*Br. J. Pharmacol.* (1988), **95**, 437–448

# Effects of 5-hydroxytryptamine agonists and antagonists on the responses of rat spinal motoneurones to raphe obscurus stimulation

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**1** The excitability of lumbar spinal motoneurones was studied in halothane-anaesthetized rats by recording with microelectrodes the amplitude of the population spike evoked antidromically by stimulation of the cut ventral roots.

**2** Electrical stimulation of the nucleus raphe obscurus for 1 min at 20 Hz increased the population spike amplitude and, as shown by intracellular recording, depolarized motoneurones. This response could be mimicked by microinjection of DL-homocysteic acid into raphe obscurus but the response was not present in animals pretreated wtih the 5-hydroxytryptamine (5-HT) neurotoxin 5,7-dihydroxytryptamine (5,7-DHT).

**3** Microiontophoretically applied 5-HT had very similar effects on the extracellularly recorded population spike to those caused by stimulation of the raphe obscurus. These responses to 5-HT were larger in 5,7-DHT-pretreated animals.

**4** The effects of 5-HT were potently mimicked by iontophoretically applied 5-carboxamidotryptamine but 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) was without effect.

**5** Antagonists were applied by microiontophoresis and also by intravenous injection. Ketanserin, the selective 5-$HT_2$ antagonist, did not antagonize the effects of 5-HT. Neither did the 5-$HT_3$-receptor antagonist MDL 72222 or the selective 5-$HT_1$ binding ligand cyanopindolol.

**6** The non-selective 5-$HT_1$/5-$HT_2$-receptor antagonist methysergide was an effective antagonist of both the effects of 5-HT and the response to raphe obscurus stimulation. Methysergide did not reduce the excitatory effects of noradrenaline.

**7** It is concluded that 5-HT application and stimulation of raphe obscurus increase the excitability of motoneurones by an action on a 5-$HT_1$-like receptor which appears to be different from the 5-$HT_{1A}$- and the 5-$HT_{1B}$-binding sites characterized by others.

## Introduction

Spinal motoneurones are densely innervated by axon terminals which contain 5-hydroxytryptamine (5-HT) (Fuxe, 1965). These arise from axons which run in the ventral and ventrolateral funiculus and the cell bodies lie in the more posterior medullary raphe nuclei, predominantly nucleus raphe obscurus (Willis, 1984; Carlton *et al.*, 1985). 5-HT increases the excitability of spinal motoneurones (Barasi & Roberts, 1974; White & Neuman, 1980) but little more is known about the pharmacological nature of the receptors involved. It is also not clear if exogenously applied 5-HT acts on the motoneurone or presynaptically upon the 5-HT axons. The receptors involved in the responses to iontophoretically applied 5-HT may be similar or very different from those activated by stimulation of raphe obscurus.

In the central nervous system, different 5-HT binding sites have been called 5-$HT_1$, 5-$HT_2$ and 5-$HT_3$ (Peroutka & Snyder, 1979; Kilpatrick *et al.*, 1987). The 5-$HT_1$ sites have been further subdivided into at least A and B subtypes which seem to have functional relevance (Deshmukh *et al.*, 1983), although less well defined C and D subtypes have also been described (Pazos *et al.*, 1984; Heuring & Peroutka, 1987). Functional receptors to 5-HT have been studied mostly in peripheral tissues and have been similarly named 5-$HT_1$-like, 5-$HT_2$ and 5-$HT_3$ (Bradley *et al.*, 1986). Although it is clear that 5-$HT_1$-like receptors are a heterogeneous group, subdivision was not attempted due to lack of definitive data. In the CNS, Davies *et al.* (1988a,b) have

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shown that 5-$HT_2$-receptors mediate the excitatory effects of 5-HT on medullary neurones and 5-$HT_1$-like receptors cause the depressant responses to 5-HT. The 5-$HT_{1A}$ selective binding ligand, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) is a potent agonist at the depressant receptor. It should be realised that the characteristics of the 5-$HT_1$-binding sites and the 5-$HT_1$-like receptors are not necessarily identical.

The present study examines the effects of antagonists and the new 5-HT agonists on spinal motoneurone excitability. These effects are compared with responses to stimulation of raphe obscurus in normal and 5,7-dihydroxytryptamine pretreated rats in which fibres containing 5-HT have been destroyed. It is concluded that 5-HT increases the excitability of motoneurones by an action on an 8-OH-DPAT insensitive 5-$HT_1$-like receptor which is antagonized by methysergide but not by cyanopindolol. These receptors are similar to those activated by stimulation of raphe obscurus and they are not presynaptic autoreceptors. A preliminary account of these data has been published (Roberts *et al.*, 1987).

## Methods

### *Surgical procedures*

Experiments were conducted on male albino Wistar rats weighing 240–290 g. Anaesthesia was induced with halothane (3% in $O_2$). Following tracheal intubation this was reduced to 1.3% and on completion of surgical procedures this was further reduced to 0.9%. At no stage could withdrawal responses or corneal reflexes be evoked. Blood pressure was monitored via a cannula in the carotid artery and was typically 110/60 mmHg. An intravenous cannula was inserted into the external jugular for systemic injection of drugs and needle electrodes were inserted subcutaneously on either side of the thorax to monitor the electrocardiogram. The head was held in a stereotaxic frame, a support placed under the pelvis and gentle traction applied to the tail. The skin of the back was incised, the dorsal musculature dissected and two lumbar vertebrae (L2–L3) clamped by wedge-shaped rods which passed through the skin. The skin flaps were tied up to form a pool into which warm paraffin was placed when surgery was complete. The dorsal laminae were removed between L1 and L6 and the dura mater was slit longitudinally and pinned back. The arachnoid was removed and L2–L5 dorsal and ventral roots were cut unilaterally. The cut ventral roots were laid across silver wire stimulating electrodes and immersed in paraffin.

### *Stimulation of raphe obscurus*

A hole was drilled through the skull above the cerebellum for the insertion of a steel guide cannula. An insulated coaxial steel stimulating electrode was lowered through the guide cannula so that the tip lay in the nucleus raphe obscurus. The atlas of Paxinos & Watson (1982) was used and the coordinates were: AP −3.0; lateral 0.0; vertical +0.5. Square wave stimuli of 5 ms duration, 20 Hz, 3.0 V were applied for a period of 1 min. Electrical stimuli are capable of exciting or blocking fibres or cell bodies in the region of the electrode. To confirm that responses from motoneurones were elicited by the excitation of cells, D,L-homocysteic acid was microinjected into the region of raphe obscurus. On these occasions, the stimulating electrode was removed from the stereotaxically positioned guide cannula and replaced by a microinjection assembly (Azami *et al.*, 1980). The microinjection needle was 90 micron o.d. glass cut to exactly the same length as the stimulating electrode; 125 nl of 0.5 M D,L-homocysteic acid was injected slowly over a 1 min period and the needle left in place for 5 min.

### *Recording*

Recordings from the ventral horn of the lumbar enlargement of the spinal cord were made with five barrelled glass microelectrodes of 7–10 micron tip diameter. The recording barrel and the current control barrel contained 4 M NaCl. The microelectrode was inserted vertically to a depth of 1000 microns into the lumbar enlargement at a point just lateral to the dorsal root entry zone. It was then advanced slowly to a maximum depth of 2000 microns where the ventral surface was encountered. Motoneurones lie between 1200 and 1800 microns from the surface. As the electrode was advanced, 0.1 ms, 0.4 V square wave stimuli at 0.3 Hz were applied to the ventral roots. The intensity was just supramaximal for activation of A fibres. This antidromic activation of motoneurones evoked a population spike which increased in amplitude with increasing intensity of ventral root stimulation. Drugs were applied iontophoretically into the ventral horn whilst recording the amplitude of the population spike. Permanent records were made by means of a gated peak detector and sample and hold circuit which drove the pen of a curvilinear polygraph. It has been demonstrated previously that the amplitude of this population spike is a useful index of the excitability of motoneurones (Barasi & Roberts, 1974; Parry & Roberts, 1980; Lipski, 1981).

Experiments were also conducted with single barrelled intracellular microelectrodes filled with 2 M potassium citrate. Recordings were made of the

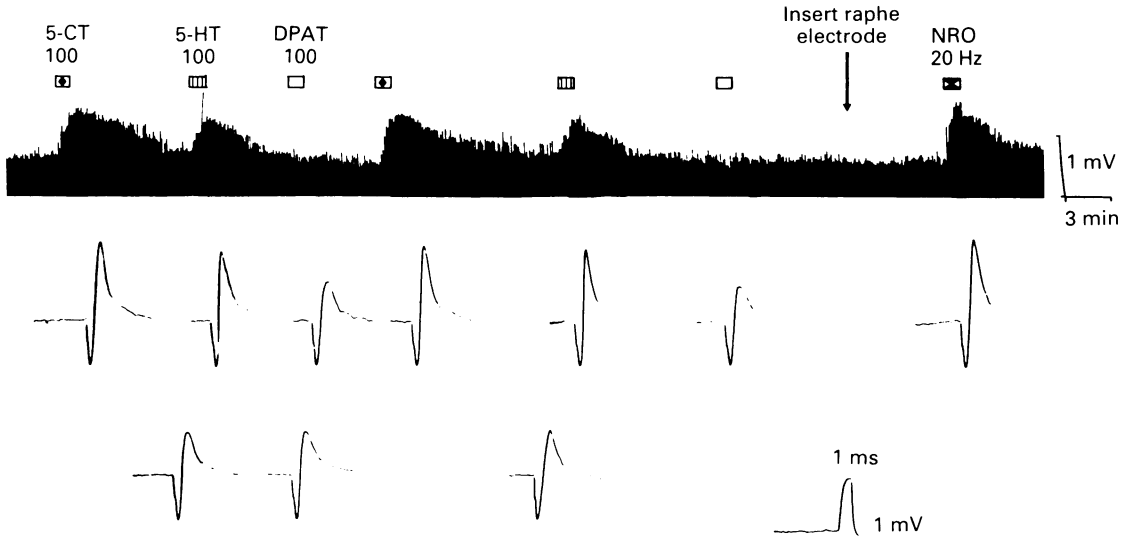


**Figure 1** The effects of 5-carboxamidotryptamine (5-CT), 5-hydroxytryptamine (5-HT) and 8-hydroxy-2-(di-n-propylamino) tetralin (DPAT) applied iontophoretically with 100 nA and electrical stimulation of nucleus raphe obscurus (NRO) on the spike potential evoked from motoneurones by stimulation of the ventral roots. The drugs and stimulation were applied for the period indicated by the bar above the record. The upper record is a polygraph trace where each vertical line represents the amplitude of spike potentials which were evoked every 3 s. The traces below this record are examples of spike potentials taken from the oscilloscope. Negativity at the electrode tip is an upward deflection. These lower records were recorded at the same time as the upper record at a point vertically above the peak of the negative spike.

membrane potential and responses of motoneurones to ventral root and raphe obscurus stimulation. These studies helped to confirm the interpretation of extracellularly recorded responses.

*Drugs*

The three drug barrels of the microelectrode contained a combination of 5-hydroxytryptamine hydrogen maleate (0.2 M, pH 4.5); noradrenaline bitartrate (0.2 M; pH 5.0); 5-carboxamidotryptamine maleate (5-CT, 0.1 M, pH 4); 8-hydroxy 2-(di-n-propylamino) tetralin hydrogen bromide (8-OH-DPAT, 0.02 M, pH 4); cyanopindolol base (0.05 M, adjusted to pH 3.0 with HCl); methysergide maleate (0.01 M, pH 4); 1aH,3a,5aH-tropan-3yl-3,5-dichlorobenzoate methanesulphonate (MDL 72222, 0.01 M, pH 5); ketanserin tartrate (0.05 M, pH 3.5–4.0). The drugs were ejected from the electrode tip by microiontophoresis and during the ejection, the current flow in the NaCl barrel was reversed to ensure no change in the net flow of current from the electrode (current balancing – Roberts & Straughan, 1967).

5-HT and noradrenaline were alternately applied by microiontophoresis into the ventral horn until stable and repeatable responses were established to each agonist. The antagonist was then applied from the same microelectrode assembly until a marked change was observed either in the baseline population spike amplitude or in the response to the agonists. If selective antagonism of the response to 5-HT was observed, the response to stimulation of raphe obscurus was studied. Application of the antagonist was then stopped and the agonist cycle continued to determine if recovery of the responses could be obtained.

Some studies were conducted on rats which had been pretreated with 5,7-dihydroxytryptamine. The creatinine sulphate salt equivalent to 150 $\mu$g of the free base was dissolved in 20 $\mu$l of 0.9% NaCl containing 0.2% ascorbic acid. This was microinjected into the lateral ventricle at least 2 weeks before electrophysiological experiments. Co-ordinates from bregma with the incisor bar set at −2.5 mm were AP + 0.8; L 1.5; V 4.6 mm below the skull surface. The completeness and permanence of the degeneration of 5-HT-containing fibres in the spinal cord, using this drug, have been confirmed both biochemically and histochemically (Foster *et al.*, 1985; 1988).

## Results

*The effects of stimulating nucleus raphe obscurus*

The response to ventral root stimulation which was recorded extracellularly from the ventral horn is

illustrated in Figure 1. This consisted of a very short latency (<0.2 ms) positive potential followed by a larger negative potential of about 0.5 ms latency and 1.5 ms duration. The amplitude of these waveforms varied with the position of the recording electrode in the ventral horn, being maximal between 1400 and 1600 microns below the surface. No drugs or central stimuli ever altered the amplitude of the positive waveform, but the amplitude of the negative component was very labile and was increased to 180–400% by stimulation of raphe obscurus. The origin of these waveforms has been extensively discussed elsewhere (for review see Lipski, 1981) and it has been concluded that the negative waveform comprises a population spike recorded from cell bodies of motoneurones.

The effects of raphe obscurus stimulation on the extracellularly recorded population spike are illustrated in Figure 1. The stimulation parameters were carefully chosen to ensure activation of small cells or fibres in raphe obscurus which may conduct action potentials slowly and only at low frequencies. Of course, such stimuli will also activate large fibres. The population spike grew in amplitude throughout the 1 min period of stimulation and continued to grow after the stimulation was stopped. The average latency to peak was $82.9 \pm 5.82$ s (mean ± s.e.mean, $n = 16$) after the beginning of the stimulus. The average response duration was $5.27 \pm 0.73$ min (mean ± s.e.mean).

The effect of raphe obscurus stimulation on the response of a single motoneurone recorded intracellularly is shown in Figure 2. The lower trace shows that the membrane potential of 72 mV depolarizes progressively during stimulation of raphe obscurus. The upper record shows three responses of the cell to ventral root stimulation. During the small depolarization induced by raphe obscurus stimulation (2 in the lower record), the full soma-dendritic action potential was recorded but before and after obscurus stimulation (1 and 3 in the lower record) only the initial segment action potential was seen.

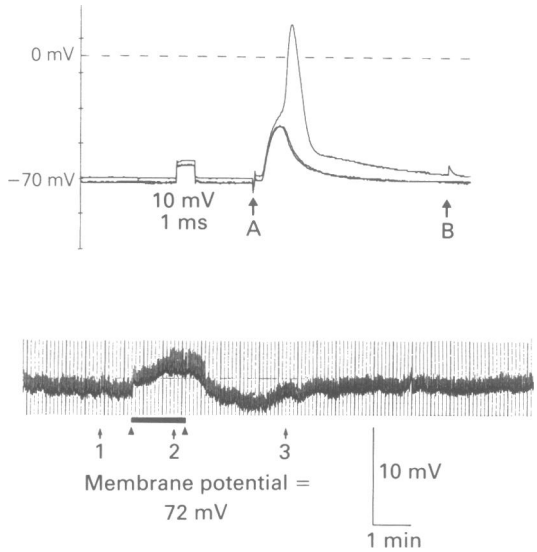


**Figure 2** Intracellularly recorded potentials from a lumbar motoneurone. The upper record shows an X-Y plot from the oscilloscope of 3 responses to stimulation of a ventral root at the time marked A. The lower trace shows a d.c.-coupled polygraph record of the membrane potential of the same motoneurone. The membrane potential of this cell was 72 mV. At the times indicated by 1, 2 and 3 on the lower record, the upper three traces were taken. For the period indicated by the black bar under the lower trace, the nucleus raphe obscurus was stimulated at 20 Hz. This caused the 3 mV depolarization of the membrane potential which can be seen both in the lower record and the upper record. The upper record shows clearly that the small depolarization of this cell was accompanied by a profound change in the amplitude of the antidromically-evoked action potential. B indicates the stimulus artifact caused by the stimulation of the raphe obscurus. Each of the upper 3 traces contains a 10 mV, 1 ms calibration pulse. The vertical upstrokes on the lower record are indications of the antidromic action potentials evoked every 2 s which were too fast to be recorded by the pen of the polygraph.

### *The microinjection of D,L-homocysteic acid into nucleus raphe obscurus*

The effect of microinjecting 125 nl of 0.5 M D,L-homocysteic acid into raphe obscurus is illustrated in Figure 3. Microinjection of similar volumes of physiological saline into raphe obscurus had no effect on the population spike recorded in the lumbar ventral horn. The latency to onset of the response in Figure 3 was 4.8 min and the duration of the response was 29 min. Responses to these injections were extremely variable in latency, duration and amplitude but the long latency and duration illustrated was typical.

### *The effects of 5-hydroxytryptamine and other agonists applied by microiontophoresis*

The iontophoretic application of 5-HT into the ventral horn with a current of 100 nA for a period of 1 min frequently increased, but never reduced, the amplitude of the population spike. The size of these effects depended upon the depth of the electrode within the ventral horn. At depths less than 1200 microns below the surface very small (<300 μV) population spikes were recorded and 5-HT rarely influenced these. Between 1200 and 1600 microns below the surface, larger responses (0.5–2.5 mV) to

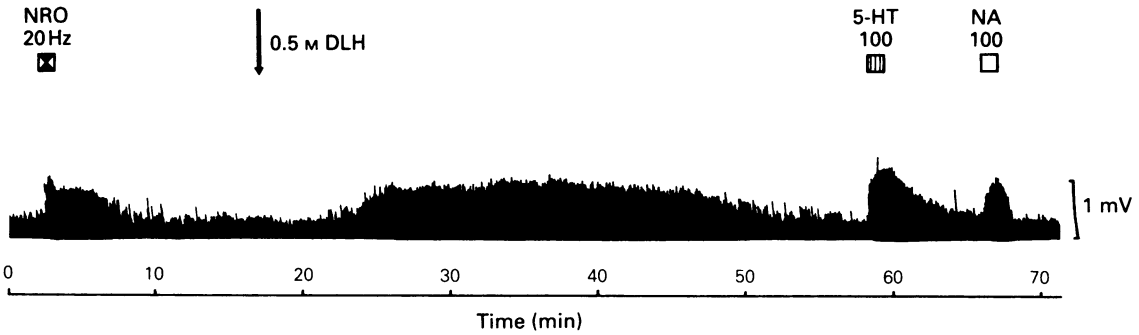


**Figure 3** The effect of raphe obscurus stimulation (NRO), microinjection of D,L-homocysteic acid (DLH) into raphe obscurus and iontophoretic application of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on the population spike amplitude. This polygraph record was obtained as described in Figure 1. Raphe obscurus stimulation, 5-HT and NA application all increased the amplitude of the population spike. At the point indicated by the arrow above the record 125 nl of 0.5 M D,L-homocysteic acid was microinjected slowly into raphe obscurus. A long latency, long duration increase in the population spike amplitude resulted.

ventral root stimulation were encountered and these were nearly always increased in amplitude by 5-HT. Although large waveforms were recorded from depths below 1600 microns these were relatively insensitive to 5-HT. A sample of 35 responses to 5-HT applied with a current of 100 nA for 1 min and recorded at depths between 1200 and 1600 micron showed that the population spike increased in amplitude by 82.9 ± 12.9% (mean ± s.e.mean) and the response lasted for 3.97 ± 0.25 min. At any particular depth, the amplitude and the duration of the response to 5-HT was dependent upon the size of the iontophoretic current.

Figure 1 illustrates a typical response to 5-HT. Responses from a particular recording site were very stable and repeatable during 2–3 h periods (see Figures 5 and 6). Tachyphylaxis to repeated applications of 5-HT was not a problem even when the interval between applications was shortened.

Microiontophoretic ejection of 5-carboxamidotryptamine (5-CT) also increased the amplitude of the population spike recorded from motoneurones (Figure 1). The effects of 5-CT (and other agonists) were always compared to the effects of 5-HT ejected from the same electrode assembly at the same location. Usually, identical iontophoretic currents and ejection periods were used for all agonists and, as it has been demonstrated that the transport numbers of 5-HT and 5-CT do not differ significantly (Davies *et al.*, 1988a), some biological relevance can be attached to the comparative size of responses to the two drugs. The ratio of response amplitudes (5-CT/5-HT) was 0.95 ± 0.004 (mean ± s.e.mean, $n = 10$) indicating that responses to 5-CT were very slightly smaller than responses to 5-HT. However, the ratio of response durations (5-CT/5-HT) was 1.42 ± 0.137 (mean ± s.e.mean, $n = 10$, $P < 0.05$) indicating that responses to 5-CT were much longer lasting than responses to 5-HT.

Similar studies were conducted with the 5-HT agonist 8-OH-DPAT (Figure 1). Although earlier studies have shown that iontophoretic release of the compound occurs from identical microelectrodes (Davies *et al.*, 1988a), 8-OH-DPAT did not affect the amplitude of the population spike even when applied with high currents (200 nA) for long periods (3–4 min).

Noradrenaline was ejected from microelectrodes into the ventral horn and was found to increase the amplitude of the population spike (Figures 3, 5, 6 and 7). This effect was not studied further but responses to noradrenaline were used in studies of antagonists to determine the ability of the antagonists to discriminate between responses to indole- and catecholamines.

*The effects of antagonists of 5-hydroxytryptamine*

The $5\text{-HT}_2$-receptor antagonist ketanserin was applied on 8 occasions with currents between 10 and 50 nA applied for between 12 and 86 min. Selective antagonism of the 5-HT response was never seen. At the higher iontophoretic currents (50 nA) a general reduction in amplitude of the population spike was observed and all responses to agonists (5-HT and noradrenaline) were reduced non-selectively. At lower iontophoretic currents of ketanserin (10–20 nA) no effect was observed for 10–20 min and then a gradual reduction of baseline population spike amplitude was seen but the responses to 5-HT were unchanged (Figure 4). Intravenously applied ketanserin with cumulative doses up to 2 mg kg$^{-1}$ was

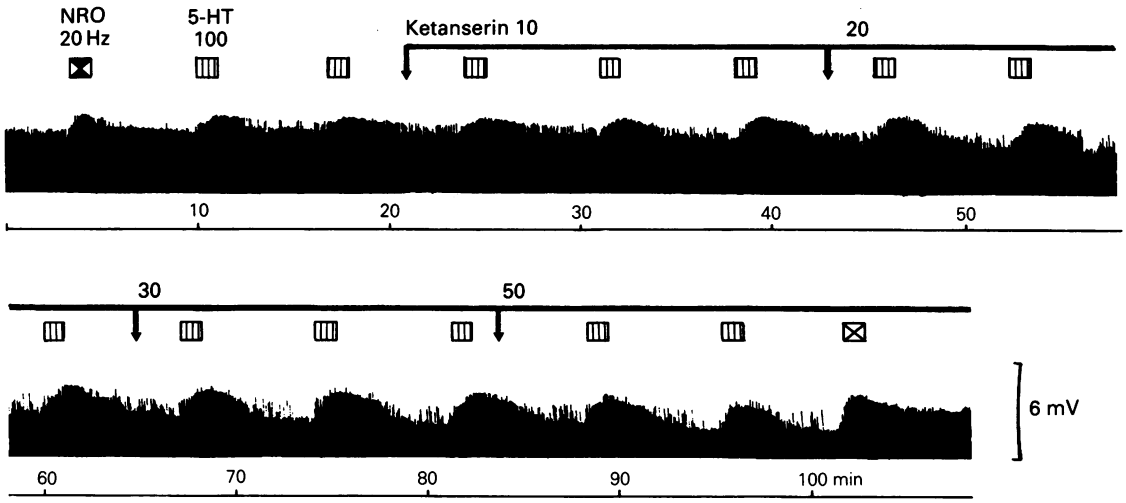


**Figure 4** The effect of the selective 5-$HT_2$-receptor antagonist, ketanserin, on responses to iontophoretically applied 5-HT. This polygraph record was obtained as described in Figure 1. Stimulation of raphe obscurus (NRO) and iontophoretic application of 5-HT with a current of 100 nA for 1 min caused a very small increase in the amplitude of the population spike. Ketanserin was applied iontophoretically for 86 min with currents of 10–50 nA without effect.

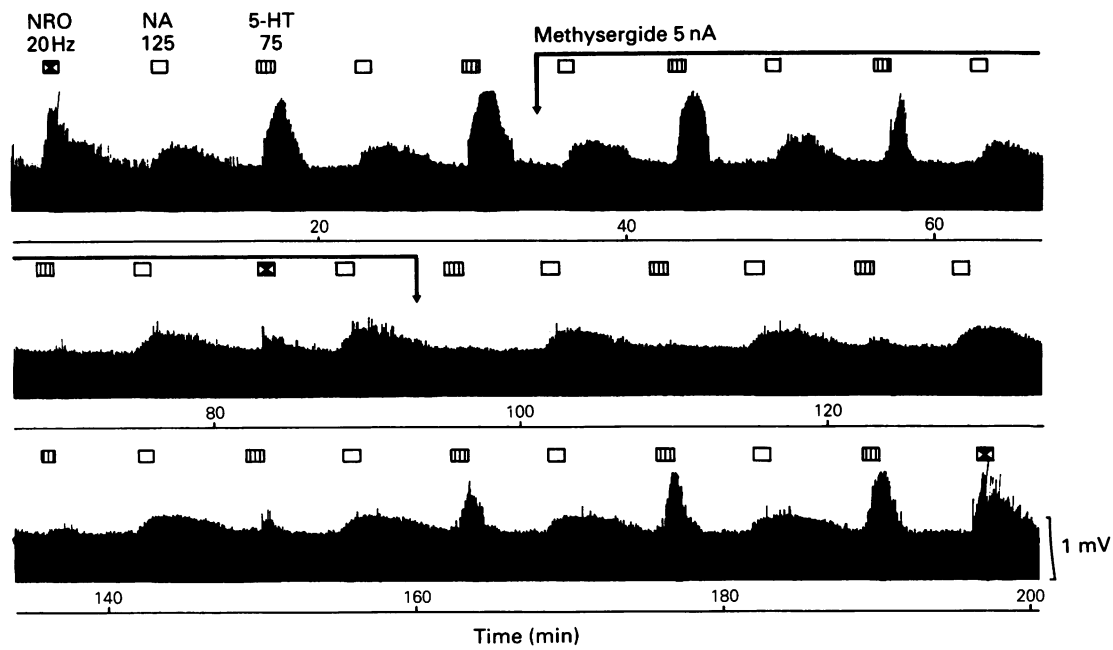


**Figure 5** The effects of iontophoretically applied methysergide on the response to stimulation of the nucleus raphe obscurus. The polygraph record was obtained as described in Figure 1. Raphe obscurus stimulation (NRO), iontophoretic application of noradrenaline (NA, 125 nA for 1 min) and 5-hydroxytryptamine (5-HT, 75 nA for 1 min) all increased the population spike amplitude. Methysergide, applied iontophoretically with a current of 5 nA for 60 min practically abolished the response to 5-HT without similarly affecting the response to noradrenaline. Towards the end of the application of methysergide, the response to raphe obscurus stimulation was nearly abolished. During the subsequent 100 min the responses to 5-HT and to raphe obscurus stimulation recovered fully. Methysergide selectively and reversibly blocked responses to 5-HT and raphe obscurus stimulation with a similar time course.

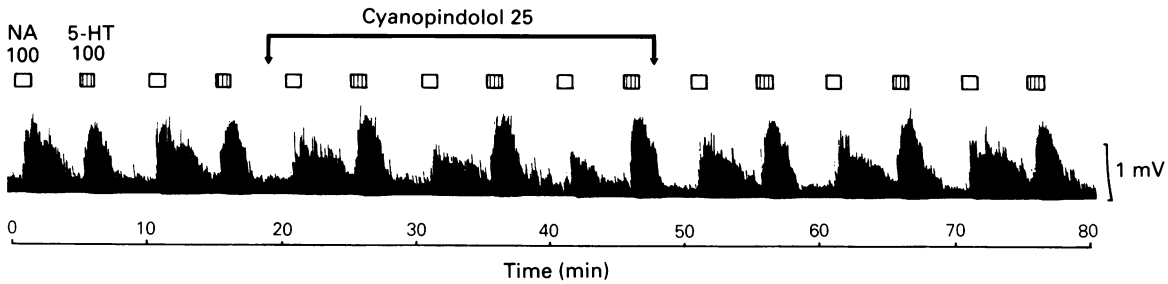


**Figure 6** The effect of the selective 5-$HT_1$-binding ligand cyanopindolol on responses to noradrenaline (NA) and 5-HT. The polygraph record was obtained as described in Figure 1. Noradrenaline and 5-HT both increased the population spike amplitude. Cyanopindolol, applied with a current of 25 nA for 28 min, had little effect on responses to 5-HT but considerably reduced responses to noradrenaline. These recovered shortly after terminating the application of cyanopindolol.

similarly without effect ($n = 5$). Responses to electrical stimulation of raphe obscurus were also unaffected by ketanserin on the six occasions these were tested.

The non-selective 5-$HT_1$/5-$HT_2$-receptor antagonist methysergide was similarly studied with iontophoretic currents between 5 and 25 nA applied for 20 to 80 min ($n = 10$). Each application was continued until some effect of the antagonist was recorded. On 8 occasions selective antagonism of the 5-HT response was seen without block of the response to noradrenaline (Figure 5). On one occasion noradrenaline was not used and the selectivity of the antagonism was not determined, and on the other occasion responses to 5-HT and noradrenaline were reduced to a similar extent. Recovery of the 5-HT responses occurred between 40 and 80 min after stopping application of the antagonist. Methysergide was not observed to have any direct action of its own on baseline population spike amplitudes. Figure 5 also shows that reduction of the response to iontophoretically applied 5-HT was accompanied by reduction of the response to stimulation of raphe obscurus. Recovery of the response to raphe obscurus stimulation was complete 100 min after termination of the antagonist application. Similar observations were made on 3 occasions with intravenous applications of methysergide, although complete recovery of the 5-HT response was not obtained with this route of administration. Cumulative doses to 1 mg $kg^{-1}$ methysergide reduced the 5-HT response to 50% of its original amplitude without affecting the noradrenaline response. Doses of 2–3 mg $kg^{-1}$ methysergide abolished the response to 5-HT with little effect on responses to noradrenaline.

The 5-$HT_3$-receptor antagonist MDL 72222 was also studied with iontophoretic currents of 15–25 nA applied for 34–55 min. No effect of these applications was seen on responses to 5-HT or noradrenaline until sudden abolition of the population spike occurred. The application of the antagonist was then terminated and recovery of the population spike took place during the next 5 min. Responses to 5-HT and noradrenaline were present immediately during this recovery ($n = 4$).

These antagonist studies showed that only methysergide, which is known to have affinity for 5-$HT_1$-binding sites in the CNS, was an effective selective antagonist of responses to 5-HT. Studies were therefore made of the effects of cyanopindolol which is also known to have high affinity for some 5-$HT_1$-binding sites. Cyanopindolol was studied on 12 occasions with iontophoretic currents between 12 and 30 nA applied for 11–65 min. On 5 occasions a selective and reversible block of responses to noradrenaline were observed (Figure 6). Cyanopindolol had marked non-selective effects on the population spike amplitude and responses to 5-HT and noradrenaline in 4 studies. In 3 studies cyanopindolol was without effect. Cyanopindolol was administered intravenously in 2 studies with doses up to 10.2 mg $kg^{-1}$ without any selective effect on the response to 5-HT.

*Responses to 5-HT and raphe obscurus stimulation in the rat depleted of 5-HT*

In animals pretreated with the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), iontophoretically applied 5-HT elicited responses which were much larger than those in normal animals (Figure 7). This was not due principally to an increase in response amplitude but rather to an increase in response duration. The response to 100 nA of 5-HT applied for 1 min in normal animals consisted of an increase of the population spike amplitude by 82.9 ± 12.9% (mean ± s.e.mean, $n = 35$); in depleted

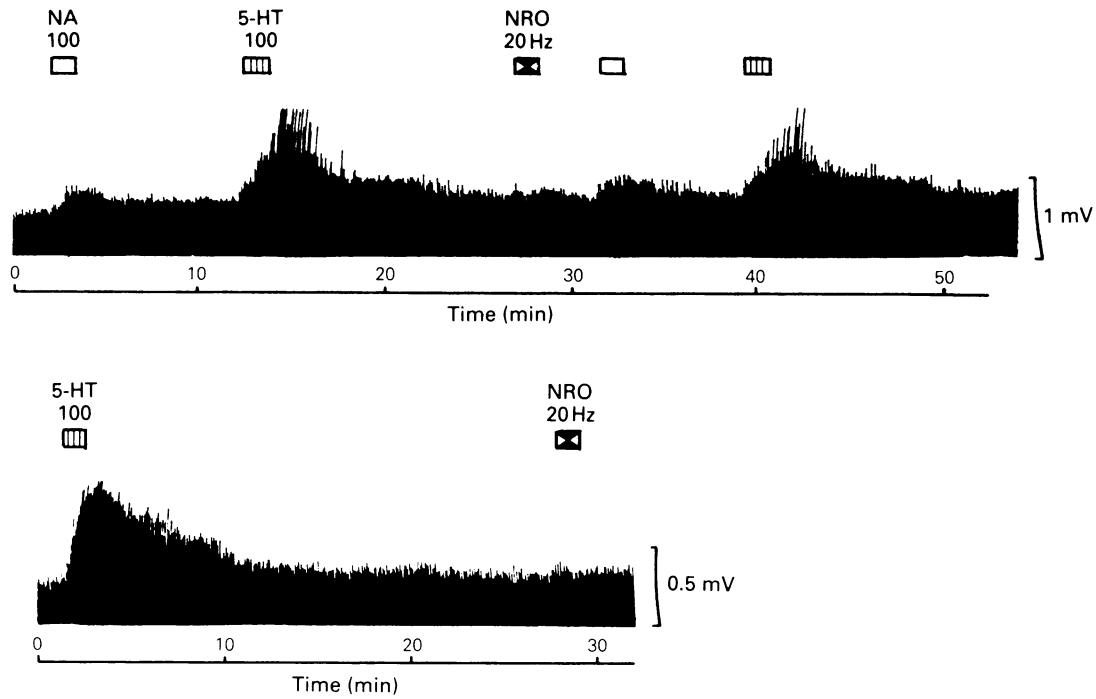


**Figure 7** The effects of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and stimulation of nucleus raphe obscurus (NRO) in animals in which 5-HT axons had previously been destroyed by intraventricular injections of 5,7-dihydroxytryptamine. The polygraph records were obtained as described in the legend to Figure 1. The upper and lower traces were obtained from studies in two different animals. In the upper record, the response to noradrenaline was very small and the response to 5-HT was big. Both drugs were applied with 100 nA for 1 min and in normal animals such applications gave responses which were not so grossly different in size (see Results). Stimulation of raphe obscurus was without effect. In the lower record, obtained from another animal, the abnormally large response to 100 nA of 5-HT for 1 min can also be seen together with the absence of any response to raphe obscurus stimulation.

animals the population spike increased by 105.57 ± 16.32% ($n = 14$, NS, $t$ test). The duration of the response in normal animals was 3.97 ± 0.24 min and in depleted animals was 10.38 ± 1.44 min ($P < 0.001$, $t$ test). This effect of depletion was selective for responses to 5-HT as there was no increase in responses to a standard application of noradrenaline at 100 nA for 1 min (increase in response amplitude: normals = 67.9 ± 9.14%, $n = 14$; depleted 42.6 ± 6.26%, $n = 5$, NS; response duration normals = 5.15 ± 0.40 min; depleted = 4.84 ± 1.24 min, NS).

The stimulation of raphe obscurus in 5,7-DHT lesioned animals was very ineffective (Figure 7) when compared to responses in control animals (Figure 1). The percentage increase in amplitude of the population spike in response to the standard parameters of stimulation in normal animals was 161.3 ± 27.7% (mean ± s.e.mean, $n = 12$) and in depleted animals was 8.1 ± 6.28% ($n = 10$, $P < 0.001$). The duration of responses was 7.0 ± 0.90 min in normals and 0.88 ± 0.48 min in depleted animals ($P < 0.001$).

## Discussion

It is well known that 5-HT increases the excitability of motoneurones (Barasi & Roberts, 1974; McCall & Aghajanian, 1979), and the present studies confirm these earlier findings. Recent studies of isolated, hemisected, neonatal spinal cords have also shown 5-HT to have potent depolarizing actions on motoneurones (Connell & Wallis, 1987a). Engberg & Ryall (1966) and Phillis *et al.* (1968) had previously obtained an inhibitory action of 5-HT but this was subsequently shown to be a non-specific effect of the amine (Engberg *et al.*, 1976). Engberg *et al.* (1979) also criticised the use of extracellular recording of the antidromically evoked response of motoneurones and showed that in the cat anaesthetized with barbi-

turate, iontophoretically applied 5-HT had little effect on intracellularly or extracellularly recorded responses of motoneurones. Lipski (1981) in a review of antidromic invasion of motoneurones and other cells, concluded that the technique was of value. This leaves the possibility that the barbiturate anaesthesia used by Engberg *et al.* (1979) significantly affected the response of motoneurones to 5-HT in much the same way as barbiturates affect the responses of cortical neurones to 5-HT (Johnson *et al.*, 1969).

Intracellular recording *in vivo* for 2–3 h is impractical with electrodes having attached iontophoresis barrels and this is the time required for identification of agonist and antagonist interactions. However, intracellular recordings were made in the present studies to identify the effects of electrical stimulation of raphe obscurus. Repetitive stimulation for 1 min caused a small depolarization (5 mV) to build up slowly and outlast the stimulation for 20–30 s. Simultaneously, the antidromically-evoked action potential which was small (30 mV), suddenly became very large (100 mV). This phenomenon was studied by Coombs *et al.* (1955; 1957a,b) who demonstrated that the antidromically-evoked action potential fails to invade the soma and dendrites of a well polarized motoneurone. Depolarization of the motoneurone close to threshold allows the action potential to pass the axon hillock and invade the whole cell. The small depolarization caused by raphe obscurus stimulation was sufficient to cause complete invasion of the cell. Raphe obscurus stimulation regularly increased the amplitude of the extracellularly-recorded, antidromically-evoked population spike. Frequently the extracellular response was unitary in nature, increasing in a series of steps (see Barasi & Roberts, 1977, Figure 1c). This is almost certainly due to motoneurones in the vicinity of the electrode depolarizing (or increasing membrane resistance) sufficiently to allow the antidromically evoked action potentials to invade the soma and dendrites.

The extracellular recording of the population spike response to ventral root stimulation is a direct test of the excitability of motoneurones. The latency of the response is about 0.5 ms and this is too short for it to be evoked by Renshaw or other polysynaptic responses to ventral root stimulation.

Although the effects of raphe obscurus stimulation closely resemble the effects of 5-HT applied by iontophoresis there was little evidence from previous studies to suggest that the same receptors were involved in both responses, and the receptor type had not been characterized. 5-CT and 8-OH-DPAT were both applied microiontophoretically at recording locations where 5-HT applied with identical currents (100 nA, 1 min) had a clear effect on the population spike. 5-CT always mimicked 5-HT and 8-OH-DPAT never displayed an agonist effect. It is not possible that the lack of 8-OH-DPAT effects were due to its failure to emerge from the electrodes as studies of the comparative mobilities of the drugs showed that both 5-CT and 8-OH-DPAT had transport numbers similar to, or slightly greater than that of 5-HT (Davies *et al.*, 1988a). Preliminary accounts of the effects of 5-CT and 8-OH-DPAT on the isolated neonatal spinal cord have similarly shown 5-CT to be a potent agonist and 8-OH-DPAT to be ineffective (Connell & Wallis, 1987b). The potent facilitatory effect of 5-CT suggests the receptor is a 5-$HT_1$-type (Bradley *et al.*, 1986) and therefore is different from the 5-$HT_2$-receptor mediating the excitatory actions of 5-HT in the brain stem (Davies *et al.*, 1988a). Although 5-CT is a weak agonist at 5-$HT_2$-receptors, the excitatory effects of 5-HT on brainstem neurones are very rarely and only weakly mimicked by 5-CT. However, the depressant effects of 5-HT on other brainstem neurones are potently mimicked by 5-CT (Davies *et al.*, 1988a), but this brainstem 5-$HT_1$-like receptor seems to be different from that on motoneurones because in the brainstem 8-OH-DPAT is also a potent agonist.

High affinity binding sites for [$^3$H]-5-HT exist in the spinal cord (Hernandez *et al.*, 1984), although levels of 5-$HT_{1A}$, 5-$HT_{1B}$ and 5-$HT_{1C}$ sites are very low (Pazos & Palacios, 1985). Huang & Peroutka (1987) have recently confirmed the existence of 1A, 1B and 1C sites in spinal cord homogenates but also demonstrated that 33% of the 5-$HT_1$-sites have characteristics which conform to none of the known subtypes. As Mitchell & Riley (1985) have also demonstrated that 5-$HT_2$ binding may be detected in spinal cord, iontophoretically applied 5-HT may bind to any of these recognition sites.

Ketanserin is a highly selective ligand displacing 5-HT from 5-$HT_2$-binding sites (Leysen *et al.*, 1981) and is also a selective antagonist of functional responses mediated via the 5-$HT_2$-receptor (Bradley *et al.*, 1986). Ketanserin did not reduce the responses of motoneurones to 5-HT even when applied with iontophoretic currents or intravenous doses considerably in excess of those required to antagonize responses at 5-$HT_2$-sites in the brainstem (Davies *et al.*, 1988a). This suggests that 5-$HT_2$-sites are not involved in the response of motoneurones to 5-HT. When applied with high currents for long periods, ketanserin reduced the baseline population spike amplitude. There are two possible explanations of this effect. The local, iontophoretic application of high currents of ketanserin into the environment of spontaneously active brainstem neurones has been shown to reduce the amplitude and prolong the duration of the action potential (Davies *et al.*, 1988a). It may have the same non-specific, membrane stabilizing effect upon motoneurones. Alternatively, the effect may be due to the $\alpha$-adrenoceptor

blocking actions of ketanserin. If the excitability of motoneurones is maintained in these preparations by a tonic release of noradrenaline, antagonism of adrenoceptors would reduce the population spike amplitude. However, ketanserin did not selectively reduce responses to iontophoretically applied noradrenaline.

5-$HT_3$-binding has recently been detected in the CNS (Kilpatrick *et al.*, 1987), but it seems unlikely that this receptor mediates the increased excitability of motoneurones by 5-HT as MDL 72222 even when applied with currents sufficient to depress the population spike directly, failed to block the response to iontophoretically applied 5-HT.

The effects of 5-CT suggest the involvement of a 5-$HT_1$-like receptor (Bradley *et al.*, 1986) and this was examined by applying cyanopindolol iontophoretically and intravenously. Cyanopindolol is a high affinity binding ligand at 5-$HT_{1A}$ and 5-$HT_{1B}$ sites in the CNS (Hoyer *et al.*, 1985). This compound failed to reduce responses to 5-HT on any occasion, although responses to noradrenaline were reduced with high currents of cyanopindolol due, probably, to the well known adrenoceptor blocking actions of pindolol and its derivatives. This suggests that the 5-HT-receptors on motoneurones do not resemble the 5-$HT_{1A}$- or 5-$HT_{1B}$-binding sites. It may of course be acting at the less well characterized 5-$HT_{1C}$ or 5-$HT_{1D}$ sites. It must also be considered that the effects of 5-HT may be due to some non-specific action on the membrane or some other receptor. However, this is unlikely because methysergide applied iontophoretically or intravenously, selectively and reversibly reduced the responses to 5-HT without affecting responses to noradrenaline. The potency of methysergide was much greater on the response of motoneurones to 5-HT (1 mg $kg^{-1}$ i.v. was effective) than on the depressant effects of 5-HT on brainstem neurones (40 mg $kg^{-1}$ i.v.). This difference in the potency of methysergide on two functional 5-$HT_1$-like receptors in the CNS may be a useful method of discriminating between them. It is possible that the receptor on motoneurones is similar to that described by Feniuk *et al.* (1985) on the dog saphenous vein.

Methysergide reduced responses to electrical stimulation of raphe obscurus in parallel with the reduction of 5-HT responses. This suggests that raphe obscurus stimulation was evoking responses by release of 5-HT from descending nerve fibres. Cells in the raphe obscurus contain 5-HT (Dahlstrom & Fuxe, 1964) and project to the ventral horn to form a dense plexus of varicosities around motoneurones (Steinbusch *et al.*, 1978). It is likely that the stimulation activated these cells because microinjections into raphe obscurus of D,L-homocysteate, which depolarizes cell bodies rather than fibres, also increased the motoneurone population spike. Further confirmation of this was obtained from studies in 5,7-DHT-pretreated animals. In these animals, raphe obscurus stimulation had small transient effects on the population spike which did not outlast the period of stimulation. Direct application of 5-HT itself, however, was much more effective in the pretreated animals. Responses were occasionally larger but always much longer lasting. This does not necessarily suggest an increase in receptor sensitivity because the uptake of 5-HT in the spinal cord of the depleted animals is nearly abolished (Foster *et al.*, 1988) and the longer responses to 5-HT probably reflect this. These studies in animals in which immunofluorescent fibres containing 5-HT are extremely rare (Foster *et al.*, 1985) also demonstrate that the increase in population spike caused by iontophoretically applied 5-HT was not due to its action upon presynaptic autoreceptors on 5-HT terminals. If this were the case 5-HT would be ineffective in depleted animals.

It is concluded from these studies that the excitability of motoneurones is increased by the action of 5-HT on a 5-$HT_1$-like receptor which is insensitive to 8-OH-DPAT and cyanopindolol but is blocked by methysergide. This functional receptor does not resemble the 5-$HT_{1A}$- or 5-$HT_{1B}$-binding site and may be one of the acknowledged but as yet not fully characterized subdivisions of the functional 5-$HT_1$-like receptor. The receptor may be physiologically important as it appears to be involved in the response of motoneurones to activity in raphe-spinal fibre systems.

We wish to acknowledge the expert assistance of Mr T. Gould. M.D. was a SERC CASE scholar with Glaxo Group Research.

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*(Received March 10, 1988*
*Revised April 24, 1988*
*Accepted May 19, 1988)*