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Pharmacological characterization of 8-OH-DPAT-induced inhibition of rat hippocampal 5-HT release *in vivo* as measured by microdialysis

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1 We have previously found that the putative 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) decreases hippocampal 5-hydroxytryptamine (5-HT) release in the anaesthetized rat, as measured by brain microdialysis. The present study attempted to characterize the receptor involved in this response using a range of monoamine receptor antagonists.

2 The classical 5-HT receptor antagonists, metergoline (5 mg kg^{-1} s.c.), methysergide (10 mg kg^{-1} s.c.) and methiothepin (10 mg kg^{-1} s.c.) each reduced dialysate levels of 5-HT which complicated their use as antagonists in these experiments. Nevertheless, pretreatment with metergoline but not methiothepin and methysergide partially reduced the 5-HT response to a maximally effective dose of 8-OH-DPAT (0.25 mg kg^{-1} s.c.).

3 The mixed 5-HT_1/β-adrenoceptor antagonist pindolol (8 mg kg^{-1} s.c.) was without effect on spontaneous 5-HT output but attenuated the effect of both maximally (0.25 mg kg^{-1} s.c.) and submaximally (0.05 mg kg^{-1} s.c.) effective dose of 8-OH-DPAT. In comparison, propranolol (10 mg kg^{-1} s.c.) did not affect 5-HT output when injected alone and did not alter the response to 8-OH-DPAT (0.25 mg kg^{-1} s.c.).

4 The 5-HT_2 receptor antagonist ritanserin (0.2 mg kg^{-1} s.c.) and the 5-HT_3 receptor antagonist BRL 43694 (0.5 mg kg^{-1} s.c.) neither altered 5-HT output alone nor significantly changed the response to 8-OH-DPAT (0.25 mg kg^{-1} s.c.).

5 The 8-OH-DPAT (0.25 mg kg^{-1} s.c.) response was not affected by pretreatment with either the dopamine D_2-receptor antagonist sulpiride (10 mg kg^{-1} s.c.) or the α_1/α_2-adrenoceptor antagonist phentolamine (10 mg kg^{-1} s.c.).

6 We conclude from these data that the decrease of hippocampal 5-HT output induced by 8-OH-DPAT does not involve 5-HT_2, 5-HT_3, adrenoceptors or dopamine D_2-receptors and that activation of a 5-HT_1 class of receptor seems probable. Full classification of the 8-OH-DPAT response awaits development of a suitably selective 5-HT_1 receptor antagonist with low intrinsic activity at the somatodendritic 5-HT autoreceptor.

Introduction

There is considerable evidence from neuropharmacological studies in animals that central 5-hydroxytryptamine (5-HT)-containing neurones are controlled by inhibitory 5-HT receptors localized both on nerve terminals in the forebrain and cell soma/dendrites in the mid-brain raphé nuclei. For example, it has been shown that exogenous 5-HT and direct acting 5-HT agonists both decrease the release of preloaded, radiolabelled 5-HT from rat brain tissue *in vitro* (Farnebo & Hamberger, 1974; Hamon *et al.*, 1974; Cerrito & Raiteri, 1979; Göthert & Weinheimer, 1979), and reduce the electrical activity of serotoninergic neurones when applied directly in the 5-HT somatodendritic area of the dorsal raphé nucleus *in vivo* (Haigler & Aghajanian, 1977; Vander Maelen *et al.*, 1986; Blier & De Montigny, 1987).

Current classification of the type of 5-HT receptor involved in 5-HT autoreceptor function relies to a large part on the association between the potencies of various 5-HT agonists in 5-HT autoreceptor

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models and their selectivity for various 5-HT receptor sub-types; 5-$HT_{1A, B, C, D,}$, 5-HT_2 and 5-HT_3. Thus, the rank-order of potency of 5-HT agonists at the nerve terminal 5-HT autoreceptor *in vitro* correlates best with the affinity of these drugs for the rat brain 5-HT_{1B} binding sites (Middlemiss, 1984a,b; Engel *et al.*, 1986). In contrast, inhibition of 5-HT cell-firing is observed following intra-raphé application of drugs with 5-HT_{1A} agonist activity, such as 8-OH-DPAT, but not those with 5-HT_{1B} agonist activity (Sprouse & Aghajanian, 1987; Sinton & Fallon, 1988). Accordingly, on the basis of such studies the somatodendritic and nerve terminal 5-HT autoreceptors are thought to be of the 5-HT_{1A} and 5-HT_{1B} subtypes, respectively.

Generally speaking, characterization of the 5-HT autoreceptor as being of the 5-HT_1 class is not well supported by studies with 5-HT antagonists. Despite having high affinity for the 5-HT_1 binding site in rat brain, the classical 5-HT antagonists metergoline and methysergide are weak or inactive as blockers of the nerve terminal 5-HT autoreceptor *in vitro*, although in this respect methiothepin is a potent antagonist (Martin & Sanders-Bush, 1982; Mounsey *et al.*, 1982; Moret, 1985). Certain β-adrenoceptor antagonists including cyanopindolol and propranolol, which bind with high affinity to brain 5-HT_1 sites, also act as nerve terminal 5-HT autoreceptor antagonists (Middlemiss, 1984c; Schlicker *et al.*, 1985). In comparison, whilst propranolol shows antagonistic activity towards the somatodendritic 5-HT autoreceptor (Sprouse & Aghajanian, 1986), metergoline, methysergide and methiothepin do not block the inhibition of serotoninergic cell firing rate by 5-HT receptor stimulation and indeed inhibit serotoninergic cell firing when administered alone (Haigler & Aghajanian, 1977).

Recently, we demonstrated that systemic administration of 8-OH-DPAT reduces 5-HT release in rat hippocampus *in vivo* as assessed by brain microdialysis (Sharp *et al.*, 1989a). Our proposal that this response was mediated via somatodendritic 5-HT_{1A} autoreceptors is directly supported by the finding that a similar effect is produced by injecting 8-OH-DPAT directly into the dorsal raphé nucleus (Sharp *et al.*, 1989b). It was therefore of interest to characterize the effect of 8-OH-DPAT on 5-HT release by the use of various monoamine receptor antagonists.

Methods

Brain microdialysis

The microdialysis methodology used in the present study is described in detail elsewhere (Sharp *et al.*, 1989a,b). Briefly, male Sprague Dawley rats (280–320 g) were anaesthetized with chloral hydrate (360 mg kg^{-1} i.p.) and a dialysis probe of the short-loop type was stereotaxically implanted into the ventral hippocampus (probe tip; rostral–caudal −4.8 mm, lateral −4.6 mm, ventral −8.5 mm, from bregma and dura surface according to Paxinos & Watson, 1982). Once secured in place with dental cement, the probe was continuously perfused (1 μl min^{-1}) with artificial CSF containing citalopram 1 μM. Every 20 min, perfusates were collected and injected immediately onto a high performance liquid chromatogram (h.p.l.c.) with electrochemical detection system (Sharp *et al.*, 1989b) for measurement of 5-HT content.

Experimental design

Dialysates were collected every 20 min until their 5-HT content was similar for 2–3 consecutive samples (typically 2–3 h post probe implantation), at which point either 8-OH-DPAT or a monoamine receptor antagonist was injected subcutaneously. For drug interaction experiments the control period was extended slightly and the antagonist was administered 30 min before injection of 8-OH-DPAT. Perfusates were collected for a further 2 h following 8-OH-DPAT or antagonist (when this was injected alone). Since several monoamine receptor antagonists were seen to decrease 5-HT output when injected alone, a maximally effective dose of 8-OH-DPAT (0.25 mg kg^{-1}; Sharp *et al.*, 1989a) was chosen for the interaction study.

The doses of methysergide, methiothepin, pindolol and propranolol were chosen on the basis of those previously shown to attenuate markedly 8-OH-DPAT-induced behaviour in rats (Tricklebank *et al.*, 1984). Similarly, on the evidence of earlier reports the doses of ritanserin (e.g. Goodwin & Green, 1985), BRL 43694 (Fake *et al.*, 1987) phentolamine (Doxey *et al.*, 1983) and sulpiride (Zetterström *et al.*, 1986) that were used would be expected to inhibit effectively *in vivo* 5-HT_2, 5-HT_3, α-adrenoceptors and dopamine D_2-receptors, respectively.

Data analysis

5-HT in dialysates is expressed as a percentage of the absolute amount of 5-HT contained in the dialysate collected immediately before administration of 8-OH-DPAT or, when injected alone, the monoamine antagonists. Areas under the curves (AUC) were calculated and statistical comparisons were made between groups on log transformed AUC data using Student's unpaired t test following one way ANOVA. A P value of less than 0.05 was considered to be statistically significant.

Drugs

Drugs from the following sources were used: 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin HBr., Research Biochemical Inc., SEMAT, St. Albans, U.K.), ritanserin (Janssen Pharmaceutical Ltd, Wantage, U.K.), BRL 43694 (endo-N-(9-methyl-9-azabicyclo [3,3,1]non-3-yl)-1-methyl-indazole-3-carboxamide HCl, Beecham Pharmaceuticals, Harlow, U.K.), metergoline (Farmitalia, Milan), methiothepin maleate (Roche, Welwyn Garden City, U.K.), methysergide maleate (Sandoz Pharmaceuticals, Feltham, U.K.), (±)-propranolol HCl (ICI Pharmaceuticals, Macclesfield, U.K.), (±)-pindolol HCl (Sandoz Pharmaceuticals, Feltham, U.K.), (±)-sulpiride (Sigma) and phentolamine mesylate (Ciba, Rogitine). Drugs were dissolved in saline (8-OH-DPAT, BRL 43694, propranolol) or alternatively a minimum amount of glacial acetic acid, then made up to volume with 5% glucose solution, and were injected subcutaneously in a volume of $1\,ml\,kg^{-1}$.

Results

Effect of 5-HT receptor antagonists on spontaneous 5-HT output in hippocampus of the anaesthetized rat

Administration of the classical 5-HT receptor antagonists methiothepin ($10\,mg\,kg^{-1}$), methysergide ($10\,mg\,kg^{-1}$) and metergoline ($5\,mg\,kg^{-1}$) reduced hippocampal dialysate levels of 5-HT over the 2 h postdrug period in comparison to saline injected controls ($P < 0.05$ in each case; Figure 1a). This effect was most pronounced for $10\,mg\,kg^{-1}$ methiothepin which decreased 5-HT levels by about 50% within 60 min of injection.

In comparison, the mixed 5-HT_1/β-adrenoceptor antagonist drugs pindolol ($8\,mg\,kg^{-1}$) and propranolol ($10\,mg\,kg^{-1}$) did not affect dialysate levels of 5-HT (Figure 1b, and 5a). Similarly, the 5-HT_2 antagonist ritanserin ($0.2\,mg\,kg^{-1}$) and the 5-HT_3 antagonist BRL 43694 ($0.5\,mg\,kg^{-1}$) had no consistent effect on basal 5-HT output (Figure 1b).

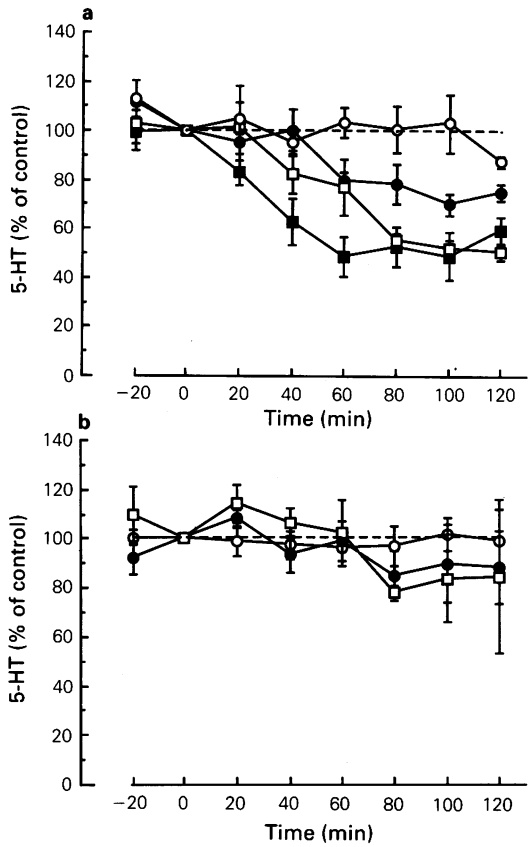

Figure 1 Effect of various 5-HT receptor antagonists on 5-hydroxytryptamine (5-HT) in ventral hippocampus dialysates of the anaesthetized rat. Drugs were injected subcutaneously at $t = 0$. (a) Saline (○, $n = 6$); metergoline $5\,mg\,kg^{-1}$ (●, $n = 4$); methysergide $10\,mg\,kg^{-1}$ (□, $n = 3$); methiothepin $10\,mg\,kg^{-1}$ (■, $n = 5$). (b) Pindolol $8\,mg\,kg^{-1}$ (○, $n = 3$), ritanserin $0.2\,mg\,kg^{-1}$ (□, $n = 3$), BRL 43694 $0.5\,mg\,kg^{-1}$ (●, $n = 3$). Mean values are shown with s.e.mean indicated by vertical bars. Each of metergoline, methysergide and methiothepin had a statistically significant effect on 5-HT compared to saline ($P < 0.05$).

Effect of metergoline, methysergide and methiothepin on the decrease of hippocampal 5-HT output induced by 8-OH-DPAT

Pretreatment with metergoline ($5\,mg\,kg^{-1}$) weakly attenuated the marked decrease of 5-HT output induced by $0.25\,mg\,kg^{-1}$ 8-OH-DPAT when compared to the effect of 8-OH-DPAT alone ($P < 0.05$; Figure 2a). A higher dose of metergoline, $10\,mg\,kg^{-1}$, also partially prevented ($P < 0.05$) the inhibitory response of 8-OH-DPAT ($0.25\,mg\,kg^{-1}$) although the effect was not greater than that observed with $5\,mg\,kg^{-1}$ ($n = 4$ rats, data not shown). Similarly $20\,mg\,kg^{-1}$ metergoline was no more effective than 5 or $10\,mg\,kg^{-1}$ of the drug ($n = 2$ rats, data not shown).

The finding that both methiothepin and methysergide induced a marked decrease in 5-HT output complicated an examination of their 8-OH-DPAT antagonist activity. Furthermore, a pilot dose-response study on methiothepin suggested that doses of the drug which would be without effect on 5-HT

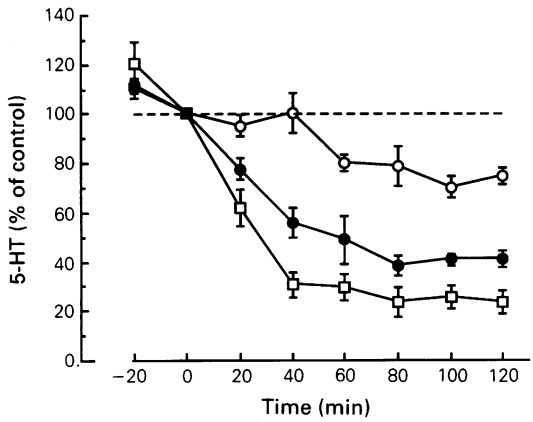

Figure 2 Effect of pretreatment with 5 mg kg^{-1} metergoline on the reduction of hippocampal 5-HT output induced by 0.25 mg kg^{-1} 8-OH-DPAT. Metergoline (○, $n = 4$) and 8-OH-DPAT (□, $n = 5$) were injected alone at $t = 0$. For combination (●, $n = 5$), metergoline was injected 30 min before 8-OH-DPAT ($t = 0$). Mean values are shown with s.e.mean indicated by vertical bars. The effect of metergoline plus 8-OH-DPAT is less than that of 8-OH-DPAT alone ($P < 0.05$).

output (<0.05 mg kg^{-1}) would have questionable 5-HT antagonist activity. Nevertheless it is clear from the data in Table 1 that a dose of methiothepin and methysergide which alone reduced 5-HT output by around 50% did not prevent an additional effect of 8-OH-DPAT.

Effect of pindolol and propranolol on the decrease of hippocampal 5-HT output induced by 8-OH-DPAT

Pretreatment with 8 mg kg^{-1} pindolol attenuated the decrease of 5-HT output induced by 0.25 mg kg^{-1} 8-OH-DPAT (Figure 3a, $P < 0.01$). In comparison, pindolol (8 mg kg^{-1}) also reduced ($P < 0.05$) the 5-HT response to a submaximally effective dose of 8-OH-DPAT (0.05 mg kg^{-1}) although the degree of inhibition was not clearly greater. A higher dose of pindolol (20 mg kg^{-1}) was no more effective in blocking the 5-HT response to 0.25 mg kg^{-1} 8-OH-DPAT than 8 mg kg^{-1} pindolol ($n = 2$, data not shown). Propranolol (10 mg kg^{-1}) did not alter the decrease in 5-HT induced by 0.25 mg kg^{-1} 8-OH-DPAT (Figure 5a).

Effect of ritanserin and BRL 43694 on the decrease of hippocampal 5-HT output induced by 8-OH-DPAT

As shown in Figure 4, pretreatment with ritanserin (0.2 mg kg^{-1}) or BRL 43694 (0.5 mg kg^{-1}) had no clear effect on the decrease of 5-HT output induced by 8-OH-DPAT (0.25 mg kg^{-1}).

Effect of phentolamine and sulpiride on the decrease of hippocampal 5-HT output induced by 8-OH-DPAT

Neither the α_1/α_2-adrenoceptor antagonist phentolamine (10 mg kg^{-1}), nor the dopamine D_2-antagonist sulpiride (10 mg kg^{-1}), altered basal 5-HT output.

Table 1 Effect of methiothepin and methysergide on 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT)-induced decrease of 5-hydroxytryptamine (5-HT) in rat hippocampal dialysates

Treatment[a] (mg kg^{-1})	*Baseline level of 5-HT immediately before treatment* (pmol per 20 μl dialysate)	*Level of 5-HT*[b] *2 h after treatment* (pmol per 20 μl dialysate)
8-OH-DPAT (0.25)	0.063 ± 0.008 (5)[c]	0.017 ± 0.004 (5) [−73.8 ± 4.5%][d]
Methiothepin (1)	0.057 ± 0.002 (5)	0.025 ± 0.007 (5) [−56.8 ± 11.3%]
8-OH-DPAT (0.25) + methiothepin (1)	0.048 ± 0.010 (5)	0.014 ± 0.004 (5) [−70.8 ± 3.0%]
Methysergide (10)	0.081 ± 0.01 (3)	0.041 ± 0.005 (3) [−49.3 ± 3.5%]
8-OH-DPAT (0.25) + methysergide (10)	0.049 ± 0.007 (5)	0.013 ± 0.001 (5) [−73.4 ± 2.2%]

[a] 8-OH-DPAT injected 30 min after methiothepin or methysergide.
[b] In all cases this was close to maximum change during the 2 h post treatment period.
[c] Mean ± s.e.mean (*n*).
[d] % reduction from pretreatment value.

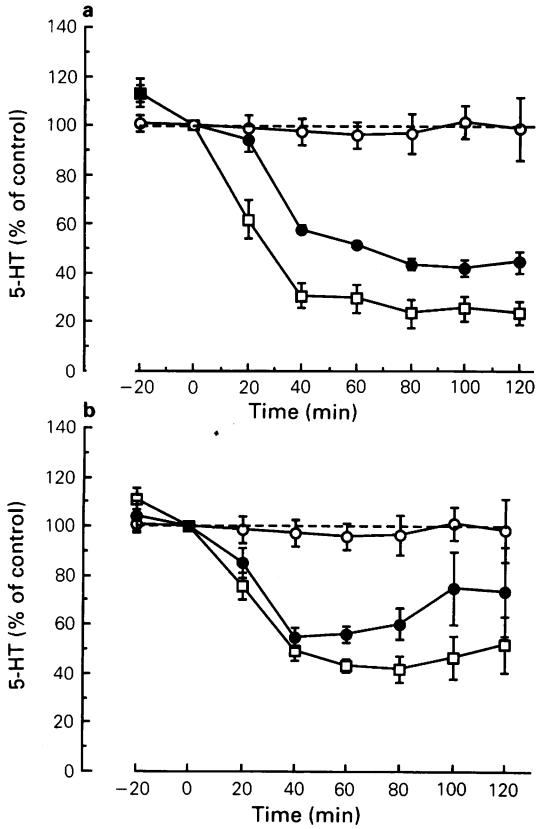

Figure 3 Effect of pretreatment with 8 mg kg^{-1} pindolol on the reduction of hippocampal 5-HT output induced by 8-OH-DPAT 0.25 mg kg^{-1} (a) and 0.05 mg kg^{-1} (b). Pindolol (○, $n = 3$) and 8-OH-DPAT (□, $n = 5$) were injected alone at $t = 0$. For combination (●, $n = 6$), pindolol was injected 30 min before 8-OH-DPAT ($t = 0$). Mean values are shown with s.e.mean indicated by vertical bars. The effect of pindolol plus 8-OH-DPAT is less than that of 8-OH-DPAT alone in both (a) ($P < 0.01$) and (b) ($P < 0.05$).

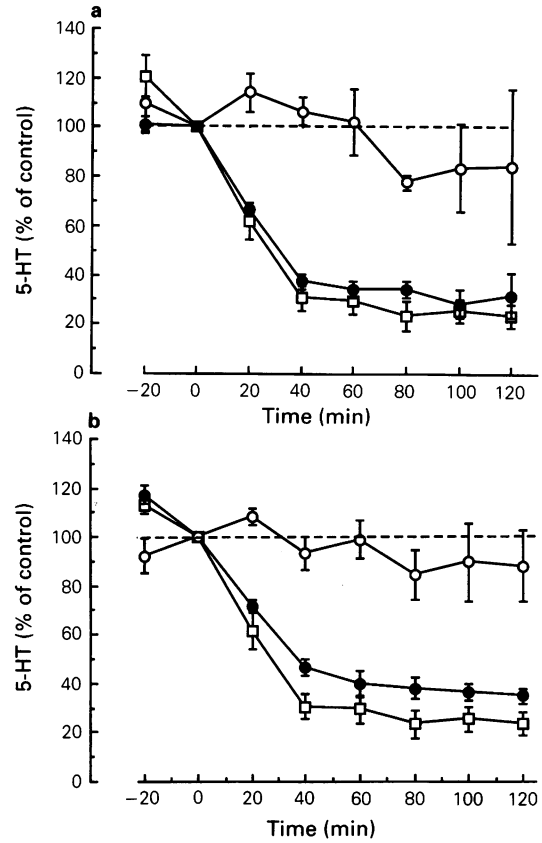

Figure 4 Effect of pretreatment with ritanserin 0.2 mg kg^{-1} (a) and BRL 43694 0.5 mg kg^{-1} (b) on reduction of 5-HT output induced by 8-OH-DPAT 0.25 mg kg^{-1}. Antagonist (○, $n = 3$) and 8-OH-DPAT (□, $n = 5$) were injected alone at $t = 0$. For combination (●, $n = 4$ ritanserin, $n = 5$ BRL 43694), antagonist was injected 30 min before 8-OH-DPAT ($t = 0$). Mean values are shown with s.e.mean indicated by vertical bars. Neither ritanserin nor BRL 43694 had a statistically significant effect on the 5-HT response to 8-OH-DPAT.

Pretreatment with either phentolamine (Figure 5) or sulpiride ($n = 4$ rats, data not shown) did not alter the 5-HT response to 8-OH-DPAT.

Discussion

We have previously shown that 8-OH-DPAT causes a marked reduction of 5-HT release in ventral hippocampus of the anaesthetized rat, as assessed by brain microdialysis (Sharp *et al.*, 1989a). For several reasons it seems likely that this effect is related to an inhibition of serotoninergic impulse flow following a direct action of the drug on somatodendritic 5-HT$_{1A}$ autoreceptors. Firstly, 8-OH-DPAT is a highly selective ligand for brain 5-HT$_{1A}$ binding sites (Middlemiss & Fozard, 1983; Hoyer 1988) which are in high concentrations on 5-HT neurones in the rat dorsal raphé nucleus (Verge *et al.*, 1985; Weissman-Nanopoulos *et al.*, 1985). Also, application of 8-OH-DPAT directly into the rat dorsal raphé nucleus causes inhibition of serotoninergic cell firing (Blier &

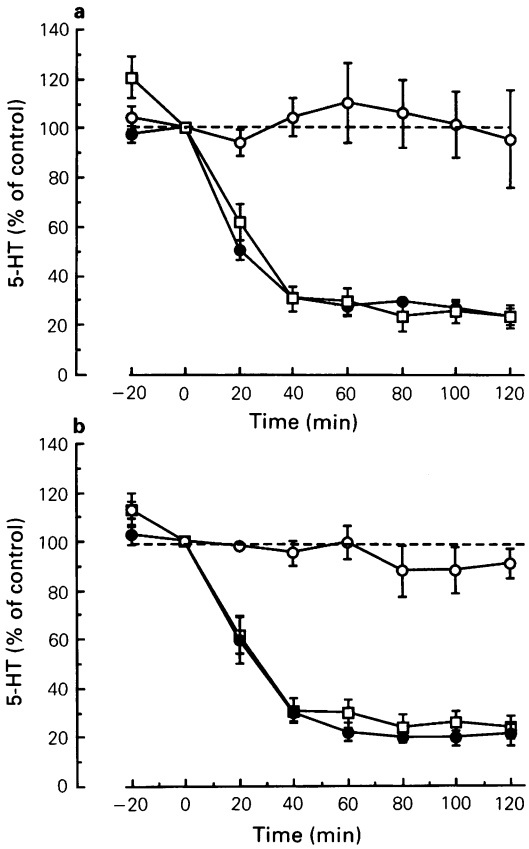

Figure 5 Effect of pretreatment with propranolol 10 mg kg^{-1} (a) and phentolamine 10 mg kg^{-1} (b) on reduction of 5-HT output induced by 8-OH-DPAT 0.25 mg kg^{-1}. Antagonist (○, $n = 3$) and 8-OH-DPAT (□, $n = 5$) were injected alone at $t = 0$. For combination (●, $n = 4$), antagonist was injected 30 min before 8-OH-DPAT ($t = 0$). Mean values are shown with s.e.mean indicated by vertical bars. Neither propranolol nor phentolamine had a statistically significant effect on the 5-HT response to 8-OH-DPAT.

De Montigny, 1987; Sprouse & Aghajanian, 1987), a reduction of regional brain 5-HT synthesis (Hjorth & Magnusson, 1989) and a fall of 5-HT output in ventral hippocampus *in vivo* (Sharp *et al.*, 1989b). Finally, 8-OH-DPAT does not decrease 5-HT release when locally applied to 5-HT nerve terminals *in vitro* (Middlemiss, 1984a; Engel *et al.*, 1986) or *in vivo* (10^{-5} M 8-OH-DPAT in microdialysis perfusion medium, unpublished observations).

The present study attempted to characterize pharmacologically the inhibitory effect of systemically administered 8-OH-DPAT on 5-HT output by use of various monoamine receptor antagonists. The main findings of the study (see Table 2) were; (i) of the three classical 5-HT receptor antagonists tested, metergoline, methysergide and methiothepin, each of which reduced 5-HT output when injected alone, only metergoline inhibited the response to 8-OH-DPAT, albeit weakly. (ii) Of the two β-adrenoceptor blockers pindolol and propranolol, both of which have high affinity for the 5-HT$_{1A}$ binding site (Hoyer, 1988), only the former drug attenuated the response to 8-OH-DPAT. This effect was weak and not clearly improved by reducing the dose of the agonist or increasing the dose of antagonist. (iii) The 5-HT response to administration of 8-OH-DPAT was not altered by pretreatment with ritanserin (5-HT$_2$ receptor antagonist), BRL 43694 (5-HT$_3$), phentolamine (α_1/α_2) or sulpiride (D_2).

The above findings, together with our previous observations, show that the receptor mediating the 8-OH-DPAT-induced reduction in hippocampal 5-HT output has some resemblance to the somatodendritic 5-HT autoreceptor characterized by electrophysiological studies. Not only is the autoreceptor modulating *in vivo* 5-HT release and serotoninergic cell-firing sensitive to 8-OH-DPAT and other putative 5-HT$_{1A}$ agonists such as gepirone, ipsapirone and buspirone (Vander Maelen *et al.*, 1986; Blier & De Montigny, 1987; Sprouse & Aghajanian 1987; Sharp *et al.*, 1989a) but methiothepin, methysergide and metergoline are poor antagonists (this study; Haigler & Aghajanian, 1977). Indeed the latter drugs reduce both 5-HT output and serotoninergic cell-firing in the anaesthetized rat (this study; Haigler & Aghajanian, 1977).

While propranolol is reported to block the inhibitory action of 8-OH-DPAT on the electrophysiologically identified somatodendritic 5-HT autoreceptor (Sprouse & Aghajanian, 1986), in this study pindolol but not propranolol attenuated the 5-HT release response to 8-OH-DPAT. Part of the reason that propranolol was inactive in this study may lie in the fact that we administered the drug systemically rather than directly onto the dorsal raphé nucleus (as done by Sprouse and Aghajanian). In this respect it is interesting that Adrien *et al.* (1989) recently found that propranolol attenuated the inhibitory effect of a putative 5-HT$_{1A}$ agonist on dorsal raphé neurones but only when locally applied *in vitro* and not when administered systemically *in vivo*. However, in the present study the inactivity of propranolol makes it unlikely that the effect of pindolol was mediated via β-adrenoceptors.

The observation that ritanserin, BRL 43694, phentolamine and sulpiride did not attenuate the 5-HT response to 8-OH-DPAT is certainly consistent with the idea that 8-OH-DPAT acts through a 5-HT$_1$ class of receptor. Further support for this idea comes

Table 2 Summary of effects of monoamine receptor antagonists on basal and 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT)-reduced hippocampal 5-hydroxytryptamine (5-HT) output

Treatment	*Dose* (mg/kg s.c.[a])	*Monoamine receptor[b] selectivity*	*Effect on basal 5-HT output*	*Effect on 8-OH-DPAT reduced 5-HT output*
Methiothepin	1	5-HT/α/DA	Reduction	No effect
Methysergide	10	5-HT/DA	Reduction	No effect
Metergoline	5	5-HT/α/DA	Reduction	Partial attenuation
Pindolol	8	$5\text{-HT}_1/\beta$	No effect	Partial attenuation
Propranolol	10	$5\text{-HT}_1/\beta$	No effect	No effect
Phentolamine	10	α_1/α_2	No effect	No effect
Ritanserin	0.2	5-HT_2	No effect	No effect
BRL 43694	0.5	5-HT_3	No effect	No effect
Sulpiride	10	DA/D_2	No effect	No effect

[a] In certain cases a range of doses were used, see text for details.
[b] Information based on references in the text.

from our observation that the effect of 8-OH-DPAT is attenuated by pindolol, a drug with high affinity for the rat brain 5-HT_{1A} binding site (Hoyer, 1988) and which effectively inhibits various 8-OH-DPAT-induced responses in the rat including the 5-HT behavioural syndrome (Tricklebank *et al.*, 1984), hypothermia (Gudelsky *et al.*, 1986) hyperphagia (Hutson *et al.*, 1987) and adrenocorticotrophic hormone secretion (Gilbert *et al.*, 1988). However, in contrast to the findings of the latter studies, pindolol appeared not to be a truly competitive antagonist of 8-OH-DPAT in our experiments. We cannot rule out the possibility that a non-5-HT_{1A} mechanism is involved in the partial blockade of 8-OH-DPAT by pindolol, although this seems less likely given our recent findings that pindolol inhibits the decrease in 5-HT output induced by other putative 5-HT_{1A} agonists.

As well as our findings with pindolol, our experiments showing no inhibition of 8-OH-DPAT by methiothepin, methysergide or propranolol also contrast with the fact that these drugs bind with high affinity to the 5-HT_{1A} binding site (Hoyer, 1988) and reports that they effectively block 8-OH-DPAT-induced behavioural responses in rats at doses close to those used in this study (Goodwin & Green, 1985; Tricklebank *et al.*, 1984). Why certain drugs should effectively prevent some but not other 8-OH-DPAT-induced responses is a matter for speculation. However, one simple explanation is that at least some of these drugs are not full antagonists but rather partial agonists at the central 5-HT_{1A} receptor. In support of this idea is evidence that both metergoline and methysergide, like 8-OH-DPAT, reduce forskolin-stimulated adenylate cyclase activity in calf hippocampus (Schoeffter & Hoyer, 1988). Furthermore, the decrease of 5-HT output induced by metergoline, methysergide and methiothepin observed in the present study, together with the inhibitory effect of these drugs on serotoninergic cell firing (Haigler & Aghajanian, 1977) is consistent with activation of 5-HT_{1A} receptors. Also, metergoline and methiothepin, like 8-OH-DPAT, are reported to reduce brain 5-HT synthesis (Bourgoin *et al.*, 1978; Long *et al.*, 1983). Decreases in 5-HT synthesis/metabolism have also been described following administration of pindolol (Hjorth & Carlsson, 1986) and propranolol (Jones & Tackett, 1988; S. Hjorth unpublished findings) although in this study we were unable to detect an effect of these drugs on basal 5-HT output.

The intrinsic activity of partial agonists will vary relative to the amount of endogenous ligand (in this case 5-HT) available at the receptor site. Thus, the efficacy of such drugs will vary according to receptor tone which will be dependent on localization of the receptor both at the synapse (pre- or postsynaptic) and within the brain. Furthermore, we cannot rule out the possibility that while drugs such as methiothepin appear to behave as 5-HT agonists in the present experiments, altering our experimental conditions to say, exclude anaesthesia, might better reveal the 5-HT_1 antagonist profile of these drugs. However, one should be equally cautious in labelling drugs like methiothepin, methysergide and metergoline as partial 5-HT_{1A} agonists on the basis of the present dialysis data since their well documented activity at other neurotransmitter receptors (e.g. Leysen *et al.*, 1981) may contribute to the observed decrease in 5-HT output.

Other contributing factors for the lack of clear-cut antagonism of 8-OH-DPAT by methysergide, methiothepin and propranolol at the doses tested could be a very high efficacy of 8-OH-DPAT at the

somatodendritic 5-HT autoreceptor and the possibility of high autoreceptor reserve (compared to the postsynaptic 5-HT_{1A} receptor). If so, it is conceivable that these drugs might reveal some somatodendritic 5-HT autoreceptor antagonist activity if the antagonist/agonist ratios were increased even further than those used in the present study.

In summary, we present experiments which suggest that the 8-OH-DPAT-induced decrease of 5-HT in hippocampal dialysates of the anaesthetized rat is mediated via a 5-HT_1 class of receptor. Classification of this receptor to be of the 5-HT_{1A} subtype, which would be entirely consistent with the selective pharmacological profile of 8-OH-DPAT, awaits the development of a selective ligand with sufficiently low intrinsic activity at the brain 5-HT_{1A} binding site. Perhaps the most suitable such candidate published in the literature to date is BMY 7378 (Yocca *et al.*, 1987). This drug, however, appears to have a 5-HT agonist-like profile in the present model (Sharp *et al.*, 1989c).

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References

ADRIEN, J., LANFUMEY, L., GOZLAN, C., FATTACCINI, C.M. & HAMON, M. (1989). Biochemical and electrophysiological evidence for an agonist action of CM 57493 at pre- and postsynaptic 5-hydroxytryptamine$_{1A}$ receptors in brain. *J. Pharmacol. Exp. Ther.*, **248**, 1222–1230.

BLIER, P. & DE MONTIGNY, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiological studies in the rat brain. *Synapse*, **1**, 470–480.

BOURGOIN, S., ARTAUD, F., BOCKAERT, H., HÉRY, F., GLOWINSKI, J. & HAMON, M. (1978). Paradoxical decrease of brain 5-HT turnover by metergoline, a central 5-HT blocker. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **302**, 313–321.

CERRITO, F. & RAITERI, M. (1979). Serotonin release is modulated by presynaptic autoreceptors. *Eur. J. Pharmacol.*, **57**, 427–430.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors. *Br. J. Pharmacol.*, **78**, 489–505.

ENGEL, G., GÖTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **332**, 1–7.

FAKE, C.S., KING, F.D. & SANGER, G.J. (1987). BRL 43694: a potent and novel 5-HT_3 antagonist. *Br. J. Pharmacol.*, **91**, 335P.

FARNEBO, L.O. & HAMBERGER, B. (1974). Regulation of ^{3}H-5-hydroxytryptamine release from rat brain slices. *J. Pharm. Pharmacol.*, **26**, 642–643.

GILBERT, F., BRAZELL, C., TRICKLEBANK, M.D. & STAHL, S.M. (1988). Activation of the 5-HT_{1A} receptor subtype increases rat plasma ACTH concentration. *Eur. J. Pharmacol.*, **147**, 431–439.

GOODWIN, G.M. & GREEN, A.R. (1985). A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. *Br. J. Pharmacol.*, **84**, 743–753.

GÖTHERT, M. & WEINHEIMER, G. (1979). Extracellular 5-hydroxytryptamine release from rat brain cortex slices. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **310**, 93–96.

GUDELSKY, G.A., KOENIG, J.I. & MELTZER, H.Y. (1986). Thermo-regulatory responses to serotonin (5-HT) receptor stimulation in the rat: Evidence for opposing roles of 5-HT_2 and 5-HT_{1A} receptors. *Neuropharmacol.*, **25**, 1307–1313.

HAIGLER, H.J. & AGHAJANIAN, G.K. (1977). Serotonin receptors in the brain. *Fed. Proc.*, **36**, 2159–2164.

HAMON, M., BOURGOIN, S., JAGGER, J. & GLOWINSKI, J. (1974). Effects of LSD on synthesis and release of 5-HT in rat brain slices. *Brain Res.*, **69**, 265–280.

HJORTH, S. & CARLSSON, A. (1986). Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors? *Eur. J. Pharmacol.*, **129**, 131–138.

HJORTH, S. & MAGNUSSON, T. (1988). The 5-HT_{1A} receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain *in vivo*. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **338**, 463–471.

HOYER, D. (1988). Functional correlates of serotonin 5-HT_1 recognition sites. *J. Receptor Res.*, **8**(1–4), 59–81.

HUTSON, P.H., DOURISH, C.T. & CURZON, G. (1987). Evidence that 8-OH-DPAT induced hyperphagia is mediated by 5-HT_{1A} receptors. *Br. J. Pharmacol.*, **92**, 645P.

JONES, L.F. & TACKETT, R.L. (1988). Interaction of propranolol with central serotonergic neurons. *Life Sci.*, **43**, 2249–2255.

LEYSEN, J.E., AWOUTERS, F., KENNIS, L., LADURON, P.W., VANDENBERK, J. & JANSSEN, P.A.J. (1981). Receptor binding profile of R 41468, a novel antagonist at 5-HT_2 receptors. *Life Sci.*, **28**, 1015–1022.

LONG, J.B., YOUNGBLOOD, W.Y. & KIZER, J.S. (1983). Regional differences in the response of serotonergic neurons in rat CNS to drugs. *Eur. J. Pharmacol.*, **88**, 89–97.

MARTIN, L.L. & SANDERS-BUSH, E. (1982). Comparison of the pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites with those of serotonin autoreceptors which modulate serotonin release. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **321**, 165–170.

MIDDLEMISS, D.N. (1984a). The putative 5-HT_1 receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. *J. Pharm. Pharmacol.*, **37**, 434–437.

MIDDLEMISS, D.N. (1984b). 8-Hydroxy-2-(di-n-propylamino)tetralin is devoid of activity at the 5-hydroxytryptamine autoreceptor in rat brain. Implications for the proposed link between the autoreceptor and the [^{3}H]-5-HT recognition site. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **327**, 18–22.

MIDDLEMISS, D.N. (1984c). Stereoselective blockade at [^{3}H] 5-HT binding sites and at the 5-HT autoreceptor by propranolol. *Eur. J. Pharmacol.*, **101**, 289–293.

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**, 151–153.

MORET, C. (1985). Pharmacology of the serotonin autoreceptor. In *Neuropharmacology of Serotonin*, ed. Green, A.R., pp. 21–49. Oxford: Oxford University Press.

MOUNSEY, I., BRADY, K.A., CARROLL, J., FISHER, R. & MIDDLEMISS, D.N. (1982). K^+-evoked ^{3}H-5-HT release from rat frontal cortex slices: the effect of 5-HT agonists and antagonists. *Biochem. Pharmacol.*, **81**, 49–53.

PAXINOS, S. & WATSON, C. (1982). *The Rat Brain in Stereotaxic Co-ordinates*. Sydney: Academic Press.

SCHOEFFTER, P. & HOYER, D. (1988). Centrally acting hypotensive agents with affinity for 5-HT$_{1A}$ binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus. *Br. J. Pharmacol.*, **95**, 975–985.

SCHLICKER, E., GÖTHERT, M. & HILLENBRAND, K. (1985). Cyano-pindolol is a highly potent and selective antagonist at the presynaptic serotonin autoreceptor in the rat brain cortex. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **331**, 398–401.

SHARP, T., BRAMWELL, S.R. & GRAHAME-SMITH, D.G. (1989a). 5-HT$_1$ agonists reduce 5-hydroxytryptamine release in rat hippocampus *in vivo* as determined by brain microdialysis. *Br. J. Pharmacol.*, **96**, 283–290.

SHARP, T., BRAMWELL, S.R., CLARK, D. & GRAHAME-SMITH, D.G. (1989b). *In vivo* measurement of extracellular 5-hydroxytryptamine in hippocampus of the anaesthetized rat using microdialysis: changes in relation to 5-hydroxytryptaminergic neuronal activity. *J. Neurochem.*, **53**, 234–240.

SHARP, T., BACKUS, L.I., BRAMWELL, S.R. & GRAHAME-SMITH, D.G. (1989c). An investigation of *in vivo* pharmacological effects of BMY 7378, a putative 5-HT$_{1A}$ antagonist. Proceedings of Serotonin Club Symposium *Serotonin: from Cell Biology to Pharmacology and Therapeutics*, Florence (Italy) March 29-April, p. 52.

SINTON, C.M. & FALLON, S.L. (1988). Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT$_1$ receptor. *Eur. J. Pharmacol.*, **157**, 173–181.

SPROUSE, J.S. & AGHAJANIAN, G.K. (1986). (−)-Propranolol blocks the inhibition of serotonergic dorsal raphé cell firing by 5-HT$_{1A}$ selective agonists. *Eur. J. Pharmacol.*, **128**, 295–298.

SPROUSE, J.S. & AGHAJANIAN, G.K. (1987). Electrophysiological responses of serotonergic dorsal raphé neurons to 5-HT$_{1A}$ and 5-HT$_{1B}$ agonists. *Synapse*, **1**, 3–9.

TRICKLEBANK, M.D., FORLER, C. & FOZARD, J.R. (1984). The involvement of subtypes of the 5-HT$_1$ receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. *Eur. J. Pharmacol.*, **106**, 271–282.

VANDER MAELEN, C.P., MATHESON, G.K., WILDERMAN, R.C. & PATTERSON, L.A. (1986). Inhibition of serotonergic dorsal raphé neurons by systemic and iontophoretic administration of buspirone, a non-benzodiazepine anxiolytic drug. *Eur. J. Pharmacol.*, **129**, 123–130.

VERGÉ, D., DAVAL, G., PATEY, A., GOZLAN, H., EL MESTIKAWY, S. & HAMON, M. (1985). Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT$_{1A}$ subtype. *Eur. J. Pharmacol.*, **113**, 463–464.

WEISSMAN-NANOPOULOS, D., MACH, E., MAGRE, J., DEMASSEY, Y. & PUJOL, J.F. (1985). Evidence for the localization of 5-HT$_{1A}$ binding sites on serotonin containing neurons in the raphé dorsalis and raphé centralis nuclei of the rat brain. *Neurochem. Int.*, **7**, 1061–1072.

YOCCA, F.D., HYSLOP, D.K., SMITH, D.W. & MAAYANI, S. (1987). BMY 7378, a buspirone analog with high affinity, selectivity and low intrinsic activity at the 5-HT$_{1A}$ receptor in rat and guinea pig hippocampal membranes. *Eur. J. Pharmacol.*, **137**, 293–294.

ZETTERSTRÖM, T., SHARP, T. & UNGERSTEDT, U. (1986). Effect of dopamine D-1 and D-2 receptor selective drugs on dopamine release and metabolism in rat striatum *in vivo*. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **334**, 117–124.

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