Psilocin
3704

Neuropharmacology, Vol. 19, pp. 465 to 472

0028-3908/80/0501-0465$02.00/0

THE ACTION OF METHYLATED DERIVATIVES OF 5-HYDROXYTRYPTAMINE AT GANGLIONIC RECEPTORS

D. I. WALLIS and H. L. NASH
Department of Physiology, University College, Cardiff CF1 1XL, Wales

(*Accepted* 27 *November* 1979)

Summary—Changes in resting membrane potential induced by 5-HT, methylated derivatives of 5-HT and DMPP were recorded from the rabbit isolated superior cervical ganglion by the sucrose-gap method. Responses were evoked by injections into the superfusion stream to the ganglion of 0.01–0.2 μmol. Whereas 5-HT and DMPP evoked a relatively brief depolarization followed by an after-hyperpolarization, *N*,*N*-dimethyl 5-HT (DM5-HT) and *N*,*N*,*N*-trimethyl 5-HT (TM5-HT) evoked depolarizations of long duration. 5-Hydroxytryptamine and DMPP were approximately equipotent; DM5-HT was 8 times and TM5-HT 70 times more active than 5-HT.

Quipazine was a selective antagonist of responses to 5-HT and hexamethonium a selective antagonist of responses to DMPP. Quipazine (1 μM) reduced in amplitude responses to 5-HT by 94%, those to DM5-HT by 37%, and those to TM5-HT by 10%; responses to DMPP increased in amplitude by 42%. When superfused over the ganglion 5-HT (10 μM) reduced responses to 5-HT by 56%, those to DM5-HT by 27%, those to TM5-HT by 25%, and those to DMPP by 9%. Hexamethonium (100 μM) reduced responses to DMPP by 84%, those to DM5-HT by 64% and those to TM5-HT by 86%; there was no reduction of responses to 5-HT which were potentiated in 7 of 13 experiments. Thus, during nicotinic receptor blockade responses to 5-HT were often enhanced, while in the presence of quipazine responses to DMPP were often enhanced.

A dual action of DM5-HT and TM5-HT at ganglionic nicotinic and 5-HT receptors is likely. Potentiation of responses mediated via one species of receptor during blockade of the other suggests that 5-HT and nicotinic receptors may be in close association in the membrane.

The cells of the superior cervical ganglion (scg) of the rabbit are depolarized by 5-hydroxytryptamine (5-HT) acting on receptor sites which have been shown to be distinct from those activated by nicotinic and muscarinic ligands (Wallis and Woodward, 1973; 1975; Wallis and North, 1978). 5-Hydroxytryptamine also has an excitatory action at a variety of other autonomic ganglia (Trendelenburg, 1967; Haefely, 1974; Wallis, 1979). Evidence of the distinctness of the receptor sites derives from observations of the selective antagonism of one set of receptors or the other. Thus, many authors have noted that the excitatory actions of 5-HT at ganglia are resistant to nicotinic receptor blocking agents, such as hexamethonium (e.g. Trendelenburg, 1956; Drakontides and Gershon, 1968; de Groat and Lalley, 1973; Wallis and Woodward, 1975), and demonstrated selective blockade of the action of nicotinic agonists such as 1-1-dimethyl-4-phenyl piperazinium (DMPP) compared to those of 5-HT (Gyermek and Bindler, 1962; Watson, 1970). Selective blockade of ganglionic 5-HT receptors has been reported less frequently. These receptors are usually regarded as falling into that category of peripheral 5-HT receptor designated "M" by Gaddum and Picarelli (1957) because of their apparent sensitivity to morphine (see Trendelenburg, 1967). Since end-organ responses were usually the means by which the assessment of blockade was determined, the specificity of this blockade can be questioned. More recently, morphine has been reported to be ineffective as a selective antagonist of the excitatory actions of 5-HT at ganglia (Wallis and Woodward, 1973; Haefely, 1974), suggesting that these 5-HT receptors need to be re-classified in a more precise manner. A search for more specific antagonists has shown that quipazine is able to antagonize selectively responses evoked by 5-HT in the rabbit scg, while leaving unaffected or even potentiating those evoked by DMPP (Lansdown, Nash, Preston and Wallis, 1979; Lansdown, Nash, Preston, Wallis and Williams, 1980).

Although there is strong evidence that 5-HT and nicotinic agonists act on different receptor sites, the responses these ligand-receptor interactions induce show several similarities. Both the responses evoked by acetylcholine (ACh), or other nicotinic agonists, and those evoked by 5-HT are rapid depolarizations, accompanied by an increase in membrane conductance (Wallis and North, 1978). The potential change induced by ACh is dependent on an increase in G_{Na} and G_K (see Nishi, 1977), whereas that induced by 5-HT is dependent on an increase in G_{Na} and possibly

Key words: rabbit superior cervical ganglion, 5-HT receptors, nicotinic receptors, dimethyl 5-hydroxytryptamine, trimethyl 5-hydroxytryptamine, dual action.

G_{Ca} (Wallis and Woodward, 1975) and probably also in G_K (Wallis and North, 1978). In both cases inward movement of Na^+ ions underlies the depolarization. The depolarizations to both ACh and 5-HT are usually followed by an after-hyperpolarization, probably the consequence of the electrogenic extrusion of Na^+ ions from the cell (Wallis and Woodward, 1975). Further, there are reports that, at cat sympathetic ganglia, some agents appear to combine nicotinic with "5-HT-like" stimulating properties. Gyermek and Bindler (1962) showed that *N,N*-dimethyl 5-hydroxytryptamine (DM5-HT, bufotenine) had an action largely via nicotinic receptors, while Haefely (1974) concluded that it acted both at nicotinic and 5-HT receptors. There is an interesting parallel with the 5-HT receptors at the terminals of postganglionic sympathetic neurones in the rabbit heart. Fozard and Mobarok Ali (1978a) suggest a dual mechanism of stimulant action on the cardiac sympathetic nerves for DM5-HT, involving activation both of receptors sensitive to 5-HT and of nicotinic receptors.

This paper reports some effects of methylated derivatives of 5-HT, including DM5-HT and *N,N,N*-trimethyl 5-hydroxytryptamine (TM5-HT), on rabbit scg cells, and suggests that the results support the idea that nicotinic and 5-HT receptor sites on these cells are in close association.

METHODS

Preparation

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, Lees and Kosterlitz (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. 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 some other agonist 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 Perspex 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.

Equipotent molar ratio

The relative activity of a methylated 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 the methylated compound was matched as closely as possible in amplitude by one evoked by an appropriate amount 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 normalised dose-response curve for the depolarization evoked by different amounts of 5-HT.

The compounds tested were derivatives of tryptamine (Fig. 1), in most cases possessing a hydroxyl group at carbon atom 5. In 5-HT R and R′ are hydrogen atoms. *N*-methyl substitution around this nitrogen atom leads in successive steps to *N*-methyl 5-HT (M5-HT), *N,N*,-dimethyl 5-HT (DM5-HT) and *N,N,N*-trimethyl 5-HT (TM5-HT). The dimethylated derivative with a hydroxyl group at carbon atom 4 is psilocin. The nicotinic agonist DMPP was used standardly. Previous work has shown that it is specific for nicotinic receptors (see Trendelenburg, 1967) and it evokes relatively constant depolarizations in the ganglion, if sufficient time is left between injections (Lansdown *et al.*, 1979). Acetylcholine was not used as a nicotinic agonist because it is so readily hydrolysed by ganglionic cholinesterase that very large amounts

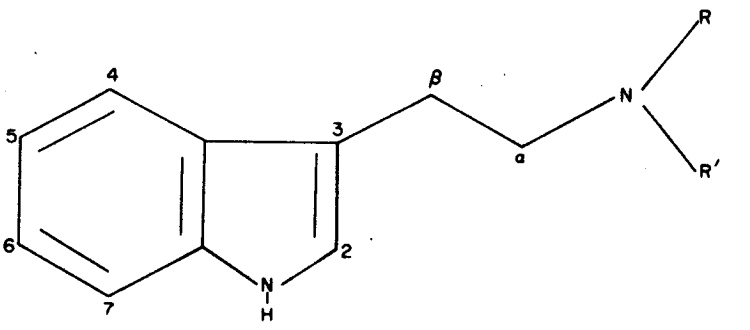

Fig. 1. Diagram to show numbering of atoms in tryptamine.

must be injected to evoke a depolarization (Wallis *et al.*, 1975; Wallis and Woodward, 1975).

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 and 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.

Antagonists at known concentrations were used to superfuse the ganglion in some of the experiments. The blocking actions of the antagonists generally required at least 30 min to reach a maximum at any given concentration. Dual or triple assessments of the degree of blockade were made in each experiment to ensure that blockade did not increase significantly over a more protracted time course.

The drugs used were 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma), *N*-methyl 5-hydroxytryptamine creatinine sulphate (M5-HT) (Upjohn), 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP) (Koch–Light), *N,N*-dimethyl 5-hydroxytryptamine monoxalate (DM5-HT, bufotenine) (Sigma), *N,N,N*-trimethyl 5-hydroxytryptamine (TM5-HT, bufotenidine) (gift of Dr R. B. Barlow), *N,N*-dimethyl tryptamine (DMT) (Sigma), *N,N,N*-trimethyl dopamine (TMD) (gift of Dr. R. B. Barlow), psilocin (Sandoz), hexamethonium bromide (Sigma), *d*-tubocurarine chloride pentahydrate (Sigma), 2-(1-piperazinyl) quinoline maleate (quipazine) (Miles Laboratories), phenoxybenzamine hydrochloride (Smith, Kline & French) and metoclopramide monohydrochloride (Beecham). All drugs were made up in Krebs solution.

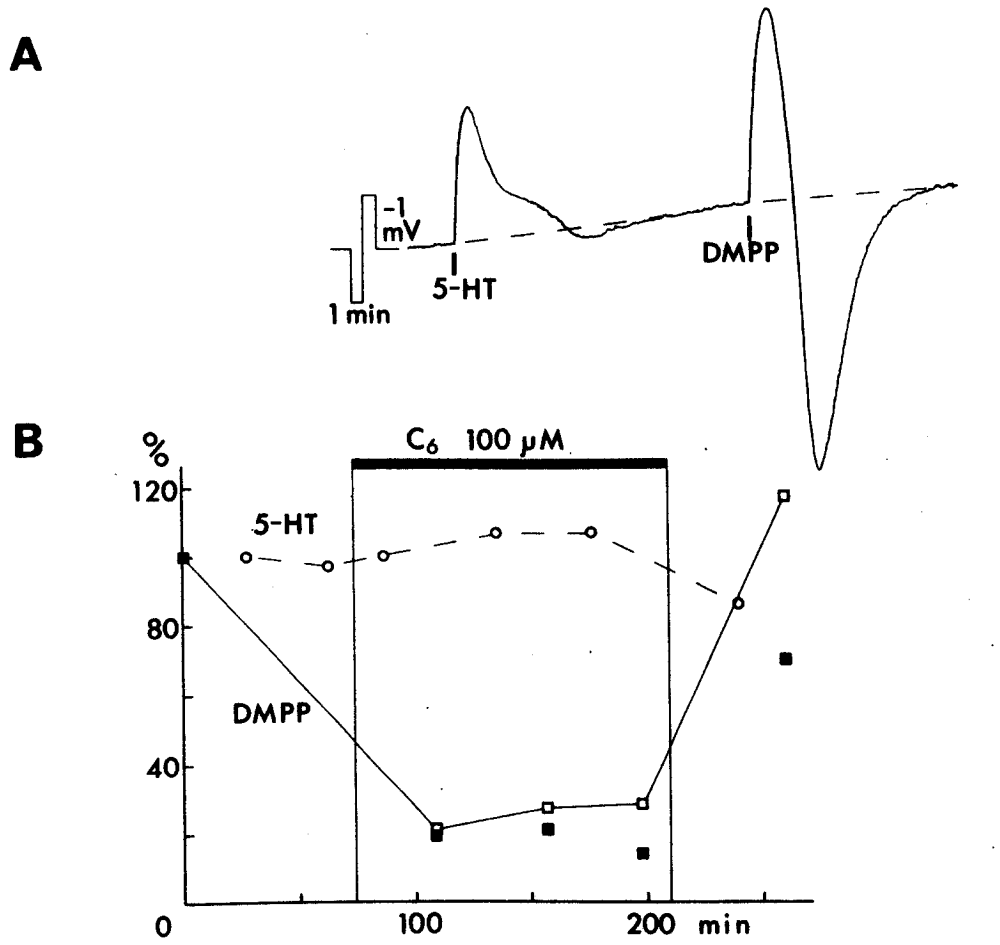

Fig. 2. Responses evoked by 5-HT and DMPP and the selective blockade of DMPP responses by hexamethonium. (A) Chart records of membrane potential change induced by 5-HT (injection of 0.2 μmol in 0.2 ml Krebs solution) and DMPP (injection of 0.1 μmol in 0.1 ml Krebs solution), depolarization of the ganglion gave upward movement of pen. The black bars indicate the duration of the injections. Dashed lines are projections of the baseline preceding the responses indicating the extent of baseline drift. (B) effect of hexamethonium (C_6, 100 μM) on the amplitude of depolarizations evoked by 5-HT and DMPP (open symbols). Solid squares show the amplitude of the after-hyperpolarizations evoked by DMPP. Ordinate, relative amplitude expressed as % of first control; abscissa, time in minutes. Black bar shows the period for which the ganglion was superfused with C_6.

RESULTS

Responses evoked by 5-HT and DMPP

Characteristically, the depolarization elicited by 5-HT was rapid and repolarization was followed by a small (Fig. 2A) or moderate after-hyperpolarization (Fig. 3A, E). The response to DMPP was typically a rapid depolarization and repolarization, followed by an after-hyperpolarization, which was often of a greater amplitude than the preceding depolarization (Fig. 2A, 3I). The activity of DMPP as a depolarizing agent at the ganglion was very similar to that of 5-HT, since the relative activity expressed as an equipotent molar ratio (epmr) was 0.93 ± 0.16 (mean $\pm$ SEM, $n = 19$).

In earlier experiments (Wallis and Woodward, 1975), it was established that responses to 5-HT remained relatively constant for several hours provided that a sufficient interval (20–25 min) was left between each injection. This is also the case with responses to DMPP. A selective depression of the responses to DMPP by an antagonist which acts at nicotinic receptors could be demonstrated. Exposure of the ganglion to hexamethonium (100 μM) caused a profound reduction in responses to DMPP; both the depolarization and the after-hyperpolarization were greatly reduced in amplitude (Fig. 2B). Responses to 5-HT, on the other hand, were not depressed but slightly enhanced in amplitude. The changes seen on exposing the ganglion to hexamethonium were essentially reversed on washing the antagonist from the tissue.

Responses evoked by methylated compounds

Chart records of the membrane potential changes evoked by methylated derivatives of 5-HT or tryptamine are shown in Figure 3. The response to each compound was a depolarization, but its character and the amount of the substance required to evoke it varied widely amongst the series of compounds. Responses of a character almost identical to those elicited by 5-HT were elicited by M5-HT (Fig. 3B). Rate of depolarization and of repolarization, as well as the magnitude of the after-hyperpolarization, were very similar to those elicited by an equimolar amount of 5-HT in the same experiment, as was the duration of the response. In 9 experiments, the epmr for M5-HT ranged from 0.5 to 3.7 and the mean epmr was 1.44 ± 0.29 (mean $\pm$ SEM). A methyl substituent on the α carbon atom, however, considerably reduces the activity of the molecule, since α methyl 5-HT has an epmr of 23 (Wallis, 1979). The compound DM5-HT was a more potent depolarizing agent than 5-HT and generated responses of much longer duration. Thus, one half of the molar amount of 5-HT which gave the control response illustrated in Figure 3(A) generated a large depolarization of prolonged time course (Fig. 3C). The rate of repolarization following exposure of the ganglion to DM5-HT was always slow, but occasionally a small and prolonged after-hyperpolarization could be detected (Fig. 3C). In 28 experiments, the epmr for DM5-HT ranged from 0.02 to 0.5 and the mean epmr was 0.13 ± 0.02 (mean $\pm$ SEM). The introduction of a third methyl group on the terminal nitrogen produces a quaternary ammo-

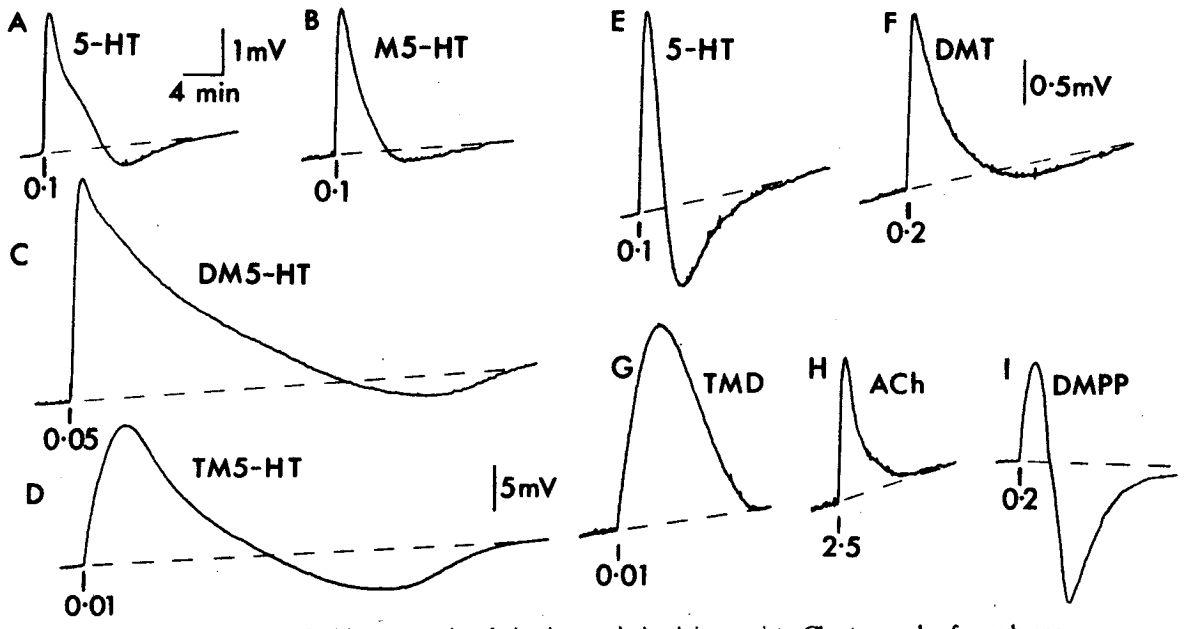

Fig. 3. Responses evoked by tryptamine derivatives and nicotinic agonists. Chart records of membrane potential change, ganglionic depolarization upwards. Black bars indicate the duration of the injections, the Figures underneath show the quantity in μmol of agonist injected. Dashed lines are projections of the baseline preceding the responses as in Figure 2. Time calibration applies to all traces, 1 mV calibration to A, B, C, G, I, 5 mV calibration to D and 0.5 mV calibration to E, F, and H.

nium compound, TM5-HT (bufotenidine). The most potent depolarizing agent of the rabbit scg proved to be TM5-HT. The response generated by one tenth of the amount of 5-HT used to elicit the control response illustrated in Figure 3(A) evoked a large depolarization (note change in vertical scale) of prolonged time course (Fig. 3D). In fact, the durations of the responses evoked by both DM5-HT and TM5-HT were similar, providing the amounts of the substances were adjusted so as to evoke depolarizations of similar magnitude. In some experiments, TM5-HT elicited a small after-hyperpolarization following the phase of slow repolarization. In 15 experiments, the epmr for TM5-HT ranged from 0.002 to 0.04 and the mean epmr was 0.014 ± 0.003 (mean ± SEM). In contrast to DM5-HT and TM5-HT, the dimethylated compound without the hydroxyl substituent at carbon atom 5, DMT, was a less potent depolarizing agent than 5-HT. It produced a depolarization of the ganglion which was characterized by a relatively slow rate of repolarization and, occasionally, a small after-hyperpolarization (Fig. 3F). In 10 experiments, the epmr ranged from 1.2 to 8 and the mean epmr was 3.07 ± 0.61 (mean ± SEM). The importance of the hydroxyl moiety at carbon atom 5 is further emphasized by the relative lack of activity displayed by the 4-hydroxyl compounds. Thus, *N,N*-dimethyl 4-HT (psilocin) produced only a small depolarization of the ganglion. In 6 experiments, the epmr ranged from 20 to 276 and the mean epmr was 117 ± 33 (mean ± SEM). Chart recordings of the responses to three agonists thought to induce ganglionic depolarization primarily, or entirely, as a result of their activity at nicotinic receptors are also shown in Figure 3. *N,N,N*-Trimethyl dopamine (TMD) evoked a large depolarization of prolonged time course (Fig. 3G). The response illustrated was evoked by 1/10th of the molar amount of 5-HT required to evoke a comparable depolarization (Fig. 3E). In 5 experiments, the epmr ranged from 0.01 to 0.15 and the mean epmr was 0.05 ± 0.02 (mean ± SEM). Acetylcholine, on the other hand, induced a depolarization, approaching in magnitude that evoked by 5-HT, only when 25 times the molar amount of 5-HT was employed (Fig. 3H), presumably because of its rapid destruction by ganglionic cholinesterase (Kosterlitz, Lees and Wallis, 1968). For this reason the epmr was not assessed, although in the presence of an anticholinesterase, 5-HT and acetylcholine display similar activity (Wallis and Woodward, 1975). The membrane potential changes generated by DMPP, including the large and characteristic after-hyperpolarization, are shown for comparison (Fig. 3I).

Effect of antagonists of 5-HT receptors

If the methylated 5-HT derivatives DM5-HT and TM5-HT have an action at both 5-HT and nicotinic receptors, then antagonists relatively specific for either one or other species of receptor should interfere with the depolarizations evoked by the methylated compounds. This was found to be the case when quipazine was used.

Table 1. Summary of the effects of superfusion with quipazine (Q), 5-HT and hexamethonium (C_6) on the relative amplitude of the depolarizations evoked by various agonists. Arrows pointing upward signify an increase, downwards a decrease

	5-HT	DM5-HT	TM5-HT	DMPP
Q 1 μM	↓94%	↓37%	↓10%	↑42%
5-HT 10 μM	↓56%	↓27%	↓25%	↓ 9%
C_6 100 μM	↑ 8%	↓64%	↓86%	↓84%

Quipazine has been reported to antagonize the depolarizing action of 5-HT at the ganglion (Lansdown *et al.*, 1979, 1980). It was found that a concentration of 0.1 μM reduced the amplitude of 5-HT depolarizations by 75%, while a concentration of 1 μM produced near total blockade. The effect of superfusion with a solution containing quipazine at a concentration of 1 μM was examined on the depolarizations evoked by 5-HT, DMPP, DM5-HT and TM5-HT. The amount of each agonist was adjusted so that depolarizations of approximately equal amplitude were induced. This concentration of quipazine is known not to depress responses to DMPP (Lansdown *et al.*, 1980); in fact, in the present series of experiments it induced a mean potentiation of responses to DMPP by 42 ± 16% (mean ± SEM, $n = 8$). In 6 ganglia in the presence of quipazine (1 μM), responses to 5-HT were depressed in amplitude by 94 ± 2%, whereas those to DM5-HT were depressed by 37 ± 5% and those to TM5-HT by 10 ± 7% (mean ± SEM in each case). These results are summarized in Table 1.

Superfusion of the ganglion with 5-HT can also substantially reduce the depolarization evoked in a single cell by microiontophoretic application of 5-HT, while leaving unaffected the fast epsp induced by orthodromic stimulation and dependent on activation of nicotinic receptors (Wallis and North, 1978). In the present experiments, superfusion with 5-HT (1 μM) reduced depolarizations evoked by injected 5-HT by 30 ± 7% ($n = 4$), while superfusion with a higher concentration (10 μM) reduced the evoked depolarizations by 56 ± 5% (means ± SEM, $n = 8$). In the two series of experiments, the effects of 5-HT superfusion on the depolarizations evoked by DMPP was variable, but the mean change in amplitude was less than 5% in the presence of 5-HT (1 μM) and less than 10% in the presence of 5-HT (10 μM). Although responses to both DM5-HT and TM5-HT were reduced in amplitude when ganglia were superfused with 5-HT (10 μM), the reductions were of similar magnitude. Responses to DM5-HT were reduced by 27 ± 11% and responses to TM5-HT by 25 ± 10% (means ± SEM, $n = 4$). These results are summarised in Table 1.

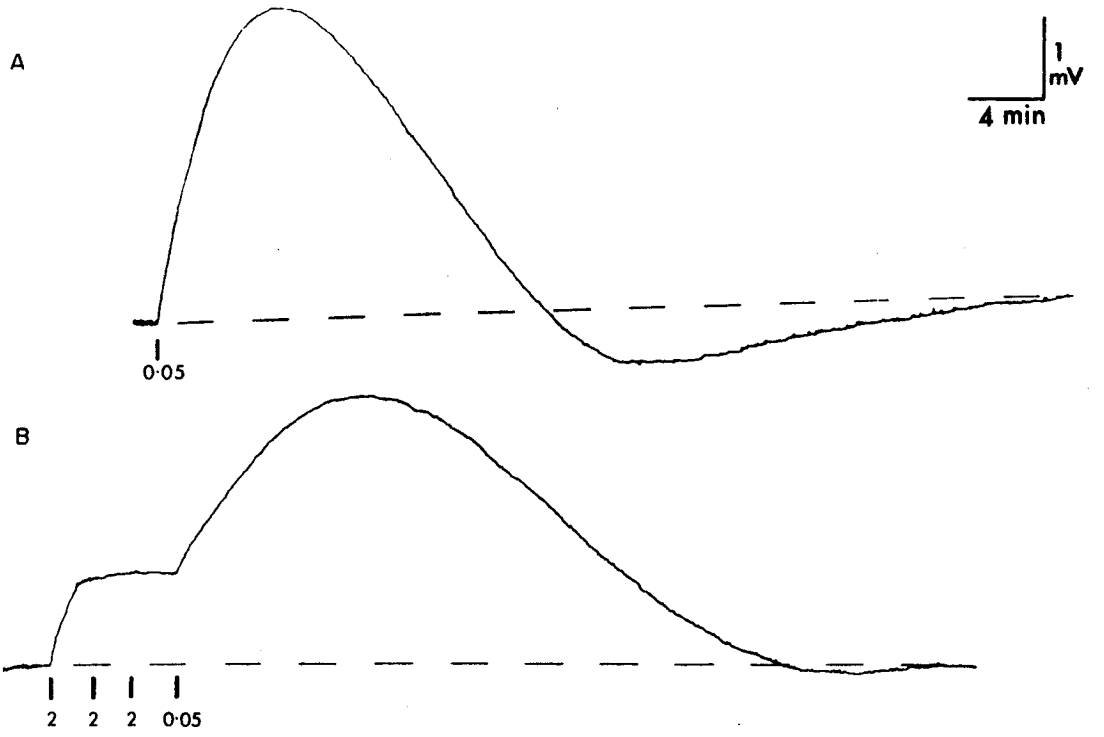

Fig. 4. Responses to DM5-HT before (A) and after (B) injection of 6 μmol 5-HT to desensitize 5-HT receptors. Black bars, Figures and dashed lines as in Figure 3.

That the action of DM5-HT is not entirely, or mainly, via 5-HT receptors can be demonstrated by an experiment where the depolarization evoked by 5-HT has been abolished by repeated injection of relatively large amounts of the amine (Fig. 4). The tachyphylaxis induced by the injection of 2 μmol 5-HT is such that subsequent injection of the same amount evoked no significant further depolarization of the ganglion, while DM5-HT evoked a very substantial depolarization, although the rate at which this depolarization occurred was markedly slowed.

Effect of antagonists at nicotinic receptors

If some of the ganglionic activity of DM5-HT and TM5-HT were due to an action at nicotinic receptors, an antagonist at these receptors should interfere with the evoked depolarizations. Hexamethonium (100 μM) reduced the amplitude of depolarizations evoked by DM5-HT and TM5-HT, but in each of 4 experiments where both compounds were tested the responses to the latter suffered the greater reduction. Responses to DM5-HT were reduced in amplitude by 64 ± 7% (n = 7) and those to TM5-HT by 86 ± 1% (n = 4) (means ± SEM). The same concentration of hexamethonium reduced the amplitude of depolarizations evoked by DMPP by 84 ± 2% (n = 7), but produced no significant reduction in responses to 5-HT, the mean change being an increase of 8 ± 12% (n = 13) (means ± SEM). These results are summarized in Table 1. Responses to DMT were also reduced in amplitude in the presence of hexamethonium by 65 ± 6% (mean ± SEM, n = 4).

d-Tubocurarine, which is known to antagonize certain responses to both 5-HT and ACh in invertebrate neurones (Gerschenfeld and Paupardin-Tritsch, 1974), failed to show selectivity in antagonizing ganglionic responses to 5-HT and DMPP. Responses to the former were reduced in amplitude by 60 ± 5% and to the latter by 69 ± 6% (means ± SEM, n = 4) in the presence of *d*-tubocurarine (10 μM). Therefore, the effect of this antagonist on responses to DM5-HT and TM5-HT was not tested.

Potentiation of responses during superfusion with antagonist drugs

If 5-HT and nicotinic receptors are in close association in the membrane, it might be anticipated that binding of an antagonist molecule at one receptor might alter the properties of the other species of receptor. Binding studies would be required to provide direct evidence of this, but some incidental observations suggest this kind of interaction.

Thus, during the non-depolarizing blockade of the ganglion by nicotine, responses to 5-HT, as well as to other non-nicotinic excitants, are enhanced while responses to DMPP are greatly depressed (Trendelenburg, 1967). It has also been reported that the depolarizations evoked by 5-HT may be enhanced in amplitude and greatly enhanced in duration in the presence of high concentrations of hexamethonium (0.5–1.4 mM) (Wallis and Woodward, 1975). In the present series of experiments, hexamethonium (50–250 μM) produced more varied effects, but potentiation of the amplitude of the depolarizations by more than 10% was seen in 7 of 13 experiments; in 3 of 13 experiments there was a reduction in amplitude of more than 10%. The overall change was a potentiation of 8%, whereas responses to DMPP were

reduced by 84% (see above and Table 1). The observation that seemed of possible significance was that during nicotinic receptor blockade responses to 5-HT were often markedly enhanced, whereas in the presence of quipazine, which is thought to be relatively specific for the receptors to 5-HT (Lansdown *et al.*, 1980), responses to DMPP were often potentiated. Mean potentiation was 42% (see above) and occurred in 6 of 8 experiments in the presence of quipazine (1 μM). At concentrations of quipazine of 0.1 μM or lower, which still reduced responses to 5-HT by 50% or more, the potentiating effect appeared weaker; the increase in the amplitude of depolarizations evoked by DMPP was 11 $\pm$ 12% (mean $\pm$ SEM, $n = 6$).

Metoclopramide, which has been shown by Fozard and Mobarok Ali (1978b) to be an effective antagonist of 5-HT receptors on the terminals of adrenergic nerves to the rabbit heart, was a less effective antagonist in these experiments than quipazine. Metoclopramide (1 μM) reduced the amplitude of depolarizations evoked by 5-HT by 69 $\pm$ 2% ($n = 4$), but the effect on responses to DMPP was variable, significant potentiation being seen in only 1 of 4 experiments; the average change was +1 $\pm$ 11% (means $\pm$ SEM, $n = 4$).

Phenoxybenzamine is relatively ineffective as an antagonist of 5-HT at ganglia (Gyermek and Bindler, 1962), but in the present experiments potentiated the depolarizations evoked by DMPP in 3 of 4 experiments. The mean increase in amplitude was 45 $\pm$ 27% (mean $\pm$ SEM, $n = 4$). The duration of responses to DMPP was increased in all 4 experiments in the presence of phenoxybenzamine, while depolarizations evoked by 5-HT were reduced in amplitude by 19 $\pm$ 7% (mean $\pm$ SEM, $n = 4$).

DISCUSSION

The *N*-methylated derivatives of 5-HT, M5-HT, DM5-HT and TM5-HT each induced a depolarization of the ganglion. Whereas M5-HT possessed a potency close to that of 5-HT itself and evoked depolarizations of very similar duration, DM5-HT and TM5-HT displayed much greater potency and induced depolarizations of much longer duration than 5-HT. Haefely (1974), in his studies on the cat scg *in vivo*, reported that injections of 0.01 μmol DM5-HT induced depolarizations of the ganglion similar in duration to those induced by 5-HT and unaffected by hexamethonium. Only larger amounts evoked depolarizations of longer duration mediated in part by nicotinic receptors. In the present studies, even though threshold quantities of DM5-HT were tested, there was no evidence either of depolarizations of shorter duration or of lack of sensitivity to hexamethonium. In its action in depressing transmission through the scg, Haefely (1974) found that DM5-HT acted in a similar fashion to 5-HT but displayed one tenth of the potency. Gyermek and Bindler (1962) studied the cat inferior mesenteric ganglion *in vivo* and reported that M5-HT, DM5-HT and TM5-HT were powerful stimulants, approximately equipotent with 5-HT. Fozard and Mobarok Ali (1976) compared the potency of 5-HT, M5-HT and DM5-HT in evoking release of noradrenaline from rabbit cardiac sympathetic nerves. The receptors studied were, therefore, also located on postganglionic sympathetic neurones. Whereas the epmr for M5-HT was found to be about 2, a value similar to that obtained in the present experiments, DM5-HT also displayed a lower activity than 5-HT and the epmr was 2.3. In these experiments, DM5-HT was about 8 times more potent than 5-HT as a depolarizing agent and TM5-HT was about 70 times more potent. This high sensitivity of the cells of the scg may, possibly, be peculiar to this ganglion and to the rabbit.

An obvious difference in the appearance of the responses evoked by the various agents is the relative magnitude of the after-hyperpolarization which follows the depolarization. This after-hyperpolarization has been shown, in the case of a nicotinic agonist, to be due to the activity of an electrogenic sodium pump (Lees and Wallis, 1974). The largest after-hyperpolarizations were induced by DMPP and, while 5-HT usually evoked moderate after-hyperpolarizations, DM5-HT and TM5-HT produced small or negligible ones whose magnitude was often obscured by the long duration of the depolarization. However, it is known that the presence or absence of an after-hyperpolarization and the magnitude of this potential are related to the rapidity with which the action of the agonist is terminated; a prolonged action is presumed to cause a continuation of the increased membrane conductance induced by the transmitter and consequent short-circuiting of the potential generated by electrogenic extrusion of Na^+ ions (Brown, Brownstein and Scholfield, 1972). Although the prolonged action of DM5-HT and TM5-HT at the ganglion might account in part for the high potency of these substances, it is clear that another factor might be the ability of these agents to interact with both 5-HT and nicotinic receptors. The evidence which supports this view consists of the observations that quipazine, 5-HT and hexamethonium were able partially to block responses evoked by DM5-HT and TM5-HT. Figures for the relative depression of responses (Table 1) suggest that TM5-HT is more active at nicotinic receptors than DM5-HT, while the latter is more active than TM5-HT at 5-HT receptors judging by the effect of quipazine. An alternative interpretation is that antagonism by 5-HT indicates that DM5-HT and TM5-HT are approximately equipotent at 5-HT receptors; the higher activity of TM5-HT at nicotinic receptors might result in quipazine potentiating the nicotinic receptor-mediated component of the response (cf. quipazine's action on responses to DMPP), so that blockade of the 5-HT receptor-mediated component by quipazine becomes difficult to assess. It was shown by Fozard and Mobarok Ali (1978a) that a combination of hexamethonium and 5-HT was necessary to abolish the action of DM5-HT on the cardiac sympathetic nerves of the rabbit. Gyermek and Bindler (1962) suggested that the sensitivity of methylated compounds to blockade by hexametho-

nium was proportional to the number of methyl substitutions. Thus, a dual action of DM5-HT and TM5-HT on the ganglion cell membrane appears likely. The N-methylated end of a molecule already bound at a 5-HT receptor through the indole nucleus might conceivably react with a neighbouring nicotinic receptor. High activity may only be associated with molecules able to bind at both 5-HT and nicotinic receptors, since psilocin, for instance, fails to show high activity. In the case of psilocin, the hydroxyl on carbon atom 4 may prevent binding at the 5-HT receptor or so distort the spatial alignment of ligand with 5-HT receptor that simultaneous binding of the *N*-terminal methyl groups with a nicotinic receptor is not possible.

This juxtaposition of 5-HT and nicotinic binding sites is also suggested by the effects of blockade of one receptor species on responses evoked via the other receptor species. It was common to observe, on blockade of 5-HT receptors by quipazine, potentiation of responses to DMPP. It has also been reported that blockade of nicotinic receptors by hexamethonium is accompanied by a potentiation of responses to 5-HT (see above). The reciprocal effects were clearest with these particular antagonists, but more variable with metoclopramide or phenoxybenzamine, perhaps because these agents are less specific or react with some different part of the receptor-ion channel complex. It is tempting to suggest that nicotinic and 5-HT receptors are in sufficiently close association in the membrane that binding of an antagonist molecule to one of the receptors might, through some induced conformation change, render the other receptor more accessible to its ligand. It is also possible in view of the similarities in the conductance changes induced by ACh and 5-HT that the ligand binding sites share at least a proportion of the ion channels which are induced to open on binding of an ACh or a 5-HT molecule. In that case, the conformation change induced by the antagonist might be interpreted as making available to the other receptor species ion channels which are normally the province of the receptor to which the antagonist is bound. An alternative explanation is that potentiation of responses is a secondary phenomenon not directly related to receptor blockade. At present the evidence is insufficient to decide between these possibilities and more searching experiments will be necessary before the situation is clarified.

Acknowledgements—We would like to thank Upjohn Ltd for the gift of *N*-methyl 5-hydroxytryptamine, Dr R. B. Barlow for the gifts of *N,N,N*-trimethyl 5-hydroxytryptamine and *N,N,N*-trimethyldopamine, Sandoz Ltd for the gift of psilocin, Dr M. H. T. Roberts for the gift of quipazine, Beecham Ltd for the gift of metoclopramide and LR Industries Ltd for the gift of rubber condoms.

REFERENCES

Brown, D. A., Brownstein, M. J. and Scholfield, C. N. (1972). Origin of the after-hyperpolarization that follows removal of depolarizing agents from the isolated superior cervical ganglion of the rat. *Br. J. Pharmac.* **44**: 651–671.

Drakontides, A. B. and Gershon, M. D. (1968). 5-hydroxytryptamine receptors in the mouse duodenum. *Br. J. Pharmac.* **33**: 480–492.

Fozard, J. R. and Mobarok Ali, A. T. M. (1976). Evidence for tryptamine receptors on cardiac sympathetic nerves. *Br. J. Pharmac.* **58**: 276–277P.

Fozard, J. R. and Mobarok Ali, A. T. M. (1978a). Dual mechanism of the stimulant action of *N,N*-dimethyl-5-hydroxytryptamine (bufotenine) on cardiac sympathetic nerves. *Eur. J. Pharmac.* **49**: 25–30.

Fozard, J. R. and Mobarok Ali, A. T. M. (1978b). Blockade of neuronal tryptamine receptors by metoclopramide. *Eur. J. Pharmac.* **49**: 109–112.

Gaddum, J. H. and Picarelli, Z. P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmac. Chemother.* **12**: 323–328.

Gerschenfeld, H. M. and Paupardin-Tritsch, D. (1974). On the transmitter function of 5-hydroxytryptamine at excitatory and inhibitory monosynaptic junctions. *J. Physiol., Lond.* **243**: 457–481.

de Groat, W. C. and Lalley, P. M. (1973). Interaction between picrotoxin and 5-hydroxytryptamine in the superior cervical ganglion of the cat. *Br. J. Pharmac.* **48**: 233–244.

Gyermek, L. and Bindler, E. (1962). Action of indolealkylamines and amidines on the inferior mesenteric ganglion of the cat. *J. Pharmac. exp. Ther.* **138**: 159–164.

Haefely, W. (1974). The effects of 5-hydroxytryptamine and some related compounds on the cat superior cervical ganglion *in situ*. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **281**: 145–165.

Kosterlitz, H. W. and Wallis D. I. (1966). The use of the sucrose-gap method for recording ganglionic potentials. *J. Physiol., Lond.* **183**: 1–3P.

Kosterlitz, H. W., Lees, G. M. and Wallis, D. I. (1968). Resting and action potentials recorded by the sucrose-gap method in the superior cervical ganglion of the rabbit. *J. Physiol., Lond.* **195**: 39–53.

Lansdown, M. R. J., Nash, H. L., Preston, P. R. and Wallis, D. I. (1979). Blockade of 5-hydroxytryptamine (5-HT) receptors by quipazine. *Br. J. Pharmac.* **66**: 80–81P.

Lansdown, M. R. J., Nash, H. L., Preston, P. R., Wallis, D. I. and Williams, R. G. (1980). Antagonism of 5-hydroxytryptamine receptors by quipazine. *Br. J. Pharmac.* In press.

Lees, G. M. and Wallis, D. I. (1974). Hyperpolarization of rabbit superior cervical ganglion cells due to activity of an electrogenic sodium pump. *Br. J. Pharmac.* **50**: 79–93.

Nishi, S. (1977). Cellular pharmacology of ganglionic transmission. In: *Advances in General and Cellular Pharmacology* (Narahashi, T. and Bianchi, C. P., Eds), Vol. 1, pp. 179–245. Plenum, New York.

Trendelenburg, U. (1956). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Br. J. Pharmac.* **11**: 74–80.

Trendelenburg, U. (1967). Some aspects of the pharmacology of autonomic ganglion cells. *Ergebn. Physiol.* **59**: 1–85.

Wallis, D. I. (1979). The apparent multiplicity of ganglionic receptors. In: *Trends in Autonomic Pharmacology* (Kalsner, S., Ed.), Vol. 1. Urban & Schwarzenberg, Baltimore.

Wallis, D. I. and North,, R. A. (1978). The action of 5-hydroxytryptamine on single neurones of the rabbit superior cervical ganglion. *Neuropharmacology* **17**: 1023–1028.

Wallis, D. I. and Woodward, B. (1973). The depolarizing action of 5-hydroxytryptamine on sympathetic ganglion cells. *Br. J. Pharmac.* **49**: 168P.

Wallis, D. I. and Woodward, B. (1975). Membrane potential changes induced by 5-hydroxytryptamine in the superior cervical ganglion of the rabbit. *Br. J. Pharmac.* **55**: 199–212.

Wallis, D. I., Lees, G. M. and Kosterlitz, H. W. (1975). Recording resting and action potentials by the sucrose-gap method. *Comp. Biochem. Physiol.* **50C**: 199–216.

Watson, P. J. (1970). Drug receptor sites in the isolated superior cervical ganglion of the rat. *Eur. J. Pharmac.* **12**: 183–193.