ELSEVIER

European Journal of Pharmacology 346 (1998) 175–180

ejp

Differential effects of 5-HT$_{1B/1D}$ receptor antagonists in dorsal and median raphe innervated brain regions

Claire Roberts [a,*], Ana Belenguer [b], Derek N. Middlemiss [a], Carol Routledge [a]

[a] *SmithKline Beecham Pharmaceuticals, Department of Neuroscience, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK*
[b] *SmithKline Beecham Pharmaceuticals, Department of Separation Science, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK*

Received 22 September 1997; revised 12 January 1998; accepted 16 January 1998

Abstract

The effect of SB-224289 (2,3,6,7-tetrahydro-1′-methyl-5-{2′-methyl-4′-[(5-methyl-1,2,4-oxadiazole-3-yl)biphenyl-4-yl]carbonyl}Furo[2,3-F]-indole-3-spiro-4′-piperidine oxalate) (4 mg/kg i.p., 5-HT$_{1B}$ receptor antagonist), GR 127935 (N-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-carboxamide) (0.3 mg/kg i.p., 5-HT$_{1B/1D}$ receptor antagonist), and paroxetine (10 mg/kg p.o.) were investigated on extracellular 5-hydroxytryptamine (5-HT) levels in the frontal cortex, striatum and dentate gyrus of the freely moving guinea-pig with microdialysis. In the frontal cortex and striatum (dorsal raphe innervated areas), GR 127935 evoked a significant decrease in extracellular 5-HT, reaching minima of 41 ± 12% and 32 ± 6% of basal, respectively. This decrease may be explained by antagonism of inhibitory 5-HT$_{1B/1D}$ receptors on raphe cell bodies, leading to a local increase in 5-HT, which, in turn, stimulated 5-HT$_{1A}$ receptors to decrease cell firing, and hence 5-HT release from terminals. In contrast, SB-224289 had no effect on 5-HT levels in either region. In the dentate gyrus (median raphe innervated area), GR 127935 and SB-224289 significantly increased extracellular 5-HT, reaching maxima of 146 ± 11% and 151 ± 19% of basal, respectively. The ability of both compounds to increase 5-HT levels in the dentate gyrus suggests a lack of 5-HT$_{1B/1D}$ receptors in the median raphe nucleus. Paroxetine produced a small but non-significant increase in extracellular 5-HT in the frontal cortex, and a small decrease in the dentate gyrus. The lack of effect of paroxetine in terminal areas may be due to the limiting effects of cell body 5-HT autoreceptors. In summary, the above data demonstrate that 5-HT$_{1B/1D}$ receptor antagonists increase 5-HT levels in the dentate gyrus, implying that acute administration of 5-HT$_{1B/1D}$ receptor antagonists will achieve a similar effect to chronic selective serotonin re-uptake inhibitor treatment in median raphe innervated areas. This, in turn, suggests that such compounds may be efficacious in the treatment of depression. © 1998 Elsevier Science B.V.

Keywords: Microdialysis; (Guinea pig); 5-HT$_{1B/1D}$ receptor antagonist

1. Introduction

5-Hydroxytryptamine (5-HT) release is subject to regulation by a negative feedback system mediated by autoreceptors located on both neuronal terminals and cell bodies. It has been established that the majority of terminal autoreceptors are of the 5-HT$_{1B}$ receptor subtype (Engel et al., 1986; Middlemiss et al., 1988; Hoyer and Middlemiss, 1988; Roberts et al., 1994b, 1996; Buhlen et al., 1996; Davidson and Stamford, 1996; Hartig et al., 1996), while somatodendritic autoreceptors encompass both 5-HT$_{1A}$ and 5-HT$_{1D}$ receptor subtypes (Starkey and Skingle, 1994; Davidson and Stamford, 1995; Pineyro et al., 1996). However, there is some preliminary evidence for the existence of a small population of 5-HT$_{1D}$ receptors at 5-HT terminal locations (Limberger et al., 1991; Davidson and Stamford, 1996) and 5-HT$_{1B}$ receptors in the raphe (Davidson and Stamford, 1995).

We have previously reported that systemic administration of both the mixed 5-HT$_{1B/1D}$ receptor antagonist, GR 127935 (N-[4-methoxy-3-(4-methyl-1-piperizinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-carboxamide), and the selective 5-HT$_{1B}$ receptor antagonist,SB-224289 (2,3,6,7-tetrahydro-1′-methyl-5-{2′-methyl-4′- [(5-methyl-1,2,4-oxadiazole-3-yl) biphenyl-4-yl]carbonyl}Furo[2,3-F]-indole-3-spiro-4′-

* Corresponding author. Tel.: +44-01279-622684; fax: +44-01279-622230; e-mail: claire_roberts-1@sbphrd.com@inet

piperidine oxalate) were unable to illicit an increase in extracellular 5-HT in the frontal cortex of the guinea-pig (Roberts et al., 1994a, 1997a,b). One should note that although GR 127935 has been reported to demonstrate partial agonism in recombinant cell lines (Pauwels and Colpaert, 1995; Watson et al., 1995), it failed to show intrinsic activity at native terminal autoreceptors (Roberts et al., 1994b, 1996). Some of these results were confirmed by another group investigating in vivo 5-HT levels in the frontal cortex (Skingle et al., 1994, 1995). However, other groups have demonstrated 5-$HT_{1B/1D}$ receptor antagonists to have no effect in the substantia nigra (Moret and Briley, 1995a,b), but to increase 5-HT release in regions such as the hypothalamus (Briley M., personal communication) and the dentate gyrus (Pullar et al., 1996). These data suggest that there are region-specific actions of 5-$HT_{1B/1D}$ receptor antagonists.

It is known that 5-HT projections arise from two main mid-brain nuclei, the dorsal and median raphe nuclei. Although many terminal regions receive projections from both nuclei, some contain a majority from one type. For example, the frontal cortex has been shown to contain a majority of dorsal raphe projections; the dentate gyrus and hypothalamus, a majority of median raphe projections (see Kosofsky and Molliver, 1987 for serotonergic morphology). Therefore, it is possible that dorsal and median raphe projections demonstrate different pharmacology. There is some evidence in the literature that suggests differences between responsiveness of 5-HT_{1A} and 5-HT_{1B} receptor ligands and selective serotonin re-uptake inhibitors in areas innervated from either the dorsal or median raphe nucleus (Blier et al., 1990a). Blier and colleagues reported that in median raphe innervated areas, 5-HT_{1B} receptors play a more important role in the control of terminal 5-HT release. In contrast, in dorsal raphe innervated areas, 5-HT_{1A} receptor control is more prevalent.

Therefore, the aim of this work was to study multiple brain regions to assess effects of: (i) the selective 5-HT_{1B} receptor antagonist, SB-224289, and the non-selective 5-$HT_{1B/1D}$ receptor antagonist, GR 127935; and (ii) systemic paroxetine on extracellular 5-HT levels, to compare the control on 5-HT release exerted by 5-HT_{1B} and 5-HT_{1D} receptor antagonism in different brain regions. Frontal cortex and striatum were used as example areas receiving 5-HT projections from the dorsal raphe nucleus and dentate gyrus as an example of median raphe nucleus projections.

2. Materials and methods

2.1. In vivo microdialysis

Male Dunkin Hartley guinea-pigs (*Porcellus*) weighing between 300–400 g were used in all experiments. Animals were maintained on a 12 h light–dark cycle at 22°C, and given free access to food and water. Guinea-pigs were anaesthetised with 5% isoflurane delivered with O_2 (3 l/min) in an induction chamber. On attaining surgical anaesthesia, the guinea-pigs were transferred to a stereotaxic frame (David Kopf), which had been adapted to accommodate an anaesthetic mask and scavenging unit (Klapwyk et al., 1995). Anaesthesia was maintained on 2–3% isoflurane with 2 l/min O_2:2 l/min N_2O.

Brain microdialysis probes were constructed as previously described in Roberts et al. (1997a). Dialysis probes were implanted either into: (i) frontal cortex (AP +4.5 mm, ML ±2.0 mm, 3.0 mm vertical from the dura); (ii) dentate gyrus (AP −4.1 mm, ML ±2.1 mm, 4.5 mm vertical from the dura), or (iii) striatum (AP +3.0 mm, ML ±3.3 mm, 7.0 mm vertical from the dura) from Bregma.

Probes were secured with two skull screws and dental acrylic cement, and the wound sealed. Animals were allowed 24 h for recovery, after which the probes were perfused with artificial cerebrospinal fluid (aCSF; 125 mM NaCl; 2.5 mM KCl; 1.18 mM $MgCl_2$; 1.26 mM $CaCl_2$; pH 7.4) at a rate of 2 μl/min. After 2 h perfusion, samples were collected every 20 min into 10 μl of aCSF. 3 samples were taken to measure basal extracellular levels of 5-HT before drug treatment. Following drug treatments, 5-HT levels were measured for 9 further samples. Guinea-pigs were injected intraperitoneally (i.p.) with vehicle or drug after the third dialysis sample (i.e., 60 min from the start of the experiment). Animals were not re-used.

2.2. High-performance liquid chromatography separation with electrochemical detection (HPLC-ECD) of 5-HT

Dialysis samples of 45 μl were injected onto an HPLC system using centre loop filling, and 5-HT separated from other substances using reverse phase, ion pair chromatography. Separation was achieved at a flow rate of 0.35 ml/min with a 3 mm × 20 cm ODS2, 3 μm column (Spherisorb), and a mobile phase consisting of 96% buffer (0.15 M NaH_2PO_4; 0.3 mM sodium octanylsulphate; 0.1 mM EDTA; pH 3.0) and 4% isopropanol. The mobile phase was filtered through a 0.22 μm GS filter and degassed with helium.

Detection of 5-HT was performed with an Antec electrochemical detector, with a glassy carbon working electrode set at +0.65 V vs. a Ag/AgCl reference electrode. The detection of 5-HT was linear over the range 2–2000 fmol with a limit of detection of 2 fmol/sample under these conditions.

2.3. Data analysis

Data from experiments were reported as area under chromatogram peaks. The first 3 samples were averaged to yield a basal level of extracellular 5-HT. All samples were expressed as percent of basal levels. Percent of basal

values for the individual time points post-treatment were accumulated and averaged, producing a value for the mean % of basal, which was an estimate of the mean area under the curve (AUC). Statistical comparisons of mean AUC following drug administration were calculated on a SAS-Research Scientist Application (v1.4, release 6.08 (1992) SAS Institute, Cary, NC 27513, USA). Analyses were performed using a one-way analysis of variance (ANOVA) followed by a post-hoc least significant difference *t*-test. Significance was taken at the 5% level.

2.4. Materials

Paroxetine, SB-224289 (Roberts et al., 1997a) and GR 127935 (Skingle et al., 1993) were synthesised at SmithKline Beecham.

The vehicle used was methyl cellulose (1%). The dose of SB-224289 was derived from a behavioural animal model of 5-HT_{1B} receptor function, i.e., antagonism of SKF 99101-induced hypothermia (Hatcher et al., 1995), and was equivalent to $2 \times ED_{50}$. The dose of GR 127935 was taken from the maximum dose shown to inhibit 5-$HT_{1B/1D}$ receptor agonist-induced contralateral turning (Skingle et al., 1993). This dose of GR 127935 was equivalent to $0.6 \times ED_{50}$ derived from the agonist-induced hypothermia model.

3. Results

Basal levels of extracellular 5-HT were 16 ± 3 ($n = 16$), 5 ± 1 ($n = 10$) and 9 ± 2 ($n = 11$) fmol/sample in the frontal cortex, striatum and dentate gyrus, respectively.

In the frontal cortex, GR 127935 (0.3 mg/kg i.p., $n = 6$) produced a significant decrease ($P < 0.05$) in extracellular 5-HT, reaching a minimum of 41 ± 12% of basal 3 h post-treatment. However, SB-224289 (4 mg/kg i.p., $n = 4$) had no significant effect (Fig. 1). The mean AUC for both compounds were 58 ± 9% ($n = 6$) and 108 ± 6% ($n = 4$), respectively. Paroxetine (10 mg/kg p.o.) had no significant effect on cortical extracellular 5-HT, with mean AUC of 103 ± 9% ($n = 5$, Fig. 2).

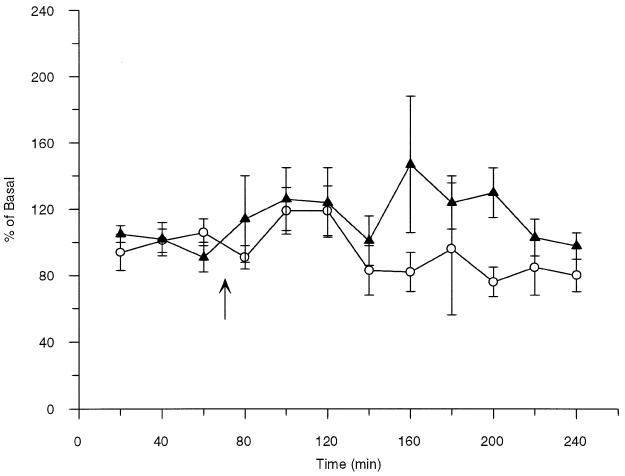

Fig. 2. Effect of (○) vehicle ($n = 5$) and (▲) paroxetine (10 mg/kg i.p., $n = 5$) on extracellular 5-HT in the frontal cortex. Arrow denotes administration of compounds.

In the striatum, GR 127935 (0.3 mg/kg i.p., $n = 3$) produced a significant decrease ($P < 0.05$) in extracellular 5-HT, reaching a minimum of 32 ± 6% of basal 3 h post-treatment. In contrast, SB-224289 (4 mg/kg i.p., $n = 4$) had no significant effect on 5-HT levels (Fig. 3). The mean AUC for both compounds were 57 ± 4% ($n = 3$) and 97 ± 3% ($n = 4$), respectively.

In the dentate gyrus, both GR 127935 (0.3 mg/kg i.p., $n = 4$) and SB-224289 (4 mg/kg i.p., $n = 4$) significantly increased extracellular 5-HT (Fig. 4, $P < 0.05$). GR 127935 reached a maximum of 146 ± 11% after 1 h and SB-224289 reached a maximum of 151 ± 19% after 2 h drug treatment. The mean AUC for both compounds were 129 ± 16%

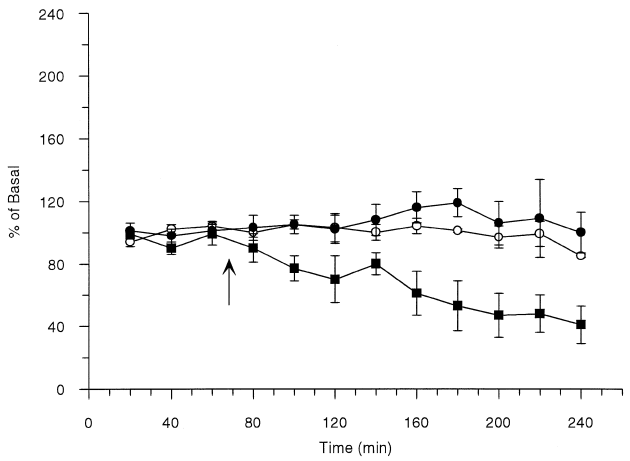

Fig. 1. Effect of (○) vehicle ($n = 6$), (■) GR 127935 (0.3 mg/kg i.p., $n = 6$) and (●) SB-224289 (4 mg/kg i.p., $n = 4$) on extracellular 5-HT in the frontal cortex. Arrow denotes administration of compounds.

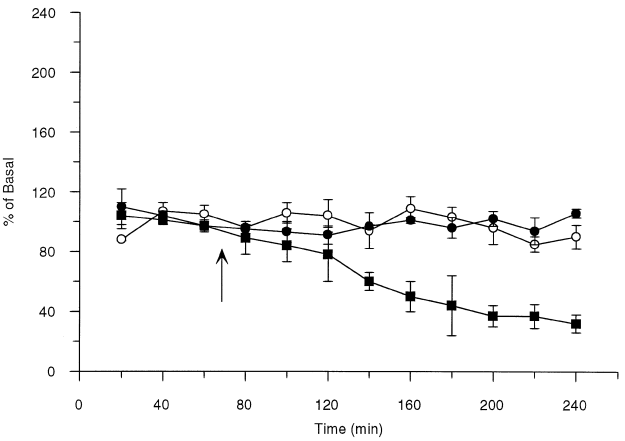

Fig. 3. Effect of (○) vehicle ($n = 3$), (■) GR 127935 (0.3 mg/kg i.p., $n = 3$) and (●) SB-224289 (4 mg/kg i.p., $n = 4$) on extracellular 5-HT in the striatum. Arrow denotes administration of compounds.

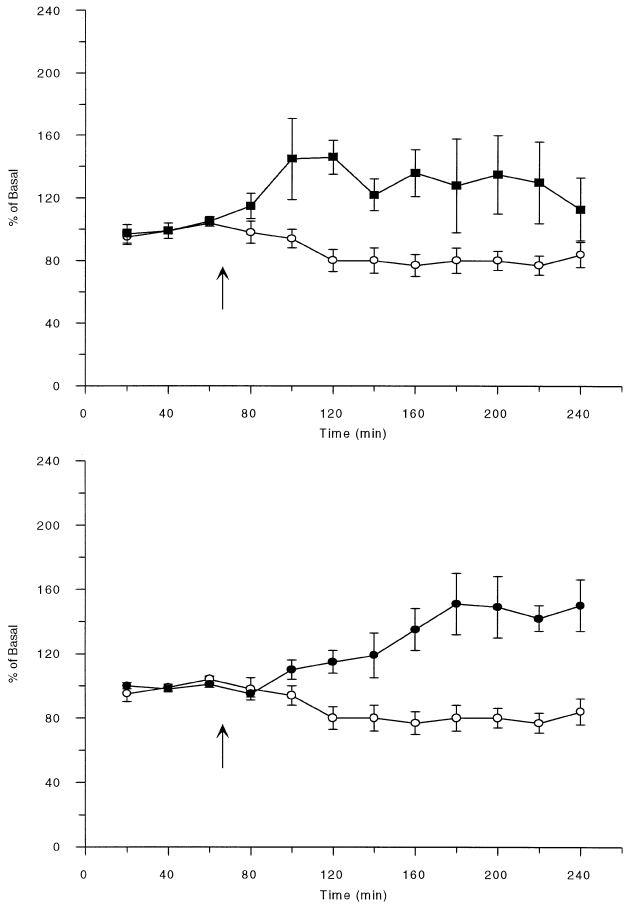

Fig. 4. (A) Effect of (○) vehicle ($n = 3$), (■) GR 127935 (0.3 mg/kg i.p., $n = 4$) and (B) (●) SB-224289 (4 mg/kg i.p., $n = 4$) on extracellular 5-HT in the dentate gyrus. Arrow denotes administration of compounds.

($n = 4$) and $129 \pm 6\%$ ($n = 4$), respectively. Paroxetine (10 mg/kg p.o.) tended to decrease extracellular 5-HT, but this did not reach significance ($P = 0.08$), with mean AUC of $66 \pm 7\%$ ($n = 3$, Fig. 5).

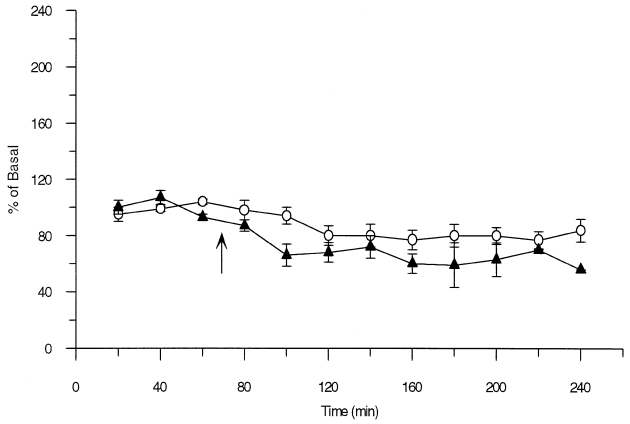

Fig. 5. Effect of (○) vehicle ($n = 5$) and (▲) paroxetine (10 mg/kg p.o., $n = 3$) on extracellular 5-HT in the dentate gyrus. Arrow denotes administration of compounds.

4. Discussion

Basal levels of extracellular 5-HT were higher in the guinea-pig frontal cortex than both the striatum and the dentate gyrus. However, 5-HT levels were not predictive of drug effects, i.e., compounds possessed different actions in the striatum and dentate gyrus although 5-HT levels were comparable in these areas.

Regarding the effects of 5-$HT_{1B/1D}$ receptor antagonists on 5-HT levels: we have previously reported (Roberts et al., 1997b) that 5-$HT_{1B/1D}$ receptor antagonists, when perfused directly into a terminal region, in this case the frontal cortex, were able to increase extracellular 5-HT levels, confirming the work of Skingle et al. (1994, 1995). These studies were interpreted to demonstrate that 5-$HT_{1B/1D}$ receptor antagonism resulted in blockade of an inhibitory 5-HT tone in the terminal area. These in vivo studies also confirmed results from in vitro [^{3}H]5-HT release assays (Roberts et al., 1996), where 5-$HT_{1B/1D}$ receptor antagonists were shown to potentiate release of [^{3}H]5-HT. Therefore, one would predict that systemic administration of 5-$HT_{1B/1D}$ receptor antagonists should increase 5-HT release. However, the data obtained in these studies suggest that 5-$HT_{1B/1D}$ receptor autoregulation is much more complex than initially proposed. The effects of 5-HT_{1B} vs. 5-$HT_{1B/1D}$ receptor antagonists in the different brain regions will be discussed in turn.

4.1. GR 127935-induced decrease in extracellular 5-HT

Differential effects of non-selective versus selective ligands on 5-HT levels were observed in the dorsal raphe innervated brain regions, i.e., the frontal cortex and striatum. The selective 5-HT_{1B} receptor antagonist, SB-224289, had no effect on 5-HT, while the mixed 5-$HT_{1B/1D}$ receptor antagonist, GR 127935, decreased 5-HT levels. The decrease may be explained by antagonism of inhibitory 5-$HT_{1B/1D}$ receptors on raphe cell bodies that leads to a local increase in 5-HT. This increase in 5-HT in turn stimulates raphe 5-HT_{1A} receptors to decrease cell firing, hence 5-HT release from terminals. Evidence to support this theory was demonstrated in a recent paper (Roberts et al., 1997b), when the GR 127935-induced decrease in 5-HT observed in the frontal cortex was attenuated on co-administration of a 5-HT_{1A} receptor antagonist, WAY 100635.

4.2. Lack of effect of SB-224289

Extrapolating from the above hypothesis, one would predict that 5-HT_{1B} receptor antagonism would also result in an increase of 5-HT in the region of the dorsal raphe nucleus, and act on 5-HT_{1A} receptors to decrease cell firing and terminal 5-HT release. The reason why a decrease was not apparent in this brain area, under the

conditions used, may be explained by either of the following.

(i) The amount of raphe 5-HT generated by cell body 5-HT_{1B} antagonism in the dorsal raphe nucleus is not as large as when both cell body 5-HT_{1B} and 5-HT_{1D} receptors are blocked. Therefore, there is a smaller degree of inhibition, via somatodendritic 5-HT_{1A} receptors, imparted on terminal 5-HT release.

(ii) Ceci et al. (1994) have eloquently demonstrated that 8-OH-DPAT inhibits dorsal raphe firing post-synaptically through fronto-cortical neurones that project onto dorsal raphe 5-HT neurones. Thus, in a similar way, it may be possible that there is an alternative projection from the 5-HT_{1D} receptors of the dorsal raphe nucleus to the frontal cortex, mediating inhibition of terminal 5-HT release.

4.3. GR 127935 and SB-224289-induced increase in dentate gyrus 5-HT

In the median raphe innervated region, both compounds had a similar effect. The ability of both compounds to increase 5-HT levels in the dentate gyrus implies the following.

(i) In this region, the limiting effects described above, i.e., from raphe cell body 5-HT_{1B} and 5-HT_{1D} receptors, may be absent. That is, 5-$HT_{1B/1D}$ receptors may not be present in the median raphe nucleus.

(ii) Endogenous 5-HT tone may differ between areas innervated by neurones projecting from the dorsal compared with the median raphe nucleus. If this were the case, then these data suggest that there is less endogenous tone in dorsal raphe innervated areas, as an increase in 5-HT levels following drug treatment was not apparent in the frontal cortex or striatum.

(iii) There exist pharmacologically distinct terminal autoreceptors in the two defined regions. It has been suggested, using the technique of in vitro [^{3}H]5-HT release, that multiple terminal autoreceptors exist in regions such as the hypothalamus (Moret and Briley, 1986), hippocampus (Wilkinson and Middlemiss, 1992) and cortex (Price et al., 1993; Roberts et al., 1996). These areas receive a mixture of median and dorsal raphe innervation. Therefore, it is possible that pharmacologically different autoreceptors exist in brain areas innervated by dorsal rather than median raphe projections. In support of this, multiple '5-HT_{1D}-like' binding sites have been demonstrated in frontal cortex, but not striatum (Mahle et al., 1991).

4.4. Effect of paroxetine

As well as potential differences between autoreceptor function, Blier et al. (1990a) have also alluded to differences in the effects of selective serotonin re-uptake inhibitors between brain areas in the rat. In contrast, this was not replicated by Sharp et al. (1994). In the present study, acutely administered paroxetine had a similar profile in both frontal cortex and dentate gyrus. These data agree with those in other studies, where the lack of effect of acutely administered paroxetine in these regions has been attributed to the limiting effects of cell body 5-HT_{1A} receptors (Arborelius et al., 1995; Hjorth, 1993; Invernizzi et al., 1996). Therefore, the similar action of paroxetine in both the dorsal and median raphe innervated areas suggests that the density of 5-HT_{1A} and/or 5-HT re-uptake sites in median vs. dorsal raphe are comparable in the guinea-pig.

4.5. Summary

We have demonstrated that in an area we have hypothesised to be devoid of 5-$HT_{1B/1D}$ receptor modulation, both selective 5-HT_{1B} and mixed 5-$HT_{1B/1D}$ receptor antagonists increased 5-HT release in vivo. This is the first demonstration that systemic administration of selective 5-$HT_{1B/1D}$ receptor antagonists increase 5-HT in some terminal regions. Increases in 5-HT levels have also been reported to be elicited by chronic administration of selective serotonin re-uptake inhibitors (Gardier et al., 1996). Selective serotonin re-uptake inhibitors are widely used as effective antidepressants, but have a delayed onset of action (Montgomery et al., 1994). The beneficial effect of selective serotonin re-uptake inhibitors has been attributed to their ability to increase 5-HT following desensitisation of raphe 5-HT_{1A} and/or terminal 5-HT_{1B} receptors, and it has been suggested that this desensitisation is responsible for their delayed onset of action (Blier et al., 1990b). These data therefore suggest that 5-$HT_{1B/1D}$ receptor antagonists will achieve an effect similar to selective serotonin re-uptake inhibitors, but have an immediate onset of action in median raphe innervated areas. However, efficacy as an antidepressant may be limited by a decrease in 5-HT release in dorsal raphe innervated areas. This possibility may be minimised by using a selective 5-HT_{1B} rather than a mixed 5-$HT_{1B/1D}$ receptor antagonist. Validation of this hypothesis now awaits clinical studies with 5-HT_{1B} receptor antagonists.

References

Arborelius, L., Nomikos, G.C., Grillner, P., Hertel, P., Hook, B.B., Hacksell, U., Svensson, T.H., 1995. 5-HT_{1A} receptor antagonists increase the activity of serotonergic cells in the dorsal raphe nucleus in rats treated acutely or chronically with citalopram. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 157–165.

Blier, P., Serrano, A., Scatton, B., 1990a. Differential responsiveness of the rat dorsal and median raphe 5-HT systems to 5-HT_1 receptor agonists and *p*-chloroamphetamine. Synapse 5, 120–133.

Blier, P., De Montigny, C., Chaput, Y., 1990b. A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. J. Clin. Psychiatry 51 (Suppl.), 14–20.

Buhlen, M., Fink, K., Böing, C., Gothert, M., 1996. Evidence for presynaptic location of inhibitory 5-$HT_{1D\beta}$-like autoreceptors in the guinea-pig cortex. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 281–289.

Ceci, A., Baschirotto, A., Borsini, F., 1994. The inhibitory effect of 8-OH-DPAT on the firing activity of dorsal raphe serotonergic neurons in rats is attenuated by lesion of the frontal cortex. Neuropharmacology 33, 709–713.

Davidson, C., Stamford, J.A., 1995. Evidence that 5-HT release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. Br. J. Pharmacol. 114, 1107–1109.

Davidson, C., Stamford, J.A., 1996. Serotonin efflux in the rat ventral lateral geniculate nucleus can be measured by fast cyclic voltammetry and is under 5-HT_{1D} as well as 5-HT_{1B} autoreceptor control. Neuropharmacology 35, 1627–1634.

Engel, G., Göthert, M., Hoyer, D., Schlicker, E., Hillenbrand, K., 1986. Identity of inhibitory pre-synaptic 5-HT autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 1–7.

Gardier, A.M., Malagie, I., Trillat, A.C., Jaquot, C., Artigas, F., 1996. Role of 5-HT_{1A} autoreceptors in the mechanism of action of serotonergic antidepressant drugs: recent findings from in vivo microdialysis studies. Fundam. Clin. Pharmacol. 10, 16–27.

Hartig, R., Hoyer, D., Humphrey, P.P.A., Martin, G.R., 1996. Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. Trends Pharmacol. Sci. 17, 103–105.

Hatcher, J.P., Slade, P.D., Roberts, C., Hagan, J.J., 1995. 5-HT_{1D} receptors mediate hypothermia in the guinea-pig. J. Psychopharmacol. 9 (3), 234–241.

Hjorth, S., 1993. Serotonin 5-HT_{1A} autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. J. Neurochem. 60, 776–779.

Hoyer, D., Middlemiss, D.N., 1988. Species differences in the pharmacology of the terminal 5-HT autoreceptor in mammalian species. Trends Pharmacol. Sci. 10, 130–132.

Invernizzi, R., Bramante, M., Samanin, R., 1996. Role of 5-HT_{1A} receptors in the effects of acute and chronic fluoxetine on extracellular serotonin in the frontal cortex. Pharmacol. Biochem. Behav. 54, 143–147.

Klapwyk, J.C., Pallett, M.J., Roberts, C., 1995. An anaesthetic mask for use on guinea-pigs (*Cavia porcellus*) in a stereotaxic frame. Lab. Anim. Sci. 29, 407–410.

Kosofsky, B.E., Molliver, M.E., 1987. The serotonergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. Synapse 1, 153–168.

Limberger, N., Deicher, R., Starke, K., 1991. Species differences in presynaptic 5-HT autoreceptors: mainly 5-HT_{1B} but possibly in addition 5-HT_{1D} in the rat, 5-HT_{1D} in the rabbit and guinea-pig brain cortex. Naunyn-Schmiedeberg's Arch. Pharmacol. 343, 353–364.

Mahle, C.D., Nowak, H.P., Mattson, R.J., Hurt, S.D., Yocca, F.D., 1991. [^{3}H]5-CT labels multiple high affinity 5-HT_{1D}-like sites in guinea-pig brain. Eur. J. Pharmacol. 205, 323–324.

Middlemiss, D.N., Bremer, M.E., Smith, S.M., 1988. A pharmacological analysis of the 5-HT receptors mediating inhibition of 5-HT release in the guinea-pig frontal cortex. Eur. J. Pharmacol. 157, 101–107.

Montgomery, S.A., Henry, J., McDonald, G., Dinan, T., Lader, M., Hindmarch, I., Clare, A., Nutt, D., 1994. Selective serotonin re-uptake inhibitors: meta-analysis of discontinuation rates. Int. Clin. Psychopharmacol. 9, 47–53.

Moret, C., Briley, M., 1986. Dihydroergocristine-induced stimulation of the 5-HT autoreceptor in the hypothalamus of the rat. Neuropharmacology 25, 169–174.

Moret, C., Briley, C., 1995a. The effect of terminal 5-HT autoreceptor antagonists on 5-HT release in the guinea-pig brain. Br. J. Pharmacol. 115, 31P.

Moret, C., Briley, M., 1995b. Comparative study of the in vivo effects of some 5-HT receptor antagonists on terminal 5-HT_{1D} autoreceptors as determined by the release of 5-HT from the substantia nigra of freely moving guinea-pigs. In: Louilot, A., Durkia, T., Spampinato, U., Cador, M. (Eds.), Proceedings of the Sixth International Conference on In Vivo Methods. Monitoring Molecules in Neurosci., pp. 197–198.

Pauwels, P.J., Colpaert, F.C., 1995. The 5-HT_{1D} receptor antagonist GR 127,935 is an agonist at cloned human 5-HT_{1D} alpha receptor sites. Neuropharmacology 34, 235–237.

Pineyro, G., De Montigny, C., Weiss, M., Blier, P., 1996. Autoregulatory properties of dorsal raphe 5-HT neurones: possible role of electronic coupling and 5-HT_{1D} receptors in the rat brain. Synapse 22, 54–62.

Price, G.W., Watson, J., Roberts, C., Jones, B.J., 1993. Multiple 5-HT terminal autoreceptors in guinea-pig cerebral cortex demonstrated by in vitro [^{3}H]5-HT release. Br. J. Pharmacol. 108, 101P.

Pullar, I.A., Colvin, E.M., Woodhouse, S.M., Osbourne, D.J., 1996. Effect of GR 127935 on the release of 5-HT in the hippocampus of the freely-moving guinea-pig. In: Gonzalez-Mora, J.L., Borges, R., Mas, M. (Eds.), Proceedings of the Seventh International Conference on in vivo Methods. Monitoring Molecules in Neuroscience, pp. 149–150.

Roberts, C., Thorn, L., Price, G.W., Middlemiss, D.N., Jones, B.J., 1994a. Effect of the selective 5-HT_{1D} receptor antagonist, GR 127935, on in vivo 5-HT release, synthesis and turnover in the guinea-pig frontal cortex. Br. J. Pharmacol. 112, 489P.

Roberts, C., Watson, J., Burton, M., Price, G.W., Jones, B.J., 1994b. The 5-HT terminal autoreceptor is a 5-HT_{1D} receptor subtype in the guinea-pig cortex. Br. J. Pharmacol. 112, 650P.

Roberts, C., Watson, J., Burton, M., Price, G.W., Jones, B.J., 1996. Functional characterisation of the 5-HT terminal autoreceptor in the guinea-pig brain cortex. Br. J. Pharmacol. 117, 384–388.

Roberts, C., Price, G.W., Gaster, L., Jones, B.J., Middlemiss, D.N., Routledge, C., 1997a. Importance of 5-HT_{1B} receptor selectivity for 5-HT terminal autoreceptor activity: an in vivo microdialysis study in the freely-moving guinea-pig. Neuropharmacology 36, 549–557.

Roberts, C., Price, G.W., Jones, B.J., 1997b. The role of 5-$HT_{1B/1D}$ receptor in modulating 5-HT levels in the frontal cortex of the conscious guinea-pig. Eur. J. Pharmacol. 326, 23–30.

Sharp, T., McQuade, R., Gartside, S., Hajos, E., White, V., Hajos, M., 1994. Microdialysis and neuropharmacology of 5-HT transmission. In: Louilot, A., Durkia, T., Spampinato, U., Cador, M. (Eds.), Proceedings of the Sixth International Conference on In Vivo Methods. Monitoring Molecules in Neuroscience, pp. 189–190.

Skingle, M., Scopes, D.I.C., Fenuik, W., Connor, H.E., Carter, M.C., Clitherow, J.W., Tyers, M.B., 1993. GR 127935: a potent orally active 5-HT_{1D} receptor antagonist. Br. J. Pharmacol. 110, 9P.

Skingle, M., Sleight, A.J., Feniuk, W., 1994. Microdialysis study investigating the effects of GR127935, a potent 5-HT_{1D} receptor antagonist, on cortical levels of 5-HT in the guinea-pig. Br. J. Pharmacol. 112, 488P.

Skingle, M., Sleight, A.J., Feniuk, W., 1995. Effects of the 5-HT_{1D} receptor antagonist GR 127935 on extracellular levels of 5-HT in the guinea-pig frontal cortex as measured by microdialysis. Neuropharmacology 34, 377–382.

Starkey, S.J., Skingle, M., 1994. 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea-pig dorsal raphe nucleus. Neuropharmacology 33, 393–394.

Watson, J., Burton, M., Price, G.W., Jones, B.J., Thomas, D., Faruq, A., Middlemiss, D.N., 1995. GR 127935 acts as a partial agonist at recombinant human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. Br. J. Pharmacol. 114, 362P.

Wilkinson, L.O., Middlemiss, D.N., 1992. Metitepine distinguishes two receptors mediating inhibition of [^{3}H]5-HT release in guinea-pig hippocampus. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 696–699.