The influence of 5-hydroxytryptamine agonists and antagonists on identified sympathetic preganglionic neurones in the rat, *in vivo*

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- 1 5-Hydroxytryptamine (5-HT) was applied by microiontophoresis in the vicinity of identified sympathetic preganglionic neurones in the upper thoracic spinal cord of the rat, *in vivo*.
- 2 Sympathetic preganglionic neurones responded in one of three ways to 5-HT: by (a) excitation (76%), (b) inhibition (4%) or (c) in a biphasic manner (5%).
- 3 The excitatory responses evoked by 5-HT were mimicked by 5-carboxamidotryptamine (5-CT) and α -methyl-5-hydroxytryptamine (α -Me-5-HT). The inhibitory and biphasic responses evoked by 5-HT were mimicked by 2-methyl-5-hydroxytryptamine (2-Me-5-HT). The observed responses evoked by 5-HT and selective agonists may be different on the same cell. In several instances a single neurone excited by one agonist was inhibited by another agonist.
- 4 The 5-HT₂-receptor antagonists, ketanserin and LY 53857, failed to abolish selectively the excitatory responses evoked by 5-HT and α -Me-5-HT, when applied by microiontophoresis. The antagonists non-selectively reduced the excitatory responses evoked by 5-HT, 5-CT, α -Me-5-HT, D,L-homocysteic acid (DLH) and noradrenaline (NA). A reduction in synaptically evoked activity was also observed.
- 5 The 5-HT₃-receptor antagonist, ICS 205-930, failed to abolish the inhibitory responses evoked by 5-HT.
- 6 It was concluded that the excitatory responses evoked by 5-HT are mediated by a receptor that is neither 5-HT₂ or 5-HT₃, but shows similarities to the 5-HT₁-like receptor profile. The inhibitory actions of 5-HT are mimicked by 2-Me-5-HT, but the receptor is not 5-HT₃, or 5-HT₁-like or 5-HT₂.

Introduction

Neuroanatomical studies have shown the existence of discrete groups of 5-hydroxytryptamine (5-HT)-containing neurones in the brainstem with extensive projections to the spinal cord (Dahlstrom & Fuxe, 1965; Loewy & McKellar, 1981), where many make direct synaptic contact with dendrites and somata of sympathetic preganglionic neurones (SPN, Bacon & Smith, 1988). Electrical or chemical stimuli applied to these brainstem regions produced either excitation or inhibition of sympathetic activity, and it is suggested that both effects may be mediated by the release of 5-HT at terminals in the spinal cord. This dual action may be achieved via two receptor subtypes located at synapses somewhere in the sympathetic spinal circuitry (Coote, 1988 for review). In a recent attempt to mimic the action of this innervation, 5-HT was given intrathecally at the T₉, T₁₀ level of the spinal cord whilst recording postganglionic sympathetic activity in a renal nerve of the rat. A sympatho-excitatory effect mediated via 5-HT₁-like receptors and a sympatho-inhibitory response mediated via 5-HT₂-like receptors was revealed (Yusof & Coote, 1988). Such an organisation could explain the results of earlier studies of cat SPN in which 5-HT, applied directly in their vicinity by iontophoresis, excited the majority but had an inhibitory effect on a significant minority of cells (Coote *et al.*, 1981; Kadzielawa, 1983). However, McCall (1983) using the cat came to the opposite conclusion, that the excitatory effect of 5-HT on SPN was mediated via 5-HT₂ receptors since it could be blocked by metergoline or methysergide, even though these are relatively unspecific antagonists.

There has been no study of this sort on rat SPN, where most of the studies of the neuroanatomy and pharmacology of 5-HT neurones have been conducted. Therefore, the present investigation was designed to determine the action of 5-HT and its agonists and antagonists, when applied directly in the

vicinity of antidromically identified single SPN in the upper thoracic spinal cord of the anaesthetized rat.

A brief account of part of this work has been published (Coote & Lewis, 1989a).

Methods

Experiments were carried out on male Sprague-Dawley rats anaesthetized with α -chloralose (35 mg kg⁻¹) and urethane (700 mg kg⁻¹), given intravenously after initial halothane induction. Blood pressure was monitored with a polyethylene cannula filled with heparinised 0.9% NaCl, placed retrogradely in the right femoral artery and connected to a transducer. An electrocardiogram (ECG) was recorded via leads connected to the right forelimb and left hindlimb, amplified (Neurolog NL103) via high impedance probe (Neurolog NL100), filtered (Neurolog NL125) and displayed on an oscilloscope (Tektronix RM565). Instantaneous heart rate was obtained from the R-R interval, using an interface unit (Neurolog NL515) and rate meter (Neurolog NL253), and displayed on a chart recorder. In some experiments a bilateral pneumothorax was performed to stabilize the recording conditions, respiration being maintained by positive pressure artificial ventilation (Harvard Instruments). Temperature of the animals was maintained at 37°C by means of a thermostatically controlled heating blanket.

The animal's head was placed in a head holder and the vertebral column firmly fixed by three metal clamps. Conventional methods were used for recording and identifying SPN in the T₂ segment. The cells were antidromically activated by stimulation of the left cervical sympathetic nerve as described elsewhere (Gilbey *et al.*, 1982). Action potentials of SPN were recorded through one barrel containing 4 M NaCl (2 M Ω) of a seven-barrel glass micropipette assembly connected to a high impedance input stage (Neurolog NL100AK), amplified (Neurolog NL104), Filtered (Neurolog NL125) and monitored

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on an oscilloscope (Gould 1425). Single unit activity was detected with a spike discriminator (Neurolog NL200), counted with a pulse integrator (Neurolog NL601) and a period generator (Neurolog NL304), and displayed on a latch counter (Neurolog NL606) and graphically on a rectilinear chart recorder (Rikadenki R-02). All data were stored on magnetic tape (Racal, Store 4).

For microiontophoresis, 5 drug-containing barrels of the micropipette assembly were connected via a silver wire to a microiontophoresis unit (Grayden Electronics), and one barrel containing 1 M NaCl was connected to an automatic current compensation module of this unit. The following drugs were dissolved in distilled water and adjusted to the required pH using either 0.1 M HCl or 0.1 M NaOH: L-glutamate 0.5 M (monosodium salt, Sigma, pH 8.0), D,L-homocysteic acid 0.1 M (DLH, Sigma, pH 8.0), 5-hydroxytryptamine creatinine sulphate 0.01 M (Sigma, pH 4.5), noradrenaline bitartrate 0.1 M (Sigma pH 4.5), 5-carboxamidotryptamine maleate 0.01 M (5-CT, Glaxo, pH 4.5), α -methyl-5-hydroxytryptamine maleate 0.01 M (α -me-5-HT, Glaxo, pH 4.5), 2-methyl-5-hydroxytryptamine maleate 0.01 M (2-me-5-HT, Glaxo, pH 4.5), ketanserin bitartrate 0.01 M (Janssen, pH 4.5), 4-isopropyl-7-methyl-9(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6a,7,8,9,10,10a-octahydroindole (4,3 Fg) quinoline maleate 0.01 M (LY 53857, Eli Lilly, pH 4.5), (3 α -tropanyl)-1-H-indole-3-carboxylic acid ester 0.01 M (ICS 205-930 HCl, Sandoz, pH 4.5).

Retaining currents (5–30 nA) were applied to the drug containing barrels between ejection periods. Compounds were ejected using positive currents (5–100 nA), apart from glutamate or DLH, which were ejected with negative currents.

5-Hydroxytryptamine receptor antagonists and analogues

To study the action of antagonists of 5-HT or its agonists, the following protocol was adopted. Three steady state cycles of responses of SPN to 5-HT and of one or more control agonists were achieved by rigorously ensuring that ejection and retention currents and their duration were the same throughout the tests. In addition, before a testing procedure, ejection currents for each of the agonists was adjusted so as to give responses of SPN that were of approximately equal magnitude. Drugs used in addition to 5-HT were, noradrenaline (NA), 5-CT, α -Me-5-HT and 2-Me-5-HT, as well as excitatory amino acids (EAA).

Results

Sympathetic preganglionic neurones (SPN) in T₂ segment of the spinal cord were identified as previously described (Gilbey *et al.*, 1982). The majority (68%) of SPN were not spontaneously active, and apart from the excitatory amino acids (EAA), no drug used in this study was able to excite silent SPN even though ejection currents up to 100 nA were applied. Therefore, the following results were obtained from both spontaneously active and previously silent SPN in which a stable firing rate was induced by continuous application of an excitatory amino acid, either glutamate or DLH. Comparisons of the action of each of the agonists used in this study showed that there was no significant difference in their effect on spontaneously active SPN and EAA-driven SPN.

5-Hydroxytryptamine

5-HT, 0–80 nA, applied in the vicinity of 118 SPN for between 15–60 s, caused 90 SPN rapidly to increase their firing rate during the application of the ejection current, reaching a maximum after the removal of this current (Figure 1). Neural activity then decreased gradually back to baseline levels over a period of 9 min. The maximum firing rate and the duration of SPN discharge increased with increasing ejection currents.

In a small number of SPN the application of 5-HT, as well as increasing the firing rate, induced a period of burst firing

Figure 1 Continuous frequency-time histograms showing the excitatory responses evoked by increasing iontophoretic currents of 5-hydroxytryptamine (5-HT) on an identified, sympathetic preganglionic neurone. The bars indicate ejection periods, with the numerals below indicating ejection currents in nanoamperes (nA). The activity of the neurones depicted in (a) is the result of leakage of D,L-homocysteic acid (DLH) in the vicinity of the neurone when no retaining current is applied to the pipette barrel. This activity is diminished on increasing the retaining current to 1 nA as shown in (b). With the higher DLH retaining current (1 nA) (b), the neurone fired in a phasic manner in response to iontophoretic 5-HT.

which ceased when the firing rate returned to control levels (Figure 1b). This pattern of activity was characterized by a burst of up to six action potentials with a maximum frequency of 8 Hz, the cell then stopped firing for some 8 s before repeating the burst of activity.

In five of the 118 SPN, application of 5-HT caused a decrease in their activity. This started during the application of the ejection current, reaching the nadir before its removal (Figure 6). The firing rate then gradually returned to baseline levels over a period of 2 min. The magnitude of these inhibitions of activity increased with increasing ejection current.

In six of the 118 SPN, 5-HT application elicited a biphasic response. This consisted of an initial decrease in firing rate which reached a maximum during the application of the ejection current. It then returned to baseline values over the next minute before increasing above this level for a further minute, and then declining over the following 4 min (Figure 2). The size of the initial inhibition and following excitation was directly related to the size of the ejection current.

No biphasic response comprising an initial excitation followed by an inhibition was observed.

5-HT₁-like receptor agonist

5-CT, 5–80 nA, applied for between 15–60 s, increased the firing rate of 44 out of 52 SPN tested (Figure 3a), in a manner

Figure 2 Continuous frequency-time histogram showing the biphasic responses evoked by increasing iontophoretic currents of 5-hydroxytryptamine (5-HT) on a spontaneously active, identified sympathetic preganglionic neurone. The neurone was unresponsive to 60 nA positive current (+ve).

Figure 3 Continuous frequency-time histograms showing the effects of 5-hydroxytryptamine agonists on three different identified sympathetic preganglionic neurones. (a) The excitatory effects of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and α -methyl-5-hydroxytryptamine (α -Me) on a non-spontaneously active neurone. No retention or ejection current was applied to the D,L-homocysteic acid (DLH) containing barrel. (b) The excitatory effect of 5-HT and the inhibitory effect of 2-methyl-5-hydroxytryptamine (2-Me) on a non-spontaneously active neurone. The neurone was 'driven' by continuous application of 3 nA DLH. The neurone was unresponsive to the iontophoretic application of 70 nA positive current (+ve). (c) The excitatory responses evoked by 5-HT and noradrenaline (NA) and the biphasic responses evoked by 2-Me-5-HT on a spontaneously active neurone.

similar to that observed with the application of 5-HT. In one SPN, 5-CT caused an inhibition and had no effect on the remaining seven cells.

The actions of both 5-HT and 5-CT on 44 neurones were compared. Both agonists were excitatory on 32 cells, and had no effect on three. 5-CT evoked excitatory responses in a further six cells, in which 5-HT had no effect on four, was inhibitory on one, and evoked a biphasic response in the remaining cell. Two cells were unresponsive to 5-CT; one being excited by 5-HT and the other being inhibited. The remaining cell was inhibited by 5-CT and excited by 5-HT.

5-HT₂ receptor agonist

α -Me-5-HT, 10–90 nA, applied for between 15–60 s, increased the firing rate of 18 out of 27 SPN (Figure 3a) and had no effect on the remaining nine SPN. Both the magnitude of the increased discharge, and its duration, were directly related to the magnitude of the ejection current.

The actions of α -Me-5-HT and 5-HT on the same cells were compared for 24 SPN. Both agonists increased the firing rate of 11 SPN, and had no effect on seven neurones. α -Me-5-HT was excitatory on four cells, of which 5-HT evoked an inhibitory response in two, a biphasic response in one and had no effect on the remaining neurone. α -Me-5-HT had no effect on the remaining two cells, whereas 5-HT was excitatory on one and inhibitory on the other.

5-HT₃ receptor agonist

2-Me-5-HT, 10–90 nA, applied for 15–60 s, decreased the firing rate of 10 out of 26 SPN (Figure 3b), caused a biphasic response (inhibition followed by excitation) in seven SPN (Figure 3c) and had no effect on the remaining nine.

The inhibitory response was characterized by an onset during the application of the ejection current, reaching a maximum immediately after its removal. The activity of the SPN then gradually returned to baseline levels over a period of 2 min. The biphasic response was characterized by an initial

rapid inhibition immediately followed by an excitatory phase, the activity then declining to baseline values over a period of 4 min. The magnitude of these effects of 2-Me-5-HT increased with increasing ejection currents.

A comparison of the actions of both 2-Me-5-HT and 5-HT on the same cell for 26 SPN showed that of the 23 SPN excited by 5-HT, 10 were inhibited by 2-Me-5-HT, six responded in a biphasic manner and seven were unaffected by the agonist. Two cells were unresponsive to 5-HT, one of which responded in a biphasic manner to 2-Me-5-HT. The remaining cell was inhibited by 5-HT but showed no response to 2-Me-5-HT.

Comparison of 5-HT receptor agonists on the same SPN

5-CT and α -Me-5-HT were both applied to eight identified SPN. Both agonists excited seven SPN, and had no effect on one. 5-CT and 2-Me-5-HT were both applied to three SPN. 5-CT excited all three, whilst 2-Me-5-HT produced a biphasic response in one and had no effect on two. α -Me-5-HT and 2-Me-5-HT were both applied to the same four SPN. α -Me-5-HT increased the activity of all four cells, whereas 2-Me-5-HT had a biphasic effect on one and no effect on three cells.

5-HT receptor antagonists

In seven rats, the 5-HT₂ receptor antagonist ketanserin failed to antagonize selectively the excitatory action of 5-HT on eight SPN, or of α -Me-5-HT on two SPN. Instead, it reduced the responses evoked by 5-HT and α -Me-5-HT, and in addition, the excitatory responses evoked by NA, 5-CT and DLH were diminished. A typical experiment is illustrated in Figure 4. Here, continuous ejection of ketanserin, with a current of 5 nA, reduced the response to NA (5 nA) and 5-HT (30 nA) and also abolished activity of the SPN, which was driven by continuous application of DLH (10 nA). All responses were reduced for almost 90 min after removal of the ketanserin ejection current. Similar non-selective effects of ketanserin were observed when tested against the excitatory response of SPN to α -Me-5-HT.

Figure 4 Continuous frequency-time histogram showing the effect of ketanserin on the excitatory responses evoked by 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on a non-spontaneously active, identified sympathetic preganglionic neurone. The neurone was 'driven' by continuous iontophoretic ejection of 10 nA D,L-homocysteic acid (DLH). Ketanserin reduced the responses evoked by both agonists and caused a reduction in the DLH-evoked basal firing rate. Partial recovery of the agonist-induced responses and full recovery of the baseline firing rate was observed approximately 90 min after removal of the antagonist ejection current.

In two other experiments, ketanserin was also shown to induce a non-selective decrease in the 5-HT-induced excitatory response of spontaneously active SPN. In one example, in which the ketanserin retaining current was reduced from 20 nA to 10 nA, an increase in firing rate to NA (10 nA) was more profoundly depressed than that to 5-HT (25 nA). The firing rate gradually returned on increasing the retention current to 20 nA.

In two rats, the actions of the 5-HT₂ receptor antagonist, LY 53857, on responses evoked by 5-HT, NA, and either 5-CT or α -Me-5-HT, were studied on two DLH-driven SPN. In both experiments, there was a virtual abolition of the responses of SPN to all the agonists, including the activity induced by DLH. In one of the experiments, partial recovery of the responses to NA, but not to 5-HT or its agonists, was observed up to 40 min after removal of the LY 53857 ejection current, after which the cell was lost. In the second experiment, there was no recovery of the response to any agonist

including DLH, even though the neurone could still be antidromically activated some 65 min after removal of the ejection current.

In an attempt to overcome the reduction in baseline activity of SPN induced by continuous application of the 5-HT receptor antagonists, ketanserin (5–45 nA) was ejected in pulses before and during the ejection of 5-HT. In one typical example shown in Figure 5, ketanserin (5 nA) ejection was started 30 s before and continued during the application of the test drug, either 5-HT (30 nA) or NA (10 nA). Both antagonist and agonist ejection currents were removed simultaneously. Five control responses of equal magnitude were first obtained before the start of the ejections of antagonist. Ketanserin, applied in this way, caused a dose- (current) dependent reduction in the response of the neurone to both 5-HT and NA which persisted up to 60 min after the final application of ketanserin. In addition, in spite of the short discontinuous ejection of ketanserin, the spontaneous activity of the SPN decreased to zero and has still not recovered 60 min after the last application of the antagonist. Interestingly, and unusually, both 5-HT and NA were able to excite this SPN despite the absence of ongoing spontaneous activity at the end of the test procedure. During the test period, with the higher ketanserin ejection currents, there was a reduction in the height of the agonist evoked spike.

Since 2-Me-5-HT had a predominantly inhibitory effect on SPN, the possibility that the 5-HT-induced reductions in SPN

Figure 5 Continuous frequency-time histogram showing the effect of the pulsatile iontophoretic ejection of ketanserin (Ket) on the excitatory responses evoked by 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on an identified spontaneously active sympathetic preganglionic neurone. The antagonist was applied 30 s before and during the agonist ejection period. With higher ejection currents, ketanserin reduced the responses evoked by both 5-HT and NA. A gradual abolition of the basal firing rate was also observed. Partial recovery of the agonist-induced responses, but not the baseline firing rate had occurred 75 min after the last antagonist ejection period.

Figure 6 Continuous frequency-time histogram showing the effect of ICS 205-930 on the inhibitory responses evoked by 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on identified, non-spontaneously active sympathetic preganglionic neurone. The neurone was 'driven' by continuous application of 6 nA D,L-homocysteic acid (DLH). The antagonist was applied 30 s before and during the agonist ejection period. ICS 205-930 had no effect on the inhibitory responses evoked by 5-HT and NA, but caused a gradual reduction in the DLH-induced firing rate. Recovery of the baseline firing rate occurred gradually after the end of the last antagonist ejection period.

firing rate were due to an action at a 5-HT₃ receptor was tested by use of the highly selective 5-HT₃ receptor antagonist, ICS 205-930.

In the experiment shown in Figure 6, three reproducible cycles of inhibitory responses were obtained to 5-HT (50 nA) and NA (40 nA) on a DLH driven SPN. ICS 205-930, ejected 30 s before and during application of either 5-HT or NA, had no effect on the inhibitory responses to each of these agonists, even though its repeated application did cause a small reduction in the baseline level of activity.

Discussion

The principal observation made in this study was that 5-HT, when applied in the vicinity of identified sympathetic preganglionic neurones, could either excite or inhibit these neurones. These actions of 5-HT could either be direct or indirect. The results are broadly comparable with similar, but more limited studies, in the cat (Coote *et al.*, 1981; Kadzielawa, 1983).

By using selective 5-HT agonists, we sought to determine the receptor subtypes which may be mediating these differing effects of 5-HT. The excitatory actions of 5-HT resembled those of 5-CT and α -Me-5-HT, the former being a selective 5-HT₁-like agonist whereas the latter is a 5-HT₂ receptor agonist which also has some actions at 5-HT₁-like receptors (Bradley *et al.*, 1986). The excitatory responses evoked by α -Me-5-HT were surprising in view of previous studies in this laboratory (Yusof & Coote, 1988) which showed that α -Me-5-HT evoked dose-dependent, ketanserin-sensitive, inhibitions of pre- and postganglionic sympathetic nerve activity when applied intrathecally in the rat. In contrast, in the present iontophoretic experiments, ketanserin and LY 53837 failed to antagonize selectively the excitatory responses evoked by α -Me-5-HT and 5-HT in SPN. Limiting the concentration of the antagonists in the vicinity of SPN, by applying them for brief periods or just by reducing the retaining current, did not reveal a selective blockade. Instead, both antagonists either had no effect or produced a marked reduction in all excitatory responses. In addition to diminishing the responses evoked by 5-HT and α -Me-5-HT, the excitatory responses evoked by 5-CT, NA and EAAs were also reduced, as was the ongoing synaptic activity. These neuronal depressant actions were unlikely to be due to current or local anaesthetic actions since neuronal spike amplitudes were unaltered; the effect came on gradually and far outlasted the period of antagonist application; there was no spike broadening; and no increase in recording noise levels was observed. Another possibility is that the antagonists were acting either pre- or postsynaptically to block a tonic 5-HT-mediated excitatory drive to these neurones (see review, Coote 1988). Depressant actions of ketanserin have been observed on cortical neurones (Lakoski & Aghajanian, 1985), raphe neurones (Davies *et al.*, 1988a,b) and spinal motoneurones (Roberts *et al.*, 1988), as have the depressant effects of methysergide, metergoline and cianserin on SPN (Kadzielawa, 1983; McCall, 1983). It is unlikely that all these different groups of neurones receive a tonic 5-HT₂ receptor-mediated excitatory input, especially as in the case of motoneurones, 5-HT itself evokes excitatory responses most likely mediated by 5-HT₁-like receptors (Roberts *et al.*, 1988).

Catecholamines have been shown to have an excitatory action on SPN (Coote & Lewis, 1989b; Shi *et al.*, 1988), and therefore ketanserin may be acting by blocking α_1 -adrenoceptors on these neurones (Leysen *et al.*, 1981). Support for this idea comes from the observation that high iontophoretic ejection currents of the α_1 -adrenoceptor antagonist, thymoxamine, reduced the spontaneous firing rate of cat SPN (Kadzielawa, 1983), however LY 53857 has been shown to have a very low affinity for α_1 -adrenoceptors (Cohen *et al.*, 1985). Therefore, the depressant effects observed with higher ejection currents of both 5-HT and α -adrenoceptor antagonists on SPN, and 5-HT antagonists on other groups of neurones, are probably due to the build up of excessive antagonist

concentrations, leading to non-specific neuronal depression by an as yet undetermined mechanism. Support for this comes from the observation that excitatory actions of EAAs are also depressed.

In view of the inability of ketanserin or LY 53857 to reduce selectivity, or abolish, the excitatory responses evoked by α -Me-5-HT and 5-HT on SPN, it seems unlikely that these responses are mediated by the 5-HT₂ receptor subtype. The present study has shown that 5-CT evoked an excitatory response when applied by iontophoresis in the vicinity of SPN, indicating that the excitatory effects of 5-HT may be mediated via the 5-HT₁-like receptor subtype. Support for this viewpoint comes from a study of Yusof & Coote (1988), where 5-CT was shown to have dose-dependent sympatho-excitatory actions when it was administered intrathecally to the rat thoracic spinal cord, an action unaffected by 5-HT₂ or 5-HT₃ receptor antagonists. In addition, α -Me-5-HT has a weak affinity for 5-HT₁-like receptors, and so its excitatory action could be explained by its effect on these receptors on SPN.

However, studies on rat spinal motoneurones have shown that whilst 5-HT, 5-CT and α -Me-5-HT evoke depolarizations of these neurones (Connell & Wallis, 1988; Roberts *et al.*, 1988), the receptor subtypes mediating these effects being neither 5-HT₂ or 5-HT₃, the receptor profile does not clearly resemble that of the 5-HT₁-like receptor as defined by Bradley *et al.* (1986). In the experiments of Connell & Wallis (1988), ketanserin was shown to have a weak antagonist action of a 5-HT-evoked depolarization, but only when administered at high concentrations (0.8×10^{-7} – 1×10^{-6} M), hence the effect was unlikely to have been due to an action at 5-HT₂ receptor sites. Therefore, in view of the similarities between the above studies and the results obtained in the present study, whilst the excitatory responses evoked by 5-HT in SPN are neither mediated by the 5-HT₂ or 5-HT₃ receptor subtypes, it would be unwise to define them rigidly as being mediated by the 5-HT₁-like receptor subtype.

2-Me-5-HT was shown to induce inhibitory responses in SPN. These responses were totally different from the excitatory responses evoked by 5-CT and α -Me-5-HT. Since 2-Me-5-HT has only a weak affinity for the 5-HT₁-like and 5-HT₂ receptor subtypes, and 5-CT and α -Me-5-HT have low affinity for 5-HT₃ receptors (Humphrey, 1984; Richardson *et al.*, 1985), it may be that these inhibitions are mediated via the 5-HT₃ receptor subtype. However, the 5-HT-induced inhibitions were not antagonized by the highly selective 5-HT₃ receptor antagonist, ICS 205-930. Therefore, it appears that 2-Me-5-HT and 5-HT can evoke inhibitory responses in SPN which are neither mediated by the 5-HT₁-like, 5-HT₂ or 5-HT₃ receptor subtypes. A similar observation was made in studies on neurones in the guinea-pig submucous plexus, where 5-HT and 2-Me-5-HT were shown to evoke slow depolarizations of these neurones via a 5-HT receptor which did not belong to the 5-HT₁-like, or the 5-HT₂ or the 5-HT₃ receptor subtypes (Surprenant & Crist, 1988).

5-HT has previously been shown to evoke long duration, 5-HT₂ receptor-mediated, inhibitions of pre- and postganglionic sympathetic nerve activity when administered intrathecally (Yusof & Coote, 1988). In contrast, the present iontophoretic studies have shown that 5-HT evokes short duration inhibitory responses which are mimicked by 2-Me-5-HT. This discrepancy may arise as the result of the route of administration of the agonist. When 5-HT is administered iontophoretically, it must, by the nature of the technique, be acting at a site near the cell soma. In contrast, when the agonist is administered intrathecally, it may be acting at a more distant spinal site, possibly via an interneurone. Therefore, the 5-HT-evoked inhibitions observed with the two different techniques may be mediated by different mechanisms and receptor subtypes.

The idea that 5-HT-mediated inhibitions of SPN may be via interneurones has previously been postulated (Gilbey *et al.*, 1981; Yusof & Coote, 1988). Such an interneurone could act by the release of glycine, a possibility not investigated in

the present experiments, but an idea for which there is increasing evidence. 5-HT, when applied microiontophoretically, induced inhibitions of SPN which had fast onsets and short duration. These characteristics would be expected of a glycine mediated response, this agonist being shown to mediate fast inhibitory postsynaptic potentials (i.p.s.ps) in rat SPN *in vitro* (Dun & Mo, 1989). Similar fast i.p.s.ps, which are blocked by strychnine, can be induced synaptically during the application of substance P (Dun & Mo, 1988) and adrenaline (Miyazaki *et al.*, 1989). Therefore, 2-Me-5-HT and 5-HT may be acting presynaptically on a glycine interneurone to cause its release. However, whilst the 5-HT-mediated inhibitions observed during intrathecal administration may also be via an interneurone, a different mechanism or receptor may exist. The

observed long duration inhibitions evoked by 5-HT and α -Me-5-HT seem inconsistent with the idea that glycine is the mediator of fast i.p.s.ps in rat SPN, and is the transmitter being released by the interneurone in this instance.

Therefore, there is now a basis for a receptor-mediated pattern of response produced by descending 5-HT pathways. We would suggest that excitatory inputs are direct and mediated by a receptor that is neither 5-HT₂ nor 5-HT₃, but shows similarities to the 5-HT₁-like receptor profile. The inhibitory inputs are indirect, being mediated both at a site distant from SPN via 5-HT₂ receptors and a second site which is presynaptic, possibly a glycine interneurone. The actions of 5-HT on this latter receptor are mimicked by 2-Me-5-HT, but the receptor is not 5-HT₁-like, or 5-HT₂ or 5-HT₃.

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