

Inhibition of reflex responses of neonate rat lumbar spinal cord by 5-hydroxytryptamine

H. Crick & D.I. Wallis

Department of Physiology, University of Wales College of Cardiff, Cardiff CF1 1SS

1 Monosynaptic (MSR) and polysynaptic (PSR) segmental reflex responses were recorded from a ventral root of the neonate rat hemisectioned spinal cord. Amplitudes of the two components were monitored with a peak height detector.

2 5-Hydroxytryptamine (5-HT) depressed the MSR and PSR in a concentration-dependent manner. The IC_{50} for MSR depression was $9.5 \pm 3.2 \mu M$ and for PSR depression was $9.0 \pm 4.8 \mu M$.

3 Blockade of neuronal uptake of 5-HT by citalopram ($0.1 \mu M$) greatly increased sensitivity to 5-HT. In the presence of citalopram, the IC_{50} for MSR depression was $30 \pm 18 nM$ and for PSR depression was $89 \pm 23 nM$.

4 5-HT did not depress the MSR or the PSR by releasing glycine since strychnine ($1 \mu M$) did not prevent these actions of 5-HT.

5 5-Carboxamidotryptamine (5-CT), 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), RU 24969, 1-[3-(trifluoromethyl)phenyl]-piperazine (TFMPP) and methysergide were full agonists for depression of the MSR. The IC_{50} for 5-CT was $3.6 \pm 0.5 nM$, for 8-OH-DPAT was $0.4 \pm 0.04 \mu M$, for TFMPP was $0.93 \pm 0.3 \mu M$ and for methysergide was $21.8 \pm 3.0 nM$. The order of potency was 5-CT > methysergide > 5-HT > 8-OH-DPAT > TFMPP.

6 8-OH-DPAT, RU 24969, TFMPP and methysergide had either no or only a minor action in reducing the PSR. 5-CT caused a 50% depression at the highest concentration tested (30 nM).

7 Neither ketanserin ($1 \mu M$) nor spiperone ($1 \mu M$) caused appreciable blockade of 5-HT depression of the MSR or 5-HT depression of the PSR.

8 Blockers of neuronal 5-HT uptake (citalopram 0.1 or $1 \mu M$, fluvoxamine $1 \mu M$) usually reduced the MSR and, to a lesser extent, the PSR. Reflex depressions were reversed by ketanserin ($1 \mu M$).

9 It was concluded that 5-HT has a potent depressant action on segmental reflexes; depression of the MSR is unrelated to depolarization of motoneurons. Although depression of the MSR was mimicked by 5-HT_{1A} receptor ligands, the action of endogenous 5-HT may be mediated through 5-HT₂ receptors. Exogenous 5-HT may act at a mixture of 5-HT receptor subtypes to depress the MSR.

Keywords: 5-HT; monosynaptic reflex; polysynaptic reflex; spinal cord; 5-HT₁-like receptors; 5-HT₂ receptors

Introduction

Descending pathways releasing 5-hydroxytryptamine (5-HT) are present in the ventral horn of the spinal cord and 5-HT has been localized in the terminal plexuses near the cell bodies and dendrites of large motoneurons in the rat (Steinbusch *et al.*, 1978; Holstege & Kuypers, 1987). Although 5-HT has a direct depolarizing action on spinal motoneurons (Wang & Dun, 1990; Elliott & Wallis, 1990a) and increases their excitability (Ahlman *et al.*, 1971; Barasi & Roberts, 1974; Roberts *et al.*, 1988), 5-HT and 5-HT agonists, such as 5-methoxy-dimethyltryptamine and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), are reported to depress reflex responses evoked by dorsal root stimulation (Saito *et al.*, 1982; Nagano *et al.*, 1988). On the other hand, facilitation of reflex responses can occur on stimulation of the nucleus raphe medianus and this effect is blocked by 5-HT antagonists (Barasi & Roberts, 1974). It is clear that the role of 5-HT in regulating motoneurone activity remains to be clarified. Whether 5-HT exerts its effects via different subtypes of 5-HT receptor is also unclear.

In the isolated spinal cord of the neonate rat, stimulation of the ipsilateral dorsal root elicits a short latency discharge in the segmental ventral root, which is assumed to be the equivalent of the monosynaptic reflex (MSR) in the adult (Otsuka & Konishi, 1974). This is followed by a longer latency polysynaptic reflex response (PSR). 5-HT has been shown to inhibit reflex responses in neonate rat isolated spinal cord, although the type of 5-HT receptor involved is unresolved (Saito *et al.*, 1982; Ohno & Warnick, 1989). Further, methy-

sergide was reported to act selectively as an agonist to depress the MSR (Saito *et al.*, 1982), an observation not always confirmed in other laboratories (Ault, 1981; Evans, 1989). The depressant effect of 5-HT on the PSR was not mimicked by methysergide nor blocked by methysergide (Saito *et al.*, 1982). 5-HT also depresses the mechanism responsible for generating the dorsal root potential on stimulation of an adjacent dorsal root (Lansdown *et al.*, 1980).

These experiments seek to clarify the action of 5-HT on reflex responses in the isolated spinal cord of the neonate rat and identify the subtype of 5-HT receptor involved. A preliminary account of this work has been presented to the British Pharmacological Society (Crick & Wallis, 1990).

Methods

Preparation

Female rats, 3–8 days old, were anaesthetized with ether. Following decapitation, the vertebral column was removed and placed in a dish containing oxygenated modified Krebs solution at room temperature (23°C). A laminectomy was performed from the ventral surface. The exposed spinal roots could now be cut as they entered and left the column, thus allowing the cord to be floated free. The cord was then pinned to a dissecting dish, the dura removed and the cord hemisectioned sagittally. The preparation was allowed to rest for an hour in oxygenated Krebs solution at room temperature (20–23°C) before experimentation began.

Recording and stimulation

Suction electrodes were used both for stimulation and recording. The hemisected cord was placed in a Sylgard-filled trough (volume 1 ml) cut into a Perspex block. The cord was superfused at $2\text{--}3\text{ ml min}^{-1}$ via a stainless steel inlet tube of 1 mm diameter and the overflow removed by suction from a drainage area connected to the preparation chamber by a piece of nappy-liner. The temperature of the superfusion fluid was maintained at 25°C . At higher temperatures, it was found that the cord was subject to large spontaneous depolarizations which left it unresponsive to stimulation. Identification of the spinal roots proved relatively simple. Dorsal roots are generally more vascularized than the ventral roots and also tend to be thicker. The ventral roots leave the cord in a fan-like manner. The root pairs used were L3 to L5 and were identified as they entered and left the cord at the lumbar enlargement.

The suction electrodes were constructed from Microcap tubes the tips of which were melted in a Bunsen flame to the correct size for the spinal roots used, i.e. so that the roots did not slip out but were not damaged by compression. A chlorided silver wire made contact with the Krebs solution in the suction tip into which the ventral root was drawn. The stimu-

lating electrode, into which the dorsal root was drawn, was of similar design except that the silver wire was not chlorided and there was an insulated copper wire, with the copper tip exposed, wound around the outside of the glass Microcap tube. A silver chloride pellet entering the side of the bath formed the reference electrode.

Supramaximal square wave pulses 0.1 ms in duration were used. The stimulus voltage was adjusted to be 50% above that required for maximal stimulation. The dorsal root was stimulated once every 15 s (0.067 Hz). Reflex responses were studied only when the monosynaptic reflex (MSR) was constant in amplitude and more than 1 mV.

The reflex response was amplified and fed into a peak height detector (Courtice, 1977), modified to provide twin windows able to sample voltages occurring during different periods after the triggering stimulus (Figure 1). The windows or gates used sample and hold circuits to detect the peak voltages occurring during the MSR and the polysynaptic reflex (PSR). Signals proportional to the peak voltages were displayed on a chart recorder. A further chart recorder was used to monitor the d.c. level. The reflex was also displayed on an oscilloscope linked to an X-Y plotter to provide a hard copy.

It could be established that depression of the reflex by 5-HT was not due to greater temporal dispersion of the compound

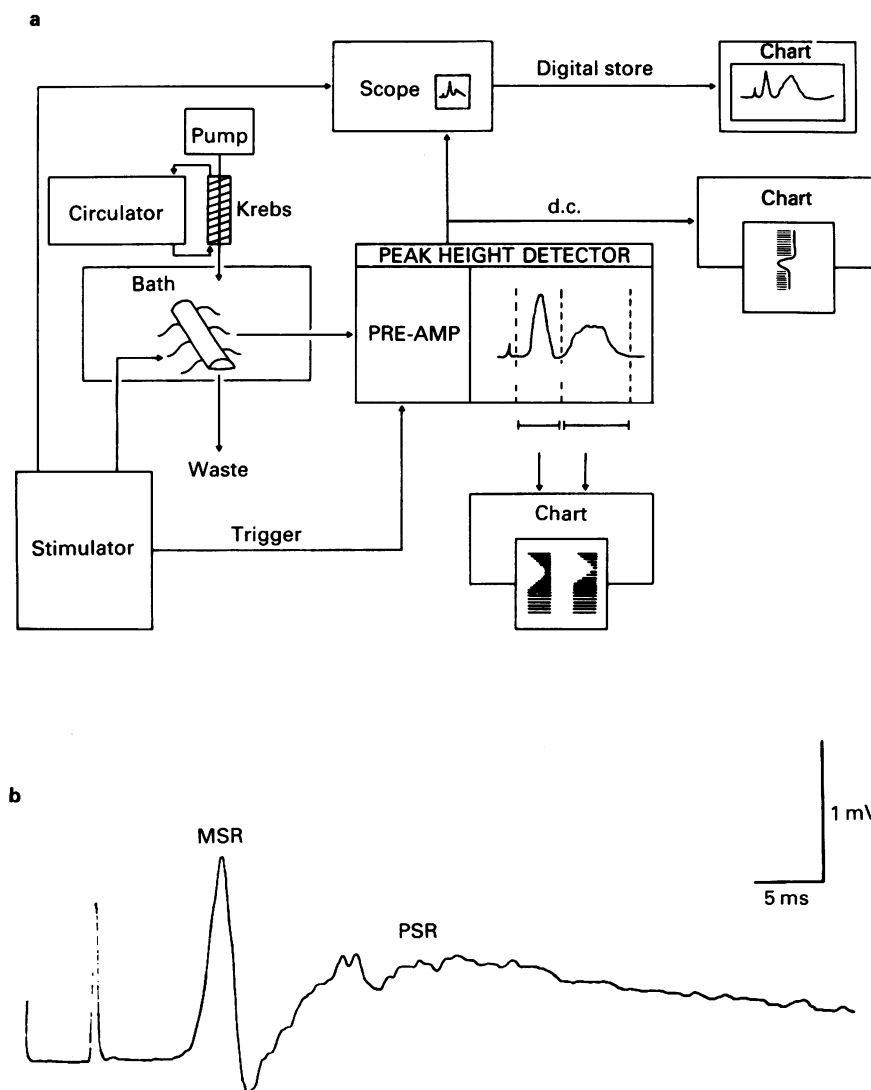


Figure 1 Arrangement for recording the segmental monosynaptic reflex (MSR) and polysynaptic reflex (PSR) from neonate rat hemisected spinal cord. (a) Block diagram of equipment showing amplification and relay of signal from ventral root to peak height detector, oscilloscope and chart recorders. (b) Chart record from oscilloscope digital store of reflex response recorded by a suction electrode from a ventral root on stimulation (10 V, 0.1 ms) of the segmental dorsal root. The initial component is the MSR and the slower component the PSR.

action potential, nor was there a change in its latency (see Figure 4). Thus, signals derived from the peak height detector were taken to be an accurate reflection of reflex depression.

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, $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ 0.6, NaHCO_3 25 and glucose 11; it was gassed with 5% CO_2 and 95% O_2 (Preston & Wallis, 1980).

The drugs used were: baclofen (BAC) (Sigma); 5-carboxamidotryptamine maleate (5-CT) (Glaxo); citalopram hydrobromide (Lundbeck Ltd); fluvoxamine maleate (Duphar); 8-hydroxy-2-(di-n-propylamino)tetralin hydroxy-bromide (8-OH-DPAT) (RBI/Semat); 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma); ketanserin tartrate (Janssen); methysergide bimalate (Sandoz); ritanserin (Janssen); RU24969 (5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]-1-H-indole succinate, Roussel Uclaf), spiperone (Janssen), strychnine (Sigma) and TFMPP (1-[3-(trifluoromethyl)phenyl]-piperazine hydrobromide, RBI/Semat).

5-CT, 5-HT and methysergide were dissolved in modified Krebs solution, while baclofen, citalopram, fluvoxamine, 8-OH-DPAT, RU 24969, ketanserin, spiperone, strychnine and TFMPP were dissolved in distilled water; a solution of ritanserin was made in 0.01% HCl before dilution in Krebs solution.

Statistical analysis

Change in MSR or PSR amplitude was measured as the % reduction in amplitude of the responses preceding application of the agonist. IC_{50} values were estimated from individual concentration-response curves. All measures of variation quoted are standard errors. Student's *t* test was used to assess the significance of differences between mean values.

Results

Effect of 5-hydroxytryptamine on reflex responses

Superfusion of the cord with concentrations of 5-HT ranging from 1–100 μM caused a concentration-related depression of both the monosynaptic and the polysynaptic reflex (Figure 2). In preliminary experiments in which the superfusion period was varied, it was established that superfusion for 3–4 min was required for blockade to reach a maximum. To allow responses to plateau and also to minimize subsequent tachyphylaxis, a standard application time of 4 min was routinely adopted for 5-HT at all concentrations except for 100 μM which was applied for 2 min. Applications were repeated after a wash period of 20–30 min, depending on the concentration of agonist applied. Other agonists were applied for 4 min except for methysergide which was applied for 3 min, following Saito *et al.* (1982). Using the standard application time, mean latency to peak depression and mean recovery times for the MSR and the PSR are given in Table 1. Latency to peak depression appeared unaffected by concentration, while recovery time increased with increasing concentration. The IC_{50} for depression of the MSR was $9.5 \pm 3.2 \mu\text{M}$ ($n = 7$), while the IC_{50} for depression of the PSR was $9.0 \pm 4.8 \mu\text{M}$

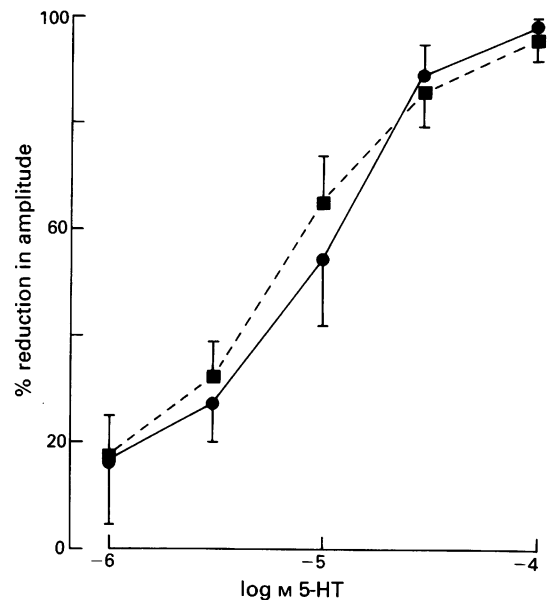


Figure 2 Effect of 5-hydroxytryptamine (5-HT) on relative amplitudes of monosynaptic (●) and polysynaptic (■) ventral root reflex. Complete abolition of the response was taken as 100%. Pooled data from 7 experiments, points show means with s.e.means indicated by vertical bars.

($n = 7$). As can be seen from Figure 2, the depressant action of 5-HT on the MSR at various concentrations closely paralleled its blocking action on the PSR. Times to peak depression and recovery for MSR and PSR were closely comparable (Table 1).

The close parallel between the two depressant actions of 5-HT was less apparent, however, after blockade of neuronal uptake of 5-HT with citalopram (0.1 μM). Under these circumstances concentration-response curves for depression of both the MSR and the PSR were shifted to the left (Figure 3). Whereas the curve for the PSR was shifted approximately 100 fold to the left, the curve for the MSR was shifted approximately 300 fold. Because citalopram itself caused a reduction of the MSR in a proportion of experiments (see below), determination of the IC_{50} after citalopram was possible in only a small number of preparations. In the presence of citalopram, the IC_{50} for depression of the MSR was $30 \pm 18 \text{ nM}$ ($n = 4$) and the IC_{50} for depression of the PSR was $89 \pm 23 \text{ nM}$ ($n = 4$).

A depolarization by 5-HT was also observed with the higher concentrations employed. However, this was not seen with other agonists active at this site (see below). Depolarizations were not studied systematically because in our hands the suction electrode technique often failed to give a satisfactory record of depolarization. Nevertheless, the depolarization was observed to have a time course different from depression of the reflex response. It was considered possible that the depressant action of 5-HT was an indirect one via release of inhibitory amino acid transmitters. In an attempt to rule out an action through release of glycine, cords were incubated with strychnine (1 μM). The results of one such experiment are

Table 1 Time course of depression by 5-hydroxytryptamine (5-HT) of monosynaptic reflex (MSR) and polysynaptic reflex (PSR): cords were superfused with 5-HT for 4 min

Conc of 5-HT (M)	MSR				PSR			
	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}
Time to peak depression (min)	3.7 ± 0.4 ($n = 7$)	4.0 ± 0.3 ($n = 7$)	4.4 ± 0.6 ($n = 7$)	3.3 ± 0.6 ($n = 5$)	3.1 ± 0.4 ($n = 6$)	3.0 ± 0.4 ($n = 7$)	3.1 ± 0.6 ($n = 7$)	2.9 ± 0.9 ($n = 5$)
Time to recovery to control value (min)	7.5 ± 0.9 ($n = 7$)	9.1 ± 0.7 ($n = 7$)	17.2 ± 2.2 ($n = 7$)	26.9 ± 3.1 ($n = 5$)	8.2 ± 0.8 ($n = 6$)	9.4 ± 0.6 ($n = 7$)	16.7 ± 2.5 ($n = 7$)	22.5 ± 2.6 ($n = 5$)

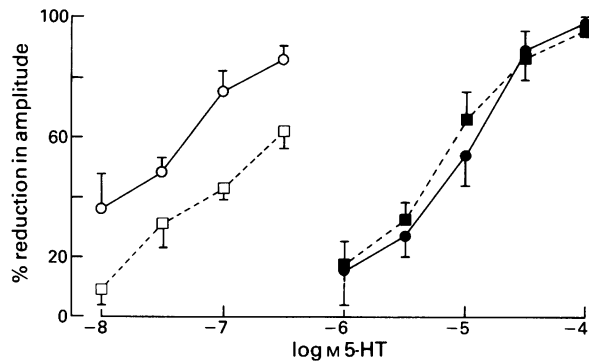


Figure 3 Effect of $0.1 \mu\text{M}$ citalopram on 5-hydroxytryptamine-induced depression of monosynaptic (MSR) and polysynaptic (PSR) ventral root reflex. Filled symbols are results from 4 cords in the absence of citalopram: (●) MSR; (■) PSR. Open symbols results from the same 4 cords after incubation with $0.1 \mu\text{M}$ citalopram for at least 1 h; (○) MSR; (□) PSR.

shown in Figure 4. It can be seen that strychnine itself facilitated later components in the PSR but did not prevent a depressant action by 5-HT. A similar result was obtained in 2 other experiments.

Agonist action of 5-hydroxytryptamine receptor ligands

A number of 5-HT receptor ligands were able to depress reflex responses. 5-CT was very potent in depressing the MSR (IC_{50} $3.6 \pm 0.5 \text{ nM}$, $n = 5$), but caused only a 50% depression of the PSR at a concentration of 30 nM (Figure 5). The effects of higher concentrations of 5-CT were not tested because of the very long-lasting depressant action on the MSR produced by

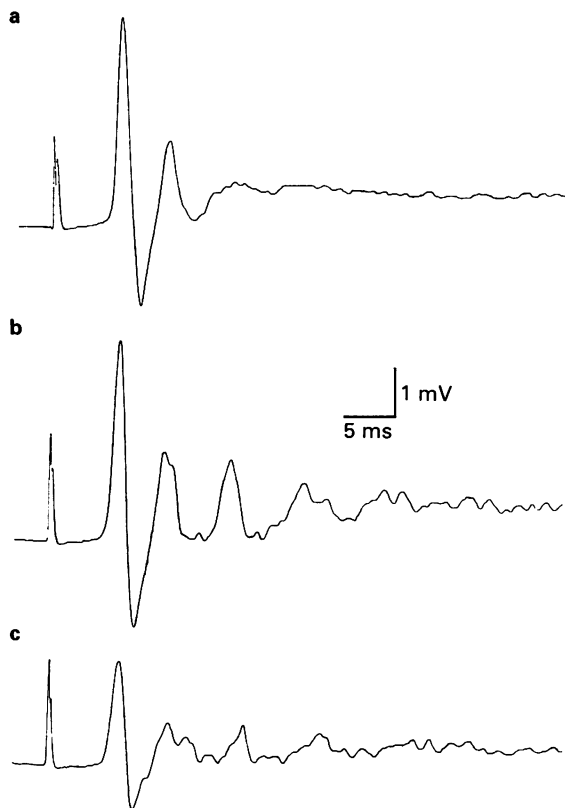


Figure 4 Effect of strychnine and 5-hydroxytryptamine (5-HT) in the presence of strychnine on reflex responses of neonate rat spinal cord: (a) control; (b) response in the presence of $1 \mu\text{M}$ strychnine and (c) depression of the response by $3 \mu\text{M}$ 5-HT in the continued presence of $1 \mu\text{M}$ strychnine.

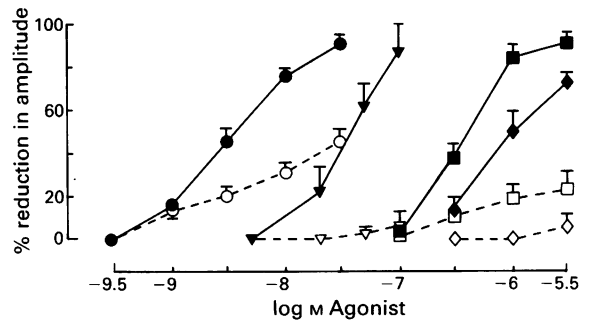


Figure 5 Concentration-response curves showing effects of 5-carboxamidotryptamine (5-CT), methysergide, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 1-[3-(trifluoromethyl)phenyl]-piperazine (TFMPP) on monosynaptic (MSR) and polysynaptic ventral root reflex (PSR). Solid lines and symbols show MSR and broken lines and open symbols show PSR. (○) 5-CT, $n = 5$; (▽) methysergide, $n = 3$; (□) 8-OH-DPAT, $n = 4$; (◇) TFMPP, $n = 3$.

this agent. Unlike 5-HT, 5-CT did not cause any detectable depolarization of motoneurons in concentrations up to 30 nM. 8-OH-DPAT was also capable of producing nearly complete inhibition of the MSR (IC_{50} $0.4 \pm 0.04 \mu\text{M}$, $n = 4$), but reduced the PSR by a maximum of only 20% (Figure 5). Similarly, TFMPP depressed the MSR (IC_{50} $0.93 \pm 0.3 \mu\text{M}$, $n = 3$, Figure 5), but had very little effect on the PSR. In confirmation of Saito *et al.* (1982), methysergide was an effective agonist for MSR depression (IC_{50} $21.8 \pm 3.0 \text{ nM}$, $n = 3$, Figure 5). Methysergide had very little effect on the PSR. RU 24969 ($0.05\text{--}10 \mu\text{M}$) also caused depression of the MSR, which was of very slow onset and which could not be reversed on washing. RU 24969 had no effect on the PSR. No attempt was made to estimate an IC_{50} for RU 24969.

Failure of ketanserin and spiperone to antagonize responses to 5-hydroxytryptamine

In control experiments, it was established that a second concentration-response curve could be constructed from the preparation following a wash period of 1 h after the last response determined for the first curve. The IC_{50} values for the depressant action of 5-HT on the MSR and PSR, determined for the second concentration-response curve for a preparation, were $10.0 \pm 3.2 \mu\text{M}$ ($n = 5$) and $3.6 \pm 0.9 \mu\text{M}$ ($n = 5$), respectively, and not significantly different from the IC_{50} values determined for the initial concentration-response curve.

The agonist action of 5-CT, 8-OH-DPAT and RU 24969 suggested that a 5-HT₁-like receptor may mediate the depression of reflex responses. In confirmation of this, the 5-HT₂ receptor antagonist ketanserin ($1 \mu\text{M}$), caused no appreciable blockade of responses to 5-HT (Figure 6). In the same experiments, baclofen ($0.3 \mu\text{M}$) was used as a control agonist mediating reflex depression. In 3 series of experiments $0.3 \mu\text{M}$ baclofen depressed the MSR by 12–36% and the PSR, on average, by 10%. Ketanserin ($1 \mu\text{M}$) did not reduce the depressant action exerted by baclofen.

Spiperone ($1 \mu\text{M}$) also failed to cause any significant blockade of these depressant actions of 5-HT (Figure 6). Neither the action of 5-HT in depressing the MSR nor its action in depressing the PSR were affected by spiperone.

Depression of reflex responses by blockers of neuronal 5-hydroxytryptamine uptake

Superfusion of the cord with a 5-HT uptake blocker such as citalopram sometimes caused a depression of reflex responses. This was seen consistently at a concentration of $1 \mu\text{M}$ citalopram and, usually, with a concentration of $0.1 \mu\text{M}$ citalopram. As can be seen from Table 2, $0.1 \mu\text{M}$ citalopram caused a reduction of the MSR by about 45% and a non-significant reduction of the PSR. That the depressant effect was due to an

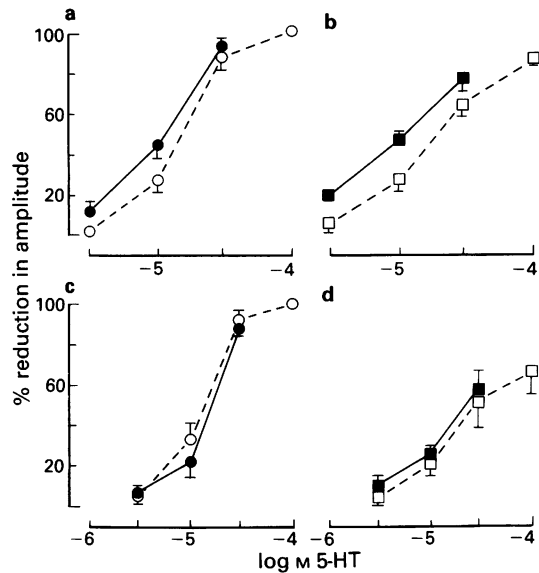


Figure 6 Effects of ketanserin (a,b) and spiperone (c,d) on depression of monosynaptic (MSR) and polysynaptic ventral root reflex (PSR) by 5-hydroxytryptamine (5-HT). Pooled data from 4 experiments with ketanserin and 4 experiments with spiperone. (a) Effect of $1 \mu\text{M}$ ketanserin on MSR; filled symbols controls and open symbols after incubation with ketanserin for at least 1 h. (b) Effect of $1 \mu\text{M}$ ketanserin on PSR in same cords; filled symbols controls and open symbols after ketanserin. (c) Effect of $1 \mu\text{M}$ spiperone on MSR; filled symbols controls and open symbols after incubation with spiperone for at least 1 h. (d) Effect of $1 \mu\text{M}$ spiperone on PSR in same cords; filled symbols controls and open symbols after spiperone.

action of citalopram itself was unlikely because a similar effect was observed with the uptake blocker, fluvoxamine ($1 \mu\text{M}$) (Table 2). Paradoxically, the 5-HT₂ receptor blocker, ketanserin at a concentration of $1 \mu\text{M}$ which did not block the depressant action of exogenous 5-HT (see above), was capable of reversing the depressant action of citalopram and fluvoxamine (Table 2). Ritanserin ($1 \mu\text{M}$) had a similar action.

In the presence of citalopram ($0.1 \mu\text{M}$) and ketanserin ($1 \mu\text{M}$), the concentration-response curve to 5-HT showed a small rightward shift such that the IC_{50} for depression of the MSR was $64.4 \pm 0.9 \text{ nM}$ ($n = 4$) and the IC_{50} for depression of the PSR was $298 \pm 96 \text{ nM}$ ($n = 4$).

Discussion

These experiments confirm that 5-HT has a potent depressant action on reflex responses generated in motoneurons by stimulation of a dorsal root. A depression of segmental transmission to neonatal rat motoneurons by 5-HT has been identified in intracellular studies on the hemisectioned cord (Elliott & Wallis, 1990a,b) and spinal cord slice (Wang *et al.*, 1990). The

use of motoneuron population responses has the advantages of averaging fluctuations in PSR amplitude seen in records from individual motoneurons (Elliott & Wallis, unpublished) and of permitting studies with agonists and antagonists of many hours duration.

Since 5-HT has a direct depolarizing action on motoneurons (Takahashi & Berger, 1990; Wang & Dun, 1990), the depolarization itself might occlude or shunt the excitatory postsynaptic potential (e.p.s.p.) and so reduce reflex responses. This seems unlikely as a principal mode of action because (a) the time courses of depolarization and reflex depression were different, (b) 5-CT caused reflex depression at concentrations that did not elicit depolarization, (c) agents such as 8-OH-DPAT and RU 24969 depressed the reflex but did not depolarize motoneurons (Elliott & Wallis, 1990b), (d) the EC_{50} for depolarization by 5-HT ($1.5 \mu\text{M}$, Elliott & Wallis, 1990b) is different from the IC_{50} for reflex depression of MSR when both are determined after blockade of neuronal 5-HT uptake (30 nM) and (e) depolarization itself which is accompanied by an increase in input resistance (Larkman *et al.*, 1989; Wang & Dun, 1990) would be expected to increase MSR amplitude (Barasi & Roberts, 1974).

5-HT can cause an increase in the frequency of inhibitory postsynaptic potentials (i.p.s.ps) arising in motoneurons (Wang & Dun, 1990; Elliott & Wallis, unpublished), due presumably to excitation of inhibitory interneurons. However, the depressant action of 5-HT on reflex responses was not due to release of glycine from interneurons because the depressant action was undiminished in the presence of strychnine. Strychnine was applied in an effective concentration since it facilitated the PSR, presumably by reducing reciprocal inhibition. This suggests but does not prove that the depressant action of 5-HT is via presynaptic receptors on the segmental pathway to motoneurons, as proposed by Wang *et al.* (1990). Assuming such presynaptic receptors are located on primary afferent terminals they will cause a consequent reduction in the monosynaptic e.p.s.p. However, the e.p.s.p. generated by polysynaptic pathways might be modulated in a more complex fashion because of interaction of 5-HT with interneurons in the pathway. This is borne out by the differences in the pharmacology of the 5-HT action on the MSR compared to its action on the PSR (see below).

Blocking neuronal uptake of 5-HT greatly affected the sensitivity of the reflexes to 5-HT. It follows that uptake of 5-HT into neurons must be particularly effective in the cord or at least in the vicinity of the 5-HT receptors responsible for the actions described here. After blockade, the IC_{50} for MSR depression was 30 nM while the IC_{50} for PSR depression was 89 nM . Blocking neuronal 5-HT uptake may possibly reveal a significant difference in sensitivity to 5-HT between MSR and PSR in a larger series of experiments. In these studies, the IC_{50} could only be determined in experiments where citalopram itself did not depress the MSR.

The agonist profile and the order of agonist potency for the depression of the MSR and depression of the PSR are shown in Table 3. 5-HT, 5-CT, methysergide, 8-OH-DPAT, RU

Table 2 Depressant effect of blockers of neuronal 5-hydroxytryptamine (5-HT) uptake on monosynaptic reflex (MSR) and polysynaptic reflex (PSR) amplitude

	MSR			PSR		
	Control	Cit	Cit + Ket	Control	Cit	Cit + Ket
Amplitude (mV)	2.00 ± 0.17	$1.11 \pm 0.20^*$	2.14 ± 0.19	0.74 ± 0.07	0.61 ± 0.06	0.72 ± 0.14
n	11	11	5	11	11	5
	MSR			PSR		
	Control	Flu	Flu + Ket	Control	Flu	Flu + Ket
Amplitude (mV)	2.11 ± 0.19	$1.14 \pm 0.15^*$	2.48 ± 0.36	0.69 ± 0.08	0.63 ± 0.09	0.73 ± 0.10
n	6	6	6	6	6	6

Cit: $0.1 \mu\text{M}$ citalopram; Flu: $1 \mu\text{M}$ fluvoxamine; Ket: $1 \mu\text{M}$ ketanserin.

* $P < 0.01$

Table 3 Pharmacology of 5-hydroxytryptamine (5-HT) responses of neonate rat spinal motoneurons

	Order of agonist potency	Inactive
Depression of monosynaptic reflex	5-CT > methysergide > 5-HT > 8-OH-DPAT > TFMPP	
Depression of polysynaptic reflex	5-HT > 5-CT	[8-OH-DPAT] RU 24969 TFMPP
Depolarization of motoneurone	5-HT > α -Me-5-HT > 5-CT > 5-MOT \gg T*	8-OH-DPAT RU 24969

5-MOT: 5-methoxytryptamine; T: tryptamine; 5-CT: 5-carboxamidotryptamine; 8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino)tetralin; TFMPP: 1-[3-(trifluoromethyl)phenyl]-piperazine

* Connell & Wallis (1989)

24969 and TFMPP caused near maximal reduction of the MSR. On the other hand, only 5-HT caused complete inhibition of the PSR, while 5-CT caused partial inhibition over the range of concentrations tested. 8-OH-DPAT, RU 24969, TFMPP and methysergide had either no or only a minor action in reducing the PSR (Table 3).

For reduction of the MSR, the IC_{50} for 5-HT (after blockade of neuronal uptake) was in the nmolar range, as was that for 5-CT and methysergide. IC_{50} values for 8-OH-DPAT and TFMPP were in the μ molar range. The order of potency was: 5-CT > methysergide > 5-HT > 8-OH-DPAT > TFMPP (Table 3). The agonist profiles for reflex depression can be contrasted with the profile for the receptor mediating motoneurone depolarization (Table 3), using data from Connell & Wallis (1989).

The results on depression of the MSR by 5-HT and 5-HT analogues may suggest mediation by a 5-HT_{1A} receptor, as suggested by Wang *et al.* (1990). A 5-HT₃ receptor is not involved because MDL 72222 is without effect on 5-HT depression of the MSR (Wang *et al.*, 1990) nor does the agonist profile suggest a 5-HT₄ receptor. However, our findings with antagonists and uptake blockers are not consistent with mediation by a 5-HT_{1A} receptor. Firstly, spiperone, which has a high affinity for 5-HT_{1A} receptors (Hoyer, 1989), did not block the effect of 5-HT and, secondly, the depression of the MSR by citalopram and fluvoxamine, presumably as a result of accumulation of endogenous 5-HT within the cord, was reversed by ketanserin. The latter observation indicates an involvement of 5-HT₂ receptors. Both spiperone and ketanserin should have blocked an action by exogenous 5-HT on 5-HT₂ receptors. Since they did not significantly reduce the actions of exogenous 5-HT before uptake blockade and ketanserin caused only a small rightward shift of the 5-HT concentration-response curve after uptake blockade, it may be concluded that exogenous 5-HT acts principally via a 5-HT₁-like receptor to depress the MSR. Perhaps the very avid uptake system or diffusional barriers limit access of exog-

enous 5-HT to the 5-HT₂ sites activated by endogenous 5-HT to reduce the MSR. Clearly, further experiments are required to resolve this paradox. It is possible that the complex pharmacology of these 5-HT actions might be the result of activation of a mixture of 5-HT receptor subtypes. Preliminary experiments indicate that the action of 8-OH-DPAT in depressing the MSR is antagonized by spiperone (Crick & Wallis, unpublished), which adds weight to this idea. Presynaptic inhibition of the synaptic input to rat locus coeruleus neurones is reported to be mediated by a mixed population of 5-HT_{1A} and 5-HT_{1B} receptors (Bobker & Williams, 1989).

It may be significant that in the spinalized cat a depression of the MSR can be induced by stimulation of the caudal raphe nuclei, perhaps by release of 5-HT (Clineschmidt & Anderson, 1970); the effect was mimicked by methysergide. In the neonate rat isolated spinal cord, the 5-HT-releaser, *p*-chloroamphetamine has also been shown to depress the MSR (Ohno & Warnick, 1989). Thus, the physiological pathway may involve a receptor at which methysergide is an agonist, a feature of 5-HT₁-like receptors (Bradley *et al.*, 1986).

At present, it is not possible to attribute depression of the PSR by 5-HT to a particular receptor subtype. Although neither 5-HT_{1A} nor 5-HT₂ receptors seem to be involved, the pharmacological profile is incomplete.

In conclusion, 5-HT has been shown to exert a potent depressant action on segmental reflexes; depression of the MSR is unrelated to the action of 5-HT in depolarizing motoneurons. Although depression of the MSR is mimicked by 5-HT_{1A} receptor ligands, the action of endogenous 5-HT may be mediated through 5-HT₂ receptors. Exogenous 5-HT may act at a mixture of 5-HT receptor subtypes to depress the MSR.

This work was supported by the Wellcome Trust. We would like to thank Sandoz Ltd. for the gift of methysergide, Glaxo Ltd for 5-CT, Roussel Uclaf Ltd for RU 24969, Janssen Ltd. for ketanserin and spiperone, Lundbeck Ltd. for citalopram and Duphar for fluvoxamine.

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(Received November 27, 1990

Revised February 7, 1991

Accepted March 7, 1991)