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Responses to 5-hydroxytryptamine evoked in the hemisected spinal cord of the neonate rat

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1 Superfusion of isolated hemisected spinal cord from neonate rats with 5-hydroxytryptamine (5-HT) (10^{-6} to 10^{-3} M) evoked concentration-related depolarizations. The maximal depolarization elicited by a concentration of 10^{-4} M was 1.0 ± 0.1 mV (mean ± s.e.mean, $n = 30$). Noradrenaline in a similar range of concentrations also elicited depolarizations.

2 The depolarizations probably originate in motoneurones as a result of direct interaction of the amines with these cells, since responses were unaltered by tetrodotoxin (10^{-7} M) or Ca^{2+}-free/Mg^{2+}-rich medium.

3 5-Carboxamidotryptamine (5-CT), S(+)-α-methyl-5-hydroxytryptamine (α-Me5-HT) and 5-methoxytryptamine (5-MeOT) evoked similar depolarizations to 5-HT. Tryptamine evoked depolarizations of smaller maximal amplitude. 5-Hydroxytryptophan, 2-methyl-5-hydroxytryptamine, 8-hydroxy-2-(di-N-propylamino) tetralin hydrobromide (8-OH-DPAT) and 5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]-1-H-indole succinate (RU 24969) had no depolarizing action.

4 Concentration-response (CR) curves were determined for 5-HT, 5-CT, α-Me5-HT, 5-MeOT and tryptamine. The ED_{50} value for 5-HT was 20.5 ± 1.2 μM. The equipotent molar ratios (EPMRs) for 5-CT and α-Me5-HT were close to unity, while 5-MeOT was approximately 3 times and tryptamine 13 to 14 times less potent than 5-HT.

5 The relative agonist potency of 5-HT with respect to other tryptamine analogues capable of depolarizing motoneurones was increased when 5-HT uptake was blocked by citalopram (10^{-7} M). In the presence of citalopram, 5-HT was 2.7 times more potent than α-Me5-HT and 16.9 times more potent than 5-CT. The apparent order of potency was 5-HT > α-Me5-HT > 5-CT (> 5-MeOT ⩾ tryptamine).

6 The monoamine oxidase inhibitor, pargyline (5×10^{-4} M), had no effect on depolarizations to 5-HT, 5-CT or α-Me5-HT.

7 Methiothepin, 1αH, 3α, 5H-tropan-3-yl-3,5-dichlorobenzoate methanesulphonate (MDL 72222) and [3α-tropanyl]-1H-indole-3-carboxylic acid ester hydrochloride (ICS 205–930) had no effect on 5-HT depolarizations elicited in motoneurones. Ketanserin (0.75×10^{-7} M to 10^{-6} M) showed modest antagonistic action and depressed maximal response amplitude; the pIC_{50} was 6.5.

8 Methysergide (10^{-8} to 10^{-7} M) was a potent antagonist of responses to 5-HT. CR curves were displaced to the right and flattened in the presence of the antagonist. The pIC_{50} assessed from the effect on depolarizations evoked by 5-HT 10^{-4} M was 7.5.

9 It is concluded that 5-HT acts directly to depolarize mammalian spinal motoneurones through receptors that are also activated by 5-CT, α-Me5-HT and 5-MeOT and are blocked by methysergide. The receptor profile, although not 5-HT_3-like, does not clearly coincide with that for either 5-HT_1-like or 5-HT_2 receptors.

Introduction

Tryptaminergic neurones which descend the mammalian spinal cord have been detected by fluorescence microscopy (Bjorklund *et al.*, 1971; Fuxe & Jonsson, 1974) and traced towards the ventral and dorsal horns. It has been suggested that 5-hydroxytrypramine (5-HT) may modulate the excitability of mammalian motoneurones (Ahlman *et al.*, 1971; Ellaway & Trott, 1975) and two related actions of 5-HT on motoneurones have been described. In rat spinal and facial motor nucleus motoneurones, 5-HT increases excitability on iontophoretic application *in vivo* (Barasi & Roberts,

1974; McCall & Aghajanian, 1979; White & Neumann, 1980) without directly evoking spike discharge. In rat facial motoneurones, 5-HT applied by iontrophoresis has been shown to evoke a slow depolarization which is accompanied by an increase in membrane input resistance (VanderMaelen & Aghajanian, 1980; 1982).

In a preliminary report, we described concentration-related depolarizations evoked by 5-HT in the isolated, hemisected spinal cord preparation from the neonate rat (Connell & Wallis, 1987a). It was concluded that the depolarizations arose from a population of motoneurones supplying axons to the ventral root in contact with the recording electrodes. This paper describes certain features of the 5-HT response recorded from neonate rat motoneurones and compares the results with studies made *in vivo* on adult rat motoneurones.

As yet the 5-HT receptor mediating the motoneurone response to 5-HT has not been fully characterized. Certain antagonists have been tested in studies in which 5-HT was applied by iontophoresis and the antagonist applied systemically and/or iontophoretically. These indicated that responses in facial nucleus and spinal motoneurones are blocked selectively by methysergide (Barasi & Roberts, 1974; McCall & Aghajanian, 1979), metergoline (McCall & Aghajanian, 1979; White & Neumann, 1983), cyproheptadine and cinanserin (McCall & Aghajanian, 1980). Since the doses of these antagonists were generally low and within the range producing blockade of 5-HT_2 receptors, it was concluded that 5-HT_2 receptors are likely to mediate the response (Fozard, 1984). However, these experiments were not able to provide any quantitative measure of the antagonism produced and the antagonists employed may have actions on 5-HT_1-like receptors. A recent study, in which ketanserin was found to be inactive as an antagonist of 5-HT-induced increases in excitability of rat spinal motoneurones, proposed that the receptor involved was 5-HT_1-like (Roberts *et al.*, 1987). In this paper, we describe the relative agonist activity of a number of compounds related structurally to 5-HT and the effects of certain selective 5-HT receptor antagonists.

Preliminary accounts of this work have been presented to the Physiological Society (Connell & Wallis, 1987a) and the British Pharmacological Society (Connell & Wallis, 1987b).

Methods

Preparation

The spinal cord was removed from rats aged between 3 and 8 days which had been anaesthetized with ether. Rats were decapitated and the entire vertebral column removed rapidly and placed in a dish containing oxygenated modified Krebs solution. The column was transected between any two vertebrae in the cervical region and a laminectomy performed from the ventral surface. When the entire length of the cord had been exposed, the dura was removed and the roots on either side were cut. The cord was then floated free and positioned on the floor of the dissecting dish so that it could be hemisected sagitally. The hemisected cord was mounted, cut surface downwards (Figure 1) on a strip of nappy liner laid over a silver grid (see also Otsuka & Konishi, 1974; Evans *et al.*, 1976; Preston & Wallis, 1980). A strip of tissue paper was laid over the upper surface of the cord and the preparation superfused with oxygenated modified Krebs solution at room temperature (18–20°C) and at a rate of 2.5 ml min^{-1}.

Recording and stimulation

Recordings were made via a pair of non-polarizable Ag/AgCl wick electrodes. One was positioned on the surface of the cord at the base of the ventral root and the other such that the distal part of the ventral root could be laid onto the wick (Figure 1). The method of recording relies on producing an air-gap around the mid-portion of the ventral root. The potential arises between the fluid-air interface at the proximal end of the root and the fluid-air interface at the distal end of the root. Dehydration of the central portion of the ventral root is prevented by the grease seal. The ventral root does not act simply as a wick to record potentials from the cord surface, since integrity of the axons in the ventral root was shown to be necessary to record either a ventral root reflex or a depolarizing response to application of 5-HT (Connell & Wallis, unpublished). Damage to the axons results in the disappearance of both these potentials.

The cord was stimulated by placing the dorsal root of the same spinal segment onto a pair of platinum wire electrodes. Square wave pulses, 0.2–0.5 ms in duration, 5–100 volts, were used to stimulate the dorsal root. The dorsal root was protected from dehydration by use of a grease seal. The grease was a mixture of paraffin and Vaseline applied through a syringe.

Changes in potential evoked by 5-HT (or noradrenaline) and evoked ventral root reflex responses were led to a low drift d.c. amplifier and thence either to a chart recorder or, in the case of reflex responses, to a digital storage oscilloscope. The amplitude of potential changes evoked by application of monoamines was measured from an extrapolation of the baseline before application of the drug (see Figure 2, etc.). The presence of an

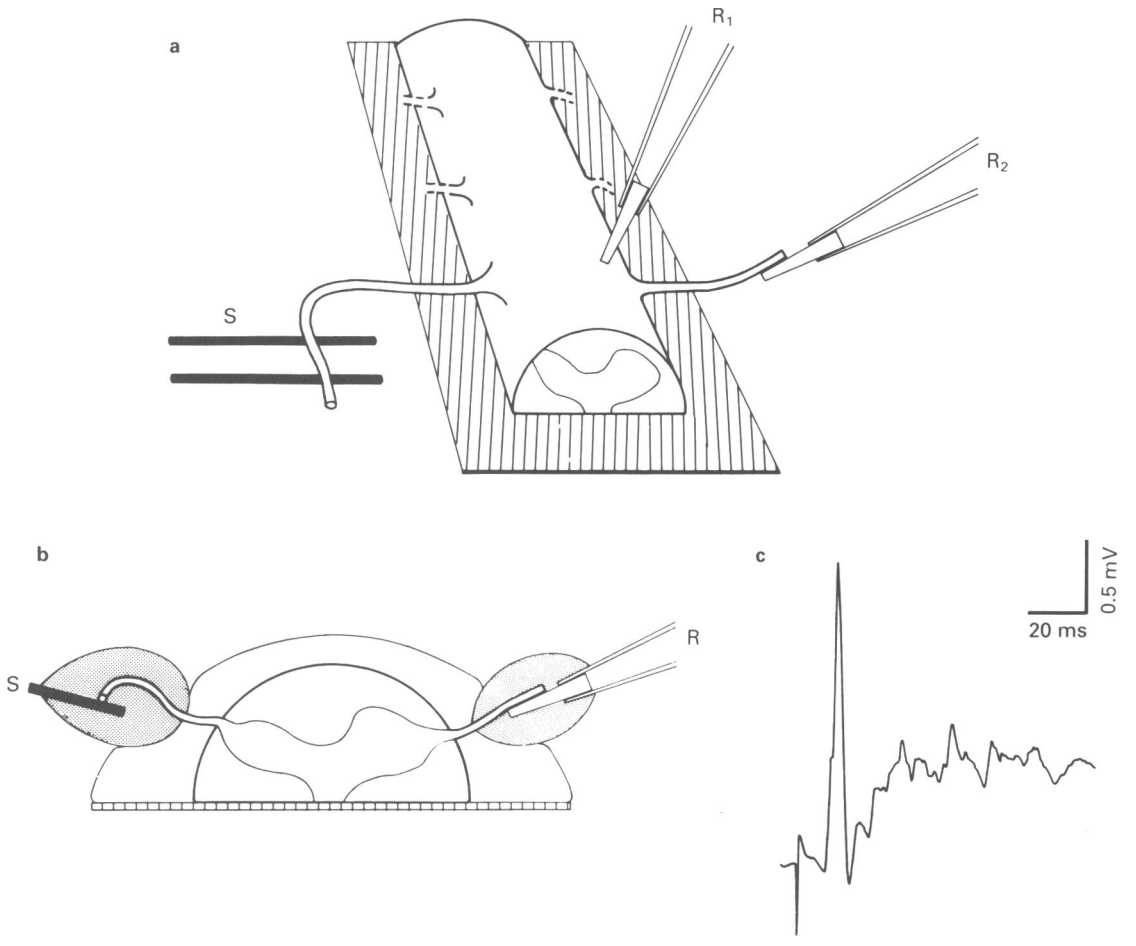

Figure 1 Recording arrangement and reflex response recorded from ventral root. (**a**) Diagrammatic view of hemisected spinal cord. A rectangle of nappy liner provides support for the cord which lies with dorsal roots to the left and ventral roots to the right. S, stimulating electrodes; R_1 and R_2, recording electrodes. (**b**) Cross-sectional view of preparation to show vaseline seals around dorsal root and stimulating electrode (left) and ventral root and recording electrode (right). The preparation was superfused continuously, the superfusion medium creating a meniscus over the surface of the tissue, as indicated approximately by the line above and to the sides of the section of cord. (**c**) Reflex response to a supramaximal stimulus delivered to the ipsilateral dorsal root (0.2 ms, 70 V), comprising short and longer latency components. Chart record from oscilloscope digital store.

evoked ventral root reflex (Figure 1) was used as a test of preparation viability in initial experiments, while in later ones the ability of 5-HT at a concentration of 10^{-4} M to evoke a depolarization of at least 0.5 mV was used. Preparations not meeting one of these criteria were discarded.

In establishing the conditions that would allow determination of repeated concentration-response (CR) curves, it became apparent that in certain preparations deterioration could occur because of a partial failure of the grease insulation. This deterioration resulted in a substantial reduction in the recorded depolarizations and was signalled by a change in the baseline noise and drift rate. When such a change occurred during the course of the experiment, the preparation was abandoned.

Solutions and drugs

A modified Krebs solution of the following composition (mM) was used: NaCl 118, KCl 3, KH_2PO_4 1.2,

$CaCl_2$ 1.2, $MgSO_4 \cdot H_2O$ 0.6, $NaHCO_3$ 25, glucose 11; it was gassed with 5% CO_2 and 95% O_2 (Preston & Wallis, 1980). 5-HT and noradrenaline were dissolved in distilled water and diluted in Krebs solution to the required final concentration just before use.

The drugs used were: 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma), (−)-noradrenaline bitartrate (Sigma), 5-methoxytryptamine hydrochloride (5-MeOT) (Sigma), S(+)-α-methyl-5-hydroxytryptamine maleate (α-Me5-HT) (Sandoz), 5-carboxamidotryptamine maleate (5-CT) (Glaxo), 2-methyl-5-hydroxytryptamine creatinine sulphate (2-Me5-HT) (Glaxo), 5-hydroxy-L-tryptophan (BDH), 8-hydroxy-2-(di-N-propylamino) tetralin hydrobromide (8-OH-DPAT) (Research Biochemicals), 5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]-1-H-indole succinate (RU 24969) (Roussel Uclaf), citalopram hydrobromide (Lundbeck), pargyline hydrochloride (Sigma), methysergide bimaleate (Sandoz), methiothepin maleate (Roche), ketanserin tartrate (Jannsen), (1αH, 3α, 5H-tropan-3-yl-3,5-dichlorobenzoate methanesulphonate (MDL 72222) (Merrell Dow), [3α-tropanyl]-1H-indole-3-carboxylic acid ester hydrochloride (ICS 205–930) (Sandoz), tetrodotoxin (Sigma).

Statistical analysis

All measures of variation quoted are standard errors. Student's *t* test was used to assess the significance of differences between mean values. The paired form of the test was used where comparison of ED_{50} values arising from a single preparation was made. In experiments with methysergide, the covariance of the pooled data was determined to assess whether the slopes and intercepts of the fitted lines for the curves in the absence or presence of antagonist were significantly different.

Results

Depolarizations evoked by 5-HT

Superfusion medium containing 5-HT was applied to the cord for periods of 30s. In concentrations of 10^{-6} to 10^{-3} M, the amine evoked depolarization of the hemisected spinal cord which was recorded via a ventral root. The amplitude of the evoked depolarization was related to the concentration of 5-HT (Figure 2a).

Initial experiments indicated that a contact time of 30s was sufficient for the depolarizations to plateau. With the higher concentrations of 5-HT (10^{-4}, 10^{-3} M), it was necessary to leave a 30min interval between applications to avoid excessive tachyphylaxis. A contact time of 15s was insufficient for the depolarization to plateau, while a contact time of 60s appeared to produce an unacceptable degree of tachyphylaxis. Thus, 30s applications were used routinely, both for 5-HT and noradrenaline.

A concentration of 10^{-6} M 5-HT was just above threshold in most preparations for evoking unequivocal depolarization. High frequency baseline noise, which is of biological origin (see below), made determination of the threshold depolarizing concentration difficult in some preparations. 5-HT, 10^{-6} M, evoked a depolarization of 0.16 ± 0.02 mV (mean ± s.e.mean, $n = 28$), while 5-HT, 10^{-4} M, evoked a maximal response of 1.00 ± 0.08 mV ($n = 30$), not significantly different from that evoked by 5-HT, 10^{-3} M, (0.88 ± 0.14 mV, $n = 12$; see also Figure 6a). On the basis of these results, in subsequent experiments where repeated CR curves were to be determined, a concentration of 5-HT of 10^{-4} M was assumed to produce a maximal response. CR curves for responses to 5-HT from pooled data are shown in Figure 6a. It can be seen that when data were normalized, i.e. when 5-HT responses were expressed as a percentage of the maximum 5-HT response from that preparation, the s.e.means were much reduced. This procedure was adopted in experiments investigating the effects of citalopram and various antagonists. ED_{50} values were measured from individual CR curves, the mean value for 5-HT being shown in Table 1.

A reduction in baseline noise was often observed at the peak of the depolarization evoked by higher concentrations of 5-HT (Figure 2a, Figure 4a), 5-carboxamidotryptamine (5-CT) (not illustrated) and 5-MeOT (Figure 4b), suggesting inhibition of the synaptic input to motoneurones.

In some preliminary experiments, we investigated whether concentration-related depolarizations could also be evoked by application of cumulative concentrations of 5-HT (Figure 2c). However, longer contact times were necessary than for application of single concentrations in order to identify the depolarization plateau. Further, a prolonged period of tachyphylaxis followed the determination of a single cumulative CR curve. In the experiments described below, application of single concentrations of 5-HT has been used to determine CR curves.

Depolarizations evoked by noradrenaline

Noradrenaline, which depolarizes toad isolated spinal cord (Tebecis & Phillis, 1967), elicited concentration-related depolarizations of the neonate

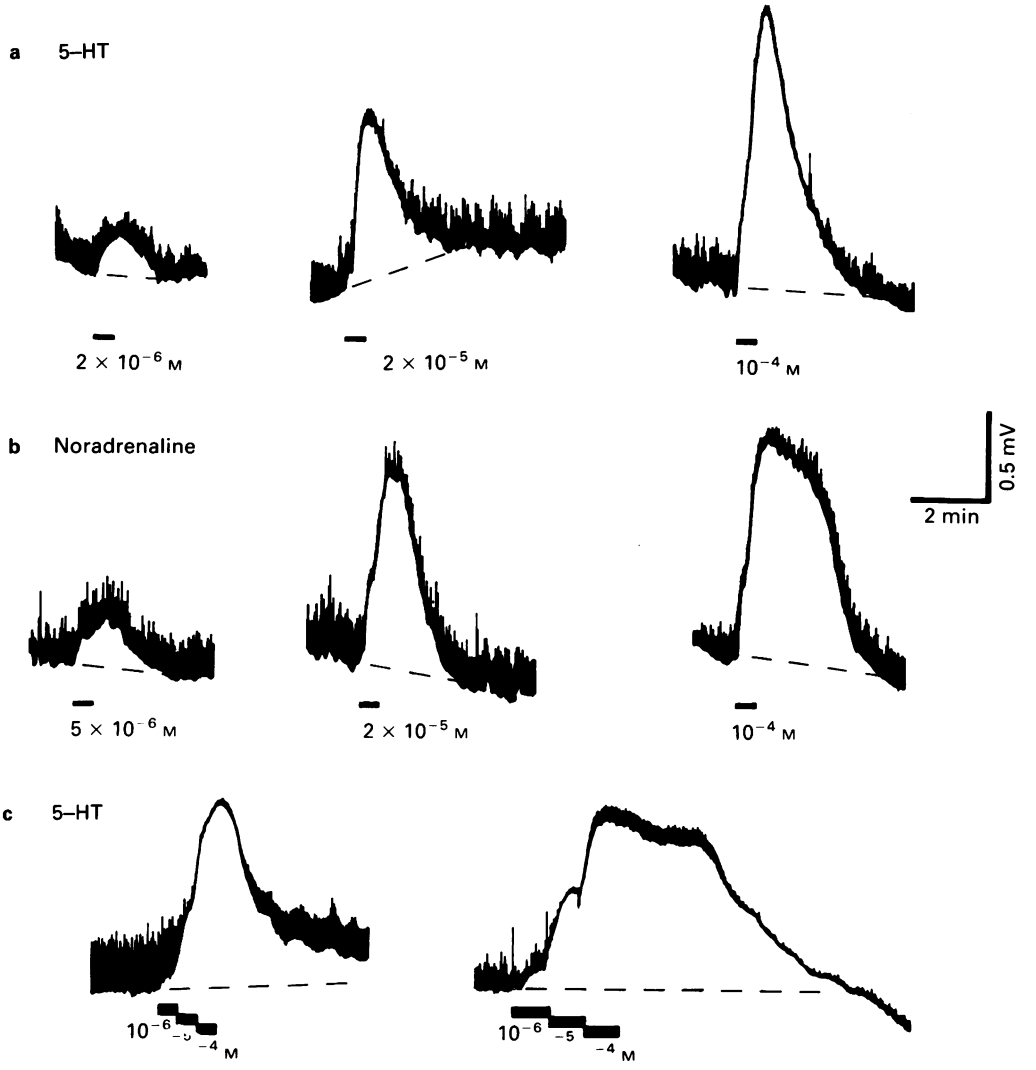

Figure 2 Concentration-related depolarizations evoked by 5-hydroxytryptamine (5-HT) and noradrenaline recorded from a ventral root. Chart records from two different preparations, depolarization caused upward movement of pen. Dashed lines indicate baseline drift as estimated from trace preceding application of drug. (**a**) Responses to 3 concentrations of 5-HT, each superfused for 30 s. Note that the higher concentrations of 5-HT reduced the baseline noise. The latter is mainly the consequence of neuronal activity, since it is abolished by tetrodotoxin (see text). (**b**) Responses to 3 concentrations of noradrenaline in the same preparation. (**c**) Concentration-related depolarizations evoked by application of cumulative concentrations of 5-HT in a second preparation. Contact time was 90 s in the left-hand trace and 150 s in the right-hand trace. Vertical calibration applies to all records, time calibration is for (**a**) and (**b**); time calibration for (**c**) is provided by superfusion durations.

rat cord (Figure 2b). It was chosen as a control agonist in these studies because the cord responded to a similar concentration range to that employed for 5-HT. Further, in experiments with selective 5-HT receptor antagonists, e.g. ketanserin, we wished to demonstrate selectivity of action against 5-HT compared to noradrenaline-evoked responses. A concentration of between 10^{-6} and 10^{-5} M was the threshold depolarizing concentration in most preparations. Noradrenaline 10^{-5} M elicited a depolarization of 0.28 ± 0.04 mV ($n = 12$), 10^{-4} M a depolarization of 0.81 ± 0.09 mV ($n = 21$) and 10^{-3} M a

Table 1 Amplitude of responses to concentrations of 10^{-4} M 5-hydroxytryptamine (5-HT) and analogues of 5-HT, ED_{50} values and equipotent molar ratios (EPMRs)

Agonists	*Mean amplitude of responses to 10^{-4} M amine* (mV)	ED_{50} (μM)
5-HT	1.00 ± 0.08 ($n = 30$)	20.5 ± 1.2 ($n = 20$)
5-HT (+ citalopram, 10^{-7} M)	1.18 ± 0.12 ($n = 13$)	3.6 ± 1.1** ($n = 10$)
		EPMR
5-CT	0.84 ± 0.05 ($n = 25$)	1.8 ± 0.3 ($n = 16$)
α-Me5-HT	0.87 ± 0.14 ($n = 9$)	1.5 ± 0.3 ($n = 8$)
5-MeOT	0.82 ± 0.11 ($n = 8$)	3.3 ± 0.9 ($n = 4$)
Tryptamine	0.43 ± 0.16* ($n = 4$)	13.4 ± 6.2 ($n = 4$)

As can be seen from Figure 6, responses to 10^{-4} M appear to be maximal. ED_{50} values for 5-HT were determined from individual concentration-response curves under control conditions and after incubation with citalopram. EPMRs were determined from the concentration required to evoke a response equivalent to 50% of the response to 10^{-4} M 5-HT from experiments in which CR curves to 5-HT and the analogue of 5-HT were constructed. 5-CT = 5-carboxamidotryptamine; α-Me5-HT = S(+)-α-methyl-5-HT; 5-MeOT = 5-methoxytryptamine.
* $P < 0.05$; ** $P < 0.001$.

depolarization of 0.74 ± 0.13 mV ($n = 6$). The response to a concentration of 10^{-4} M was a maximal response to noradrenaline.

Origin of 5-HT responses in the spinal cord

Although the recorded depolarization was assumed to arise in motoneurones (see above), the possibility that 5-HT was exciting interneurones or nerve terminals so as to release excitatory neurotransmitters onto the motoneurones had to be considered. If 5-HT was depolarizing motoneurones in an indirect fashion, suppression of electrical activity by tetrodotoxin (TTX) ought to attenuate or abolish the responses. Superfusion of the cord with TTX (10^{-7} M) caused a progressive reduction of the ventral root reflex (Figure 3a), which was abolished after less than 30 min exposure to TTX. TTX also abolished all, or nearly all, baseline noise, indicating a suppression of the synaptic input. In 5 experiments, neither depolarizations evoked by 5-HT nor those evoked by noradrenaline were altered in cords that had been incubated with TTX (10^{-7} M) for at least 30 min. The responses recorded in one of these experiments are illustrated in Figure 3b. Note, however, that the high frequency noise at the peaks of the depolarizations to 5-HT and noradrenaline, which was seen in this experiment and was due, presumably, to electrical activity, was abolished by TTX (Figure 3b). Thus, both 5-HT and noradrenaline appear to elicit a direct depolarization of motoneurones. In further support of this contention, a modified Krebs solution, from which Ca ions had been omitted and which contained 2.4 mM Mg ions, did not alter the amplitude of depolarizations to 5-HT and noradrenaline; the baseline noise was also abolished by this solution (not illustrated).

Depolarizing ability of selective 5-HT receptor agonists

A number of selective 5-HT receptor agonists and analogues of 5-HT were tested to see whether they were able to depolarize the isolated spinal cord. Certain agents appeared to produce depolarizations similar in form to those elicited by 5-HT. These were 5-CT, α-Me5-HT and 5-MeOT. 5-CT, α-Me5-HT and 5-MeOT evoked concentration-related depolarizations; response amplitudes to 10^{-4} M concentrations of these amines were not significantly different from that for 5-HT (Table 1). Typical responses evoked by 5-HT and 5-MeOT in the same preparation are shown in Figure 4. Responses to 10^{-4} M tryptamine had a lesser amplitude than those to 5-HT (Table 1, Figure 5).

5-Hydroxytryptophan (up to 10^{-3} M) and 2Me5-HT (10^{-4} M) possessed no unequivocal depolarizing ability. 8-OH-DPAT (10^{-7}–10^{-4} M) and RU 24969 (10^{-7}–10^{-4} M) caused a small hyperpolarization and either no depolarization or a low amplitude depolarization of long duration following the hyperpolarization.

CR curves were determined for 5-HT, 5-CT, α-Me5-HT, 5-MeOT and tryptamine using a concentration range of 10^{-6} to 10^{-3} M (except in the case of α-Me5-HT where shortage of material did not allow testing of the highest concentration). In an individual experiment, responses to 5-HT and some other agonist were evoked alternately, the order of presentation of the various concentrations being random-

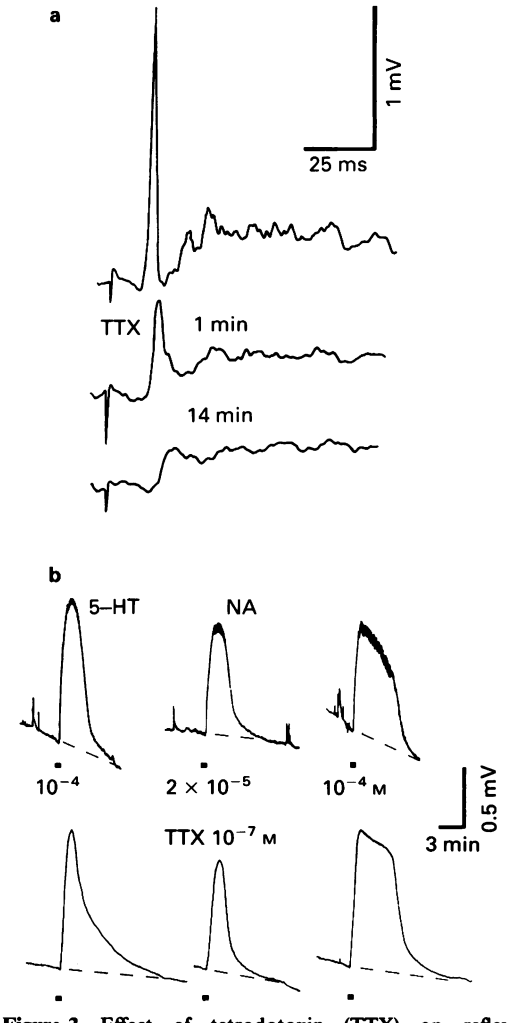

Figure 3 Effect of tetrodotoxin (TTX) on reflex responses and depolarizations to 5-hydroxtryptamine (5-HT) and noradrenaline (NA) recorded from a ventral root. (**a**) Short and longer latency reflex responses evoked by supramaximal stimulus (0.2 ms, 70 V) to ipsilateral dorsal root. Chart records from oscilloscope digital store. The effect of 10^{-7} M TTX was to cause a progressive reduction of both responses. After 30 min superfusion the response was abolished (not illustrated). (**b**) Chart records of depolarizations evoked by 30 s superfusion of 10^{-4} M 5-HT, 2×10^{-5} M or 10^{-4} M noradrenaline. The lower records were obtained after incubation of the preparation for at least 30 min with TTX (10^{-7} M). Note that the noise associated with the peak depolarization evoked by noradrenaline, and assumed to represent neuronal discharge, was abolished by TTX.

ized. The pooled data are shown in Figure 5; apart from tryptamine, 10^{-4} M and 10^{-3} M concentrations of the amines evoked maximal responses. The supramaximal concentration of 10^{-3} M 5-HT caused substantial tachyphylaxis and this concentration was not normally used in determining the first of repeated CR curves. CR curves from pooled data for 5-HT, 5-CT, α-Me5-HT and 5-MeOT were similar, whereas the CR curve for tryptamine was displaced to the right (Figure 5).

Equipotent molar ratios (EPMRs) were estimated from individual experiments with 5-HT and other indoleamines, the EPMR being derived from the concentration of indoleamine which evoked a response 50% of the maximal 5-HT response in that particular preparation. It can be seen from Table 1 that EPMRs for 5-CT and α-Me5-HT were not far from unity, while 5-MeOT was approximately 3 times less potent and tryptamine 13 to 14 times less potent than 5-HT.

Effect of citalopram and pargyline on responses to 5-HT

The EPMRs for 5-HT, 5-CT and α-Me5-HT suggest that these amines are roughly equipotent on the motoneuronal 5-HT receptor. However, to substantiate this point it is necessary to show that active uptake mechanisms for 5-HT or destruction by monoamine oxidase (MAO) have not altered the effective concentration of indoleamine within the cord. Differential effects of this kind might distort the true relative potency of 5-HT, 5-CT and α-Me5-HT.

In order to block neuronal and non-neuronal uptake of 5-HT, citalopram (10^{-7} M) was used (Pawlowski *et al.*, 1981). At higher concentrations, e.g. 10^{-6} M, citalopram causes depression of depolarizations evoked by 5-HT in vagal sensory neurones (Round & Wallis, 1987). The effect of citalopram (10^{-7} M) was to potentiate the depolarizations evoked by submaximal concentrations of 5-HT (Figure 4). The results from a single experiment are shown in this figure. It is noticeable that while the depolarizations evoked by 10^{-6} and 10^{-5} M 5-HT were considerably enhanced in amplitude, those to 10^{-6} and 10^{-5} M 5-MeOT were not. The response to 10^{-4} M 5-MeOT was depressed by citalopram.

CR curves of pooled data for responses to 5-HT before exposure to citalopram and after incubation with the uptake inhibitor for 1 h are shown in Figure 5b. There is a marked displacement of the curve to the left, although the curve maxima are superimposed. ED_{50} values were calculated from CR curves obtained in individual experiments. The ED_{50} for 5-HT in the presence of citalopram was significantly lower than that in its absence (Table 1).

The effects of citalopram on responses to 5-CT and α-Me5-HT were more equivocal. Depolar-

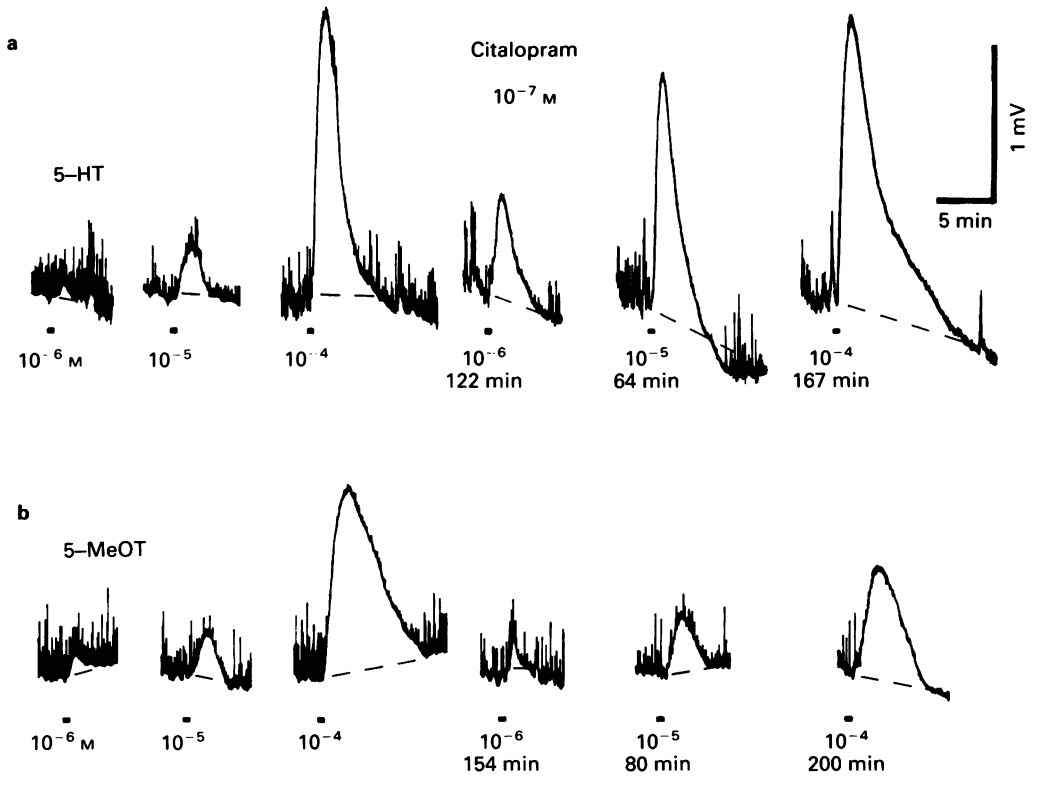

Figure 4 Concentration-related depolarizations evoked by 5-hydroxytryptamine (5-HT) and 5-methoxytryptamine (5-MeOT) recorded from a ventral root and the effect of the uptake inhibitor, citalopram. All records from the same preparation. (**a**) Chart records of responses to 3 concentrations of 5-HT, each superfused for 30 s. Responses evoked by the lower concentrations of 5-HT were enhanced after incubation of the tissue with citalopram (10^{-7} M). Times indicate duration of exposure to citalopram. (**b**) Chart records of responses to 3 concentrations of 5-MeOT, each superfused for 30 s. After equilibration of the cord with citalopram (10^{-7} M) for the times indicated, there was no potentiation of responses to 5-MeOT.

izations evoked by both 10^{-5} M 5-CT or 10^{-5} M α-Me5-HT were potentiated, although to a lesser extent than those of 5-HT. After incubation with citalopram, the EPMR for 5-CT relative to 5-HT was 16.9 ± 3.9 ($n = 6$), while the EPMR for α-Me5-HT relative to 5-HT was 2.7 ± 0.5 ($n = 3$).

Pargyline was used to prevent destruction of indoleamines by MAO. In the presence of pargyline (5×10^{-4} M), the amplitude of depolarizations evoked by 10^{-6} and 10^{-5} M 5-HT was not altered significantly in 7 experiments. The mean ED_{50} obtained after incubation with pargyline was not significantly different from that obtained before incubation. The effect of pargyline on responses to 5-CT was examined in 4 experiments and on responses to α-Me5-HT in 3 experiments. The depolarizations elicited by either 10^{-6} and 10^{-5} M 5-CT or the same concentrations of α-Me5-HT were unaffected by the MAO inhibitor.

Effect of antagonists on depolarizing responses to 5-HT

Antagonists were tested after first eliciting a control CR curve to 5-HT from a cord preparation. Preliminary experiments had established that a second CR curve could be determined from a preparation after allowing a wash period of 60 min between curves. Before the degree of antagonism could be assessed, it was necessary to determine to what degree a second control CR curve measured from the same preparation was displaced to the right. The pooled results from a series of control experiments are shown in Figure 6b. The mean CR curve determined 1–2 h after an initial CR curve shows neglible displacement to the right, but values for maximal depolarization were depressed by around 14%. The mean ED_{50} values measured from individual 1st and 2nd CR curves did not differ significantly (Table 2).

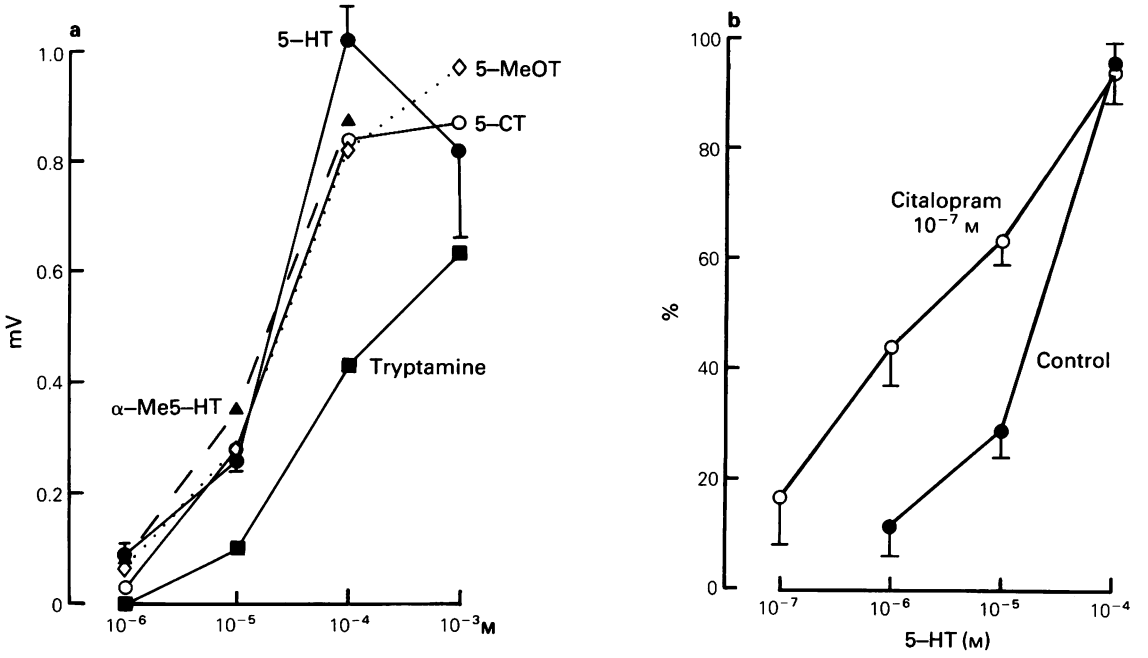

Figure 5 Concentration-response (CR) curves for indoleamines related to 5-hydroxytryptamine (5-HT) and the effect of citalopram on the CR curve for 5-HT. (**a**) CR curves to 5-HT ($n = 6$ to 30), 5-carboxamidotryptamine (5-CT; $n = 7$ to 25), α-methyl 5-HT (α-Me5-HT; $n = 8$ to 9), 5-methoxytryptamine (5-MeOT; $n = 5$ to 8) and tryptamine ($n = 4$). Ordinate scale, amplitude (mV); abscissa scale, concentration of amine. The s.e. bars for indoleamines other than 5-HT have been omitted for clarity. (**b**) CR curves from pooled data for 5-HT before (●) and, in the same preparation, after incubation for at least 1 h with citalopram (10^{-7} M) (○). Bars show s.e.means, $n = 10$. Ordinate scale, depolarization amplitude relative to response to 10^{-4} M 5-HT taken as 100; abscissa scale, concentration of 5-HT.

Ketanserin, methiothepin, MDL 72222, ICS 205–930 and methysergide were tested for antagonist action against the depolarizations evoked by 5-HT in the isolated spinal cord.

The selective 5-HT_2 receptor antagonist, ketanserin, appeared to display some blocking action at a concentration of 0.75×10^{-7} M (Figure 7a), although an unequivocal effect was discernible in only 10 of 15 experiments. The CR curve, determined in the presence of ketanserin and derived from pooled data from 15 experiments, was shifted to the right of the control curve and maximal response amplitude was depressed. Responses to 10^{-4} M 5-HT were reduced, on average, by $40.5 \pm 4\%$ ($n = 15$). The ED_{50} for 5-HT was increased about 2 fold in the presence of ketanserin (0.75×10^{-7} M) (Table 2). However, a concentration of 10^{-8} M ketanserin produced no change in ED_{50} values for 5-HT either with or without blockade of 5-HT uptake by citalopram (Table 2). In 2 experiments, 10^{-6} M ketanserin showed a marked blocking action. The pIC_{50}, defined as the negative logarithm of the concentration of antagonist reducing 5-HT responses by 50%, was estimated graphically from the pooled effects of various concentrations. Compensation was made for the reduction of responses to 10^{-4} M 5-HT under control conditions. The pIC_{50} assessed from the effect of the antagonist on responses to 10^{-4} M 5-HT was 6.5.

In the presence of ketanserin (0.75×10^{-7} M), responses to 10^{-4} M noradrenaline were not reduced in amplitude (amplitude relative to control: $122 \pm 10\%$, $n = 15$). At higher concentrations, ketanserin (10^{-7} and 10^{-6} M) caused a reduction of $27 \pm 9\%$ ($n = 5$) of responses to 10^{-4} M noradrenaline. It was possible to demonstrate selective blockade of responses to noradrenaline by prazosin and phentolamine. Prazosin (10^{-7} M) reduced by about 75% responses to noradrenaline (10^{-5} and 10^{-4} M), but had no effect on 5-HT responses in 2 experiments. A similar selective blockade was seen with phentolamine (10^{-6} M) in 4 experiments in which

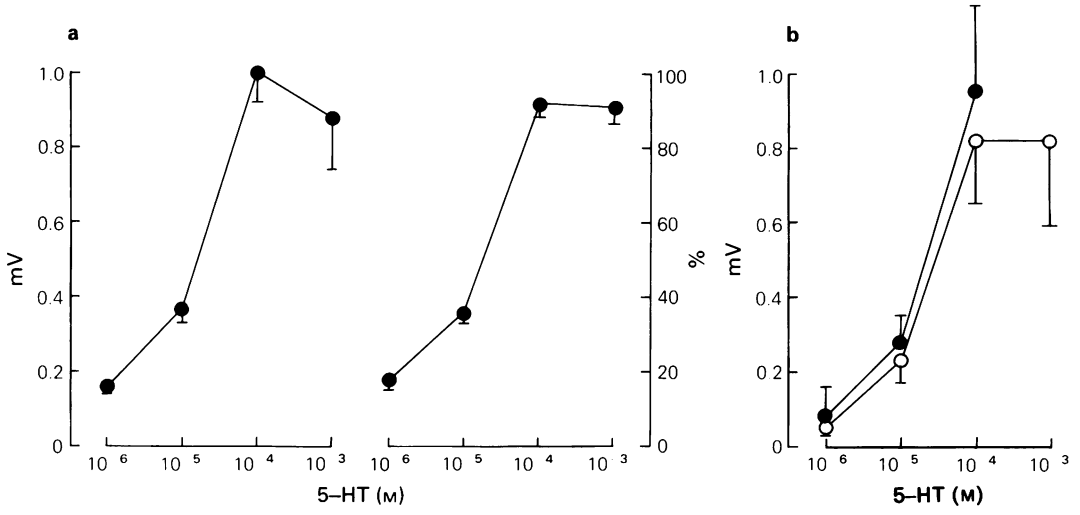

Figure 6 Concentration-response (CR) curves to 5-HT showing the change in depolarization amplitude with increasing concentration of 5-HT. (**a**) CR curves from pooled data (n = 12 to 30), depolarization plotted as absolute amplitude (left-hand graph, ordinate scale in mV) and after normalizing data (right-hand graph, ordinate scale as relative amplitude, amplitude of largest response to 5-HT from a particular preparation taken as 100). (**b**) First (●) and second (○) control CR curves to 5-HT. Pooled data from 7 experiments in which a second CR curve was obtained after washing for 1 h. Bars show s.e.means; abscissae, concentration of 5-HT.

responses to noradrenaline (2×10^{-5} and 10^{-4} M) were reduced by 36 ± 7%, but responses to 5-HT were unaffected.

Methiothepin (10^{-7} M), which is an antagonist at 5-HT_1 and 5-HT_2 receptors (Bradley *et al.*, 1986), had no effect on depolarization of motoneurones by 5-HT (Table 2). Further, neither of the 5-HT_3 receptor antagonists tested, MDL 72222 (10^{-7} M) and ICS 205–930 (10^{-9} M), had any effect on these responses (Table 2).

Methysergide (10^{-8} and 10^{-7} M) was a potent antagonist of 5-HT responses in the spinal cord. CR

Table 2 ED_{50} or ED_{25} values for 5-hydroxytryptamine (5-HT) before and after incubation with various antagonists

Antagonists	*Control (1st CR curve)* ED_{50} (μM)	*2nd CR curve* ED_{50} (μM)
None	20.2 ± 4.2 (n = 6)	20.5 ± 3.1 (n = 6)
Ketanserin		
0.75×10^{-7} M	15.5 ± 2 (n = 14)	31.1 ± 8.1 (n = 14)*
10^{-8} M	23.4 ± 1.5 (n = 3)	31.2 ± 2.9 (n = 3)
10^{-8} M (in citalopram)	12.4 ± 5.0 (n = 6)	3.6 ± 0.7 (n = 6)
Methiothepin		
10^{-7} M	22.3 ± 2.9 (n = 6)	23.5 ± 1.2 (n = 6)
MDL 72222		
10^{-7} M	20.2 ± 2 (n = 4)	21.1 ± 2.1 (n = 4)
ICS 205–930		
10^{-9} M	19.6 ± 2.7 (n = 4)	18.9 ± 3.0 (n = 4)
Methysergide	ED_{25}	ED_{25}
10^{-8} M	3.6 ± 1.2 (n = 7)	14.0 ± 2.7 (n = 7)**
10^{-7} M	2.7 ± 0.9 (n = 4)	296 ± 236 (n = 4)

* $P < 0.05$; ** $P < 0.01$.

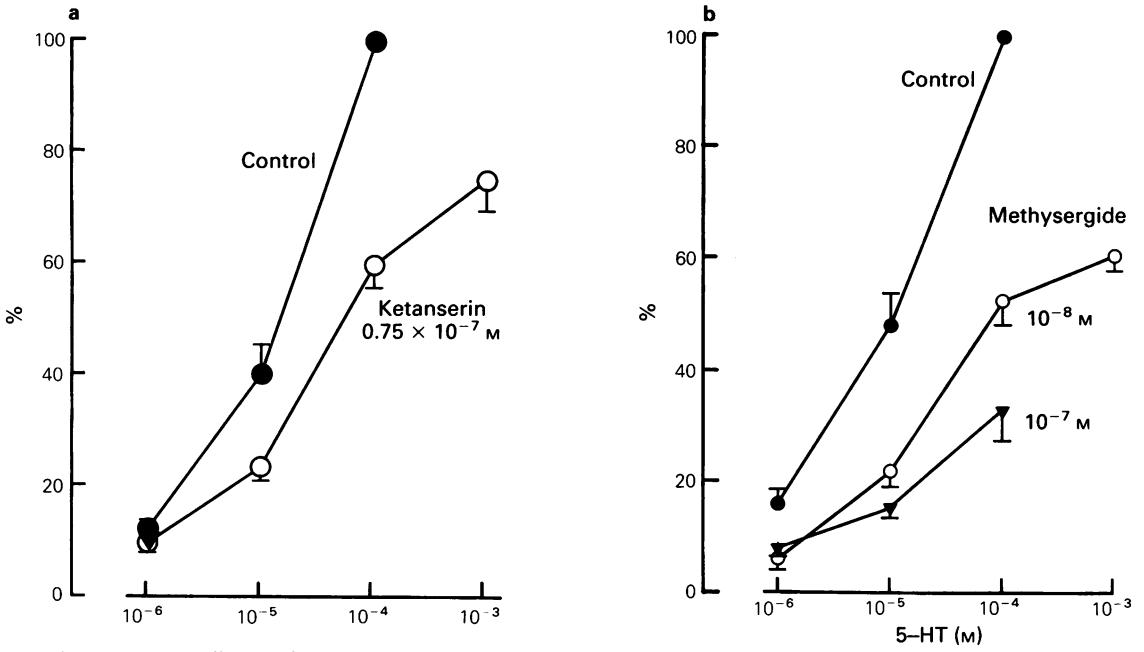

Figure 7 The effects of ketanserin and methysergide on concentration-response (CR) curves to 5-hydroxytryptamine (5-HT). (**a**) The effect of ketanserin (0.75×10^{-7} M) on responses to 5-HT. The tissue was equilibrated with the antagonist for at least 1 h before re-testing. Bars show s.e.means, $n = 15$. Ordinate scale, depolarization amplitude relative to response to 10^{-4} M 5-HT; abscissa scale, concentration of 5-HT. (**b**) The effect of methysergide on depolarizations evoked by 5-HT. The two concentrations of methysergide were tested in two separate series of experiments. Control values for 5-HT responses in the two series have been pooled ($n = 10$). Methysergide (10^{-8} M) shifted the CR curve to the right and depressed maximum response amplitude ($n = 7$). Methysergide (10^{-7} M) produced a further shift of the curve ($n = 3$).

curves were displaced to the right and there was a depression of the maximum (difference in slope, $P < 0.001$) (Figure 7b). The flattening of the CR curve in the presence of methysergide made it more appropriate to indicate the antagonist activity of methysergide by using ED_{25} values (Table 2). The mean dose-ratio during blockade by 10^{-8} M methysergide was 5.6. The pIC_{50} assessed from responses to 10^{-4} M 5-HT after compensation for the reduction seen in control experiments was 7.5.

Discussion

The results demonstrate that 5-HT elicits concentration-related depolarizations in the neonate rat isolated spinal cord. Since these responses were led from a ventral root whose axons must be viable for the response to be recorded, it may be concluded that the depolarization arose in the motoneurones supplying axons to that root as a result of either a direct or an indirect excitation by 5-HT. Since the depolarizations were not reduced by incubation of the cord with TTX or with a Ca^{2+}-free/enhanced Mg^{2+} medium, it can be concluded that 5-HT acts directly to depolarize motoneurones, as reported by VanderMaelen & Aghajanian (1980, 1982) for facial motoneurones in the adult rat. A depolarizing action of 5-HT measured intracellularly has also been reported for preganglionic sympathetic neurones (Ma & Dun, 1986). The mean amplitude of the maximal depolarization recorded in response to 5-HT was similar to that elicited by noradrenaline, although the two amines act via different receptors (see below).

Using a contact time of depolarizing amine with tissue of 30 s and an appropriate wash period, the conditions were established whereby repeated CR curves could be elicited from the preparation. Some reduction in maximal response amplitude (14%) was seen in the second CR curve. This is likely to be the consequence of tachyphylaxis, which is a pronounced feature of certain neuronal depolarizing responses to 5-HT (Wallis, 1981).

The ability of certain selective 5-HT receptor agonists to depolarize motoneurones was examined.

The results showed that 5-CT, α-Me5-HT and 5-MeOT evoked responses from the cord which were similar to those elicited by 5-HT. Tryptamine also had a depolarizing action, although the intrinsic activity of this agent appeared to be less. 2Me5-HT, 8-OH-DPAT and RU 24969 were inactive as depolarizing agents. The relative potency of these agents can only be assessed properly under circumstances where the concentration of amine in the extracellular space is the same as that in the superfusion medium. Removal of amine by an uptake process or destruction by monoamine oxidase (MAO) might significantly reduce its concentration around the motoneurones. Further, if these processes affected 5-HT selectively, the relative potency of 5-HT would be underestimated. The results showed that MAO activity did not seem to reduce the effective concentration in the extracellular space, since in the presence of pargyline the responses to 5-HT and the other amines were unaltered. On the other hand, the results suggested that an active uptake process for 5-HT was significantly reducing the effective concentration of 5-HT around the motoneurones with a consequent underestimate of the potency of the indoleamine. In the presence of citalopram to block 5-HT uptake, the sensitivity to 5-HT was increased about 6 fold (see ED_{50} values in Table 1). It seems likely that the uptake system was saturated at a concentration of 10^{-4} M 5-HT in the superfusion medium, since the response to this concentration was not potentiated by citalopram.

When active uptake of 5-HT was blocked with citalopram, 5-HT was 2.7 times more potent than α-Me5-HT and 16.9 times more potent than 5-CT. Although the effect of citalopram on responses to 5-MeOT was not quantified, the apparent order of potency of the agonists in depolarizing motoneurones was 5-HT > α-Me5-HT > 5-CT > (5-MeOT ≫ tryptamine). This order of potency has similarities to that reported for the 5-HT_1-like receptor on endothelial cells of rabbit jugular vein (Leff *et al.*, 1987).

The potentiation of responses to 10^{-5} M 5-CT and 10^{-5} M α-Me5-HT may reflect some active uptake of these indoleamines. Alternatively, it is possible that the depolarizations evoked by submaximal concentrations of these agents were facilitated by the presence of endogenous 5-HT within the cord, which accumulated when active uptake was blocked.

The activity of certain of the selective agonists and the inactivity of others at the motoneurone 5-HT receptor give some indication of the nature of this receptor. First, it seems unlikely that it is a 5-HT_3 receptor. 2Me5-HT which is selective for 5-HT_3 receptors (Bradley *et al.*, 1986) was inactive, while 5-CT which is inactive at 5-HT_3 receptors (Richardson & Engel, 1986) was an effective depolarizing agent of motoneurones. 5-CT is selective for 5-HT_1 receptors (Bradley *et al.*, 1986), but certain other 5-HT_1 receptor agonists (8-OH-DPAT, RU 24969) were ineffective in depolarizing motoneurones. Of the other indoleamines that mimicked the action of 5-HT, such as α-Me5-HT and 5-MeOT, the former has low activity at 5-HT_3 receptors (Wallis & Nash, 1981), while 5-MeOT is inactive at 5-HT_3 sites (Wallis, 1981). Both α-Me5-HT and 5-MeOT may be agonists at 5-HT_1 receptors and both, especially the former, can activate 5-HT_2 receptors (Humphrey, 1984; Richardson & Engel, 1986; Engel *et al.*, 1986). Interestingly, 5-MeOT has been shown to mimic the excitatory action of 5-HT on brainstem neurones of cat and rat *in vivo*, although the 5-HT receptor involved was not identified (Bradley & Briggs, 1974).

It is clear from the results that 5-HT and noradrenaline act through different receptors to depolarize motoneurones. Thus, responses to noradrenaline were antagonized by prazosin and phentolamine, while responses to 5-HT were unaffected by these antagonists. Further, ketanserin which had a modest antagonistic action on 5-HT responses at a concentration of 0.75×10^{-7} M, did not reduce responses to noradrenaline.

The results with selective agonists, although suggesting an involvement of a 5-HT_1-like receptor because of the relatively high activity of 5-CT, are not definitive. Experiments with antagonists might be expected to provide more conclusive evidence. The results with the selective 5-HT_3 receptor antagonists confirmed that a 5-HT_3 receptor was not involved. An insensitivity to ketanserin should be displayed by a 5-HT_1-like receptor (Bradley *et al.*, 1986). Motoneurone responses to 5-HT were antagonized in a non-competitive fashion by relatively high concentrations of ketanserin. The pA_2 values for the antagonist action of ketanserin at 5-HT_2 receptors on vascular smooth muscle range from 8.7–9.1 (Humphrey, 1984). Since in our experiments the pIC_{50} was about 6.5, a much higher concentration of ketanserin was required for blockade compared to the action on identified functional 5-HT_2 sites. Further, although ketanserin is often regarded as inactive at 5-HT_1-like receptors, it has been reported to display a pIC_{50} of 7.0 at 5-HT_{1C} sites in pig choroid plexus (Hoyer & Kalkman, 1986).

Since methysergide is an antagonist at 5-HT_1 and 5-HT_2 receptors, although not at 5-HT_3 receptors (Bradley *et al.*, 1986), the sensitivity of motoneuronal 5-HT responses to methysergide is consistent with the presence of a 5-HT_1-like or a 5-HT_2 receptor. The depolarizations of lateral horn sympathetic neurones were also blocked by methysergide (Ma & Dun, 1986).

Thus, although the results with 5-CT suggest that

receptors on motoneurones might be considered 5-HT_1-like (see also Roberts *et al.*, 1987), the evidence for this view is inconclusive. The weak activity of ketanserin may not be inconsistent with this view, especially as ritanserin shows no antagonist activity (Connell & Wallis, unpublished). However, a puzzling feature was the inactivity of methiothepin, which is regarded as a prototypical antagonist of 5-HT_1-like receptors (Bradley *et al.*, 1986), but also expresses a high affinity for both 5-HT_1 and 5-HT_2 binding sites in rat brain cortex (Leysen *et al.*, 1981). A resistance to methiothepin is displayed by certain neuronal responses mediated by 5-HT_1-like receptors, e.g. facilitation of transmitter release in the mouse urinary bladder by 5-HT (Holt *et al.*, 1986) and hyperpolarization of sympathetic ganglion cells by 5-CT (Ireland & Jordan, 1987). Nevertheless, since a sensitivity to methiothepin in the case of 5-HT_1-like receptors or a sensitivity to ketanserin in the case of 5-HT_2 receptors is regarded as a defining feature in the present classification of 5-HT receptors (Bradley *et al.*, 1986), the profile for the motoneurone 5-HT receptor does not clearly coincide with that for 5-HT_1 or that for 5-HT_2 receptors. We conclude that 5-HT acts directly to depolarize spinal motoneurones through unidentified receptors, which are also activated by 5-CT, α-Me5-HT and 5-MeOT and are blocked by methysergide.

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