British Journal of Pharmacology (1997) 121, 941–946

Effect of a selective 5-HT reuptake inhibitor in combination with 5-HT_{1A} and 5-HT_{1B} receptor antagonists on extracellular 5-HT in rat frontal cortex *in vivo*

[1]T. Sharp, V. Umbers & S.E. Gartside

University of Oxford Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

1 Selective 5-hydroxytryptamine (5-HT; serotonin) reuptake inhibitors (SSRIs) cause a greater increase in extracellular 5-HT in the forebrain when the somatodendritic 5-HT_{1A} autoreceptor is blocked. Here, we investigated whether blockade of the terminal 5-HT_{1B} autoreceptor influences a selective 5-HT reuptake inhibitor in the same way, and whether there is an additional effect of blocking both the 5-HT_{1A} and 5-HT_{1B} autoreceptors.

2 Extracellular 5-HT was measured in frontal cortex of the anaesthetized rat by use of brain microdialysis. *In vivo* extracellular recordings of 5-HT neuronal activity in the dorsal raphe nucleus (DRN) were also carried out.

3 The selective 5-HT reuptake inhibitor, paroxetine (0.8 mg kg^{-1}, i.v.), increased extracellular 5-HT about 2 fold in rats pretreated with the 5-HT_{1A} receptor antagonist, WAY100635. When administered alone neither paroxetine (0.8 mg kg^{-1}, i.v.) nor WAY100635 (0.1 mg kg^{-1}, i.v.) altered extracellular 5-HT levels.

4 Paroxetine (0.8 mg kg^{-1}, i.v.) did not increase 5-HT in rats pretreated with the 5-$HT_{1B/D}$ receptor antagonist, GR127935 (1 mg kg^{-1}, i.v.). GR127935 (1 and 5 mg kg^{-1}, i.v.) had no effect on extracellular 5-HT when administered alone.

5 Interestingly, paroxetine (0.8 mg kg^{-1}, i.v.) caused the greatest increase in 5-HT (up to 5 fold) when GR127935 (1 or 5 mg kg^{-1}, i.v.) was administered in combination with WAY100635 (0.1 mg kg^{-1}, i.v.). Administration of GR127935 (5 mg kg^{-1}, i.v.) plus WAY100635 (0.1 mg kg^{-1}, i.v.) without paroxetine, had no effect on extracellular 5-HT in the frontal cortex.

6 Despite the lack of effect of GR127935 on 5-HT under basal conditions, when 5-HT output was elevated about 3 fold (by adding 1 μM paroxetine to the perfusion medium), the drug caused a dose-related (1 and 5 mg kg^{-1}, i.v.) increase in 5-HT.

7 By itself, GR127935 slightly but significantly decreased 5-HT cell firing in the DRN at higher doses (2.0–5.0 mg kg^{-1}, i.v.), but did not prevent the inhibition of 5-HT cell firing induced by paroxetine.

8 In summary, our results suggest that selective 5-HT reuptake inhibitors may cause a large increase in 5-HT in the frontal cortex when 5-HT autoreceptors on both the somatodendrites (5-HT_{1A}) and nerve terminals (5-HT_{1B}) are blocked. This increase is greater than when either set of autoreceptors are blocked separately. The failure of a 5-HT_{1B} receptor antagonist alone to enhance the effect of the selective 5-HT reuptake inhibitor in our experiments may be related to a lack of tone on the terminal 5-HT_{1B} autoreceptor due to a continued inhibition of 5-HT cell firing. These results are discussed in relation to the use of 5-HT autoreceptor antagonists to augment the antidepressant effect of selective 5-HT reuptake inhibitors.

Keywords: 5-HT; 5-HT_{1A} receptors; 5-HT_{1B} receptors; WAY100635; GR127935; paroxetine; antidepressants; dorsal raphe nucleus; microdialysis; electrophysiology

Introduction

It is established that selective 5-hydroxytryptamine (5-HT; serotonin) reuptake inhibitors (SSRIs) are effective in the treatment of depression although, as with other antidepressants, they take several weeks to produce their full therapeutic effect (Åsberg *et al*., 1986; Schatzberg *et al*., 1987). Pharmacological strategies aimed at speeding up the antidepressant effect of SSRIs have recently focused on the co-administration of SSRIs and 5-HT autoreceptor antagonists (Artigas *et al*., 1996).

Preclinical studies show that selective 5-HT_{1A} receptor antagonists potentiate the effect of SSRIs on extracellular 5-HT in the forebrain (Hjorth *et al*., 1993; Gartside *et al*., 1995; Invernizzi *et al*., 1996). This effect is probably associated with the blockade of somatodendritic 5-HT_{1A} autoreceptors (Invernizzi *et al*., 1992). Thus, normally SSRIs (indirectly) activate these receptors leading to an inhibition of the firing of raphe 5-HT neurones (eg. Chaput *et al*., 1986; Hajós *et al*., 1995a) and a fall in 5-HT release in the terminal regions (Auerbach *et al*., 1995). However, when the 5-HT_{1A} autoreceptors are blocked, the inhibition of 5-HT neuronal activity is prevented (Hajós *et al*., 1995a; Gartside *et al*., 1995; Arborelius *et al*., 1995) and the effect of SSRIs on extracellular 5-HT in the specific regions of forebrain is facilitated.

In addition to the somatodendritic 5-HT_{1A} autoreceptor, the 5-HT_{1B} autoreceptor located on the 5-HT nerve terminal also regulates 5-HT release (for review see Middlemiss & Hutson, 1990). It seems likely that this autoreceptor would also offset the ability of SSRIs to increase extracellular 5-HT at the nerve terminal and, hence, a 5-HT_{1B} receptor antagonist might potentiate the effect of the SSRI. However, a potentiation may not occur if the SSRI-induced inhibition of 5-HT cell firing persists. Indeed, since 5-$HT_{1B/1D}$ autoreceptor antagonists alone have been shown to increase extracellular 5-HT in the dorsal raphe nucleus (DRN) (Starkey & Skingle, 1994; Davidson & Stamford, 1995), such drugs may inhibit 5-HT cell firing through indirect activation of the somatodendritic 5-HT_{1A} autoreceptor (Skingle *et al*., 1995). Nonetheless, combined blockade of the somatodendritic 5-HT_{1A} autoreceptor and nerve terminal 5-HT_{1B} autoreceptor may be a more powerful way to potentiate the effects of an SSRI than blockade of the two autoreceptors separately.

[1]Author for correspondence:

In the present study we have tested the effect on extracellular 5-HT in the rat frontal cortex of an SSRI (paroxetine) administered with a selective 5-$HT_{1B/D}$ receptor antagonist (GR127935; Skingle *et al*., 1996). Measurements of 5-HT cell firing in the DRN were also made in parallel experiments. The effect on extracellular 5-HT of paroxetine in combination with GR127935 and a selective 5-HT_{1A} receptor antagonist (WAY100635; Fletcher *et al*., 1996) was also tested. A preliminary account of these findings was presented to the European Society for Neurochemistry (Sharp *et al*., 1996). The 5-HT receptor nomenclature used in this paper follows recent revisions made by the Serotonin Club Nomenclature Committee (Hartig *et al*., 1996).

Methods

Animals

Male Sprague-Dawley rats (270–310 g, Harlan-Olac, Bicester, U.K.) were housed in groups of up to 6 under controlled conditions of temperature (21°C) and humidity (50%), in a 12 h light/dark cycle (lights on 8 h 00 min). Animals were allowed food and water *ad libitum*.

General stereotaxic surgical procedures

Rats were anaesthetized with chloral hydrate (initially 400–500 mg kg^{-1}, i.p.) and placed in a stereotaxic frame (Kopf) with the incisor bar set at −3.3 mm. The skull was exposed and a burr hole drilled for the implantation of a recording electrode or microdialysis probe. A lateral tail vein was cannulated for i.v. administration of drugs. Supplementary doses of chloral hydrate were administered (i.v.) as required to maintain full general anaesthesia. Core temperature was maintained at 35–36°C throughout the experiment by a thermoregulated blanket connected to a rectal thermometer.

Microdialysis studies

Single cannula microdialysis probes (Cordis Dow GFE9 membrane, 250 μm in diameter, 3 mm tip) were stereotaxicaly implanted into the frontal cortex (AP + 3.2 mm; ML + 3.0 mm; DV −4.5 mm; from bregma and dura surface, Paxinos & Watson, 1986). The probe was constantly perfused at 2 μl min^{-1} with artificial cerebrospinal fluid (composition (mM): NaCl 140, KCl 3, $CaCl_2$ 2.4, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.27, glucose 7.2, at pH 7.4). In some experiments, paroxetine (1 μM) was present in the perfusion medium throughout the experiment. Dialysates were collected every 20 min and assayed immediately for 5-HT by high performance liquid chromatography (h.p.l.c.) with electrochemical detection as described previously (Gartside *et al*., 1995). Basal levels of 5-HT in dialysates from the frontal cortex were typically 0.01–0.02 pmol (see Figure legends for individual group means).

Drugs were administered (i.v.) after baseline levels of 5-HT had stabilized (typically 2–3 h post probe implantation), and the response was followed for a further 2 h. At the end of each experiment, the brain was removed, post-fixed in 4% paraformaldehyde, and the location of the microdialysis probe was subsequently verified in brain sections.

Electrophysiological studies

Spontaneously active 5-HT neurones in the DRN were recorded extracellularly by use of methods described previously (Hajós *et al*., 1995a,b). In brief, single barrelled glass microelectrodes filled with 2 M NaCl containing 2% Pontamine Sky Blue (3–8 MΩ *in vitro* impedance), were implanted above the DRN (AP −7.8 mm; ML 0 mm; DV −4.5 mm) and then lowered under the control of a microdriver. Signals were amplified (× 1000) and filtered (300–3000 Hz band-pass), and fed to an audio speaker, an oscilloscope and a chart recorder. The signal was also recorded on digital audio tape for off-line analysis.

5-HT neurones were identified on the basis of their electrophysiological characteristics (broad action potentials with positive-negative or positive-negative-positive deflections, regular firing pattern, 0.5–3 Hz firing rate). Burst-firing, presumed 5-HT neurones (Hajós *et al*., 1995b) were not recorded in this study.

Extracellular recordings were made from one 5-HT cell per animal. Baseline firing activity was recorded for at least 3 min, after which time vehicle or drugs were administered. Drugs were injected intravenously in doubling doses at 2 min intervals.

In one group of rats ($n = 7$), GR127935 was administered in doses of 0.5, 0.5, 1, 2 and then 1 mg kg^{-1} to give a final total dose of 5 mg kg^{-1}, i.v. Paroxetine was then administered to 4 of these animals in accumulating doses (0.1, 0.1, 0.2, 0.4 and 0.8 mg kg^{-1}, i.v.) until cells were inhibited completely. In a second group of rats ($n = 4$), vehicle (5% glucose, pH 4–5) was first injected in accumulating volume (0.1, 0.1, 0.2, 0.4, 0.2 ml i.e same volume as solution of GR127935), and then paroxetine was administered. All rats injected with paroxetine received a subsequent injection of WAY100635.

At the end of each experiment a small amount of Pontamine Sky Blue was iontophoretically ejected from the tip of the electrode. The brain was removed, post-fixed in 4% paraformaldehyde, and the position of the electrode tip subsequently determined by microscopic inspection of slide-mounted sections.

Data presentation and statistical analysis

For the microdialysis experiments, data in figures are expressed as mean ± s.e.mean % of the amount of 5-HT in the 20 min sample collected immediately before the last drug injected.

For the electrophysiological studies, a percentage inhibition induced by each total dose of drug was calculated as the firing rate (in a 30 s period) after drug, relative to the firing rate (100%) in a 60 s baseline period immediately before administration of the first dose of the drug.

Data (% of control) were analysed statistically by 1- or 2-way ANOVA with repeated measures as appropriate. Statistical significance at the 95% level is presented.

Drugs and chemicals

GR127935 (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide HCl.H_2O), WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclo-hexanecarboxamide 3HCl) and paroxetine HCl were generous gifts of GlaxoWellcome plc (Greenford, U.K.), Wyeth Research (Maidenhead, U.K.) and SmithKline Beecham (Harlow, U.K.), respectively. Chloral hydrate (Sigma) was dissolved in distilled/deionised water. WAY100635 and paroxetine were dissolved in 5% glucose solution. GR127935 was dissolved in a few drops of glacial acetic acid, then 5% glucose and adjusted to pH 4–5 with sodium hydroxide.

Drugs were administered i.v. either in a single dose at 1.0 ml kg^{-1} (microdialysis experiments) or in increasing doses with an initial volume of 0.1 ml (electrophysiological experiments).

Results

Effect of paroxetine in combination with the 5-HT_{1A} receptor antagonist, WAY100635

Paroxetine (0.8 mg kg^{-1}, i.v.) increased 5-HT levels in frontal cortex dialysates about 2 fold above baseline in animals pretreated 10 min previously with 0.1 mg kg^{-1}, i.v., WAY100635 (Figure 1). When administered alone, neither paroxetine

(0.8 mg kg^{-1}, i.v.) nor WAY 100635 (0.1 mg kg^{-1}, i.v.) increased 5-HT levels in frontal cortex dialysates relative to vehicle (5% glucose)-injected controls (Figure 1).

Effect of paroxetine in combination with the 5-HT$_{1B/D}$ receptor antagonist, GR127935

Paroxetine (0.8 mg kg^{-1}, i.v.) did not increase 5-HT in rats treated 10 min previously with 1 mg kg^{-1}, i.v. GR127935 (Figure 2b). Administration of GR127935 alone (1 and 5 mg kg^{-1}, i.v.) did not alter frontal cortex 5-HT (Figure 2a).

Effect of paroxetine in combination with both WAY100635 and GR127935

A striking result was that paroxetine (0.8 mg kg^{-1}, i.v.) caused a 5 fold increase in 5-HT in the frontal cortex in rats pretreated 10 min previously with GR127935 (5 mg kg^{-1}, i.v.) in combination with WAY100635 (0.1 mg kg^{-1}, i.v.) (Figure 3). This increase was significantly greater ($F_{(8,72)}=3.9$; $P<0.005$; 2-way ANOVA) than the 2 fold increase in 5-HT obtained after administration of paroxetine (0.8 mg kg^{-1}, i.v.) and WAY100635 (0.1 mg kg^{-1}, i.v.). Administration of a lower dose of GR127935 (1 mg kg^{-1}, i.v.) plus WAY100635 (0.1 mg kg^{-1}, i.v.) also significantly ($F_{(8,72)}=2.2$; $P<0.05$; 2-way ANOVA) potentiated the effect of paroxetine (0.8 mg kg^{-1}, i.v.), but to a lesser extent than the higher dose of GR127935 (5 mg kg^{-1}, i.v.; Figure 3).

Administration of GR127935 (5 mg kg^{-1}, i.v.) plus WAY-100635 (0.1 mg kg^{-1}, i.v.) without paroxetine, did not increase 5-HT (Figure 3).

Effect of locally applied paroxetine in combination with GR127935

The ability of GR127935 to potentiate the effect of paroxetine plus WAY100635 on frontal cortex 5-HT output (Figure 3) might be explained by GR127935 blocking tone at the terminal 5-HT autoreceptor. Experiments were carried out to test the effect of GR127935 when extracellular levels of 5-HT in the frontal cortex were elevated.

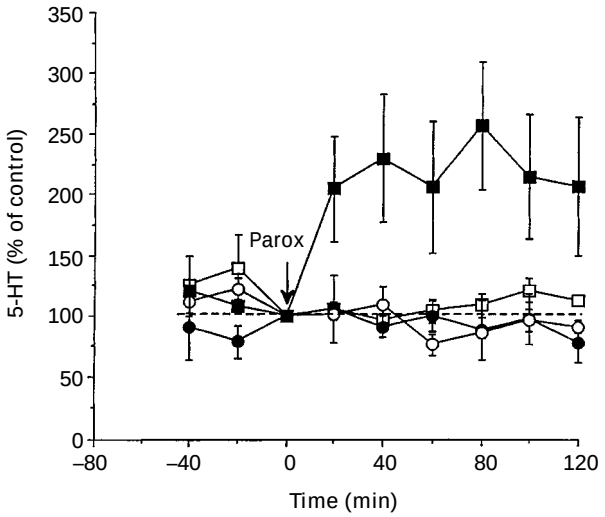

Figure 1 Effect of paroxetine alone or in combination with WAY100635 on levels of 5-HT in microdialysates from the frontal cortex of the anaesthetized rats. (○) Effect of vehicle; (●) WAY100635 (0.1 mg kg^{-1}); (□) paroxetine (0.8 mg kg^{-1}); (■) paroxetine (0.8 mg kg^{-1}) + WAY100635 (0.1 mg kg^{-1}). WAY100635 was injected 10 min before paroxetine. Mean±s.e.mean (n=number of rats) amounts of 5-HT in the perfusates at t=0 were 27±5 (n=6) (vehicle), 18±2 (n=6) (paroxetine), 10±2 (n=4) (WAY100635) and 16±3 (n=5) (paroxetine plus WAY100635) fmol/sample, respectively. Each point represents a mean and vertical lines show s.e.mean.

Perfusate levels of 5-HT were increased 2–3 fold by addition of paroxetine (1 μM) to the perfusion medium (compare basal 5-HT data in Figure 4 with those in Figures 1–3). In the presence of locally applied paroxetine, GR127935 caused an immediate and dose-related (1 and 5 mg kg^{-1}, i.v.) increase in 5-HT (Figure 4). Injection of vehicle (5% glucose, pH 4–5) had no effect on 5-HT (Figure 4).

Effect of GR127935 and paroxetine on 5-HT cell firing

Acute administration of GR127935 (0.5–5 mg kg^{-1}, i.v.) caused a slight (10–20%) but statistically significant decrease in the firing rate of 5-hydroxytryptaminergic neurones (Figure 5a) when compared to pre-drug firing rate ($F_{(4,36)}=3.9$; $P<0.01$; 1-way ANOVA). In comparison, acute injection of vehicle (5% glucose, pH 4–5) had not consistent effect (Figure

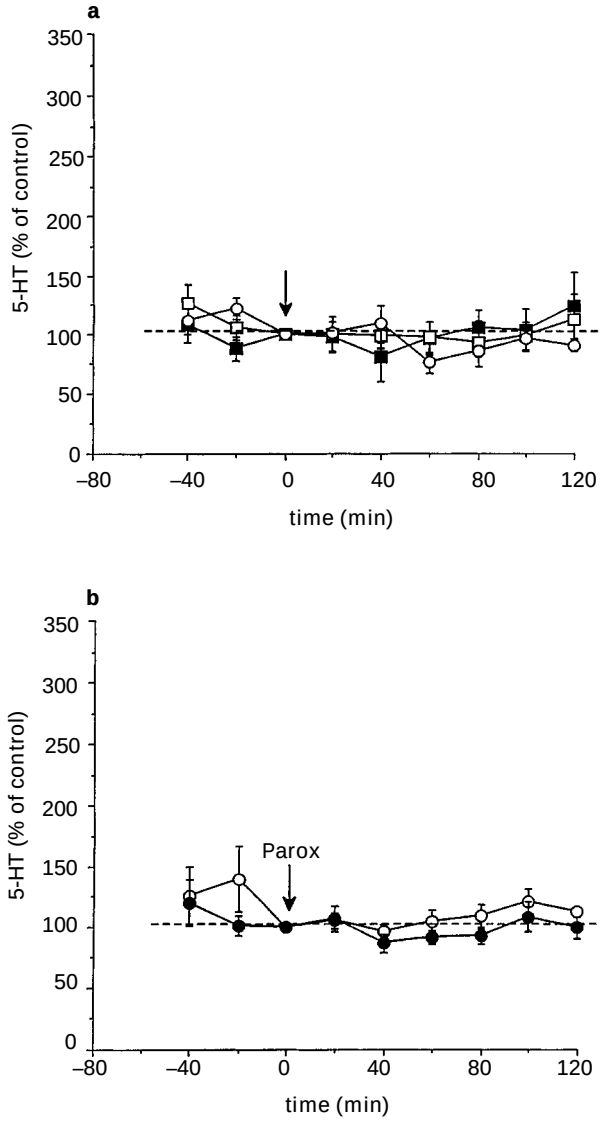

Figure 2 Effect of GR127935 injected either (a) alone or (b) in combination with paroxetine on levels of 5-HT in microdialysates from the rat frontal cortex. In (a), (○) effect of vehicle; effect of GR127935 1 mg kg^{-1} (■) and 5 mg kg^{-1} (□). In (b), effect of paroxetine (0.8 mg kg^{-1}) in the absence (○) and presence (●) of GR127935 (1 mg kg^{-1}). In (b) GR127935 was injected 10 min before paroxetine. Mean±s.e.mean (n=number of rats) amounts of 5-HT in the perfusates at t=0 were 27±5 (n=6) (vehicle), 13±4 (n=4) (1 mg kg^{-1} GR127935), 25±11 (n=6) (5 mg kg^{-1} GR127935), 18±2 (n=6) (paroxetine) and 22±4 (n=5) (paroxetine and GR127935) fmol/sample, respectively. Data for vehicle and paroxetine alone are the same as in Figure 1. Each point represents a mean and vertical lines show s.e.mean.

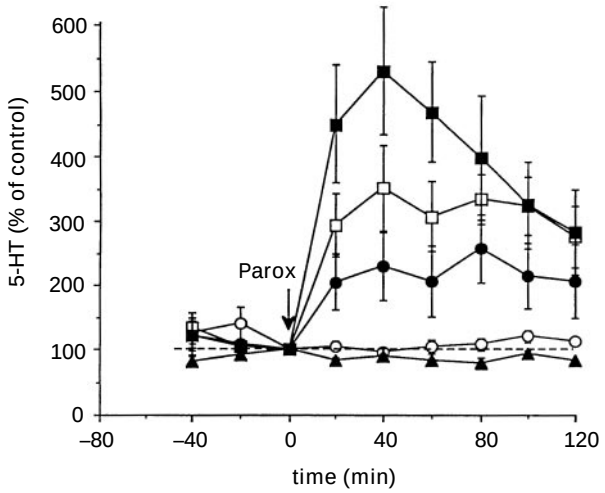

Figure 3 Effect of paroxetine (0.8 mg kg^{-1}) alone (○), in combination with WAY100635 (0.1 mg kg^{-1}) (●) or WAY100635 (0.1 mg kg^{-1}) plus GR127935 (■, 5 mg kg^{-1} or □, 1 mg kg^{-1}), on levels of 5-HT in microdialysates from the rat frontal cortex. (▲) Effect WAY100635 (0.1 mg kg^{-1}) plus GR127935 (5 mg kg^{-1}). Antagonists were injected 10 min before paroxetine. Mean ± s.e.mean (n = number of rats) amounts of 5-HT in the perfusates at t = 0 were 18 ± 2 (n = 6) (paroxetine), 16 ± 3 (n = 5) (paroxetine plus WAY100635), 14 ± 2 (n = 6) (paroxetine plus WAY100635/1 mg kg^{-1} GR127935) and 14 ± 3 (n = 6) (paroxetine plus WAY100635/ 5 mg kg^{-1} GR127935), 25 ± 4 (n = 4) (WAY100635 plus 5 mg kg^{-1} GR127935) fmol/sample, respectively. Data for paroxetine alone and paroxetine plus WAY100635 are the same as in Figure 1. Each point represents a mean and vertical lines show s.e.mean.

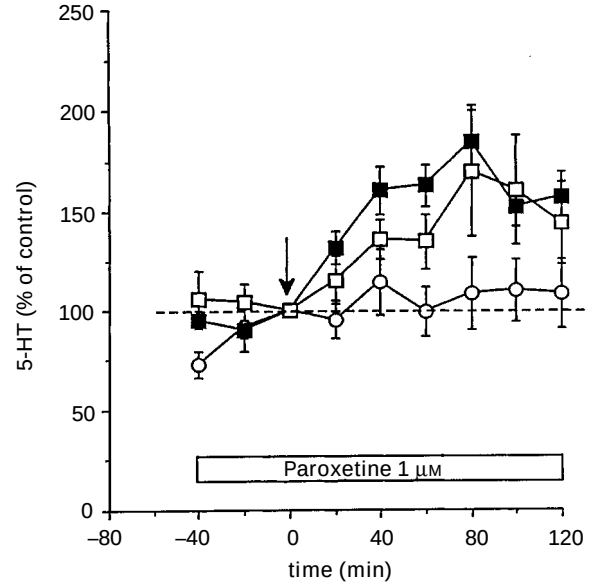

Figure 4 Effect of GR127935 (■, 1 mg kg^{-1} or □, 5 mg kg^{-1}) or vehicle (○) on frontal cortex extracellular 5-HT measured with 1 μM paroxetine present in the perfusion medium throughout the experiment. GR127935/vehicle was injected i.v. at t = 0. Mean ± s.e.mean (n = number of rats) amounts of 5-HT in the perfusates at t = 0 were 55 ± 17 (n = 4) (vehicle), 49 ± 14 (n = 6) (1 mg kg^{-1} GR127935) and 41 ± 10 (n = 5) (5 mg kg^{-1} GR127935) fmol/sample, respectively. Each point represents a mean and vertical lines show s.e.mean.

5a). Subsequent injection of paroxetine (0.1 – 0.4 mg kg^{-1}, i.v.) caused a dose-related and complete inhibition of 5-HT cell firing in the DRN in all cases (Figure 5b). The potency with which paroxetine inhibited 5-HT cell firing was not different between rats treated with vehicle (n = 4) and those treated with GR127935 (n = 4; total dose of 5 mg kg^{-1}, i.v.) (Figure 5b). Of

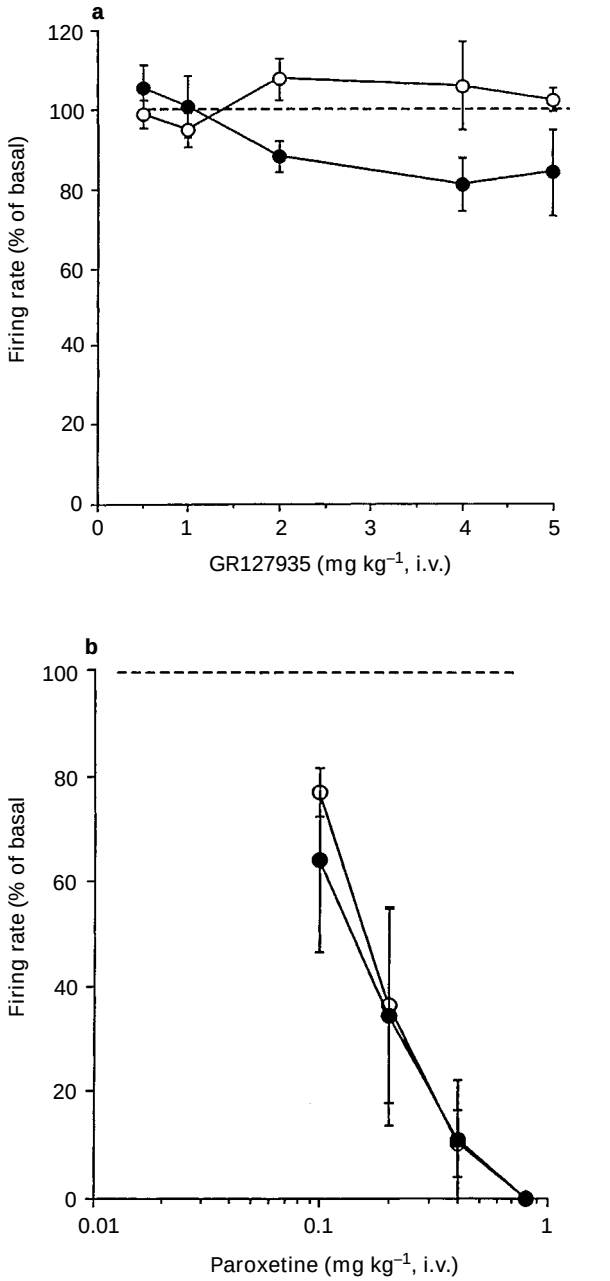

Figure 5 Effect of GR127935 injected alone (a) or in combination with paroxetine (b) on 5-HT cell firing in the DRN of the anaesthetized rat. GR127935 (●, n = 7 rats) or vehicle (○, n = 4 rats) were first injected in accumulating amounts (effects shown in (a)) and then paroxetine followed about 10 min later (effects shown in (b)). Only 4 rats treated with GR127935 subsequently received paroxetine. Each point represents a mean and vertical lines show s.e.mean.

the 8 cells inhibited by paroxetine, 7 were reversed by WAY100635 (0.1 mg kg^{-1}, i.v.).

Discussion

When an SSRI is administered acutely, any increase in synaptic 5-HT caused by inhibition of 5-HT reuptake is likely to be offset by the effects of activation of 5-HT autoreceptors on the somatodendrites and nerve terminals (i.e. decreased 5-HT cell firing and release). These is now much evidence from microdialysis studies that SSRIs cause a greater increase in extracellular 5-HT in the specific forebrain regions when combined with 5-HT_{1A} receptor antagonists (Invernizzi *et al.*, 1992; 1996; Hjorth, 1993; Gartside *et al.*, 1995). This effect is

probably due to the blockade of somatodendritic 5-HT_{1A} autoreceptors preventing the SSRI from inhibiting 5-HT neuronal activity (Gartside *et al*., 1995).

In the present study we confirm our recent observations that the selective 5-HT_{1A} receptor antagonist, WAY100635, potentiates the effect of the SSRI, paroxetine, on extracellular 5-HT in the frontal cortex (Gartside *et al*., 1995). Previously we found that the dose of WAY100635 which has this effect (0.1 mg kg^{-1}, i.v.), completely blocks the paroxetine-induced inhibition of 5-HT cell firing.

In contrast to the effect of the 5-HT_{1A} receptor antagonist, here we find that the selective 5-$HT_{1B/D}$ receptor antagonist, GR127935 (Skingle *et al*., 1996), did not potentiate the effect of systemically administered paroxetine on 5-HT in the frontal cortex. Furthermore, GR127935 by itself did not alter basal extracellular 5-HT in frontal cortex and caused only a minor (10–20%) decrease in 5-HT cell firing in the DRN. The latter results suggest that under our experimental conditions there is a lack of tone on the 5-$HT_{1B/D}$ autoreceptors (see later discussion). A likely reason for the lack of effect of GR127935 in combination with systemically administered paroxetine is that GR127935 (unlike WAY100635, cf Gartside *et al*., 1995) clearly did not prevent the inhibition of 5-HT cell firing induced by paroxetine. One would expect that under conditions of reduced 5-HT cell firing, terminal 5-HT release would be even lower than that under baseline conditions, and any effect of blocking 5-HT_{1B} autoreceptors located at the nerve terminal would be minimal.

A striking result in the present study is that paroxetine caused a much greater increase in 5-HT in the frontal cortex of rats pretreated with both GR127935 and WAY100635 than in those pretreated with WAY100635 alone (or indeed GR127935 alone). Thus, the SSRI would appear to have a greater effect on extracellular 5-HT when both the somatodendritic 5-HT_{1A} autoreceptors and the nerve terminal 5-HT_{1B} autoreceptors are blocked compared to when either receptor population is blocked separately.

The present data obtained in frontal cortex appear to concur with those of Hjorth (1993) who found that in rat ventral hippocampus, the increase in extracellular 5-HT induced by the SSRI, citalopram, could be increased by the 5-$HT_{1A/1B}$ antagonist (−)-penbutolol and that this effect was greater than that of the selective 5-HT_{1A} receptor antagonist (**S**)-UH-301 (albeit only marginally so). However, attribution of the greater effect of (−)-penbutolol to the combined blockade of 5-HT_{1A} and 5-HT_{1B} autoreceptors could be complicated by the non-selective pharmacology of this drug. In addition, the relative influence of 5-HT_{1A} and 5-HT_{1B} autoreceptors on 5-HT release in frontal cortex may not be the same as in ventral hippocampus. However, at least in the case of selective 5-HT_{1A} receptor antagonists, these drugs facilitate the effect of SSRIs on extracellular 5-HT in both frontal cortex and ventral hippocampus, although probably not dorsal hippocampus (Hjorth, 1993; Gartside *et al*., 1995; Gundlah *et al*., 1997; Invernizzi *et al*., 1997). The possibility that 5-HT_{1A} and 5-HT_{1B} autoreceptor function may vary from region to region is in need of further investigation.

It seems likely that GR127935 becomes 'active' when interacted with paroxetine and WAY100635 because paroxetine no longer inhibits 5-HT cell firing due to the blockade of the 5-HT_{1A} autoreceptor by the 5-HT_{1A} receptor antagonist (see above). Because of this interaction between paroxetine and WAY100635, extracellular 5-HT becomes elevated in the terminal region, thus increasing the tone on the inhibitory 5-HT_{1B} autoreceptor (which is removed by GR127935). Indeed, we found that when 5-HT tone was increased in the terminal region by applying paroxetine locally to the frontal cortex (and thereby avoiding an action of the drug at the level of the DRN), GR127935 now caused a clear cut increase in 5-HT levels.

Throughout this study, paroxetine was used at a dose (0.8 mg kg^{-1}, i.v.) which by itself did not increase extracellular 5-HT in the frontal cortex. Our previous data show that at this dose, paroxetine causes a significant blockade of 5-HT reuptake, in that it increases extracellular 5-HT in the DRN and causes a complete cessation of 5-HT neuronal activity in this region (Gartside *et al*., 1995). However, a higher dose of paroxetine (2.4 mg kg^{-1}, i.v.) will increase extracellular 5-HT in the frontal cortex (although the mechanism underlying this effect is rather unclear, see Gartside *et al*., 1995). Our data predict that GR127935 would increase the effect of paroxetine on extracellular 5-HT, if the latter were injected systemically at a dose which by itself increased 5-HT.

During the preparation of this manuscript we became aware of a study showing that in the awake guinea-pig, GR127935 (0.3 and 5 mg kg^{-1}, s.c.) facilitates the increase in hypothalamic extracellular 5-HT induced by the SSRI, sertraline, when the latter is administered at a dose which by itself increases 5-HT (Rollema *et al*., 1996). Thus, notwithstanding the methodological differences between the present study and that of Rollema *et al*. (species, anaesthesia and particularly, brain region – see above), the effect of SSRIs on extracellular 5-HT may be potentiated by a 5-$HT_{1B/1D}$ receptor antagonist (or at least GR127935) but this may only occur at high doses of the SSRI. This appears to contrast with the effects 5-HT_{1A} receptor antagonists, which are able to potentiate the effects of low doses of SSRIs that by themselves do not increase extracellular 5-HT in the forebrain (Invernizzi *et al*., 1992; Gartside *et al*., 1995; see Figure 1).

A role for the 5-HT_{1B} autoreceptor in the effects of GR127935 described here seems likely, on the basis of evidence that it is pharmacologically selective for 5-$HT_{1B/D}$ receptors versus other 5-HT receptor subtypes and other neurotransmitter receptors (Skingle *et al*., 1996). Furthermore, in the rat 5-HT_{1D} receptors are few in number compared to 5-HT_{1B} receptors (Bruinvels *et al*., 1993), and are not likely to play a significant role in the regulation of 5-HT release from the nerve terminals. GR127935 has been shown to have partial 5-$HT_{1B/D}$ receptor agonist activity in transfected cells (Pauwels & Colpaert, 1995). However, to our knowledge this action has not been detected in brain tissue and even so, a 5-$HT_{1B/D}$ receptor agonist effect is not likely to account for the increase in 5-HT that we observed. A pharmacokinetic effect of GR127935 (ie. increased plasma levels of WAY100635) cannot be ruled out, although these two drugs have quite different chemical structures and we are not aware of any evidence that they share common metabolic routes.

It should be possible to resolve without doubt whether the effects of GR127935 are mediated by 5-HT_{1B} autoreceptors once selective and silent 5-HT_{1B} receptor antagonists become available. Data on one such candidate compound have recently been described (Price *et al*., 1996). These drugs would also be very useful to determine whether the slight decrease in 5-HT cell firing induced by GR127935 is mediated via blockade of 5-HT_{1B} autoreceptors (possibly in the DRN) or some other pharmacological effect of the drug (eg. 5-HT_{1A} receptor agonism; Pauwels & Palmier, 1995).

Recent clinical studies indicate that the delay in onset of antidepressant action of an SSRI can be reduced by co-administration of low doses of the 5-HT_{1A}/β-adrenoceptor antagonist, pindolol (Artigas *et al*., 1994; Blier & Bergeron, 1995). This finding is currently thought to be associated with the ability of pindolol to block the somatodendritic 5-HT_{1A} autoreceptor and thereby potentiate the effect of the SSRI on extracellular 5-HT in the forebrain (Artigas *et al*., 1996). On this basis, the present data suggest that an SSRI may have an even better therapeutic effect under conditions when both the somatodendritic (5-HT_{1A}) and nerve terminal (5-HT_{1B}) 5-HT autoreceptors are blocked.

This work was supported by a grant (T.S.) and training fellowship (S.E.G.) from the Medical Research Council (U.K.). We are grateful to GlaxoWellcome, Wyeth and SmithKline Beecham for the generous gifts of drugs.

References

ARBORELIUS, L., NOMIKOS, G.G., GRILLNER, P., HERTEL, P., HÖÖK, 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.

ARTIGAS, F., PEREZ, V. & ALVAREZ, E. (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch. Gen. Psychiatry*, **51,** 248–251.

ARTIGAS, F., ROMERO, L., DE MONTIGNY, C. & BLIER, P. (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. *Trends Neurosci.*, **19,** 378–383.

ÅSBERG, M., ERIKSSON, B., MATENSON, B., TRASKMAN-BENDZ, L. & WAGNER, A. (1986). Therapeutic effects of serotonin uptake inhibitors in depression. *J. Clin. Psychiatry*, **47,** 23–35.

AUERBACH, S.B., LUNDBERG, J.F. & HJORTH, S. (1995). Differential inhibition of serotonin release by 5-HT and NA reuptake blockers after systemic administration. *Neuropharmacology*, **34,** 89–96.

BLIER, P. & BERGERON, R. (1995). Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J. Clin. Psychopharmacol.*, **15,** 217–222.

BRUINVELS, A.T., PALACIOS, J.M. & HOYER, D. (1993). Autoradiographic characterisation and localisation of 5-HT_{1D} compared to 5-HT_{1B} binding sites in rat brain. *Naunyn-Schmeideberg's Arch. Pharmacol.*, **347,** 569–582.

CHAPUT, Y., BLIER, P. & DE MONTIGNY, C. (1986). Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. *Naunyn-Schmeideberg's Arch. Pharmacol.*, **333,** 342–348.

DAVIDSON, C. & STAMFORD, J.A. (1995). Evidence that 5-hydroxytryptamine (5-HT) release was monitored in slices of rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. *Br. J. Pharmacol.*, **114,** 1107–1109.

FLETCHER, A., FORSTER, E.A., BILL, D.J., BROWN, G., CLIFFE, I.A., HARTLEY, J.E., JONES, D.E., MCLENACHAN, A., STANHOPE, K.J., CRITCHLEY, D.J.P., CHILDS, K.J., MIDDLEFELL, V.C., LANFUMEY, L., CORRADETTI, R., LAPORTE, A.-M., GOZLAN, H., HAMON, M. & DOURISH, C.T. (1996). Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. *Behav. Brain Res.*, **73,** 337–353.

GARTSIDE, S.E., UMBERS, V., HAJÓS, M. & SHARP, T. (1995). Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI *in vivo*: effects on 5-HT cell firing and extracellular 5-HT. *Br. J. Pharmacol.*, **115,** 1064–1070.

GUNDLAH, C., HJORTH, S. & AUERBACH, S.B. (1997). Autoreceptor antagonists enhance the effect of the reuptake inhibitor citalopram on extracellular 5-HT: This effect persists after repeated citalopram treatment. *Neuropharmacology*, (in press).

HAJÓS, M., GARTSIDE, S.E. & SHARP, T. (1995a). Inhibition of median and dorsal raphe neurones following administration of the selective serotonin reuptake inhibitor paroxetine. *Naunyn-Schmeideberg's Arch. Pharmacol.*, **351,** 624–629.

HAJÓS, M., GARTSIDE, S.E., VILLA, A.E.P. & SHARP, T. (1995b). Evidence for a repetitive (burst) firing pattern in a sub-population of 5-hydroxytryptamine neurones in the dorsal and median raphe nuclei of the rat. *Neuroscience*, **69,** 189–197.

HARTIG, P.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.

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.

INVERNIZZI, R., BELLI, S. & SAMANIN, R. (1992). Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphé prevents the drug's effect in the frontal cortex. *Brain Res.*, **584,** 322–324.

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.

INVERNIZZI, R., VELASCO, C., BRAMANTE, M., LONGO, A. & SAMANIN, R. (1997). Effect of 5-HT_{1A} receptor antagonists on citalopram-induced increase in serotonin in the frontal cortex, striatum and dorsal hippocampus. *Neuropharmacology*, (in press).

MIDDLEMISS, D.N. & HUTSON, P.H. (1990). The 5-HT_{1B} receptors. *Ann. New York Acad. Sci.*, **600,** 132–147.

PAUWELS, P.J. & COLPAERT, F.C. (1995). The 5-HT_{1D} antagonist GR127935 is an agonist at cloned human 5-$HT_{1D\alpha}$ receptor sites. *Neuropharmacology*, **34,** 235–237.

PAUWELS, P.J. & PALMIER, C. (1995). Functional effects of the 5-HT_{1D} receptor antagonist GR 127,935 at human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1A} and opossum 5-HT_{1B} receptors. *Eur. J. Pharmacol.*, **290,** 95–103.

PAXINOS, G. & WATSON, C. (1986). In *The Rat Brain in Stereotaxic Co-ordinates*. Sydney: Academic Press.

PRICE, G.W., BURTON, M.J., ROBERTS, C., WATSON, J., DUCKWORTH, M., GASTER, L., MIDDLEMISS, D.N. & JONES, B.J. (1996). SB 216641 and BRL 15572 pharmacologically discriminate between h5-HT_{1B} and h5-HT_{1D} receptors. *Br. J. Pharmacol.*, **119,** 301P.

ROLLEMA, H., CLARKE, T., SPROUSE, J.S. & SCHULZ, D.W. (1996). Combined administration of a 5-hydroxytryptamine (5-HT)$_{1D}$ antagonist and a 5-HT reuptake inhibitor synergistically increases 5-HT release in guinea pig hypothalamus in vivo. *J. Neurochem.*, **67,** 2204–2207.

SCHATZBERG, A.F., DESSAIN, E., O'NEIL, P., KATZ, D.L. & COLE, J.O. (1987). Recent studies on selective serotonergic antidepressants: trazodone, fluoxetine and fluvoxamine. *J. Clin. Psychopharmacol.*, **7,** 44S–49S.

SHARP, T., MCQUADE, R., GARTSIDE, S.E., UMBERS, V. & HAJÓS, M. (1996). Antidepressant action on anatomically distinct 5-HT pathways: combined electrophysiological and neurochemical studies. *J. Neurochem.*, **66** (suppl.2), S35B.

SKINGLE, M., SLEIGHT, A.J. & FENIUK, W. (1995). Effects of the 5-HT_{1D} receptor antagonist GR127935 on extracellular levels of 5-HT in the guinea-pig frontal cortex as measured by microdialysis. *Neuropharmacology*, **34,** 377–382.

SKINGLE, M., BEATTIE, D.T., SCOPES, D.I.C., STARKEY, S.J., CONNOR, H.E., FENIUK, W. & TYERS, M. (1996). GR127935: a potent and selective 5-HT_{1D} receptor antagonist. *Behav. Brain Res.*, **73,** 157–161.

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,** 393–402.

(Received January 20, 1997
Revised March 27, 1997
Accepted April 7, 1997)