUPERIOR CERVICAL GANGLION CELLS

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Membrane potential changes evoked by 5-HT and related substances were recorded by the sucrose-gap method from rabbit ganglia superfused with Krebs solution at 20°C. A solution of the substance under test was injected into the superfusion stream. The activity of 23 substances was compared to that of 5-HT in respect of depolarizing capacity. 0.01 μmol 5-HT produced a near-threshold depolarization, while 0.6-0.8 μmol induced a maximal one. Some 5-HT analogues evoked prolonged responses distinctly different from the rapid depolarization and repolarization characteristic of 5-HT, while others were inactive. Compounds di- or trimethylated at the side-chain nitrogen atom were capable in addition of activating nicotinic receptors. The results suggest that: (1) the optimal requirements for activating ganglionic 5-HT receptors are a hydroxyl group at position 5 on the indole nucleus and a side-chain bearing an ethylamine amino group; (2) methyl substituents around the terminal nitrogen atom are well tolerated and a quaternary nitrogen may increase activity at the 5-HT receptor; and (3) substitution of a methyl group at carbon atom 2 of the indole nucleus reduces activity. A limitation of the technique is the difficulty of obtaining more than one dose-response curve from a particular preparation; a reduction in potency due to lower affinity cannot be readily distinguished from one due to lower intrinsic activity.

Superior cervical ganglion 5-HT receptors 5-HT analogues

1. Introduction

The presence of receptors for 5-hydroxytryptamine (5-HT) on ganglion cells in the superior cervical ganglion is well established (Trendelenburg, 1967; Wallis and Woodward, 1973; Haefely, 1974). 5-HT receptors are distinct from nicotinic and muscarinic receptors (Gyermek and Bindler, 1962; Wallis and North, 1978; Wallis, 1979), and have recently been shown to be blocked selectively by quipazine (Lansdown et al., 1980). The response evoked by 5-HT is a rapid depolarization (Wallis and Woodward, 1975), accompanied by an increase in membrane conductance. Increases in G_{Na} and probably also in G_{Ca} and G_K underly the potential change (Wallis and Woodward, 1975; Wallis and North, 1978). The depolarization is followed by a rapid repolarization and usually by an after-hyperpolarization. The latter appears to be the result of the electrogenic extrusion of Na^+ ions from the cell (Wallis and Woodward, 1975).

There have been several recent reports on the properties of other peripheral, neuronal (Drakontides and Gershon, 1968; Hirst and Silinsky, 1975; Fozard and Mobarok Ali, 1976) and non-neuronal 5-HT receptors (Apperley et al., 1976; Cooper and Wyllie, 1979; Dalton, 1979; Graf and Pletscher, 1979), as well as on central 5-HT receptors (Carlström et al., 1973; Bradley and Briggs, 1974; Haigler and Aghajanian, 1977). It was of interest, therefore, to compare these findings with those for ganglionic 5-HT

receptors whose properties are the subject of this paper. An attempt has been made to characterize the ganglionic 5-HT receptor using a range of substances related to 5-HT and, in addition, mescaline and phenyl biguanide.

The sucrose-gap technique which was employed in these experiments allows a direct measure of the potency of ganglion excitants, since it permits an estimate of change in resting membrane potential. Although accurate determinations of the equipotent molar ratio (epmr) are often difficult, it is important to attempt to express the results in a quantitative rather than a qualitative manner (see Methods). A comparison of the depolarizing ability of analogues of 5-HT can yield precise information about activity at 5-HT receptors only if the ligands examined interact solely with 5-HT receptors. Since it has been reported that the N-methylated compounds, N,N-dimethyltryptamine (DMT), N,N-dimethyl-5-hydroxytryptamine (DM5-HT) and N,N,N-trimethyl-5-hydroxytryptamine (TM5-HT) are capable of activating nicotinic receptors in cat sympathetic ganglia (Gyermek and Bindler, 1962; Haefely, 1974), preliminary experiments using hexamethonium (100 μmol/l) were performed to establish whether there was a nicotinic component in the response to methylated analogues of 5-HT. Such a component was only identified in the responses to DMT, DM5-HT and TM5-HT, but not, for instance, in the response to N-methyl-5-hydroxytryptamine (M5-HT). Responses to M5-HT were blocked by quipazine. The activity of DMT, DM5-HT and TM5-HT was assessed in the presence and absence of hexamethonium.

2. Materials and methods

2.1. Preparation and recording

Superior cervical ganglia were removed from adult rabbits anaesthetized with urethane (1.25-1.5 g/kg i.v. as a 25% w/v solution) and prepared as described by Wallis et al. (1975) for insertion into a sucrose-gap apparatus. In this version of the apparatus, the sucrose compartment is separated from adjacent chambers by rubber membranes (Kosterlitz and Wallis, 1966; Wallis et al., 1975). Potential changes induced by 5-HT or other substances were amplified and displayed on a potentiometric chart recorder (Servoscribe R.E. 511-20). Ganglia were superfused with Krebs solution at 20-22°C at a rate of 2-3 ml/min. Using this technique, the tissue may be maintained in vitro for many hours.

2.2. Test of depolarizing action

To avoid the tachyphylaxis that follows superfusion of the tissue with a solution of 5-HT in an effective concentration, injections of 5-HT dissolved in a small volume of Krebs solution were made into the superfusion stream to the ganglion (Wallis and Woodward, 1975). A depolarization, approximately two-thirds maximum is produced by 0.2 μmol (81 μg) of 5-HT and this quantity was used to evoke a standard response. Reproducible responses could be obtained if the flow of Krebs solution to the ganglion was controlled with the aid of a perfusion pump and drop chamber, and if the rate of injection was kept relatively constant at 1.5 ml/min. Injections of 5-HT or of related compounds were made from one of three 1 ml syringes mounted in a Perspex block through which the superfusion medium flowed. The syringe needles penetrated this block and were positioned so that their tips ejected material into the superfusion stream just upstream of the ganglion. All agonists were dissolved in Krebs solution.

2.3. Equipotent molar ratio

The relative activity of a compound with respect to 5-HT was expressed in terms of the molar amount equiactive with 5-HT, giving an equipotent molar ratio (epmr). The depolarization evoked by an agonist was matched as closely as possible in amplitude by one

evoked by an appropriate amount of 5-HT. It was not possible to construct full dose-response curves for analogues of 5-HT. Depolarization amplitude was measured from a projection of the trace preceding the response. In some experiments where close matching was not achieved, an adjustment was made by reference to a dose-response curve derived from pooled values from many experiments for the depolarization evoked by different amounts of 5-HT. When the analogue failed to produce any response or one too small to measure against the baseline noise, the compound was described as inactive; an epmr greater than a certain value could be assigned to it by comparison with the amount of 5-HT generating a threshold response (values in square brackets in table 1).

When a substance apparently failed to elicit a measurable response in a minority of preparations, the epmr was estimated by comparison with an amount of 5-HT generating a threshold response in these preparations and was pooled with other determinations to give a mean epmr. The frequency distribution of the values of epmr displayed a strong positive skew in the sets of observations where n was above 10 and approximated to log normal distributions. On the basis that all epmr values are log normally distributed, the geometric mean was used to express the mean epmr and the S.E.M. was calculated after transformation into log normal distribution as a measure of the scatter.

Most compounds tested were derivatives of tryptamine. Fig. 1 shows the numbering of atoms in the indolealkylamine molecule; the structure of these compounds may be read from this figure in conjunction with table 1.

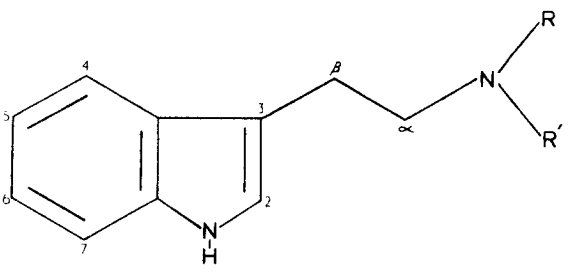

Fig. 1. Diagram to illustrate the numbering of atoms in the indolealkylamine molecule. R and R', substituents on terminal nitrogen atom.

2.4. Solutions and drugs

All solutions were made from distilled water passed through a deionizer. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.2; $NaHCO_3$ 25, $MgSO_4$ 1.2, glucose 11; it was gassed with 5% CO_2 and 95% O_2. The concentration of the sucrose solution superfusing part of the internal carotid nerve was 315 mM and taken to be isotonic.

The drugs used were 5-hydroxytryptamine creatinine sulphate (Sigma) or bimaleate (Koch-Light) (5-HT), tryptamine hydrochloride (BDH), 6-hydroxytryptamine creatinine sulphate (6-HT) (Sigma), 5,6-dihydroxytryptamine creatinine sulphate (5,6-DHT) (Sigma), 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT) (Sigma), N-methyl-5-hydroxytryptamine creatinine sulphate (M5-HT) (Upjohn), N-methyltryptamine (MT) (Sigma), α-methyl-5-hydroxytryptamine creatinine sulphate (αM5-HT) (Upjohn), N,N-dimethyl-5-hydroxytryptamine mono-oxalate (DM5-HT, bufotenine) (Sigma), N,N,N,-trimethyl-5-hydroxytryptamine bromide (TM5-HT, bufotenidine) (gift of Dr. R.B. Barlow), N,N-dimethyltryptamine (DMT) (Sigma), N,N-dimethyl-4-hydroxytryptamine (psilocin) (Sandoz), N,N-dimethyl-4-hydroxytryptamine dihydrogen phosphate ester (psilocybin) (Sandoz), N,N-dimethyl-3-(2-aminomethyl)-indole (gramine) (Sigma), 5-methoxytryptamine hydrochloride (5-MOT) (Sigma), N,N-dimethyl-5-methoxytryptamine (DM5-MOT) (Sigma), 2-methyl-N,N-dimethyltryptamine hydrochloride (gift of Dr. R.B. Barlow), 5-hydroxy-L-tryptophan (BDH), DL-tryptophan (Koch-Light), 5-hydroxyindole-3-acetic acid (5-HIAA) (Sigma), N-acetyl-5-methoxytryptamine (melatonin) (Sigma), D-lysergic acid diethylamide (LSD) (Sandoz), mescaline (Sigma), phenyl biguanide (gift of Dr. A.S. Paintal and Dr. J. Walker),

dopamine hydrochloride (Koch-Light), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Koch-Light), hexamethonium bromide (Sigma). All drugs were made up in Krebs solution immediately before the experiment.

3. Results

3.1. Responses evoked by 5-HT

Characteristically, the depolarization evoked by 5-HT was rapid and repolarization was followed by a small or moderate after-hyperpolarization (figs. 2A, 3A,B). Ganglia were normally responsive to 0.01 μmol 5-HT injected into the superfusion stream, which was approximately the amount required to evoke a threshold response. This was injected as a 100 μmol/l solution of 5-HT in Krebs. Between 0.6 and 0.8 μmol, injected as a 1 mmol/l solution in Krebs, was required to produce a maximal depolarization mediated via 5-HT receptors. It was noted that depolarizing agents acting via nicotinic receptors were capable of evoking depolarizations of greater maximal amplitude than those evoked by 5-HT. To check that neither the anion species associated with the amine nor the creatinine modified the responsiveness of the tissue, in several experiments the activity of 5-HT bimaleate was compared with that of 5-HT creatinine sulphate, the salt normally used. No difference in the depolarizing ability of the two salts could be detected.

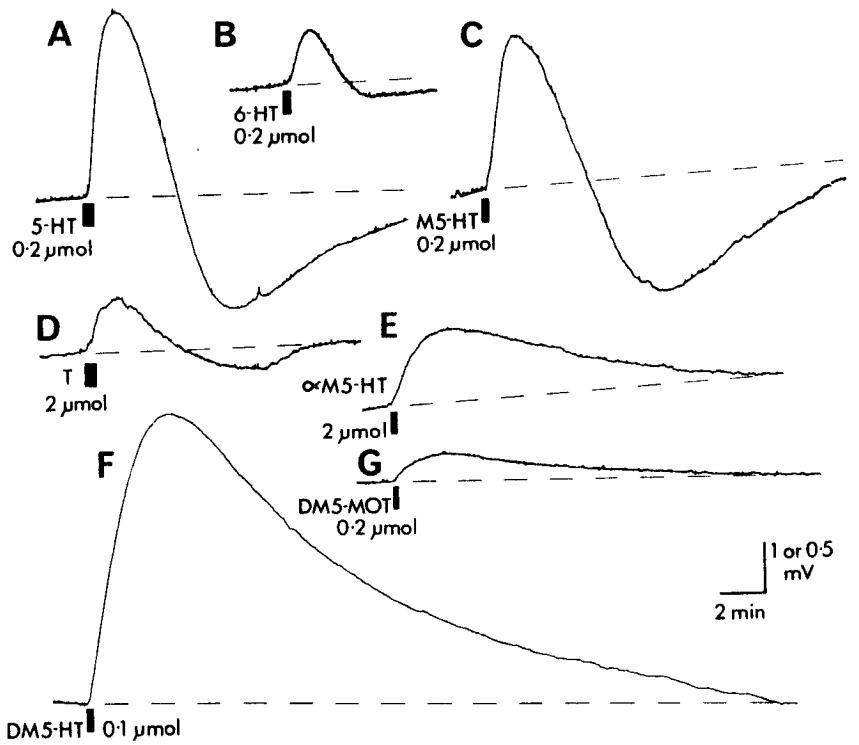

Fig. 2. Responses evoked by 5-HT and other tryptamine derivatives. Chart records of membrane potential change, ganglionic depolarization upwards. Black bars indicate the duration of the injections into the superfusion stream and the figures underneath show the quantity in μmol of agonist injected. Dashed lines are projections of the baseline preceding the responses. 0.5 mV calibration applies to D only. The records were obtained from more than one preparation.

3.2. Responses evoked by related indoles

Small modifications to the 5-HT molecule usually severely reduced the ability to depolarize the ganglion. In some cases, 5-HT analogues produced prolonged responses (see table 1), distinct in character from the rapid depolarization and repolarization induced by 5-HT or by the nicotinic agonist, 1-1-dimethyl-4-phenylpiperazinium (DMPP) (Lansdown et al., 1980).

The presence of a hydroxyl substituent at carbon atom 5 seemed crucial to activity at the ganglionic receptor. Tryptamine failed to evoke a measurable depolarization in some experiments and in others evoked only a small depolarization, even when 2 μmol was tested (fig. 2D). As an approximate measure of activity, the epmr was estimated by matching in amplitude the response to tryptamine with one evoked by an appropriate amount of 5-HT (see Methods). Tryptamine had about $\frac{1}{115}$th the potency of 5-HT (table 1). That the hydroxyl group is important for activity is indicated by the fact that its presence in a different position, as in 6-HT, increases the potency relative to tryptamine. 6-HT consistently evoked small depolarizations (fig. 2B) and its potency was about $\frac{1}{35}$th that of 5-HT. The addition of a second hydroxyl group in either position 6 or 7, as in 5,6-DHT or 5,7-DHT, reduced potency to approximately the same extent. Both compounds were about $\frac{1}{5}$th to $\frac{1}{4}$th as potent as 5-HT (table 1).

Unlike receptors on brainstem neurones in cats and rats (Bradley and Briggs, 1974) and neuronal receptors in the intrinsic nerve plexus of the guinea-pig ileum (Fozard and Mobarok Ali, 1978a), ganglionic 5-HT receptors were not depolarized by the 5-methoxy-derivative of tryptamine. 5-MOT, like certain non-indole amines, e.g. dopamine, induced a small hyperpolarization of the ganglion (fig. 3E,F), although dopamine was considerably more potent in this respect (fig. 3H).

The nature of the side chain attached to carbon 3 appeared also to be important. A carboxyl group at the α carbon atom, as in 5-hydroxytryptophan, reduced activity by somewhat less than the loss of the hydroxyl group in position 5 (table 1). However, in about one third of the ganglia tested, 5-hydroxytryptophan was inactive. Tryptophan was entirely without activity, as was 5-hydroxyindole acetic acid. Melatonin, in which the nitrogen is acetylated and the indole nucleus carries a methoxy group in position 5, was almost without activity (table 1). This substance is the N-acetylated analogue of 5-MOT, but unlike 5-MOT it produced no hyperpolarization of the ganglion.

3.3. Methylated analogues of 5-HT

Methylation of the side chain is of especial interest, since several of the N-methylated derivatives are potent hallucinogens. The di- and trimethylated derivatives (DM5-HT,TM5-HT) are capable of a dual action, activating both 5-HT and nicotinic receptors in cat ganglia (Haefely, 1974), in rabbit ganglia Wallis and Nash, 1980) and in terminals of sympathetic nerves (Fozard and Mobarok Ali, 1978b). Blockade of both species of receptor is required to suppress the responses. The high potencies of DM5-HT and TM5-HT which were observed (table 1) are, therefore, partly a consequence of activation of both 5-HT and nicotinic receptors. A more accurate index of the activity of N-methylated compounds at 5-HT receptors should be obtained if the nicotinic receptors are subjected to blockade. Quantitative comparison is still problematical, however, since hexamethonium is known to potentiate 5-HT depolarization of the ganglion (Wallis and Woodward, 1975; Wallis and Nash, 1980), while d-tubocurarine is relatively non-selective in antagonizing the depolarizing actions of 5-HT and the nicotinic agonist, DMPP (Wallis and Nash, unpublished). Nevertheless, by inducing nearly complete suppression of the depolarizations evoked by DMPP with hexamethonium (100 μmol/l), it was possible to obtain some estimate of the residual depolarizing activity of the N-methylated compounds. For compounds where

TABLE 1

Summary of structure and relative activities of tryptamine analogues as depolarizing agents on the superior cervical ganglion. Figures for epmr show geometric mean ± S.E.M. (log units) after transformation into log normal distribution; figures in square brackets derived from comparison with a threshold depolarizing amount of 5-HT; epmr values for DM5-HT, TM5-HT and DMT in parentheses are estimates in the presence of hexamethonium (100 μmol/l).

Name	Structure	Depolarizing (+) or hyperpolarizing (—) or no response (NR)	epmr	n	Prolonged response	Nicotinic component to response
5-hydroxytryptamine (5-HT)	5 = OH, R = H, R′ = H	+	1			
Tryptamine	R = H, R′ = H	+ or NR	113 (±0.09)	9		
6-hydroxytryptamine (6-HT)	6 = OH, R = H, R′ = H	+ or NR	35 (±0.14)	7		
5,6-dihydroxytryptamine (5,6-DHT)	5 = OH, 6 = OH, R = H, R′ = H	+	4.9 (±0.05)	7		
5,7-dihydroxytryptamine (5,7-DHT)	5 = OH, 7 = OH R = H, R′ = H	+	4.0 (±0.14)	4		
N-methyl-5-hydroxytryptamine (M5-HT)	5 = OH, R = CH_3 R′ = H	+	1.3 (±0.08)	9		
N-methyl-tryptamine (MT)	R = CH_3, R′ = H	+	35 (±0.22)	6		
α-methyl-5-hydroxytryptamine (αM5-HT)	5 = OH, α = CH_3, R = H, R′ = H	+	21 (±0.08)	6	+	
Bufotenine (DM5-HT)	5 = OH, R = CH_3, R′ = CH_3	+	0.08 (±0.09) (0.8 (±0.11))	28 (6)	+	+
Bufotenidine (TM5-HT)	5 = OH, R = $(CH_3)_2$ R′ = CH_3	+	0.01 (±0.09) (0.16 (±0.07))	15 (4)	+	++
Dimethyltryptamine (DMT)	R = CH_3, R′ = CH_3	+	2.6 (±0.08) (7.1 (±0.04))	10 (4)	(+)	+
Psilocin (DM4-HT)	4 = OH, R = CH_3, R′ = CH_3	+	89 (±0.16)	6	+	
Psilocybin	4 = H_2PO_4, R = CH_3, R′ = CH_3	+	59 (±0.23)	6	+	
Gramine	β = $N(CH_3)_2$	+	98 (±0.09)	5	+	
5-methoxytryptamine (5-MOT)	5 = CH_3O, R = H, R′ = H	—	[>500]	6		
Dimethyl-5-methoxytryptamine (DM5-MOT)	5 = CH_3O, R = CH_3 R′ = CH_3	+	53 (±0.09)	7	+	
2-methyl-N, N-dimethyltryptamine	2 = CH_3, R = CH_3 R′ = CH_3	+	112 (±0.1)	4	+	
5-hydroxytryptophan	5 = OH, α = COOH	+ or NR	74 (±0.15)	16		
Tryptophan	α = COOH	NR	[>600]	4		
5-hydroxyindole acetic acid (5HIAA)	5 = OH, β = COOH	NR	[>450]	4		
Melatonin	5 = CH_3O, R = H R′ = HOOC, CH_3	+ or NR	283 (±0.16)	6	+	
Lysergic acid diethylamide (LSD)		+ or — or NR	43 (±0.13)	7		
Mescaline		+ or —	[>200]	5		
Phenyl biguanide		+	10.6 ± 0.15	6		

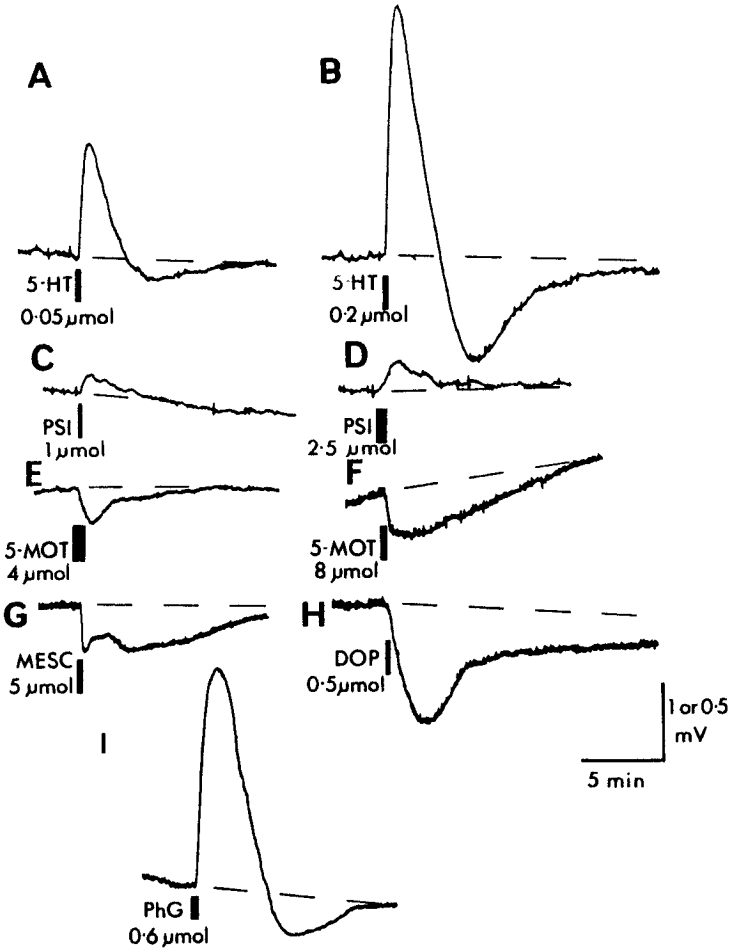

Fig. 3. Responses evoked by 5-HT, tryptamine derivatives and related compounds. Chart records of membrane potential changes, as in fig. 2. 0.5 mV calibration applies to traces F, G and H, 1 mV calibration to A, B, C, D, E and I. Psi, psilocin; mesc, mescaline; dop, dopamine; PhG, phenyl biguanide. Note hyperpolarizations induced by some compounds. Records A-D from same preparation, other records from different preparations.

preliminary experiments established that there was a nicotinic component to the response, the epmr was estimated both in the absence and the presence of hexamethonium; table 1 gives both values.

Methylation of the α-carbon atom reduced activity at the ganglionic 5-HT receptor. The response to 2 μmol αM5-HT was a prolonged, low amplitude depolarization (fig. 2E). αM5-HT had about $\frac{1}{20}$th the potency of 5-HT.

Methylation of the amino nitrogen, however, did not reduce activity significantly. The response to N-methyl 5-HT (M5-HT) was very similar to that induced by 5-HT itself (fig. 2C) and its potency was similar to that of 5-HT (epmr 1.3). Depolarizations evoked by M5-HT were not depressed by hexamethonium (100 μmol/l), but were blocked by quipazine (10 μmol/l). N-methylation of tryptamine increased activity three-fold relative to tryptamine. The N,N-dimethylated derivative of 5-HT (DM5-HT, bufotenine) gave responses of greater magnitude and duration than 5-HT. Thus 0.1 μmol generated a larger depolariza-

tion than 0.2 μmol 5-HT in the same experiment (fig. 2A) and the response displayed a notably prolonged time course (fig. 2F). The rate of repolarization following the peak of the response was always slow, but occasionally a small and prolonged after-hyperpolarization was also seen. DM-5HT was about 12 times more potent than 5-HT (table 1). The N,N,N-trimethylated derivative of 5-HT is a quaternary ammonium compound (TM5-HT, bufotenidine) and proved to be the most potent depolarizing agent tested on the superior cervical ganglion (see also Wallis and Nash, 1980). When injected in quantities equiactive with DM5-HT, it evoked large depolarizations of similar duration to those induced by the dimethylated compound. TM5-HT was about 100 times more potent than 5-HT (table 1).

The dimethylated tryptamine derivative (DMT) was somewhat less active than 5-HT. It evoked depolarizations of moderate duration and was about $\frac{1}{2}$ to $\frac{1}{3}$rd as potent as 5-HT; notable, however, was the increase in activity compared to N-methyltryptamine (table 1).

Introduction of a methyl substituent in the indole nucleus at position 2 as in 2-methyl-N,N-dimethyltryptamine greatly reduced activity. This substance had about $\frac{1}{110}$th the potency of 5-HT (table 1) and about $\frac{1}{45}$th that of DMT.

In the presence of hexamethonium (100 μmol/l), responses to DM5-HT, TM5-HT and DMT were all reduced, but the estimated potency of TM5-HT at 5-HT receptors was still much greater than that of 5-HT itself (epmr 0.16, table 1). DM5-HT, however, had an activity similar to that of 5-HT, the estimated epmr being 0.8 (table 1). The activity of DMT was reduced to about $\frac{1}{7}$th that of 5-HT (epmr 7.1, table 1).

3.4. The hydroxyl substituent in N-methylated compounds

The importance of the hydroxyl substituent at carbon atom 5 was again emphasized by the changes in activity observed in N-methylated compounds where it is displaced, methoxylated or absent. Thus, DM4-HT (psilocin), unlike DM5-HT, had an activity about $\frac{1}{90}$th of 5-HT and produced small, prolonged depolarizations (fig. 3C,D). Psilocybin had similar but possibly somewhat greater activity and evoked responses of a similar character. N,N-Dimethyl-5-methoxytryptamine (DM5-MOT) produced low amplitude, prolonged responses (fig. 2G). It displayed about $\frac{1}{50}$th the potency of 5-HT (table 1) and less than $\frac{1}{500}$th that of DM5-HT.

3.5. Other compounds tested

Amongst other compounds tested were those in which the side chain is shortened. Thus, gramine has a methylamine side-chain and the terminal nitrogen has two methyl substituents. That the length of the side chain is significant was indicated by the fall in activity associated with a reduction in its length. This substance was about 30 times less potent than DMT and 100 times less potent than 5-HT (table 1).

Hallucinogens such as LSD and mescaline may act at 5-HT receptors in the CNS. The indole moiety of LSD is fused within a heterocyclic ring system; it had only about $\frac{1}{45}$th the activity of 5-HT and often produced a biphasic response. The diethylamide moiety was essential for activity, since lysergic acid itself was inactive. Mescaline, a phenylethylamine structurally related to dopamine and not to 5-HT, had a feeble often hyperpolarizing effect at the ganglion not unlike that of 5-MOT or dopamine. Responses could be clearly biphasic in character (fig. 3G).

Phenyl biguanide, reported to be a potent excitant at 5-HT receptors on sensory nerve endings (Paintal, 1954) and on myenteric plexus neurones in the duodenum (Drakontides and Gershon, 1968) induced depolarization of ganglion cells. The response had the relatively short duration displayed by 5-HT (fig. 3I). However, phenyl biguanide had only about $\frac{1}{10}$th the activity of 5-HT.

4. Discussion

Unlike much earlier work, which relied on indirect measures of ganglion cell excitation such as contraction of the nictitating membrane or frequency of action potential discharge, the technique employed in this study allows a direct measure of the potency as ganglion excitants of a range of substances related to 5-HT. The responses are uninfluenced by presynaptic actions of 5-HT (Haefely, 1974; Wallis and Woodward, 1975), which complicate the effects of the amine on ganglionic transmission. The responses arise from a population of neurones at or near the Krebs solution—sucrose solution interface (Wallis et al., 1975), but it is not certain whether all, or merely some, of the cells in this population are depolarized by 5-HT (Wallis and North, 1978; Skok and Selyanko, 1979). A limitation of the technique is that it is usually not possible to construct full dose-response curves or, at most, only a single full curve from one preparation, principally because of the intervals that must be left between injections to avoid tachyphylaxis. Thus, we cannot distinguish between a reduction in potency due to lower affinity from one due to lowered intrinsic activity.

Responses evoked by various agonists varied in the rate of rise of the depolarization, in duration and in the presence or absence of a phase of after-hyperpolarization. Occasionally, no depolarization was recorded but a hyperpolarization. Although some of this variability might be a consequence of activation of a mixed population of receptors, e.g. nicotinic in addition to 5-HT receptors, this did not seem to be the case with the majority of compounds tested. In our experience, 5-HT has never been observed to induce an initial hyperpolarization (see also Wallis and Woodward, 1975) as reported by Haefely (1974) for the cat s.c.g. in situ. The after-hyperpolarizations observed are the consequence of the inward movement of sodium ions activating an electrogenic sodium pump; this inward flux of sodium ions underlies the depolarization evoked by 5-HT (Wallis and Woodward, 1975). Absence of an after-hyperpolarization does not necessarily point to the involvement of other receptors, nor is its presence a positive indication of activation of 5-HT receptors. Thus, nicotinic agents may evoke large after-hyperpolarizations (Lees and Wallis, 1974). Further, the presence or absence of an after-hyperpolarization and the magnitude of this potential are related to the rapidity with which the action of an agonist is terminated. Prolonged activation of receptors results in a prolonged membrane conductance increase and this may effectively short circuit the potential generated by electrogenic extrusion of Na^+ ions (Brown et al., 1972).

Most of the compounds investigated in this study were close analogues of 5-HT and are therefore likely to interact principally with 5-HT receptors. In cases where an analogue was relatively potent, preliminary experiments were done to exclude a nicotinic component to the response and to confirm sensitivity of the response to quipazine. It would have been impracticable, however, to test analogues in the presence of a larger range of antagonists. If interaction with 5-HT receptors may be assumed, then the results suggest that certain features of an indole molecule are particularly important for the activation of ganglionic 5-HT receptors.

(1) The molecule should have a hydroxyl group at the 5 position on the indole nucleus. Substitution of this group by a methoxyl group renders the compound inactive at the 5-HT receptor and the weak hyperpolarizing action observed seems most likely to arise as a result of stimulation of ganglionic dopamine or noradrenaline receptors. Mescaline, which also possesses methoxyl substituents on the benzene ring, sometimes induced similar small amplitude hyperpolarizations. Absence of the hydroxyl group, as in tryptamine, leads to a great loss in activity, but a depolarizing response is usually still observed. It is possible that the 5-hydroxyl group is involved with some part of the receptor molecule through

hydrogen bonding, its hydrogen atom acting as acceptor; in addition, the bulk of the methoxyl group might prevent access of the ligand to the receptor (see Horn, 1973). Tryptamine may be a useful ligand for differentiating certain indole-alkylamine receptors, since in several tissues, e.g. rabbit ear and *Helix* heart (Bertaccini and Zamboni, 1961), cerebral cortical neurones (Krnjevic and Phillis, 1963), it is a relatively potent agonist, while in other tissues it is much less active or devoid of agonist activity, e.g. s.c.g. (see above); inferior mesenteric ganglion (Gyermek and Bindler, 1962); and lateral geniculate neurones (Curtis and Davis, 1962).

The work of Fozard and Mobarok Ali (1978a) suggests that 5-MOT may also be a useful discriminator between sub-categories of 5-HT receptor. They found that 5-MOT is a potent agonist at the cholinergic intramural ganglion cells of the ileum, as it is on a number of other tissues, e.g. dorsal horn of neonate rat spinal cord (Preston and Wallis, unpublished), brain stem neurones of cat and rat (Bradley and Briggs, 1974), frog skin epithelial cells (Dalton, 1979), mid-brain raphe nuclei (De Montigny and Aghajanian, 1977), and rat stomach strip where 5-MOT is 20 times more active than tryptamine (Vane, 1959). On the other hand, 5-MOT displays little or no activity on sympathetic ganglion cells (Gyermek and Bindler, 1962; this paper), on sympathetic nerve terminals in the heart (Fozard and Mobarok Ali, 1978a), at the 5-HT uptake site of rat hypothalamus (Horn, 1973), and is much less active in the rat lateral geniculate and amygdala than in the mid-brain raphe (De Montigny and Aghajanian, 1977).

(2) The side-chain must bear a terminal amino group and cannot be shortened without loss of activity. Analogues with longer side chains than ethylamine were unavailable for testing. It is possible, according to Carlström et al. (1973) that certain indole compounds may have a side-chain whose preferred conformation is the extended form, while in others the folded conformation is preferred. They suggest that compounds which adopt similar conformations in crystal may display similar conformations when bound at a 5-HT receptor. Many analogues of 5-HT inactive at the ganglion and inactive also as hallucinogens possess folded side-chains according to X-ray crystallographic measurements, i.e. the amino nitrogen atom is rotated inward and is in the gauche position relative to the indole ring. These include tryptamine, tryptophans and 5-MOT. Other analogues possess extended side-chains in crystal and Carlström et al. (1973) suggest that this is a necessary requirement for an indole compound to act in the CNS as an hallucinogen. These compounds include DMT, DM5-HT, DM5-MOT, psilocybin and LSD, which are all hallucinogens, but also melatonin, which has no hallucinogenic activity. 5-HT itself may exist in both conformations in the crystalline state and in solution (Carlström et al., 1973). Although an extended side-chain may be a requirement for activity at ganglionic receptors, not all compounds with an extended side-chain were highly active, e.g. DM5-MOT, psilocybin and melatonin. This might be attributable, however, to the absence of a hydroxyl grou' on carbon atom 5. The ganglionic 5-HT receptor may, therefore, be less permissive of small changes to the interacting ligand than the CNS receptor at which hallucinogens act.

Methylation at the α carbon atom increases the affinity of a compound for the 5-HT uptake site in the hypothalamus (Horn, 1973), but in our experiments α-methylation reduced rather than increased activity. A reduction in activity was also observed by Walker and Woodruff (1972) in studies on snail (*Helix aspersa*) neurones, whilst Haefely (1974) reported that αM5-HT had a marked affinity for, but no intrinsic activity at, ganglionic 5-HT receptors, blocking the action of 5-HT itself. However, substitution of a single methyl group at the terminal nitrogen atom of 5-HT had little effect, suggesting that the receptor region interacting with this atom can tolerate small substituents. Among tryptamine analogues, N-methylation as in MT increased

activity compared to tryptamine itself. The increase in activity, seen when compounds di- or tri-methylated at the terminal nitrogen atom were tested, is difficult to interpret because of the ability of these compounds to activate nicotinic receptors (Wallis and Nash, 1980). However, even during nicotinic receptor blockade, TM5-HT was considerably more active than 5-HT, and DM5-HT, which in the hypothalamus and at sympathetic nerve terminals is less active than 5-HT (Horn, 1973; Fozard and Mobarok Ali, 1976), was approximately equiactive with 5-HT. Thus, these results suggest (3) that methyl substituents on the nitrogen atom do not impede access to the receptor and that a quaternary nitrogen may increase the activity of a compound at 5-HT receptors.

Finally (4), substitution of a methyl group at carbon atom 2 of the indole nucleus reduces activity, as can be seen by comparing the activity of 2-methyl-N,N-dimethyltryptamine with that of DMT.

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