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Evidence for depressant 5-HT_1-like receptors on rat brainstem neurones

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1 The technique of microiontophoresis was used to evaluate the contribution of 5-HT_1-like, 5-HT_2- and 5-HT_3-receptors to the depressant effects of 5-hydroxytryptamine (5-HT) on neurones in the midline of the medullary brainstem of the rat *in vivo*.

2 Depressant responses to 5-HT were resistant to antagonism by the 5-HT_2-receptor antagonist ketanserin and the 5-HT_3-receptor antagonist MDL 72222 applied either microiontophoretically or administered systemically.

3 Microiontophoretic or systemic administration of the 5-HT antagonist metergoline, which shows nanomolar affinity for the 5-HT_1-binding site, also failed to attenuate the depressant responses to 5-HT.

4 Systemic administration of high doses of methysergide (30–40 mg kg^{-1}) attenuated the depressant responses to 5-HT but did not block depressant responses to GABA or excitatory responses to glutamate.

5 The depressant effects of 5-HT were potently mimicked by the 5-HT_1-like receptor agonists 5-carboxamidotryptamine and 8-OH-DPAT.

6 These results indicate that neither 5-HT_2-receptors nor 5-HT_3-receptors are involved in the depressant effects of 5-HT on midline brainstem neurones. The depressant effects of 5-carboxamidotryptamine (5-CT) and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and blockade of the response to 5-HT by high doses of methysergide suggests the involvement of 5-HT_1-like receptors. The lack of effect of metergoline, however, indicates that this receptor may be different from any of the 5-HT_1 binding sites yet described.

Introduction

Microiontophoretically applied 5-hydroxytryptamine (5-HT) has both excitatory and depressant effects on the firing rate of central neurones (Roberts & Straughan, 1967; Boakes *et al.*, 1970; Bradley & Briggs, 1974; Haigler & Aghajanian, 1974a). In many brain areas, however, 5-HT appears to be predominantly depressant (Haigler & Aghajanian, 1977). Indeed, in the secondary visual areas and those areas forming the limbic system 5-HT appears to be exclusively depressant (Aghajanian, 1981). Although a wide variety of peripheral 5-HT antagonists have been tested, including cinanserin, cyproheptadine, methysergide and methiothepin, none has been shown consistently and selectively to block the depressant effects of 5-HT on central neurones (Roberts & Straughan, 1967; Boakes *et al.*, 1970; Haigler & Aghajanian, 1974b). Consequently, the receptor mediating the depressant effects of 5-HT is ill-defined.

On peripheral tissues at least three types of 5-HT receptor have been identified, termed 5-HT_1-like, 5-HT_2 and 5-HT_3 (see Bradley *et al.*, 1986). Whether one or more of these receptor types is involved in neuronal depression by 5-HT is uncertain. In this study we have used the 5-HT_1-like receptor agonist 5-carboxamidotryptamine (5-CT), the 5-HT_2- receptor antagonist ketanserin and the 5-HT_3- receptor antagonist MDL 72222 to investigate the nature of the receptor mediating the depressant effects of 5-HT on midline medullary brainstem neurones.

Although no potent and selective 5-HT_1 antagonist has yet been described, Bradley *et al.* (1986) have suggested that methysergide may be of some value in defining 5-HT_1-like receptors. Although methysergide preferentially acts at 5-HT_2-receptors, at higher doses or concentrations methysergide also blocks the actions of 5-HT on 5-HT_1-like receptors (Bradley *et al.*, 1986). We have, therefore, also examined the

potential 5-HT blocking properties of methysergide. It is apparent from radioligand binding studies that the 5-HT antagonist metergoline shows high affinity for the 5-HT$_1$-recognition site (Peroutka & Snyder, 1979; Leysen *et al.*, 1981). Furthermore, metergoline has been shown to attenuate depressant responses to 5-HT on cortical neurones (Sastry & Phillis, 1977; Jones, 1982). Thus, the actions of metergoline have also been examined.

It is evident from both functional and radioligand binding studies that the 5-HT$_1$-like receptor does not form a homogeneous population (Pedigo *et al.*, 1981; Bradley *et al.*, 1986). The 5-HT$_1$-binding site has been divided into at least two distinct populations which seem functionally relevant, 5-HT$_{1A}$ and 5-HT$_{1B}$ (Deshmukh *et al.*, 1982). The centrally active 5-HT agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (Arvidsson *et al.*, 1981) potently displaces binding from the 5-HT$_{1A}$-site but has little affinity for the 5-HT$_{1B}$-site (Middlemiss & Fozard, 1983). In order to examine the relevance of these subtypes of the binding sites to the functional effects of 5-HT on central neurones we have also investigated the actions of 8-OH-DPAT.

Methods

Male Wistar rats (250 to 400 g) were anaesthetized with halothane (2–4% in oxygen). Tracheal, carotid and external jugular cannulae were inserted. Anaesthesia was subsequently maintained with halothane (0.8–1.2% in oxygen).

Five-barrelled microelectrodes were broken back to a tip diameter of 5 to 7 μm. One of the barrels was filled with NaCl (4 M) for the extracellular recording of action potentials as described previously (Davies *et al.*, 1988). A second barrel in each electrode was filled with Pontamine Sky Blue (PSB) (2% in 0.5 M sodium acetate) and used to mark the recording site at the end of each study. The PSB barrel was also used for current balancing. The remaining three barrels were filled with drug solutions.

The agonistic actions of 5-CT and 8-OH-DPAT were examined on cells that gave consistent depressant responses to 5-HT. A cell was considered to be unresponsive to the agonists if no change in spontaneous firing rate was observed following its application with ejecting currents up to 100 nA for 1 min.

The effects of the antagonists were examined on cells giving consistent depressant responses to 5-HT. When a unit was encountered 5-HT was applied at constant intervals. Between applications, retaining currents of 15 nA were passed in order to prevent diffusional release. Intervals between applications were kept constant to standardize the effects of retaining current on subsequent iontophoretic release (Bradshaw *et al.*, 1973). When consistent repeatable responses to 5-HT were obtained, the antagonist was applied continuously from another barrel of the micropipette by the passage of a small ejecting current (5 to 20 nA) without interrupting the 5-HT cycle.

The effects of intravenous administration of the antagonists were also investigated (one study only in each animal). When constant repeatable responses to 5-HT were obtained, the antagonists were administered via a cannula in the left external jugular vein.

At the end of each study the recording site was marked by the ejection of PSB. The animal was perfused with formal saline and the brain removed and stored in formal saline. Sections, 50 μm thick, were cut on a freezing microtome, mounted and stained with neutral red. Only those recording sites identified as being within 1 mm of the midline and between 0.8 and 2.3 mm behind the interaural line were included for analysis.

Drugs

The following drug solutions were used: 5-hydroxytryptamine bimaleate (0.1 or 0.2 M, pH 4), 5-carboxamidotryptamine maleate (0.1 M, pH 4), 8-OH-DPAT hydrogenbromide (0.02 M, pH 4), ketanserin tartrate (0.01 M, pH 3.5–4), MDL 72222 (1αH,3α,5αH - tropan - 3yl - 3,5 - dichlorobenzoate, 0.01 M, pH 5–5.5), metergoline (0.01 M, pH 4), sodium L-glutamate (0.2 M, pH 8) and γ-amino-n-butyric acid (GABA) (0.2 M, pH 3.5).

Results

Effects of the antagonists ketanserin, MDL 72222, metergoline and methysergide on depressant responses to 5-HT

Ketanserin Microiontophoretic application of ketanserin with ejecting currents up to 15 nA for up to 40 min failed to attenuate the depressant responses to 5-HT on any of the 8 cells studied. Application of ketanserin with higher ejecting currents invariably resulted in a decrease in the spontaneous firing rate accompanied by a reduction in the amplitude of the spike.

On a further 5 cells the effects of intravenously administered ketanserin were also evaluated. Ketanserin was administered in small aliquots of 50 to 100 μg kg^{-1} in order to minimize effects on blood pressure. In this way, high cumulative doses could be

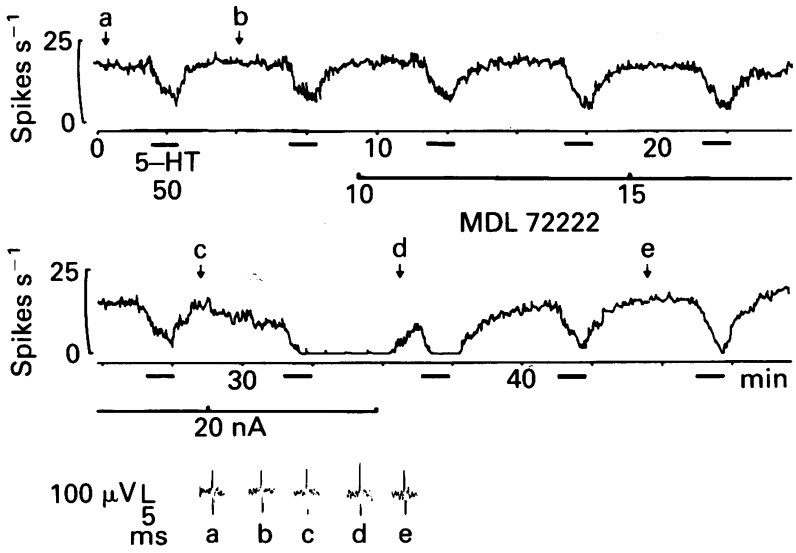

Figure 1 Continuous ratemeter record showing the effect of MDL 72222 on the depressant response of a single brainstem neurone to 5-hydroxytryptamine (5-HT). The horizontal bars below the ratemeter record indicate the iontophoretic application of drugs. The numbers refer to the intensity of the ejecting current in nA. Below the ratemeter record are samples of extracellularly recorded action potentials taken at those points in time indicated by the arrows. MDL 72222 failed to attenuate the depressant response to 5-HT. At higher ejecting currents, however, MDL 72222 did depress the spontaneous activity of the cell. It can be seen from the spike records that this effect of MDL 72222 was associated with an increase in spike amplitude.

achieved without affecting the relationship between the cell and electrode as judged by the spike amplitude. Thus, cumulative doses of $2\,mg\,kg^{-1}$ ketanserin ($n = 5$) did not attenuate the depressant effects of 5-HT.

MDL 72222 Microiontophoretic application of MDL 72222 (5–20 nA, $n = 6$) did not attenuate the depressant responses to 5-HT. When applied with higher ejecting currents MDL 72222 caused a reduction in the spontaneous activity of the cell. Interestingly, on half of these cells this depressant effect of MDL 72222 was not associated with a decrease in spike amplitude. Rather, as illustrated in Figure 1, there was an increase in the spike amplitude associated with this effect. Systemically administered MDL 72222 ($1\,mg\,kg^{-1}$, $n = 4$) did not attenuate the depressant effects of 5-HT. Neither was there any consistent effect on the spontaneous firing rate of the cells.

Metergoline Microiontophoretically applied metergoline (5–20 nA, $n = 8$) also failed to attenuate the depressant responses to 5-HT. Application of metergoline with higher currents usually resulted in a decrease in the spontaneous firing rate associated with a decrease in the spike amplitude. Systemic administration of metergoline in divided doses up to $5\,mg\,kg^{-1}$ ($n = 5$) did not attenuate the depressant responses to 5-HT.

Methysergide The effects of systemically administered methysergide were examined on 3 cells giving consistent depressant responses to both 5-HT and GABA, and 2 cells giving consistent depressant responses to 5-HT and excitatory responses to glutamate. Methysergide was given in aliquots of 0.5–$1\,mg\,kg^{-1}$ over periods of up to 1 h. On each

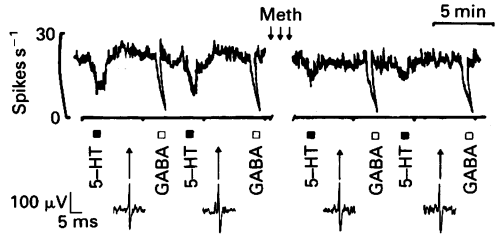

Figure 2 The effects of systemically administered methysergide on the depressant responses of a single brainstem neurone to 5-hydroxytryptamine (5-HT). Methysergide (Meth) was administered in a cumulative dose of $30\,mg\,kg^{-1}$ i.v. over a period of 45 min. This resulted in a marked reduction in the response to 5-HT but no change in the response to γ-aminobutyric acid (GABA). There was no discernible change in spike amplitude during this study.

occasion, methysergide (30–40 mg kg^{-1}) attenuated the depressant response to 5-HT. Even at such high doses, however, methysergide had little or no effect on the depressant responses to GABA or the excitatory responses to glutamate. Recovery from the effects of methysergide was not observed although on no occasion was there any change in the amplitude of the action potential. An example of the effects of methysergide is illustrated in Figure 2.

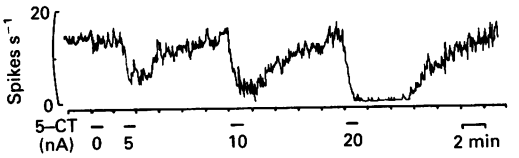

Figure 3 Continuous ratemeter record showing the effects of increasing currents of 5-carboxamidotryptamine (5-CT) on the spontaneous activity of a single brainstem neurone.

Effects of selective 5-HT receptor agonists

Comparison of the effects of 5-HT and 5-CT The effects of 5-CT were examined on 16 neurones that gave consistent depressant responses to 5-HT. 5-CT (5–50 nA) had marked depressant effects on all 16 cells. The size of the depressant effects of 5-CT were proportional to the magnitude of the ejecting current as illustrated in Figure 3. These effects of 5-CT were never associated with a decrease in spike amplitude, rather, the effects of 5-CT were frequently associated with an increased spike amplitude.

The relative potencies of 5-HT and 5-CT were compared on 12 cells. This comparison is valid since it has been shown that the iontophoretic mobility of 5-HT and 5-CT are very similar. When applied with equivalent ejecting currents the mean ratio of the maximum percentage decrease from baseline firing and the duration of the responses (5-HT : 5-CT) were 1 : 1.51 and 1 : 6.75 respectively, showing that the effects of 5-CT were more profound and much longer lasting. As a measure of the total response the areas of the individual responses were also measured. This gave a mean ratio (5-HT : 5-CT) of 1 : 15.9. All differences between 5-HT and 5-CT were statistically significant ($P < 0.01$, paired t test). A comparison of the effects of 5-HT and 5-CT are illustrated in Figure 4.

Comparison of the effects of 5-HT and 8-OH-DPAT The effects of 8-OH-DPAT were examined on 29 cells giving consistent depressant responses to 5-HT. Depressant responses to 8-OH-DPAT were observed on 24 of these cells. On the remaining 5

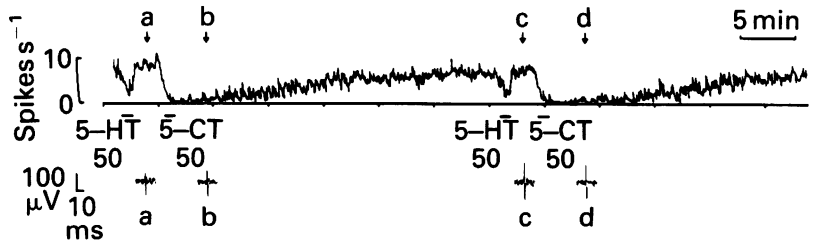

Figure 4 Comparison of the effects of 5-hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) on the spontaneous activity of a single brainstem neurone. When both compounds were applied with identical ejecting currents 5-CT caused a greater maximal depression of firing rate and had much longer-lasting effects. The depressant effects of 5-CT were not associated with a decrease in the spike amplitude as shown below the ratemeter record.

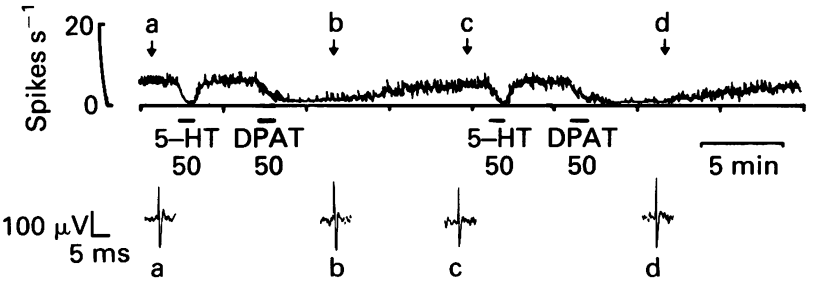

Figure 5 Comparison of the effects of 5-hydroxytryptamine (5-HT) and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) on the spontaneous activity of a single brainstem neurone. When applied with identical ejecting currents 5-HT and 8-OH-DPAT caused a similar maximal depression of firing rate but 8-OH-DPAT had longer-lasting effects. The depressant responses to 8-OH-DPAT were associated with an increased spike amplitude as shown below the ratemeter record.

cells 8-OH-DPAT evoked no response when applied with ejecting currents up to 100 nA, for 1 min. The depressant effects of 8-OH-DPAT were not associated with any decrease in spike amplitude, rather, as illustrated in Figure 5, an increase in spike amplitude was frequently observed.

The relative potencies of 5-HT and 8-OH-DPAT were compared on 15 cells. When applied with equivalent ejecting currents 5-HT and 8-OH-DPAT caused a similar maximal depression of firing rate (5-HT : 8-OH-DPAT; 1 : 0.98, $P > 0.1$) but 8-OH-DPAT had longer lasting effects (1 : 3.3, $P < 0.01$). A comparison of the areas of the individual responses gave a ratio of 1 : 3.2 ($P < 0.01$). A comparison of the effects of 5-HT and 8-OH-DPAT is illustrated in Figure 5.

Discussion

Microiontophoretic or systemic administration of high doses of ketanserin failed to attenuate the depressant effects of 5-HT. Ketanserin has a high affinity for 5-HT_2-binding sites (Leysen *et al.*, 1981) and is a potent antagonist of 5-HT at 5-HT_2- receptors on peripheral tissues (Leysen *et al.*, 1984). Systemic administration of ketanserin at doses lower than those used in this study have been shown to displace binding from central 5-HT_2-binding sites (Laduron *et al.*, 1982) and block many of the behavioural effects arising from the activation of central 5-HT systems (Yap & Taylor, 1983; Lucki *et al.*, 1984). These results are in accord with recent studies which have shown that the depressant effects of 5-HT on cortical, hippocampal and dorsal raphe neurones are not amenable to antagonism by ketanserin (Lakoski & Aghajanian, 1985; Mason 1985). It would seem unlikely, therefore, that the depressant responses to 5-HT in the medullary brainstem are mediated by 5-HT_2-receptors.

Neither microiontophoretic nor systemic administration of MDL 72222 reduced the depressant responses to 5-HT. MDL 72222 shows negligible affinity for either 5-HT_1- or 5-HT_2-recognition sites (Fozard, 1984). It is, however, a potent antagonist at 5-HT_3-receptors on peripheral tissues (Fozard, 1984; Azami *et al.*, 1985). The systemic dose used in this study is well in excess of the intravenous dose, 39 $\mu g\,kg^{-1}$, necessary to block the Bezold-Jarish reflex (Fozard, 1984).

Recent behavioural studies suggest that 5-HT_3-receptors are located in the CNS (Tyers *et al.*, 1987). It is of interest to note, therefore, that microiontophoretically applied MDL 72222 frequently resulted in a decrease in the spontaneous firing rate of cells. This effect was occasionally associated with an increase in the spike amplitude which may be indicative of a hyperpolarizing effect of the drug. It is currently unclear whether this is due to its 5-HT_3 blocking properties, as these effects only appeared with high currents and were not apparent when MDL 72222 was administered intravenously. It would seem unlikely from these results, however, that 5-HT_3-receptors are involved in the depressant effects of 5-HT on brainstem neurones.

Both 5-CT and 8-OH-DPAT had marked depressant effects upon the firing rate of brainstem neurones. These effects of 5-CT and 8-OH-DPAT are unlikely to result from a membrane stabilizing or local anaesthetic action, since neither compound depressed the amplitude of the action potential. Indeed, the depressant effects of both compounds were often associated with an increase in spike amplitude, which would be consistent with hyperpolarizing effects of the drugs. Whilst it is true that both 5-CT and 8-OH-DPAT depressed the majority of cells depressed by 5-HT, a high correlation between the effects of 5-HT, 5-CT and 8-OH-DPAT is not necessarily proof that these compounds interact at the same receptor type. Indeed, GABA, for example would also depress the majority of cells depressed by 5-HT. In this regard, however, it is important to note that 5-CT did not, and 8-OH-DPAT only occasionally, depressed cells which gave consistent excitatory responses to 5-HT (Davies *et al.*, 1988).

When 5-CT and 5-HT were applied with similar ejecting currents 5-CT caused a greater maximal depression of the firing rate and had much longer lasting effects. It seems likely that the greater apparent potency of 5-CT is a genuine biological phenomenon and not an artefact of the release from the electrode, since the mobilities of 5-HT and 5-CT, when tested *in vitro*, are not significantly different (Davies *et al.*, 1988). The long-lasting effects of 5-CT may also reflect a low affinity for the 5-HT uptake system. On peripheral tissues 5-CT shows a marked selectivity for 5-HT_1-like receptors. Thus, upon 5-HT_1-like receptors mediating vasodilatation and tachycardia in the cat, 5-CT is at least ten times more potent than 5-HT (Saxena *et al.*, 1985; Connor *et al.*, 1986). On 5-HT_2-receptors which mediate bronchoconstriction in the cat (Connor *et al.*, 1986) and 5-HT_3-receptors on the guinea-pig ileum (Humphrey, 1984) 5-CT is up to fifty times less potent than 5-HT. The binding profile of 5-CT shows similar selectivity. 5-CT has a somewhat higher affinity than 5-HT for the 5-HT_1-recognition site but a lower affinity than 5-HT for the 5-HT_2-recognition site (Engel *et al.*, 1983). Thus, the potent depressant effects of 5-CT seen in this study would be consistent with an action upon 5-HT_1-like receptors.

8-OH-DPAT caused a similar maximal depression

of firing rate to that seen with equal currents of 5-HT. The mobility of 8-OH-DPAT when tested *in vitro* is not greatly different from that of 5-HT (Davies *et al.*, 1988). Thus, the effects of the two agonists will not be unduly distorted by their release from the microelectrodes. The longer-lasting effects of 8-OH-DPAT may also be due to its low affinity for the 5-HT uptake system (Hamon *et al.*, 1984). 8-OH-DPAT is able to discriminate between 5-HT$_1$-like receptor subtypes in functional studies. Thus, 8-OH-DPAT inhibits transmitter release from guinea-pig enteric cholinergic neurones (Fozard & Kilbinger, 1985) but does not inhibit 5-HT release from cortical slices (Engel *et al.*, 1986). Similarly, 8-OH-DPAT discriminates between central 5-HT-recognition sites, showing a high affinity for the 5-HT$_{1A}$-site with at least a thousand fold less affinity for the 5-HT$_{1B}$-site (Middlemiss & Fozard, 1983). The data obtained with metergoline, however, tend to rule out the possibility that the 5-HT$_{1A}$-site is involved in the response to 5-HT. Metergoline has a high affinity for the 5-HT$_{1A}$-binding site (pKd = 8.1 – Engel *et al.*, 1986) yet failed to attenuate the depressant response to 5-HT. However, whilst it seems unlikely that the depressant effects of 5-HT involve 5-HT$_{1A}$-binding sites the data described here do not exclude the possibility that 8-OH-DPAT itself acts via 5-HT$_{1A}$-receptors, although, as discussed earlier, 8-OH-DPAT tends to depress only those cells depressed by 5-HT, suggesting that the two compounds act via the same receptor.

Metergoline shows a high affinity for each of the various 5-HT$_1$-binding site subtypes (Engel *et al.*, 1986; Heuring & Peroutka, 1987). Its failure to attenuate the depressant effects of 5-HT would, therefore, suggest that the receptor involved in this response is not identical to any of the 5-HT$_1$-binding sites yet described. The lack of antagonism by metergoline, however, is not necessarily inconsistent with the postulate that the depressant effects of 5-HT are mediated via a 5-HT$_1$-like receptor (as defined by Bradley *et al.*, 1986). Thus, for example, the contraction of the dog saphenous vein by 5-HT is potently mimicked by 5-CT, is not blocked by ketanserin but may be attenuated by methysergide, thus suggesting the involvement of a 5-HT$_1$-like receptor (Apperley *et al.*, 1980; Feniuk *et al.*, 1985). Metergoline, however, is not an effective antagonist of 5-HT on the dog saphenous vein (Feniuk, personal communication).

Sastry & Phillis (1977) have shown that metergoline attenuates the depressant effects of 5-HT on cortical neurones without reducing responses to other monoamines. Jones (1982) also noted that the depressant effects of 5-HT on cortical neurones could be blocked by metergoline. Neither systemically nor microiontophoretically applied metergoline blocked the responses to 5-HT on brainstem neurones. It is tempting, therefore, to suggest that the depressant effects of 5-HT on cortical and brainstem neurones are mediated by different receptor types.

The lack of antagonism of the depressant response of brainstem neurones to 5-HT by metergoline may indicate that this effect of 5-HT does not involve a 5-HT receptor. However, the depressant response to 5-HT can be blocked by high doses of methysergide. Methysergide has affinity for the 5-HT$_1$-binding site which is less than two orders of magnitude lower than its affinity for the 5-HT$_2$-binding site (Leysen *et al.*, 1981). It has also been shown to block 5-HT$_1$-like receptors on peripheral tissues and in behavioural tests, but at much higher doses than those required to block 5-HT$_2$-receptors (Apperley *et al.*, 1980; Blackburn *et al.*, 1984; Saxena & Lawang, 1985). Although very high doses of methysergide (30–40 mg kg^{-1}) were necessary to block the depressant effects of 5-HT, we have shown that this effect of methysergide is selective for 5-HT. Thus, at the same doses methysergide did not attenuate depressant responses to GABA or excitatory responses to glutamate. Indeed, Saxena & Lawang (1985) found it necessary to administer methysergide in doses up to 25 mg kg^{-1} in order to block completely the hypotensive effects of 5-HT in the anaesthetized rat. Similarly, doses of 10 mg kg^{-1} or more of methysergide have been found necessary to block a number of 5-HT$_1$-like receptor mediated behavioural responses (Green *et al.*, 1981; Blackburn *et al.*, 1984). Thus, although at such high doses other explanations are possible, the effects of methysergide described here are compatible with the suggestion that the depressant effects of 5-HT are mediated by 5-HT$_1$-like receptors.

In conclusion, we have found no evidence to suggest that either 5-HT$_2$-receptors or 5-HT$_3$- receptors are involved in the depressant responses of midline medullary brainstem neurones to 5-HT. The potent depressant effects of 5-CT and 8-OH-DPAT and the blockade of the response to 5-HT by high doses of methysergide suggest the involvement of 5-HT$_1$-like receptors. The lack of antagonism by metergoline, however, suggests that this receptor is not identical to any of the 5-HT$_1$-binding sites yet described.

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