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Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI *in vivo*: effects on 5-HT cell firing and extracellular 5-HT

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1 The acute inhibitory effect of selective 5-hydroxytryptamine (serotonin) reuptake inhibitors (SSRIs) on 5-HT neuronal activity may offset their ability to increase synaptic 5-HT in the forebrain.

2 Here, we determined the effects of the SSRI, paroxetine, and a novel selective 5-HT_{1A} receptor antagonist, WAY 100635, on 5-HT cell firing in the dorsal raphé nucleus (DRN), and on extracellular 5-HT in both the DRN and the frontal cortex (FCx). Extracellular electrophysiological recording and brain microdialysis were used in parallel experiments, in anaesthetized rats.

3 Paroxetine dose-dependently inhibited the firing of 5-HT neurones in the DRN, with a maximally effective dose of approximately 0.8 mg kg^{-1}, i.v. WAY 100635 (0.1 mg kg^{-1}, i.v.) both reversed the inhibitory effect of paroxetine and, when used as a pretreatment, caused a pronounced shift to the right of the paroxetine dose-response curve.

4 Paroxetine (0.8 mg kg^{-1}, i.v.), doubled extracellular 5-HT in the DRN, but did not alter extracellular 5-HT in the FCx. A higher dose of paroxetine (2.4 mg kg^{-1}, i.v.) did increase extracellular 5-HT in the FCx, but to a lesser extent than in the DRN. Whereas 0.8 mg kg^{-1}, i.v. paroxetine alone had no effect on extracellular 5-HT in the FCx, in rats pretreated with WAY 100635 (0.1 mg kg^{-1}), paroxetine (0.8 mg kg^{-1}, i.v.) markedly increased extracellular 5-HT in the FCx.

5 In conclusion, pretreatment with the selective 5-HT_{1A} receptor antagonist, WAY 100635, blocked the inhibitory effect of paroxetine on 5-HT neuronal activity in the DRN and, at the same time, markedly enhanced the effect of paroxetine on extracellular 5-HT in the FCx. These results may be relevant to recent clinical observations that 5-HT_{1A} receptor antagonists in combination with SSRIs have a rapid onset of antidepressant effect.

Keywords: SSRI; 5-HT_{1A} receptor antagonist; WAY 100635; microdialysis; 5-HT cell firing; dorsal raphé nucleus; frontal cortex

Introduction

It is established that selective 5-hydroxytryptamine (5-HT, serotonin) reuptake inhibitors (SSRIs) are effective in the treatment of depression. However, as with other antidepressants, several weeks of treatment with SSRIs is usually required before their therapeutic benefit becomes apparent (Åsberg *et al.*, 1986; Schatzberg *et al.*, 1987). Whilst the mechanism underlying the delay in antidepressant effect of SSRIs is not clear, there is evidence that it is only following repeated administration that SSRIs enhance 5-HT neurotransmission (Artigas *et al.*, 1994; Blier & de Montigny, 1994). Present strategies aimed at accelerating the onset of the antidepressant effect of SSRIs are focussed on shortening the time to produce this enhancement of 5-HT neurotransmission.

Although the acute administration of SSRIs might be expected to enhance the action of 5-HT on its postsynaptic receptors in the forebrain through blockade of 5-HT reuptake, it is well known that, when administered acutely, SRRIs inhibit the firing of 5-HT neurones in the midbrain raphé nuclei (Sheard *et al.*, 1972; Chaput *et al.*, 1986; Hajós *et al.*, 1995). This inhibitory effect is probably the result of a local increase in extracellular 5-HT in the raphé region and the resultant activation of somatodendritic 5-HT_{1A} autoreceptors (Rigdon & Wang, 1991). The inhibition of 5-HT cell firing induced by SSRIs would be expected to decrease 5-HT release in the forebrain. Thus, in the intact brain the acute effect of SSRIs on synaptic levels of 5-HT is likely to reflect a balance between blockade of 5-HT reuptake at the nerve terminal, and inhibition of firing of the 5-HT neurone (Fuller, 1994).

Interestingly, it has recently been reported that drugs with 5-HT_{1A} receptor antagonist properties are able to enhance the effect of SSRIs on forebrain extracellular 5-HT, as measured *in vivo* by microdialysis (Invernizzi *et al.*, 1992a; Hjorth, 1993). However, the mechanism underlying this effect has not yet been fully elucidated. In particular, it is unclear whether the doses of SSRIs used in the above studies would result in significant inhibition of 5-HT cell firing and, if so, whether the doses of 5-HT_{1A} antagonists used would block this effect. Furthermore, confirmation of the role of the 5-HT_{1A} receptor in this interaction has been precluded by the lack of availability of 5-HT_{1A} receptor antagonists which are both silent and selective.

In the present study the effects of an SSRI (paroxetine) and a putative selective 5-HT_{1A} receptor antagonist (WAY 100635) (Fletcher *et al.*, 1994) on 5-HT neuronal firing and on the extracellular 5-HT have been compared. These drugs were administered both alone and in combination. Measurements of 5-HT neuronal firing were made in the DRN. Extracellular 5-HT was measured both in the DRN itself and in the frontal cortex (FCx) which receives the majority of its 5-HT innervation from the DRN (O'Hearn & Molliver, 1984). A preliminary account of these findings has been presented to the British Pharmacological Society (Gartside *et al.*, 1995).

Methods

Animals

Male Sprague-Dawley rats (270–310 g; Harlan-Olac, Bicester, UK) 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 08 h 00 min). Animals were allowed food and water *ad libitum*.

General stereotaxic surgical procedures

Rats were anaesthetized with chloral hydrate (400–500 mg kg^{-1}, i.p.) and placed in a stereotaxic frame (Kopf) with the incisor bar set at −3.3 mm. Proper surgical anaes-

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thesia was maintained throughout the experiment by administration of supplementary doses of the anaesthetic. The body temperature of the animals was maintained at 36.0 ± 0.5°C by means of a homeothermic blanket with rectal probe. The skull was exposed and a burr hole drilled for the implantation of the recording electrode or microdialysis probe. A lateral tail vein was cannulated, with a 25 gauge hypodermic needle, for intravenous administration of drugs.

Electrophysiological studies

A glass capillary tube (external diameter 2.5 mm; Clarke Electromedical) was drawn out, with a pipette puller, to form a single barrelled glass microelectrode. The electrode was broken back to obtain an external tip diameter of ≈2 μm, and was then filled with a solution of 2 M NaCl with 2% Pontamine Sky Blue. Electrodes had *in vitro* impedances of 3–8 MΩ. An electrode was implanted to a depth of 4.5 mm though a burr hole drilled over the DRN (AP −7.6 mm; ML 0 mm; Paxinos & Watson, 1983) and subsequently lowered by means of a hydraulic microdriver (Kopf).

Spontaneously active presumed 5-HT neurones in the DRN, identified on the basis of their electrophysiological characteristics (see Results), were encountered between 5.0 and 6.5 mm below the dura surface. The signal was amplified (× 1000) and filtered (low frequency ≤300 Hz; high frequency ≥3000 Hz), and was displayed on an oscilloscope and chart recorder, and recorded on DAT tape for off-line analysis.

Extracellular recordings were made from one neurone per animal. The baseline firing activity of the neurone was recorded for at least 3 min, after which time drugs were administered intravenously. One group of animals received paroxetine, in doubling doses (initial dose 0.1 mg kg^{-1}, i.v.) at ≈2 min intervals, followed (after at least 65% inhibition had been achieved) by WAY 100635 (0.1 mg kg^{-1}, i.v.). A further group of animals received WAY 100635 (0.1 mg kg^{-1}, i.v.) and, approximately 6 min later, some of this group of animals received paroxetine in doubling doses (initial dose 0.4 mg kg^{-1}, i.v.) at ≈2 min intervals.

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, stored in 4% paraformaldehyde, and subsequently sectioned with a vibratome. Sections were stained with cresyl violet and the position of the electrode tip determined by microscopic inspection. Only those neurones found to be located within the DRN are included in the study.

Microdialysis studies

A concentric microdialysis probe with a 3 mm tip of exposed dialysis membrane (Cordis Dow GFE9 membrane, 250 μm in diameter), was stereotaxicaly implanted in either the DRN (AP −7.5 mm; ML 2.0 mm; DV −6.3 mm, from bregma and dura surface, implanted at 18° to the vertical), or the right frontal cortex (AP +3.2 mm; ML +3.0 mm; DV −4.5 mm; Paxinos & Watson, 1983). The probe was constantly perfused at 2 μl min^{-1} with artificial cerebro-spinal fluid (aCSF) (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). Dialysates were collected over 20 min, and were assayed immediately for 5-HT by h.p.l.c. with electrochemical detection as described elsewhere (Sharp & Zetterström, 1992).

Drugs were administered only after a stable baseline level of 5-HT had been established (typically 2–3 h post probe implantation). Two groups of animals with dialysis probes in the DRN, and 2 groups with probes in the FCx, received paroxetine (0.8 or 2.4 mg kg^{-1}, i.v.). A further 3 groups of animals, all with dialysis probes in the FCx, received vehicle injection (1 ml kg^{-1}, body weight), WAY 100635 (0.1 mg kg^{-1}, i.v.) alone, or WAY 100635 (0.1 mg kg^{-1}, i.v.), followed 10 min later by paroxetine (0.8 mg kg^{-1}, i.v.). Response to administration of drugs was determined over a 2 h period.

At the end of each experiment, the brain was removed, stored in 4% paraformaldehyde and subsequently sectioned with a freezing microtome, and the location of the microdialysis probe verified by inspection. Only experiments in which the probe was found to be correctly located in the DRN or FCx are included.

Drugs and chemicals

WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide.3HCl) and paroxetine HCl were generous gifts of Wyeth Research, Maidenhead, U.K. and SmithKline Beecham, Harlow, U.K., respectively. Chloral hydrate (Sigma) was dissolved in distilled/deionised water. WAY 100635 and paroxetine were dissolved in sterile 5% glucose solution. Chemicals used for h.p.l.c. were of analytical grade.

Data presentation and statistical analysis

For the electrophysiological studies, mean firing rate (spikes, 10 s^{-1}) were determined in periods of 60 s (pre-drug) or 30 s (post-drug). The percentage inhibition or increase quoted represents the difference between the post-drug firing rate and the pre-drug firing rate, as a percentage of the pre-drug firing rate. Individual ED_{50} values were determined by interpolation of percentage inhibition curves on a semi-log plot. Differences between groups were assessed by unpaired or single sample Student's *t* test.

Microdialysis data are presented as % of baseline ($t=0$) values. Statistical analysis of absolute 5-HT levels was by 1- or 2-way ANOVA with *post-hoc* Tukey test. Statistical significance at the 95% level is reported.

Results

Effects of paroxetine on 5-HT cell firing in the DRN

Presumed 5-HT neurones recorded in the DRN showed the electrophysiological characteristics of immunohistologically identified 5-HT neurones (Aghajanian & VanderMaelen, 1982). Thus, they fired spontaneously and regularly, with discharge rates of between 0.4 and 2.6 spikes s^{-1}. The action potential was characterized by a large positive deflection followed by a negative or negative/positive deflection, and had a duration of around 2 ms.

Acute i.v. administration of paroxetine potently and dose-dependently inhibited the spontaneous firing of the DRN

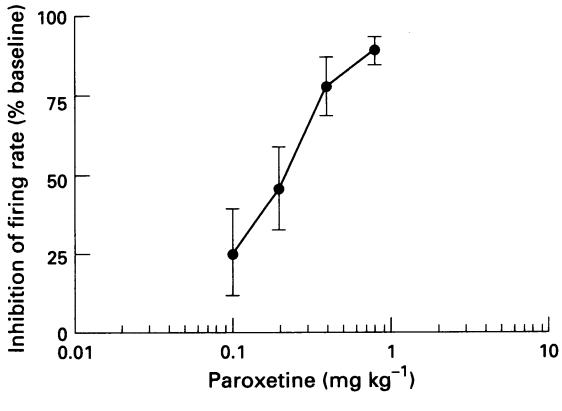

Figure 1 The relationship between increasing doses of paroxetine and changes in 5-HT cell firing in the DRN. Paroxetine was administered in cumulative doses as described in Methods. The inhibition of cell firing is expressed as a percentage of cell firing immediately before injection of the first dose of paroxetine. Each point is a mean ± s.e.mean of 7–9 determinations.

neurones (Figure 1 and Figure 2a). The mean ED_{50} calculated from individual inhibition curves for 9 cells was 0.252 ± 0.063 mg kg^{-1}, i.v. and the maximally effective dose (i.e. that dose inducing 100% inhibition) was estimated to be ≈ 0.8 mg kg^{-1}, i.v.

Effect of WAY 100635 on 5-HT cell firing in the DRN

In total, 10 presumed 5-HT neurones in the DRN were tested with a single injection of WAY 100635 (0.1 mg kg^{-1}, i.v.). In 6 of these cells, administration of WAY 100635 (0.1 mg kg^{-1}, i.v.) increased the firing rate. This effect was manifest within 120 s and persisted for at least 6 min, such that in the period 300–360 s after administration of WAY 100635, the mean firing rate in these 6 cells was 43.4 (± 10.7)% above the baseline firing rate. Three other cells showed a marked but transient inhibition of cell firing after WAY 100635, and one showed a decrease followed by an increase. Overall, in the 10 cells tested, the firing rate 300–360 s after administration of WAY 100635 was not statistically different from baseline (21.1 ± 13.3% above baseline (100%): single sample t test).

Effect of WAY 100635 and paroxetine in combination on 5-HT cell firing in the DRN

Of 9 cells inhibited by paroxetine (see above), subsequent administration of WAY 100635 (0.1 mg kg^{-1}, i.v.) almost completely reversed the effect of paroxetine in 7 (Figure 2a and b). It is of note however, that in some cases the reversal was quite short-lived and that, in these cells, further administration of WAY 100635 was apparently without effect. In the 2 cases of cells inhibited by paroxetine but not reversed by WAY 100635, it was unclear whether the cell was 'lost' before administration of the antagonist, or whether the firing remained totally inhibited even in the presence of WAY 100635.

Pretreatment with WAY 100635 (0.1 mg kg^{-1}, i.v.) caused a shift to the right of the paroxetine dose-response curve which

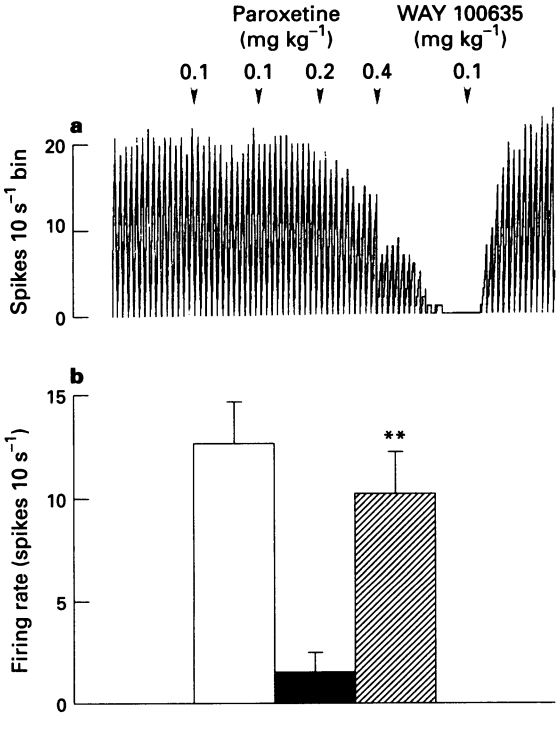

Figure 2 (a) An original rate meter recording of a single DRN cell showing inhibition of cell firing by cumulative doses of paroxetine, and reversal of the effect of paroxetine by subsequent administration of WAY 100635. Drugs were administered as indicated by the arrows. (b) Graph illustrating mean firing rates of DRN cells during 60 s periods: open column, before paroxetine; solid column, after administration of various total doses of paroxetine; and hatched column after subsequent administration of WAY 100635. **$P<0.01$ *vs* after paroxetine administration.

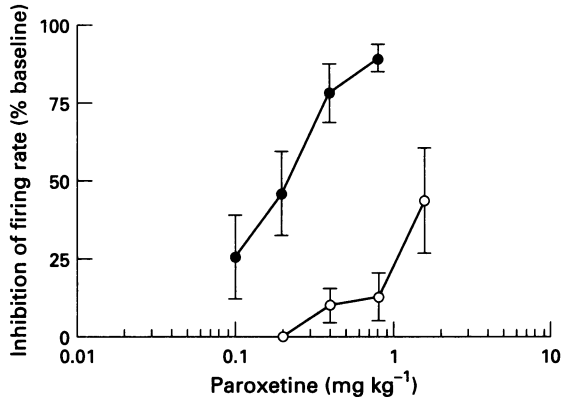

Figure 3 Mean percentage inhibition of 5-HT cells in the DRN induced by cumulative doses of paroxetine in (●) naïve rats (taken from Figure 1) and (○) rats pretreated with WAY 100635 (0.1 mg kg^{-1}, i.v.) Each point represents the mean ± s.e.mean of 5–7 determinations.

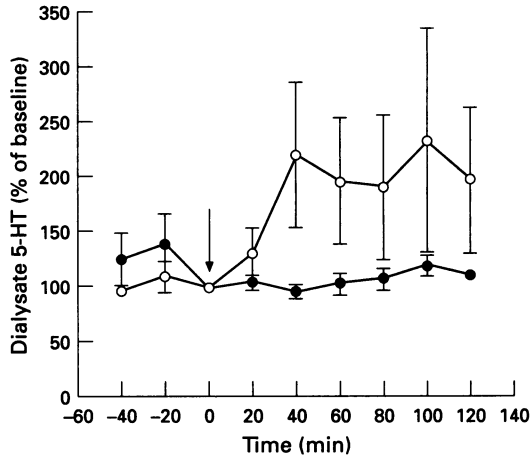

Figure 4 The effect of paroxetine (0.8 mg kg^{-1}, i.v.) on 5-HT in dialysates of (○) the DRN, and (●) the FCx. Paroxetine was given at $t=0$. Data are mean ± s.e.mean, $n=4-7$. See Results for statistical analysis.

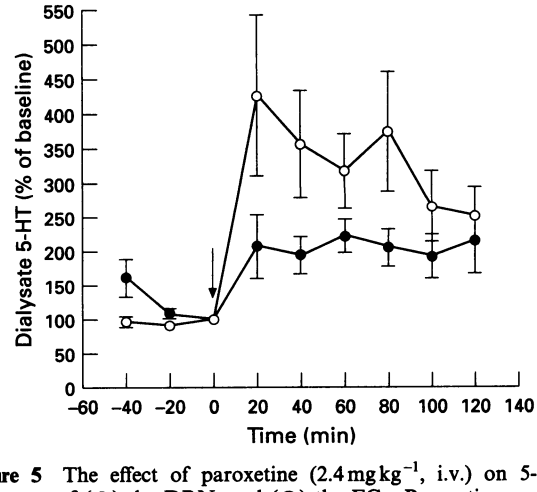

Figure 5 The effect of paroxetine (2.4 mg kg^{-1}, i.v.) on 5-HT in dialysates of (○) the DRN, and (●) the FCx. Paroxetine was given at $t=0$. Data are mean ± s.e.mean, $n=5-6$. See Results for statistical analysis.

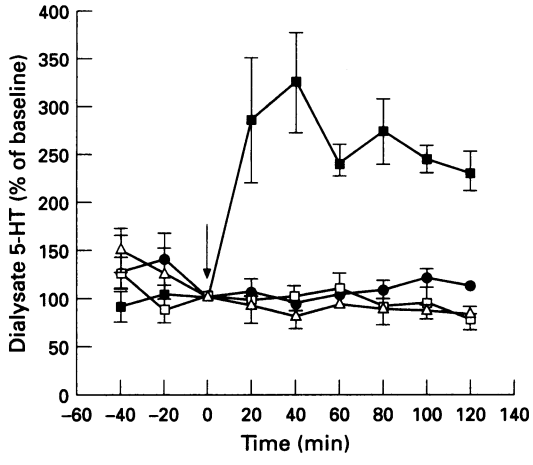

Figure 6 Time course of the effect of (△) vehicle, (□) WAY 100635 (0.1 mg kg^{-1}, i.v.), (●) paroxetine (0.8 mg kg^{-1}, i.v.) (taken from Figure 4), or (■) WAY 100635 (0.1 mg kg^{-1}, i.v.) followed by paroxetine (0.8 mg kg^{-1}, i.v.), on 5-HT in dialysates of the FCx. Paroxetine was given at $t=0$. Data are mean ± s.e.mean, $n=4-7$. See Results for statistical analysis.

was of such magnitude that it was not possible to determine an ED_{50} for paroxetine in the presence of WAY 100635 (Figure 3). However, the administration of 0.8 mg kg^{-1}, i.v. paroxetine, a dose which was maximally effective in naïve rats, caused only a small inhibition of cell firing of 12.5 ± 7.9 (7)% in animals pretreated with 0.1 mg kg^{-1}, i.v. WAY 100635.

Effect of paroxetine on extracellular 5-HT in the DRN and FCx

A dose of paroxetine (0.8 mg kg^{-1}, i.v.), which maximally suppressed firing in the DRN, increased 5-HT levels in dialysates of the DRN approximately 200% within 40 min of its administration (Figure 4). In contrast, this same dose of paroxetine (0.8 mg kg^{-1}, i.v.) did not increase 5-HT levels in dialysates of the FCx, at any time over the following 2 h.

Statistical analysis of the DRN and FCx time-course data by 2-way ANOVA showed significant main effects of brain region and time point, as well as a significant (brain region × time point) interaction ($F_{(6,42)}=3.3$; $P<0.01$). *Post-hoc* analysis revealed that 5-HT levels in dialysates of the DRN were significantly ($P<0.05$) higher than in the FCx at all time points from 20 to 80 min post-paroxetine. Basal levels of 5-HT in the DRN and FCx before injection of 0.8 mg kg^{-1} paroxetine were not significantly different (12.6 ± 3 (4) *vs* 11.2 ± 1.7 (7) fmol per sample; DRN vs FCx:NS).

A higher dose of paroxetine (2.4 mg kg^{-1}, i.v.) caused a large increase (to 400% of baseline) in 5-HT levels in dialysates of the DRN, but also an increase (to 200% of baseline) in dialysates of the FCx (Figure 5). Both responses were long-lasting (>2 h). Two-way ANOVA revealed significant main effects of brain region and time point and a significant (brain region × time point interaction) ($F_{(6,54)}=3.2$; $P<0.01$). 5-HT levels in DRN dialysates were significantly higher than those in FCx dialysates from 20 to 80 min after administration of 2.4 mg kg^{-1} paroxetine. Basal levels of 5-HT in the DRN and FCx of animals administered 2.4 mg kg^{-1} paroxetine were not significantly different (17.0 ± 4 (5) and 8.7 ± 2.0 (6) fmol per sample; DRN *vs* FCx: NS).

Effect of WAY 100635 on extracellular 5-HT in the FCx

Neither vehicle injection (1 ml kg^{-1} body weight, i.v.), nor administration of WAY 100635 (0.1 mg kg^{-1}, i.v.) alone, altered 5-HT levels in the FCx over the 2 h following the injection (Figure 6). Analysis of the time-course data by 1-way ANOVA revealed no significant effect of either treatment.

Effect of WAY 100635 and paroxetine in combination on extracellular 5-HT in the FCx

Administration of either paroxetine (0.8 mg kg^{-1}, i.v.) or WAY 100635 (0.1 mg kg^{-1}, i.v.) alone did not alter 5-HT levels in dialysates of the FCx (see above). However, in animals pretreated with WAY 100635 (0.1 mg kg^{-1}, i.v.), administration of paroxetine (0.8 mg kg^{-1}, i.v.) increased 5-HT levels in FCx dialysates to about 300% of baseline (Figure 6). Statistical analysis of the time-course by 2-way ANOVA revealed significant main effects of drug treatment and time point, and a significant interaction ($F_{(6,48)}=4.6$; $P=0.001$). 5-HT levels in the WAY 100635 pretreated group were significantly higher than in those receiving paroxetine alone at all time points between 20 and 120 min ($P<0.05$). Basal levels of 5-HT in FCx dialysates from the two groups did not differ significantly (6.8 ± 1.2 (7) *vs* 11.2 ± 1.7 (7) fmol per sample; WAY-100635 + paroxetine *vs* paroxetine: NS).

Discussion

In the intact brain the net effect of acute administration of SSRIs on synaptic levels of 5-HT will be influenced by both blockade of 5-HT reuptake at the nerve terminal, and any effect the SSRI may have on the release of 5-HT (Fuller, 1994). SSRIs, are known to inhibit the firing of DRN 5-HT neurones (Sheard *et al.*, 1972; Chaput *et al.*, 1986; Hajós *et al.*, 1995), and thus may inhibit terminal 5-HT release. Here we have investigated the inhibitory action of the SSRI, paroxetine, on 5-HT neuronal activity in the DRN. In parallel experiments, we have determined the effects of paroxetine on extracellular 5-HT in both the DRN and the FCx. Using a novel selective 5-HT$_{1A}$ receptor antagonist, WAY 100635, the consequence of preventing the inhibition of 5-HT cell firing, on extracellular 5-HT in the FCx, was determined. Measurements of 5-HT neuronal activity and extracellular 5-HT were carried out in anaesthetized rats by use of electrophysiological recording and brain microdialysis.

Effect of paroxetine on 5-HT cell firing in relation to extracellular 5-HT

Here we have shown that, in common with other SSRIs (Sheard *et al.*, 1972; Chaput *et al.*, 1986; Rigdon & Wang, 1991), paroxetine inhibits the firing of DRN neurones: a cumulative total dose of 0.8 mg kg^{-1}, i.v. causing a near total inhibition of 5-HT neuronal activity. In a recent study we observed that paroxetine also inhibited 5-HT cell firing in the median raphé nucleus (MRN) and did so at doses similar to those inhibiting firing of DRN cells (Hajós *et al.*, 1995). Since 5-HT neurones of the DRN and MRN together comprise the principle source of 5-HT innervation to the forebrain (Dahström & Fuxe, 1964; Ungerstedt, 1971), our data suggest that in the rat, paroxetine (at doses of 0.8 mg kg^{-1}, i.v. and above) causes a near total cessation of impulse flow in 5-HT neurones projecting to the forebrain.

In parallel experiments, 0.8 mg kg^{-1}, i.v. paroxetine increased extracellular 5-HT in the DRN but had no measurable effect in the FCx. A higher dose of paroxetine (2.4 mg kg^{-1}, i.v.) did increase extracellular 5-HT in the FCx, but to a lesser extent than in the DRN. In common with paroxetine, there is evidence that the SSRIs, citalopram, sertraline and fluvoxamine, also preferentially increase extracellular 5-HT in the cell body region compared to nerve terminal regions (Adell & Artigas, 1991; Bel & Artigas, 1992; Invernizzi *et al.*, 1992a,b). It has been suggested that this apparent regional selectivity may be due to 5-HT neurones responding to the increases in extracellular 5-HT in the cell body region, reducing their firing rate, so that any increase in synaptic 5-HT in the terminal region is small (Fuller, 1994). Data in the present study are consistent with this view. Thus, the dose of paroxetine which

increased extracellular 5-HT in the DRN, was that which caused a cessation of 5-HT cell firing in the DRN and, at the same time had little effect on extracellular 5-HT in the FCx. Furthermore, when this inhibition of 5-HT cell firing was blocked pharmacologically, extracellular 5-HT in the FCx increased markedly (see later).

It is perhaps surprising that paroxetine, at doses causing a near total inhibition of 5-HT neuronal activity, resulted in increased extracellular 5-HT in the DRN and the FCx. Extracellular levels of 5-HT depend principally on the rate of 5-HT release into the extracellular space, and the rate of reuptake (Fuller, 1994). When 5-HT neuronal activity is low (and hence neuronal release of 5-HT is low), it would be predicted that blockade of 5-HT reuptake would not increase extracellular 5-HT to any marked degree. In the DRN, the apparent dissociation between 5-HT cell firing and extracellular 5-HT after paroxetine might be explained on the basis of evidence that somatodendritic 5-HT release continues in the absence of 5-HT neuronal activity (Adell *et al.*, 1993). However, since the release of 5-HT from the nerve terminals is generally assumed to be dependent on 5-HT impulse flow (cf. Sharp *et al.*, 1989), our observation of a sustained increase in extracellular 5-HT in the FCx following the higher dose of paroxetine is more difficult to explain. It is possible that even at the nerve terminal a component of 5-HT release is independent of impulse flow, and that the increase in extracellular 5-HT following the higher dose of paroxetine results from a greater degree of 5-HT reuptake blockade than is achieved at the lower dose of paroxetine. Another possibility is that paroxetine at higher doses has an amphetamine-like 5-HT releasing action. Whatever the mechanism, this effect may not be unique to paroxetine in that a variety of SSRIs increase extracellular 5-HT forebrain regions when given in relatively high doses (Carboni & DiChiara, 1989; Rutter & Auerbach, 1993; Hjorth, 1993; Fuller, 1994).

A selective 5-HT$_{1A}$ receptor antagonist reverses the inhibitory effect of paroxetine on 5-HT cell firing

Here we show that the inhibition of DRN firing induced by paroxetine is prevented by the novel selective 5-HT$_{1A}$ receptor antagonist, WAY 100635 (Fletcher *et al.*, 1994). WAY 100635 (0.1 mg kg^{-1}, i.v.) both reversed the inhibitory effect of paroxetine and, when used as pretreatment, caused a pronounced shift to the right of the paroxetine dose-response curve. We have recently reported (Hajós *et al.*, 1995) that the inhibition of DRN and MRN cell-firing by paroxetine is reversed by i.v. administration of spiperone and WAY 100135, both of which have 5-HT$_{1A}$ receptor antagonist properties (Escandon *et al.*, 1994; Lanfumey *et al.*, 1994). Taken together, these findings suggest that the inhibition of raphé firing by paroxetine involves activation of 5-HT$_{1A}$ receptors. It should be noted that whilst WAY 100635 almost completely reversed the effect of paroxetine in the majority of cases, in some cells no reversal, or only a partial reversal was observed. Previously we noted that the paroxetine-induced inhibition was not reversed by spiperone or WAY 100135 in a number of cells (Hajós *et al.*, 1995). While these findings may reflect technical problems (e.g. loss of neurone during recording), the involvement of 5-HT receptors other than the 5-HT$_{1A}$ receptor might be implicated. In this respect it is relevant that, in the guinea-pig, 5-HT$_{1B/D}$ receptors have been shown to modulate 5-HT release in the DRN (Starkey & Skingle, 1994). However, a recent *in vitro* electrophysiological study suggested that 5-HT$_{1D}$ receptors have no role in the inhibition of firing of DRN 5-HT neurones by exogenous 5-HT (Craven *et al.*, 1994).

The present study does not address the site of the interaction between paroxetine and WAY 100635; however, several pieces of evidence point toward the involvement of the somatodendritic 5-HT$_{1A}$ autoreceptor in the DRN. Thus, paroxetine increases extracellular 5-HT in the DRN (this study) where 5-HT$_{1A}$ receptors, located on cell bodies and/or dendrites of 5-HT neurones (Vergé *et al.*, 1985; Sotelo *et al.*, 1990), are known to inhibit 5-HT cell firing (Sprouse & Aghajanian, 1987). Furthermore, SSRIs inhibit the firing of DRN neurones *in vitro* (Rigdon & Wang, 1991), and this effect is prevented by local application of 5-HT$_{1A}$ receptor antagonists (Jolas *et al.*, 1994).

WAY 100635 potentiates the effect of paroxetine on extracellular 5-HT in the FCx

Whereas injection of 0.8 mg kg^{-1}, i.v. paroxetine alone had no effect on extracellular 5-HT in the FCx, in rats pretreated with WAY 100635 (0.1 mg kg^{-1}, i.v.), the same dose of paroxetine induced a striking and sustained increase. A recent microdialysis study showed that pretreatment with (-)-penbutolol, or (S)-UH-301 potentiates the effects of the SSRI, citalopram, on extracellular 5-HT in the hippocampus (Hjorth, 1993). (-)-Penbutolol and (S)-UH-301 share 5-HT$_{1A}$ receptor blocking properties. When terminal 5-HT reuptake is blocked by the presence of an SSRI in the perfusion medium, systemic administration of SSRIs decreases extracellular 5-HT in the hippocampus. Using this model, Hjorth & Auerbach (1994) showed that the 5-HT$_{1A}$ receptor antagonists, pindodol and WAY 100135, block this decrease. The use of a highly selective 5-HT$_{1A}$ receptor antagonist in the present study provides further evidence that the results of the Hjorth study (1993) can indeed be attributed to 5-HT$_{1A}$ receptor blockade. That this effect of 5-HT$_{1A}$ receptor antagonists is mediated by somatodendritic 5-HT$_{1A}$ autoreceptors seems likely on the basis of evidence that injection of the non-selective 5-HT$_{1A}$ receptor antagonist methiothepin into the DRN enhanced the effect of citalopram on extracellular 5-HT in the cortex (Invernizzi *et al.*, 1992b). Importantly, our data also provide evidence of an association between the 5-HT-enhancing actions of the 5-HT$_{1A}$ receptor antagonists, and their ability to block the inhibitory effect of the SSRI on 5-HT cell-firing. Thus, in the present study WAY 100635 enhanced the effect of paroxetine on extracellular 5-HT at a dose that almost completely blocked the inhibitory effect of paroxetine on DRN firing.

Evidence that WAY 100635 is a silent 5-HT$_{1A}$ receptor antagonist

In the present study WAY 100635 (0.1 mg kg^{-1}, i.v.) alone moderately increased the firing rate (~50%) of some, but not all (6/10), DRN 5-HT neurones tested. Increases in 5-HT neuronal firing after WAY 100635 may reflect some tonic 5-HT-mediated inhibition in this population of neurones; however, the mechanism underlying the transient inhibition of firing observed in some neurones in the present study remains unclear. Two previous studies in other species (guinea-pig and cat) (Mundey *et al.*, 1994; Fornal *et al.*, 1994) have reported increased 5-HT cell firing after WAY 100635, although one other study in the rat found no increase (Fletcher *et al.*, 1994). In accord with Gurling *et al.* (1994), in the present study WAY 100635 had no effect on extracellular 5-HT in the FCx. That WAY 100635 neither consistently inhibits 5-HT cell firing nor decreases extracellular 5-HT in the forebrain confirms its lack of intrinsic activity at the 5-HT$_{1A}$ autoreceptor. Many putative selective 5-HT$_{1A}$ receptor antagonists (e.g. BMY 7378, NAN-190, MDL 73005) tested to date decrease both DRN firing and extracellular 5-HT (Sharp & Hjorth, 1990; Gartside *et al.*, 1990; Van den Hooff & Galzan, 1991; Greuel & Glaser, 1992), and as such have proved to be imperfect tools for the study of effects mediated by the 5-HT$_{1A}$ autoreceptor (for review see Fletcher *et al.*, 1993).

5-HT$_{1A}$ receptor antagonists and SSRIs in the treatment of depression

There is evidence that, although SSRIs inhibit 5-HT cell firing when given acutely, tolerance to this effect develops following long term administration, because of desensitization of the somatodendritic 5-HT$_{1A}$ autoreceptor (de Montigny *et al.*, 1990). It has been suggested that this desensitization may un-

derlie the antidepressant effect of SSRIs, since its onset coincides with the onset of the therapeutic effect (Blier & de Montigny, 1994). On this basis, the combination of a 5-HT$_{1A}$ receptor antagonist with an SSRI might, in the short term, produce a therapeutic effect equivalent to that observed after chronic administration of the SSRI alone. Interestingly, a recent open trail demonstrated that the antidepressant effect of paroxetine can be accelerated by co-administration of low doses of pindolol (Artigas *et al.*, 1994). Pindolol, like WAY 100635, has 5-HT$_{1A}$ autoreceptor antagonist properties (Sharp & Hjorth, 1990) and there is preliminary evidence that pindolol enhances the ability of an SSRI to increase extracellular 5-HT in the forebrain (Bel *et al.*, 1994). If the latter effect of pindolol underlies its antidepressant action, the present data indicate that WAY 100635 and other selective 5-HT$_{1A}$ antagonists like it, may have therapeutic use in the treatment of depression.

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