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Evidence for excitatory 5-HT_2-receptors on rat brainstem neurones

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1 The technique of microiontophoresis was used to investigate the identity of the receptor mediating the excitatory effects of 5-hydroxytryptamine (5-HT) upon neurones in the midline of the medullary brainstem of the rat *in vivo*.

2 The 5-HT_1-like receptor agonists 5-carboxamidotryptamine (5-CT) and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) failed to excite the majority of neurones excited by 5-HT. The mobilities of 5-CT and 8-OH-DPAT when tested *in vitro* were found not to differ significantly from that of 5-HT, suggesting that the lack of effect of these agonists was not due to a lower rate of release from the microelectrodes.

3 The excitatory responses to 5-HT were attenuated by the 5-HT_2-receptor antagonists ketanserin and methysergide when applied microiontophoretically or administered intravenously (0.3 and 1 mg kg^{-1} respectively). Excitatory responses to glutamate and noradrenaline were not reduced.

4 The 5-HT_3-receptor antagonist MDL 72222 failed to attenuate selectively the excitatory response to 5-HT when applied either by microiontophoresis or administered intravenously (1 mg kg^{-1}).

5 Microiontophoretic application of the α_1-adrenoceptor antagonist prazosin did not attenuate excitatory responses to either 5-HT or noradrenaline. Intravenously administered prazosin (0.8 mg kg^{-1}) also failed to attenuate excitatory responses to 5-HT, but did block excitatory responses to noradrenaline.

6 These results suggest that 5-HT_2-receptors, but not 5-HT_1-like receptors, 5-HT_3-receptors or α_1-adrenoceptors, are involved in the excitatory response of midline medullary neurones to 5-HT.

Introduction

It is evident from electrophysiological (Roberts & Straughan, 1967), behavioural (Peroutka *et al.*, 1981; Green, 1984) and radioligand binding studies (Peroutka & Snyder, 1979) that there are multiple 5-hydroxytryptamine (5-HT)-receptor types in the central nervous system. In a recent review article Bradley *et al.* (1986) suggested that 5-HT-receptors may be divided into three distinct populations, termed 5-HT_1-like, 5-HT_2 and 5-HT_3. 5-HT_2- receptors have been defined in terms of the high antagonistic potency of compounds such as ketanserin and methysergide (Leysen *et al.*, 1981). Similarly, 5-HT_3-receptors may be identified by the potent antagonistic activity of MDL 72222 (Fozard, 1984) and ICS 205-930 (Donatsch *et al.*, 1984). The 5-HT_1-like receptor has not been defined by the actions of a selective antagonist. One of the criteria suggested by Bradley *et al.* (1986) for the identification of 5-HT_1-like receptors is that the actions of 5-HT should be mimicked by 5-carboxamidotryptamine (5-CT) at concentrations equal to or less than those of 5-HT.

It is well established that 5-HT applied by microiontophoresis has both excitatory and depressant effects on the firing rate of central neurones and that the excitatory, but not depressant, effects of 5-HT are amenable to antagonism by traditional 5-HT-receptor antagonists like methysergide (Roberts & Straughan, 1967; Boakes *et al.*, 1970; Haigler & Aghajanian, 1974; McCall & Aghajanian, 1980). However, the relationship between the receptors mediating these central actions of 5-HT and peripheral 5-HT-receptors remains unclear. In particular, it has yet to be established whether the receptor subtypes outlined by Bradley *et al.* (1986) are involved in these neuronal actions of 5-HT.

Previous studies have shown that the predominant effect of 5-HT on neurones in the midline of the

medullary brainstem of the rat is excitatory (Couch, 1970; 1976; Llewelyn *et al.*, 1983). In this study, therefore, we have used the 5-HT_1-like receptor agonists 5-CT and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), the 5-HT_2-receptor antagonists ketanserin and methysergide and the 5-HT_3-receptor antagonist MDL 72222 in order to determine whether one or more of these receptor subtypes are involved in the excitatory effects of 5-HT in the brainstem.

In addition to being a potent 5-HT_2-receptor antagonist, ketanserin also has powerful α_1-adrenoceptor blocking effects (Fozard, 1982; McCall & Schuette, 1984). Indeed, the possibility of wrongly attributing an effect of 5-HT to an action on 5- HT_2-receptors based solely on data obtained with ketanserin has been noted by several authors (Bradley *et al.*, 1986). In order to avoid this possibility, we have also examined the effects of the α_1-adrenoceptor antagonist prazosin on the excitatory responses to 5-HT.

Methods

Male Wistar rats (250 to 350 g) were anaesthetized with halothane (2% in oxygen). Tracheal, carotid and external jugular cannulae were inserted and the animal placed in a stereotaxic frame. Upon completion of the surgery, anaesthesia was maintained with halothane (0.8–1.2% in oxygen) delivered via the tracheal cannula. Blood pressure was continually monitored and body temperature was maintained between 37°C and 38°C. All animals respired spontaneously.

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. 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.

Action potentials from single spontaneously active neurones in the midline of the medullary brainstem were counted using a window discriminator and spike processor and displayed as a continuous record on a polygraph. The action potentials were simultaneously displayed on oscilloscopes to monitor their shapes and amplitudes. Permanent records of a sample of action potentials were made throughout each study by means of an output from a digital memory oscilloscope to an X-Y plotter.

The agonistic actions of 5-CT and 8-OH-DPAT were examined on cells that gave consistent excitatory responses to 5-HT. A cell was considered to be unresponsive to 5-CT and 8-OH-DPAT if no change in spontaneous firing rate was observed following its application with ejecting currents up to 100 nA for 1 min. It has been established that iontophoretic currents are carried by solutions of 5-CT and 8-OH-DPAT in a quantitatively similar manner to solutions of 5-HT (see transport number determination below).

The effects of the antagonists were examined on cells giving consistent excitatory responses to both 5-HT and a control agonist which was either noradrenaline or glutamate. When a unit was encountered, the agonists were applied alternately at regular 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 the agonists were obtained, the antagonist was applied continuously from another barrel by the passage of a small ejecting current (5 to 20 nA) without interrupting the agonist cycle. Application of the antagonist was continued after antagonism of the 5-HT response had occurred, until a further response to the control agonist had been evoked. The application of the antagonist was then discontinued and the application of the agonists continued until recovery was observed.

Responses to 5-HT throughout each study were quantified by measuring the area of the response and converting to total spike number as described by Bradshaw *et al.* (1974). A response was considered to be affected by the antagonist if there was a decrease of at least 50% in the size of the response compared to the size of the mean control. Antagonist studies were only performed on cells where the agonist responses varied by less than 10% before the antagonist application.

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.

Transport number determination in vitro

To allow any conclusions to be drawn concerning the relative effects of 5-HT, 5-CT and 8-OH-DPAT on neuronal activity, it is necessary to establish whether the transport numbers of 5-CT and 8-OH-DPAT differ significantly from that for 5-HT. Therefore, the relative mobilities of 5-HT and 5-CT were compared for 5 microelectrodes using the competition technique described by Bradshaw *et al.*

(1981). Two barrels of each electrode were filled with [^{3}H]-5-HT creatinine sulphate (0.05 M, 4 mCi mmol^{-1}) and 5-HT bimaleate (0.05 M) and two barrels were filled with [^{3}H]-5-HT creatinine sulphate (0.05 M, 4 mCi mmol^{-1}) and 5-CT bimaleate (0.05 M). The efflux of radioactive material into a series of small vials containing 0.5 ml of 0.9% saline was measured over 10 min periods. Firstly, the rate of spontaneous efflux was determined (two samples). Then the release of radioactive material from the barrels containing 5-HT was measured for a range of ejecting currents (25, 50, 75 and 100 nA, 2 samples for each current). After a further collection of 2 samples of spontaneous efflux the procedure was repeated for the barrels containing 5-CT. Finally, two further samples of spontaneous efflux were obtained. The amount of radiolabelled material released during each sample was determined by liquid scintillation spectrophotometry. For each of the 5 electrodes the transport number of 5-HT creatinine sulphate in the presence of 5-HT and 5-CT was then determined as described by Bradshaw *et al.* (1981).

Similar studies were conducted on a further 5 electrodes to establish the relative transport numbers of 5-HT and 8-OH-DPAT. These studies were conducted as described for 5-CT with the exception that release was only measured for a 50 nA ejecting current.

Drugs

The following drug solutions were used: 5-hydroxytryptamine bimaleate (0.1 or 0.2 M, pH 4), noradrenaline bitartrate (0.2 M, pH 4), 5-carboxamidotryptamine bimaleate (0.1 M, pH 4), 8-hydroxy-2-(di-n-propylamino) tetralin hydrogenbromide (0.02 M, pH 4) sodium-L-glutamate (0.2 M, pH 8), ketanserin tartrate (0.01 M, pH 3.5–4), methysergide maleate (0.01 M, pH 3.5–4.5), prazosin hydrochloride (0.001 M, pH 5–5.5) and MDL 72222 (1αH, 3α,5αH-tropan-3yl-3,5-dichlorobenzoate) (0.01 M, pH 5–5.5).

Results

Location of recording sites

A total of 115 cells was studied. From the PSB dye spots it was determined that recording sites lay in the medullary brainstem between 0.8 mm and 2.3 mm behind the interaural line and within 1 mm of the midline, with the majority (91%) located within 0.5 mm of the midline.

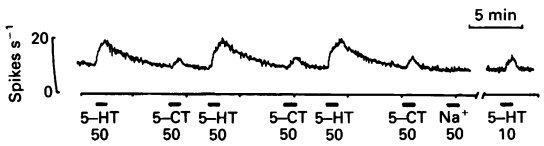

Figure 1 The effects of 5-carboxamidotryptamine (5-CT) on the spontaneous activity of a single brainstem neurone. Ordinate scale: firing rate (spikes s^{-1}), abscissa scale: running time. The horizontal bars below the trace refer to iontophoretic drug applications and the numbers refer to the intensity of the ejecting current in nA. When applied with identical ejecting currents 5-CT evoked much smaller responses than 5-hydroxytryptamine (5-HT). Similarly it was only necessary to apply 5-HT with one fifth of the current used to apply 5-CT in order to produce responses of approximately equal magnitude. Ejection of sodium ions had no discernible effect.

Relative mobilities of 5-HT, 5-CT and 8-OH-DPAT

For 5 electrodes the relative mobilities of 5-HT and 5-CT were assessed. The transport number of [^{3}H]-5-HT creatinine sulphate was 0.087 ± 0.008 (mean $\pm$ s.e. mean) in the presence of equimolar amounts of cold 5-HT and 0.079 ± 0.002 (mean $\pm$ s.e. mean) in the presence of equimolar amounts of cold 5-CT. There was no statistically significant difference in the transport number of [^{3}H]-5-HT in the presence of cold 5-HT and cold 5-CT ($P > 0.4$, t test).

For a further 5 electrodes the relative mobilities of 5-HT and 8-OH-DPAT were compared. The transport number of [^{3}H]-5-HT creatinine sulphate was 0.093 ± 0.011 (mean $\pm$ s.e. mean) in the presence of equimolar amounts of cold 5-HT and 0.069 ± 0.008 (mean $\pm$ s.e. mean) in the presence of equimolar amounts of cold 8-OH-DPAT. These results suggest that the mobility of 8-OH-DPAT is somewhat higher than that of 5-HT, though no statistically significant difference was found ($P > 0.1$, t test).

Effects of 5-CT and 8-OH-DPAT

The effects of 5-CT were examined on 31 cells giving consistent excitatory responses to 5-HT (25–100 nA, 20–60 s). On 22 of these cells no response to 5-CT was observed when it was applied with ejecting currents of up to 100 nA for 1 min. On the remaining 9 cells weak excitatory responses to 5-CT were observed. On each of these cells, however, it was necessary to apply 5-CT with ejecting currents up to 5 times greater than those used to eject 5-HT in order to produce responses of equal magnitude. An example is illustrated in Figure 1.

The effects of 8-OH-DPAT were examined on 26 cells which gave consistent excitatory responses to

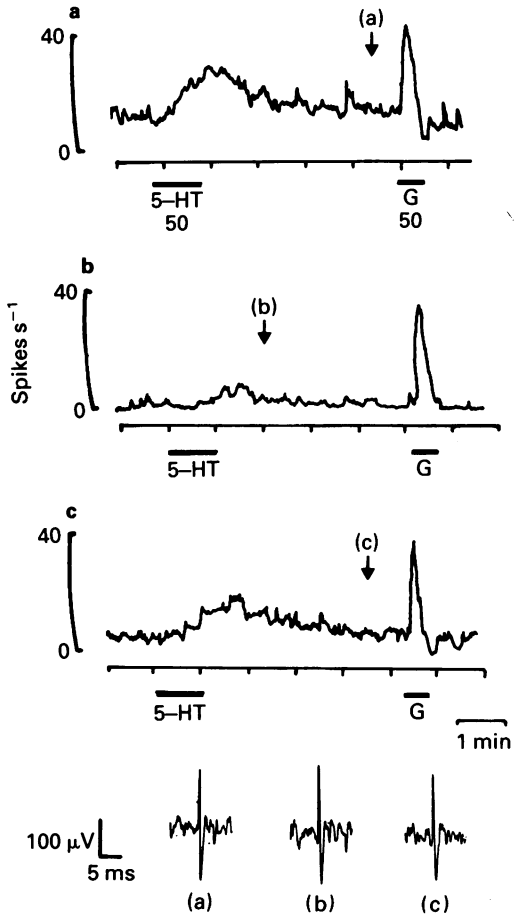

Figure 2 The effect of ketanserin on excitatory responses to 5-hydroxytryptamine (5-HT) and glutamate (G) on a single brainstem neurone. (a) Control responses to the agonists. (b) Responses to the agonists during the continuous application of ketanserin. Ketanserin was applied with a current of 5 nA and had been applied continuously for 28 min at the start of trace (b). Ketanserin blocked the response to 5-HT without reducing the response to glutamate. (c) Recovery of the responses to 5-HT 42 min after the application of ketanserin had been terminated. Below the ratemeter records are a sample of extracellularly recorded action potentials taken at those times indicated by the arrows. It can be seen that antagonism of the 5-HT response was not accompanied by any change in spike amplitude.

5-HT. On 16 of these cells 8-OH-DPAT failed to evoke any response when applied with currents up to 100 nA for 1 min. On 4 cells depressant responses to 8-OH-DPAT were observed. On the remaining 6 cells it was possible to evoke excitatory effects to 8-OH-DPAT. It was necessary, however, to apply 8-OH-DPAT with approximately 4 times the current used to apply 5-HT in order to evoke responses of similar magnitude.

Effects of ketanserin, methysergide, MDL 72222 and prazosin on excitatory responses to 5-HT

Ketanserin The effects of ketanserin were examined on cells giving consistent excitatory responses to both 5-HT and glutamate ($n = 8$) or 5-HT and noradrenaline ($n = 4$). With 8 of these 12 cells ketanserin (5–15 nA) reduced the excitatory responses to 5-HT without altering spike characteristics and with little or no effect on the responses to the control agonists. Blockade of the response to 5-HT was usually associated with a decrease in the spontaneous firing rate of the cell. An example is shown in Figure 2. On the remaining 4 cells, ketanserin also markedly reduced the response to 5-HT but on these occasions it also decreased the amplitude of the spike. This made interpretation of the studies difficult.

The effects of intravenously administered ketanserin were also examined on cells giving consistent excitatory responses to either 5-HT and glutamate ($n = 5$) or 5-HT and noradrenaline ($n = 2$). Ketanserin (0.2–0.5 mg kg^{-1}) reversibly reduced the responses to 5-HT on all 7 occasions. Responses to glutamate were reduced in only 2 studies and responses to noradrenaline were not affected. Examples are shown in Figures 3 and 4.

Methysergide The antagonistic actions of microiontophoretically applied methysergide were examined on 9 cells giving repeatable excitatory responses to 5-HT and glutamate. On each occasion methysergide (5 to 10 nA) reversibly attenuated the excitatory response to 5-HT without reducing the excitatory response to glutamate. On 6 of the 9 cells this effect of methysergide was accompanied by a decrease in the spontaneous firing rate. An example of the effects of methysergide is illustrated in Figure 5.

The effects of intravenously administered methysergide were also evaluated on 6 cells giving consistent excitatory responses to 5-HT and glutamate. Methysergide (0.8–1.3 mg kg^{-1}) reversibly reduced the responses to 5-HT on each occasion without reducing responses to glutamate (Figure 6).

MDL 72222 The effects of microiontophoretically applied MDL 72222 were examined on 8 cells giving consistent excitatory responses to 5-HT and glutamate. When applied with low ejecting currents (5–20 nA) MDL 72222 had little or no effect on the responses to the agonists. When applied with higher ejecting currents (20–50 nA) MDL 72222 reversibly reduced the excitatory responses to both 5-HT and glutamate. This effect, however, was invariably

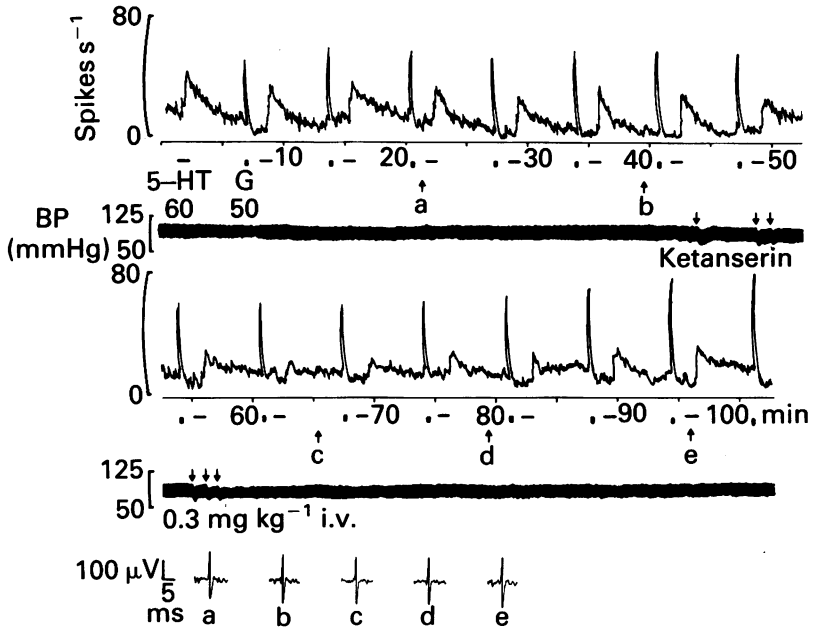

Figure 3 A continuous ratemeter record showing the effects of intravenously administered ketanserin on the excitatory responses to 5-hydroxytryptamine (5-HT) and glutamate (G) on a single brainstem neurone. The trace below the ratemeter record shows the blood pressure (BP) of the animal (mmHg). Ketanserin was administered in divided doses (indicated by the arrows) to a cumulative dose of 0.3 mg kg^{-1}. Ketanserin attenuated the excitatory responses to 5-HT without reducing the responses to glutamate. It can also be seen that there was little change in spike amplitude throughout the course of the study.

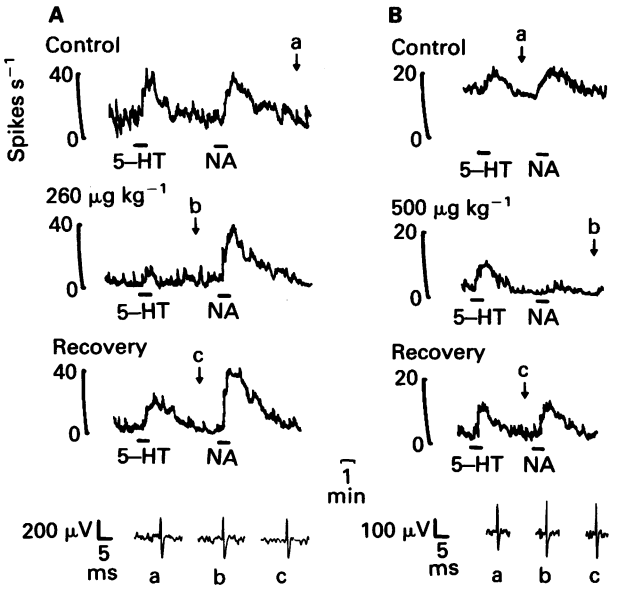

Figure 4 Comparison of the effects of intravenously administered ketanserin (260 μg kg^{-1}) (A) and prazosin (500 μg kg^{-1}) (B) on the excitatory responses to 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on two different brainstem neurones. The records labelled recovery were obtained 66 min after the administration of ketanserin and 50 min after the administration of prazosin.

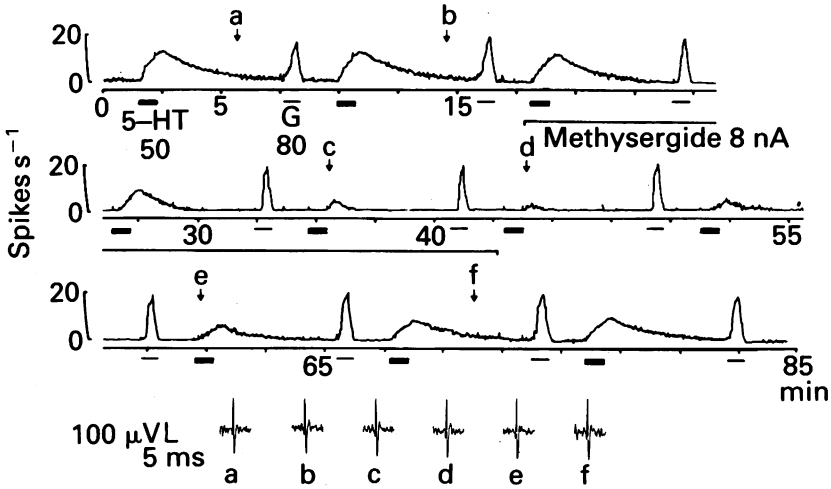

Figure 5 A continuous ratemeter record showing the effects of methysergide on the excitatory responses to 5-hydroxytryptamine (5-HT) and glutamate (G) on a single brainstem neurone. Continuous application of methysergide (8 nA) caused a marked reduction in the excitatory response to 5-HT with little effect on the excitatory response to glutamate. Antagonism of 5-HT was not accompanied by any decrease in spike amplitude.

accompanied by a decrease in the spike amplitude. Systemic administration of MDL 72222 (1 mg kg^{-1} i.v., $n = 5$) also failed to reduce responses to 5-HT.

Prazosin The effects of microiontophoretically applied prazosin were examined on 6 cells giving consistent excitatory responses to both 5-HT and noradrenaline. Prazosin, applied with a current of 10 nA for up to 40 min did not attenuate the responses to either agonist. When applied with higher currents prazosin, invariably caused a marked reduction in spike amplitude.

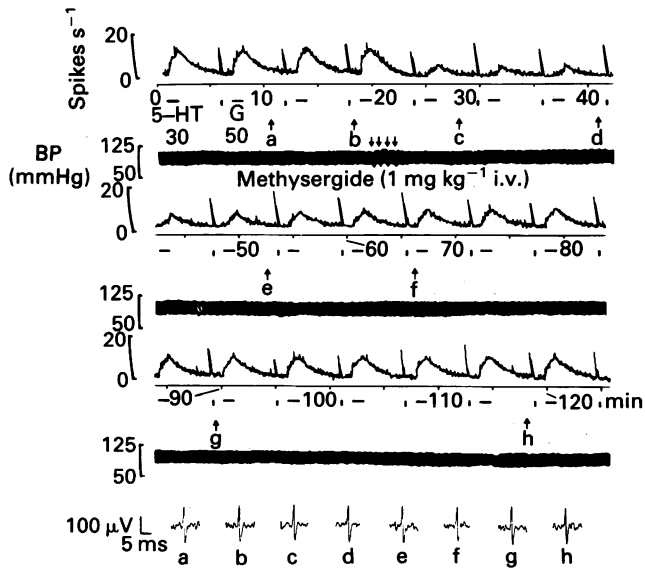

Figure 6 A continuous ratemeter record showing the effects of intravenously administered methysergide on the excitatory responses to 5-hydroxytryptamine (5-HT) and glutamate (G) on a single brainstem neurone. Methysergide was administered in divided doses (indicated by the arrows) to a cumulative dose of 1 mg kg^{-1}. Methysergide attenuated the excitatory responses to 5-HT without reducing the responses to glutamate. It can also be seen that there was little change in spike amplitude throughout the course of the study.

Intravenously administered prazosin (0.5 to 1 mg kg^{-1}, $n = 5$) reversibly attenuated excitatory responses to noradrenaline but had little or no effect on excitatory responses to 5-HT. This effect of prazosin was always accompanied by a marked reduction in the spontaneous firing rate of the cell. An example is shown in Figure 4.

Discussion

5-CT failed to excite the majority of neurones giving a consistent excitatory response to 5-HT. Indeed, even on those cells where excitatory responses to 5-CT were observed, these were much smaller responses than those evoked on the same cell by the same current of 5-HT. The lack of effect of 5-CT is unlikely to be due to inadequate release from the microelectrodes since the mobility of 5-CT did not significantly differ from that of 5-HT. 5-CT displaces [^{3}H]-5-HT (5-HT$_1$) binding with a somewhat greater potency than 5-HT (Engel *et al.*, 1986). In functional studies on peripheral tissues containing 5-HT$_1$-like receptors, 5-CT displays a potency between 2 and 50 times greater than that of 5-HT (Feniuk *et al.*, 1985; Charlton *et al.*, 1986; Trevethick *et al.*, 1986). Thus, it would seem unlikely that 5-HT$_1$-like receptors are involved in the excitatory response to 5-HT on brainstem neurones.

5-CT has a lower affinity for 5-HT$_2$-binding sites than 5-HT, but this difference is less than ten fold (Engel *et al.*, 1986). 5-CT also acts upon 5-HT$_2$-receptors in the rabbit aorta, although it is up to 26 times less active than 5-HT (Feniuk *et al.*, 1985). The occasional weak excitatory responses seen with 5-CT may, therefore, be due to a weak action on 5-HT$_2$-receptors.

The results obtained with 8-OH-DPAT were very similar to those obtained with 5-CT. Thus, 8-OH-DPAT failed to excite the majority of neurones excited by 5-HT. Moreover, on those cells where excitatory responses to 8-OH-DPAT were observed the responses were much smaller than those observed with the same current of 5-HT. The transport studies *in vitro* indicate that the mobility of 8-OH-DPAT is at least as great as that of 5-HT. Thus its lack of potent excitatory actions are not due to an inadequate release from the electrode. Although 8-OH-DPAT acts only upon a subpopulation of 5-HT$_1$-like receptors (Middlemiss & Fozard, 1983; Bradley *et al.*, 1986), its lack of any potent excitatory action does support the results obtained with 5-CT, suggesting that 5-HT$_1$-like receptors are not involved in the excitatory effects of 5-HT on midline brainstem neurones.

The 5-HT$_3$-receptor antagonist MDL 72222 failed to attenuate selectively the excitatory response to 5-HT, either when applied microiontophoretically or administered intravenously (1 mg kg^{-1}). MDL 72222 did occasionally have effects upon spike characteristics when applied with high ejecting currents. These effects were suggestive of a membrane stabilizing action. Fozard (1984) also noted local anaesthetic properties of MDL 72222 in the guinea-pig wheal test when it was used in high concentrations. These results suggest that 5-HT$_3$-receptors are not involved in the excitatory effects of 5-HT upon midline brainstem neurones.

On certain vascular smooth muscle preparations, 5-HT is thought to evoke some of its effects via α_1-adrenoceptors (Apperley *et al.*, 1976; Black *et al.*, 1981). Thus, we examined the effects of the α_1-adrenoceptor antagonist prazosin on the excitatory responses to 5-HT. Prazosin failed to attenuate responses to 5-HT whether given locally or applied systemically, thereby failing to support a role for α_1-adrenoceptors in this response to 5-HT. Indeed, there is some doubt as to whether the excitatory effects of noradrenaline itself are mediated via α_1-adrenoceptors in this brain area. Previous studies on both medullary raphe and reticular neurones have shown the noradrenaline response to be resistant to blockade by a wide range of α-adrenoceptor antagonists (Boakes *et al.*, 1971; Behbehani *et al.*, 1981). In this study we found that prazosin, given microiontophoretically, failed to block the effects of noradrenaline at currents less than those which caused a decrease in spike amplitude. Interestingly, systemically administered prazosin did selectively attenuate the excitatory effects of noradrenaline. The reason for the different effects of systemic and locally applied prazosin is unclear. It is possible that a more even distribution of prazosin was achieved by the systemic route, though effects elsewhere in the CNS or periphery cannot be excluded.

The excitation of brainstem neurones by 5-HT was blocked by the 5-HT$_2$-receptor antagonists ketanserin and methysergide applied locally. In addition to its 5-HT blocking properties, on several cells ketanserin also had effects on the spike indicative of a membrane stabilizing action. Antagonism of the 5-HT responses, however, seems unlikely to be due to its membrane stabilizing effects since, on the majority of cells studied, it was possible to block the response to 5-HT without reducing the response to the control agonist and without affecting the spike amplitude. It seems likely, therefore, that these results simply reflect the fact that ketanserin has potent membrane stabilizing effects on brainstem neurones which, on occasions, cannot be dissociated from its 5-HT blocking properties. Indeed, similar problems have been noted with other antagonists, in particular, atropine and propranolol (Curtis & Phillis, 1960; Johnson *et al.*, 1969).

Ketanserin shows a high degree of selectivity between 5-HT receptor subtypes. Thus, ketanserin shows a high affinity for 5-HT_2-receptors but a thousand fold less affinity for 5-HT_1-like and 5-HT_3-receptors (Leysen *et al.*, 1981; Bradley *et al.*, 1986). Ketanserin does, however, show appreciable affinity for α_1-adrenoceptors (Leysen *et al.*, 1981). A role for α_1-receptors in the excitatory effects of 5-HT would, however, seem highly unlikely. Firstly, the responses to 5-HT were not blocked by the α_1-adrenoceptor antagonist prazosin. Secondly, the responses to 5-HT were blocked by the 5-HT_2-receptor antagonist methysergide, which shows negligible affinity for α_1-receptors. Thus, the most likely explanation of these results would seem to be that the excitatory effects of 5-HT on brainstem neurones are mediated via 5-HT_2-receptors but that in addition to its 5-HT blocking properties ketanserin also has membrane stabilizing properties at higher concentrations.

This conclusion is supported by the results of the systemic experiments with ketanserin and methysergide, where both antagonists effectively blocked the excitatory responses to 5-HT. It must be recognised, however, that when given systemically the antagonists will be acting widely throughout both central and peripheral nervous systems. However, systemically administered ketanserin and methysergide did not attenuate responses to the control agonists. Thus whilst a distal site of action cannot be excluded it would not seem the most parsimonious explanation. The doses of ketanserin and methysergide used in this study are in a similar range to those required to block central 5-HT_2-receptors in behavioural studies. Thus, Yap & Taylor (1983), for example, found that methysergide dose-dependently blocked 5-hydroxytryptophan-induced wet dog shakes in the rat in the range 0.25 to 5 $mg\,kg^{-1}$. Ketanserin was similarly effective in the range 0.01 to 1 $mg\,kg^{-1}$.

In conclusion, these results suggest that 5-HT_1-like receptors, 5-HT_3-receptors and α_1-adrenoceptors are not involved in the excitatory effects of 5-HT on midline medullary brainstem neurones. Rather, the results indicate that neuronal excitation by 5-HT in this brain area is mediated by 5-HT_2-receptors.

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References

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1976). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. *Br. J. Pharmacol.*, **58**, 211–221.

BEHBEHANI, M.M., POMEROY, S.L. & MACK, C.E. (1981). Interaction between central grey and nucleus raphe magnus: role for norepinephrine. *Brain Res. Bull.*, **6**, 361–364.

BLACK, J.L., FRENCH, R.J. & MYLECHARANE, E.J. (1981). Receptor mechanisms for 5-hydroxytryptamine in rabbit arteries. *Br. J. Pharmacol.*, **74**, 619–629.

BOAKES, R.J., BRADLEY, P.B., BRIGGS, I. & DRAY, A. (1970). Antagonism of 5-hydroxytryptamine by LSD-25 in the central nervous system: a possible basis for the actions of LSD-25. *Br. J. Pharmacol.*, **40**, 202–218.

BOAKES, R.J., BRADLEY, P.B., BROOKES, N., CANDY, J.M. & WOLSTENCROFT, J.H. (1971). Actions of noradrenaline and other sympathomimetic amines and antagonists on neurones in the brain stem of the cat. *Br. J. Pharmacol.*, **41**, 462–479.

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLENCHARANE, B.P., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1973). Kinetics of the release of noradrenaline from micropipettes: Interaction between ejecting and retaining currents. *Br. J. Pharmacol.*, **49**, 667–677.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1974). Effects of imipramine and desipramine on the responses of single cortical neurones to noradrenaline and 5-hydroxytryptamine. *Br. J. Pharmacol.*, **52**, 349–358.

BRADSHAW, C.M., PUN, R.Y.K., SLATER, N.T. & SZABADI, E. (1981). A procedure for comparing the mobilities of unlabelled drugs used in microelectrophoresis experiments. *J. Pharmacol. Meth.*, **5**, 67–74.

CHARLTON, K.G., BOND, R.A. & CLARKE, D.E. (1986). An inhibitory prejunctional 5-HT_1-like receptor in the isolated perfused rat kidney: apparent distinction from the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subtypes. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **332**, 8–15.

COUCH, J.R. (1970). Responses of neurons in the raphe nuclei to serotonin, norepinephrine and acetylcholine and their correlation with an excitatory synaptic input. *Brain Res.*, **19**, 137–150.

COUCH, J.R. (1976). Further evidence for a possible excitatory serotonergic synapse on raphe neurons of pons and lower midbrain. *Life Sci.*, **19**, 761–768.

CURTIS, D.W. & PHILLIS, J.W. (1960). The action of procaine and atropine on spinal neurones. *J. Physiol.*, **153**, 17–34.

DONATSCH, P., ENGEL, G., RICHARDSON, B.P. & STADLER, P.A. (1984). ICS 205-930: a highly selective and potent antagonist at peripheral neuronal 5-hydroxytryptamine (5-HT) receptors. *Br. J. Pharmacol.*, **81**, 34P.

ENGEL, G., GOTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presy-

naptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT$_{1B}$ binding sites. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **332**, 1–7.

FENIUK, W., HUMPHREY, P.P.A., PERREN, M.J. & WATTS, A.D. (1985). A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. *Br. J. Pharmacol.*, **86**, 697–704.

FOZARD, J.R. (1982). Mechanisms of the hypotensive effect of ketanserin. *J. Cardiovasc. Pharmacol.*, **4**, 829–838.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 36–44.

GREEN, A.R. (1984). 5-HT-mediated behaviour. *Neuropharmacology*, **23**, 1521–1528.

HAIGLER, H.J. & AGHAJANIAN, G.K. (1974). Peripheral serotonin antagonists: failure to antagonise serotonin in brain areas receiving a prominent serotonergic input. *J. Neural Transm.*, **35**, 257–273.

JOHNSON, E.S., ROBERTS, M.H.T., SOBIESZEK, A. & STRAUGHAN, D.W. (1969). Noradrenaline sensitive cells in cerebral cortex. *Int. J. Neuropharmacol.*, **8**, 549–566.

LEYSEN, J.E., AWOUTERS, F., KENNIS, L., LADURON, P.M., VANDENBERK, J. & JANSSEN, P.A.J. (1981). Receptor binding profile of R 41 468, a novel antagonist at 5-HT$_2$ receptors. *Life Sci.*, **28**, 1015–1022.

LLEWELYN, M.B., AZAMI, J. & ROBERTS, M.H.T. (1983). Effects of 5-hydroxytryptamine applied into nucleus raphe magnus on nociceptive thresholds and neuronal firing rate. *Brain Res.*, **258**, 59–68.

McCALL, R.B. & AGHAJANIAN, G.K. (1980). Pharmacological characterization of serotonin receptors in the facial motor nucleus: a microiontophoretic study. *Eur. J. Pharmacol.*, **65**, 175–183.

McCALL, R.B. & SCHUETTE, M.R. (1984). Evidence for an alpha$_1$ receptor-mediated central sympathoinhibitory action of ketanserin. *J. Pharmacol. Exp. Ther.*, **228**, 704–710.

MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-hydroxy-2-(Di-n-propylamino) tetralin discriminates between subtypes of the 5-HT$_1$ recognition site. *Eur. J. Pharmacol.*, **90**, 151–152.

PEROUTKA, S.J., LEBOWITZ, R.M. & SNYDER, S.H. (1981). Two distinct central serotonin receptors with different physiological functions. *Science*, **212**, 827–829.

PEROUTKA, S.J. & SNYDER, S.H. (1979). Multiple serotonin receptors: Differential binding of ^{3}H-5-hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. *Molec. Pharmacol.*, **16**, 687–699.

ROBERTS, M.H.T. & STRAUGHAN, D.W. (1967). Excitation and depression of cortical neurones by 5-hydroxytryptamine. *J. Physiol.*, **193**, 269–294.

TREVETHICK, M.A., FENIUK, W. & HUMPHREY, P.P.A. (1986). 5-Carboxamidotryptamine is a potent agonist mediating relaxation and elevation of cyclic AMP in the isolated neonatal porcine vena cava. *Life Sci.*, **38**, 1521–1528.

YAP, C.Y. & TAYLOR, D.A. (1983). Involvement of 5-HT$_2$ receptors in the wet-dog shake behaviour induced by 5-hydroxytryptophan in the rat. *Neuropharmacology*, **22**, 801–804.

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