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European Journal of Pharmacology 372 (1999) 127–134

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5-HT_{1A} receptor antagonists neither potentiate nor inhibit the effects of fluoxetine and befloxatone in the forced swim test in rats

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Received 12 February 1999; received in revised form 17 March 1999; accepted 23 March 1999

Abstract

Recent clinical data suggest that coadministration of pindolol with an antidepressant, particularly the 5-hydroxytryptamine (5-HT) reuptake inhibitor fluoxetine, can shorten the time to onset of clinical activity and increase the proportion of responders. We have examined the interaction of antidepressants with 5-HT_{1A} receptors using the forced swim test in rats using both (±)-pindolol and the selective 5-HT_{1A} receptor antagonist WAY 100,635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl) cyclohexanecarboxamide trihydrochloride) in combination with either fluoxetine or the selective monoamine oxidase-A inhibitor befloxatone. 8-Hydroxy-dipropylaminotetralin (8-OH-DPAT; 0.125–1 mg/kg s.c.), used as a reference for 5-HT_{1A} agonist activity, reduced immobility in the forced swim test and this effect was significantly antagonised by WAY 100,635. WAY 100,635 alone (0.01–0.1 mg/kg s.c.) was without effect, although a higher dose, 0.3 mg/kg s.c., had a nonsignificant tendency to increase immobility. In contrast, (±)-pindolol (1–16 mg/kg s.c.) significantly reduced immobility, but to a lesser extent than 8-OH-DPAT. As expected, the antidepressants fluoxetine (10–80 mg/kg p.o.) and befloxatone (0.03–1 mg/kg p.o.) dose-dependently reduced immobility time. When the antidepressants were combined with WAY 100,635 (0.1 mg/kg), WAY 100,635 either had no effect or, at relatively high doses, significantly reduced their activity in this test. Combination of the antidepressants with (±)-pindolol (2 or 4 mg/kg s.c.) failed to reveal a significant interaction. These results demonstrate that the anti-immobility effects of fluoxetine and befloxatone are neither facilitated nor antagonised by doses of WAY 100,635 that completely reverse the effects of 8-OH-DPAT. Furthermore, there was no evidence that coadministration of the antidepressants with (±)-pindolol was able to facilitate their antidepressant-like effects. Thus, whereas direct agonist activity at 5-HT_{1A} receptors can modulate immobility in the forced swim test, this receptor subtype does not appear to play a major role in the antidepressant-like effects of fluoxetine or befloxatone under the conditions used in this study.

Keywords: Fluoxetine; Befloxatone; (±)-Pindolol; WAY 100,635; Forced swim test; 5-HT_{1A} receptor

1. Introduction

Most antidepressant drugs interact, more or less selectively, with different monaminergic systems, in particular the noradrenergic and the serotoninergic systems. This interaction usually takes the form of inhibition of metabolising enzymes (e.g., monamine oxidase inhibitors) or inhibition of reuptake systems. Despite these different mechanisms of action, all antidepressants seem to share a slow onset of activity in man, resulting in a delay of several weeks before maximal antidepressant efficacy can be attained (Burke and Preskorn, 1995; Parker, 1996; Stassen et al., 1996). This is despite the fact that their interaction with monoamine systems occurs immediately after the first administration. In addition to this slow onset of activity, an additional problem is that not all patients respond to a particular antidepressant drug (Thase and Rush, 1995). These two drawbacks of antidepressant therapy act synergistically in that determination of nonresponse is often not possible for several weeks, thus effectively increasing the period during which symptoms go untreated. The major challenge for the development of novel antidepressant agents is to reduce the time to maximal effect and to increase the proportion of responders. Several agents have been combined with antidepressant treatment in an attempt to increase the rate of response, increase efficacy or increase the number of responders. These include methylphenidate (Gwirtsman et al., 1994; Stoll et al., 1996), lithium (McCance-Katz et al., 1992),

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00202-2

triiodothryonine and neuroleptics (reviewed in Thase and Rush, 1995) as well as combinations of antidepressants, with mixed results (Thase and Rush, 1995; Nemeroff, 1996; Schweitzer et al., 1997).

Recent clinical data suggest that coadministration of (±)-pindolol and antidepressant treatment, particularly a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor such as fluoxetine, can shorten the time to onset of clinical activity and increase the proportion of responders (Artigas et al., 1994; Blier and Bergeron, 1995; Maes et al., 1996; Bakish et al., 1997; Blier et al., 1997; Perez et al., 1997; Tome et al., 1997a,b). It has been hypothesised that this action of (±)-pindolol results from its antagonism of somatodendritic 5-HT_{1A} receptors in the raphe nuclei, thereby mimicking the desensitisation of these receptors that occurs after chronic antidepressant treatment (Hjorth and Auerbach, 1994; Rutter et al., 1994; Gardier et al., 1996; Hjorth and Auerbach, 1996) and preventing the reduction in raphe cell activity that occurs after acute antidepressant treatment (Arborelius et al., 1995; Haddjeri et al., 1998). According to this theory, acute treatment with antidepressants initially results in an increase in serotoninergic activity at both presynaptic and postsynaptic sites, the former resulting in an inhibitory effect on cell body activity via somatodendritically located 5-HT_{1A} receptors. It has been suggested that (±)-pindolol has some selectivity for the somatodendritically located 5-HT_{1A} receptors (Blier and Bergeron, 1995; Artigas et al., 1996; Gardier et al., 1996) although this remains controversial.

Although most animal tests use acute treatment to demonstrate antidepressant activity, it remains possible that blockade of somatodendritic 5-HT_{1A} receptors may potentiate these effects. One of the most widely used preclinical tests for antidepressant activity is the forced swim test (Porsolt et al., 1978) and previous studies have suggested a role for postsynaptic 5-HT_{1A} receptors in the anti-immobility effects of 5-HT_{1A} ligands and also of several antidepressant compounds in this test (Luscombe et al., 1993; Borsini, 1995; Detke et al., 1995). To date, however, only preliminary reports have been published concerning the interaction of antidepressant compounds with pindolol in animal models of depression with the aim of demonstrating a facilitation and in most cases these have been selective 5-HT reuptake inhibitors (Detke et al., 1996; Jackson et al., 1996; Sluzewska and Szczawinska, 1996). In addition, few studies have examined the effects of the recently available 'silent' antagonists of the 5-HT_{1A} receptor.

In order to further study the interaction between 5-HT_{1A} receptor antagonists and antidepressant compounds, we examined various combinations of either (±)-pindolol or the selective 5-HT_{1A} receptor antagonist WAY 100, 635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl) cyclohexanecarboxamide trihydrochloride; Forster et al., 1995) in combination with either fluoxetine or the selective and reversible monoamine oxidase-A inhibitor befloxatone (Caille et al., 1996; Curet et al., 1996) using the forced swim test in rats. Befloxatone was included in the present experiments as no studies appear to have been carried out on the role of 5-HT_{1A} receptors in the effects of monoamine oxidase inhibitors in the forced swim test, although somatodendritic receptors appear to be involved in controlling their effects on 5-HT release (Gartside et al., 1995; Haddjeri et al., 1998) and there are some limited evidence that the clinical effectiveness of the monoamine oxidase inhibitors moclobemide and phenelzine can be enhanced by pindolol (Artigas et al., 1994; Blier and Bergeron, 1995).

2. Methods

2.1. Subjects

Adult male Sprague–Dawley rats (Iffa-Credo, Lyon, France) weighing 150–170 g at the time of testing were used throughout. On arrival from the suppliers, they were housed under standard conditions in groups of ten in temperature (21 ± 2°C) and humidity (50 ± 10% RH) controlled animal quarters with lights on between 0700 and 1900 (light intensity in the home cages varied between 40 and 130 lux). One day prior to, and throughout, testing, the animals were housed singly under similar conditions. Food and water were freely available at all times except during testing.

2.2. Apparatus and experimental protocol

Testing was carried out in two similar plexiglass cylinders (diameter 18 cm, height 38 cm) filled to a depth of 15 cm with water at 25°C. Two rats were observed at a time during all phases of the experiment. On the first experimental day, rats were gently placed in the water for a 15 min period of habituation. On removal from the water, they were placed in a plexiglass box under a 60 W bulb for 30 min to dry. The next day, they were replaced in the cylinders and observed for 5 min. During this period, the total time spent immobile (i.e., making only the movements necessary to remain afloat) was measured. At the end of the 5 min period, the rats were removed from the water and killed by i.p. injection of a lethal dose of pentobarbitone.

2.3. Compounds and drug administration

All treatments were administered three times, 25 h (i.e., immediately after the habituation session), 5 and 1 h prior to testing via the oral or subcutaneous route as indicated below. Where necessary, additional p.o. or s.c. injections of vehicle were administered to control for any additional stress of two injections prior to testing. Compounds were dissolved in sterile water or 0.9% NaCl for p.o. and s.c. injections, respectively, except for pindolol and befloxa-

tone which were suspended in a solution of tween 80 (0.5%)/methocel (0.5%). The antidepressant compounds (fluoxetine and befloxatone) were administered p.o.; all other compounds were administered s.c. All compounds were administered in a dose–volume of 5 ml/kg and all doses refer to the base, except those of 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) which are expressed as the salt. Compounds were obtained from the following sources: 8-OH-DPAT HBr and (±)-pindolol from Sigma Chemicals, La Verpillière, France; WAY 100,635 HCl, fluoxetine HCl and befloxatone were all synthesised at Synthélabo Recherche.

2.4. Statistics

In all experiments, the group size was 11 or 12 rats. In some cases, where wider dose ranges were desired, the results from two experiments have been combined (following statistical verification that the data from the two experiments was comparable). Comparisons between groups were carried out with a one- or two-way analysis of variance (ANOVA) as appropriate followed by intergroup comparisons using a Newman–Keuls analysis. Results were considered as significant for values of $P < 0.05$ (two-tailed). All statistical procedures were carried out using SAS version 6.08.

3. Results

3.1. Effect of test compounds given alone

Both fluoxetine and befloxatone induced a dose-dependent and statistically significant reduction in time spent immobile in the forced swim test (Fig. 1), as did 8-OH-DPAT (Fig. 2). The magnitude of the effect of 8-OH-DPAT appeared to be greater than that of the antidepressant compounds. The selective 5-HT_{1A} receptor antagonist WAY 100,635 had no significant effects on immobility time, although there was a tendency for it to increase at the highest dose tested of 0.3 mg/kg s.c. (Fig. 2). In contrast to this selective and 'silent' 5-HT_{1A} receptor antagonist, (±)-pindolol dose-dependently reduced immobility time with a maximum effect comparable to that of the antidepressants (Fig. 2).

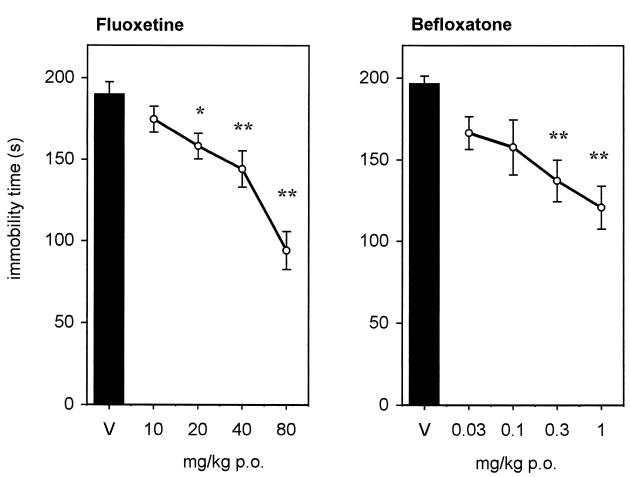

Fig. 1. The effect of fluoxetine and befloxatone on the time spent immobile in the forced swim test in rats. All data are from an observation period of 5 min duration, 1 h after compound administration. All values are mean ± S.E.M. for $n = 11$ or 12. $^{*}P < 0.05$; $^{**}P < 0.01$ compared to vehicle-treated controls (shaded column) using the Newman–Keuls test following a significant ANOVA (Fluoxetine: $F(4,54) = 15.78$, $P < 0.0001$; befloxatone: $F(4,55) = 5.56$, $P < 0.001$).

3.2. Effect of way 100,635 on the anti-immobility effects of 8-OH-DPAT and (±)-pindolol

The ability of 8-OH-DPAT to reduce time spent immobile was dose-dependently and completely antagonised by WAY 100,635 (Fig. 3). The dose–range over which antagonism occurred (0.003–0.1 mg/kg s.c.) was consistent with that required to antagonise other 8-OH-DPAT-induced effects such as hypothermia in the rat (unpublished data). The effect of (±)-pindolol also appeared to be attenuated by the 5-HT_{1A} receptor antagonist (Fig. 3) but this did not reach statistical significance, probably because of the small magnitude of the (±)-pindolol effect.

3.3. Interaction of antidepressants with way 100,635

The first series of experiments investigated the ability of WAY 100,635 (0.1 mg/kg s.c.) to affect the anti-immobility effect of fluoxetine or befloxatone. The dose of WAY 100,635 was chosen from previous experiments as a dose that completely antagonised all behavioural responses to 8-OH-DPAT and which had no effect on immobility time (see Fig. 2). Two doses were chosen from the dose–response curves in Fig. 1 for each of the antidepressants to give an anti-immobility time from the lower and upper ends of the dose–response curve.

The results, presented in Fig. 4, show that cotreatment with WAY 100,635 did not potentiate the effects of either fluoxetine or befloxatone. Indeed, the antagonist appears to have attenuated the anti-immobility effects of the two antidepressants, a result supported by the analysis of variance. In both cases, the overall ANOVA was highly significant (fluoxetine: $F(4,54) = 15.65$, $P < 0.001$; befloxatone: $F(4,55) = 4.56$, $P < 0.01$) and post-hoc Newman–Keuls comparisons of the groups receiving vehicle and antidepressant alone revealed significant effects of both fluoxetine and befloxatone (see Fig. 4). In all cases, the combination of antidepressant with WAY 100,635 (0.1 mg/kg s.c.) resulted in no significant differences compared to vehicle-treated animals, suggesting an attenuation of their antidepressant-like effects in the forced swim test. This was supported by the Newman–Keuls comparisons of antidepressant treatment alone and in combination with WAY 100,635. The effect of the higher fluoxetine dose was significantly reduced by the 5-HT_{1A} receptor antago-

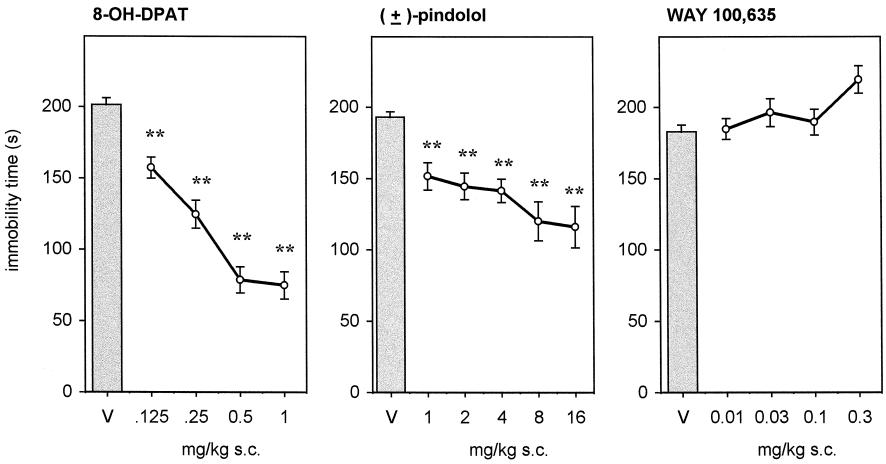

Fig. 2. The effect of 8-OH-DPAT, (±)-pindolol and WAY 100,635 on the time spent immobile in the forced swim test in rats. All other details as for Fig. 1 except $n = 12–24$ for the pindolol dose–response experiment. $^{*}P < 0.05$; $^{**}P < 0.01$ compared to vehicle-treated controls (shaded column) using the Newman–Keuls test following a significant ANOVA (8-OH-DPAT: $F(4,55) = 41.38$, $P < 0.0001$; (±)-pindolol: $F(5,102) = 9.09$, $P < 0.001$; WAY 100635: $F(4,55) = 1.92$, ns).

nist and the difference between animals administered the higher dose of befloxatone with or without WAY 100,635 just failed to reach statistical significance ($P = 0.06$).

An additional experiment was carried out using a ten-fold lower dose of WAY 100,635 (0.01 mg/kg s.c.) in combination with either fluoxetine (10 mg/kg p.o.) or befloxatone (0.1 mg/kg p.o.) in order to determine if less antagonism at 5-HT_{1A} receptors (particularly at post-synaptic receptors which might mediate the anti-immobility effects of antidepressants in this test) might reveal a facilitation of the antidepressant effects. None of these treatments differed significantly from vehicle treatment ($F(4,55) = 0.35$, ns; results not shown).

In view of the above results, a second series of experiments was carried out to more fully characterise the antagonism of fluoxetine and befloxatone by WAY 100,635. A range of doses of WAY 100,635 which were effective in antagonising 8-OH-DPAT (see Fig. 3) were tested against the anti-immobility effects of fluoxetine (40 mg/kg p.o.) or befloxatone (0.3 mg/kg p.o.). The results, shown in Fig. 5, demonstrate the ability of WAY 100,635 to attenuate the antidepressant-like effects of fluoxetine and befloxatone in this test. In both experiments, the result of the ANOVA was significant (fluoxetine: $F(4,55) = 7.67$, $P < 0.0001$; befloxatone: $F(4,55) = 3.02$, $P < 0.05$). Subsequent post-hoc comparisons demonstrated significant ef-

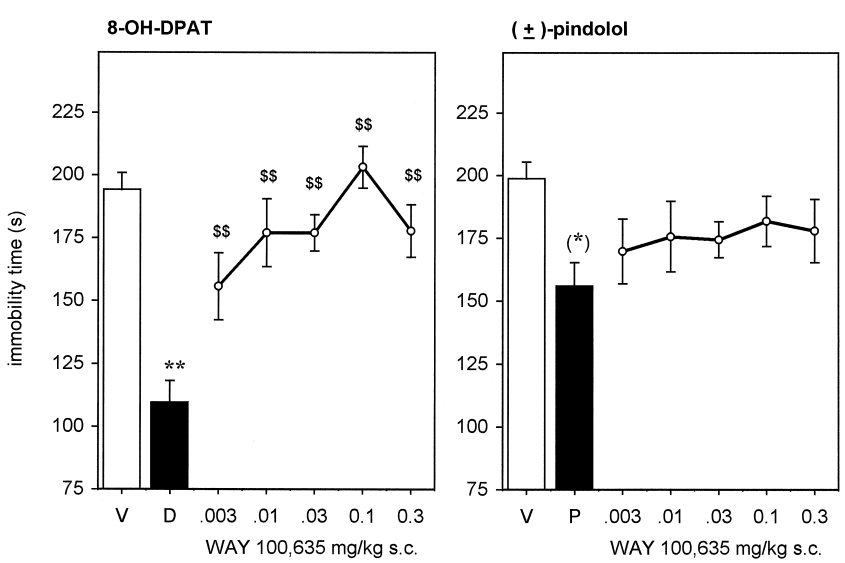

Fig. 3. Antagonism of the effects of 8-OH-DPAT (0.25 mg/kg s.c.) and (±)-pindolol (8 mg/kg s.c.) by WAY 100,635. All values are mean ± S.E.M. for $n = 12–24$. Values for vehicle-treated controls are represented by the white columns, values for animals receiving 8-OH-DPAT (D) or (±)-pindolol (P) are represented by the grey columns. $(^{*})P = 0.06$, $^{**}P < 0.01$ compared to vehicle (V)-treated control group; $\$\$P < 0.01$ compared to 8-OH-DPAT-treated group, Newman–Keuls test after significant ANOVA (8-OH-DPAT: $F(6,113) = 13.47$, $P < 0.0001$; (±)-pindolol: $F(6,112) = 2.33$, $P < 0.05$).

fects of both fluoxetine and befloxatone compared to vehicle treatment. In the fluoxetine experiment, all treatment groups were significantly different from the vehicle-treated group but the highest dose of WAY 100,635 significantly attenuated the effects of fluoxetine. In the befloxatone experiment, only befloxatone alone differed significantly from the group receiving vehicle, suggesting an attenuation of its effects by WAY 100,635.

3.4. The interaction of fluoxetine and befloxatone with (±)-pindolol

A final experiment was carried out to examine the interaction between fluoxetine or befloxatone and (±)-pindolol to determine if the effects of these compounds might be additive. For this reason, doses of fluoxetine (10 mg/kg p.o.) and befloxatone (0.1 mg/kg p.o.) were chosen from the lower end of the dose–response relationship shown in Fig. 1, as were the doses of (±)-pindolol (2 and 4 mg/kg s.c.; only 2 mg/kg was tested in combination with befloxatone).

Neither fluoxetine, (±)-pindolol nor the combination of fluoxetine with either of the two doses of (±)-pindolol differed significantly from vehicle-treated rats (ANOVA: $F(5,54) = 2;21$, ns). In the experiment evaluating the effects of befloxatone and (±)-pindolol in combination, only the (±)-pindolol-treated group differed from saline (ANOVA: $F(3,52) = 3.04$, $P < 0.05$; post-hoc Newman–Keuls test: vehicle vs. (±)-pindolol, $P < 0.05$). The effect of a combination of befloxatone and (±)-pindolol did not differ significantly from that of the vehicle.

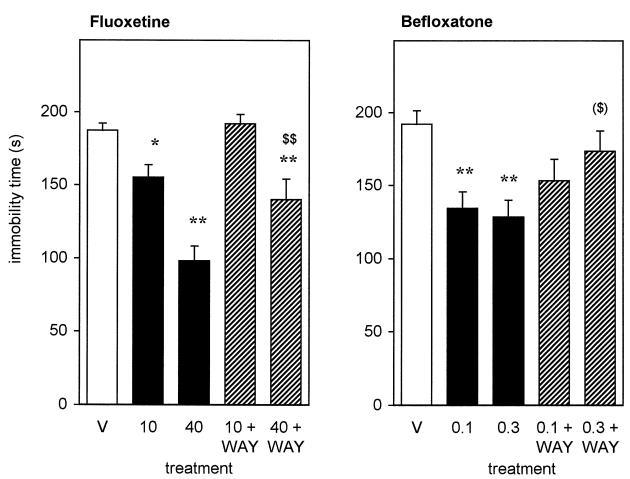

Fig. 4. Interaction of WAY 100,635 (0.1 mg/kg s.c.) with fluoxetine (10 or 40 mg/kg p.o.) and befloxatone (0.1 or 0.3 mg/kg p.o.). All values are mean ± S.E.M. for $n = 11$ or 12. Values for vehicle-treated controls are represented by the white columns, values for animals receiving antidepressant alone are represented by the grey columns and values for groups receiving antidepressant plus WAY 100,635 are represented by the hatched columns. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle (V)-treated control group; ($)$P = 0.06$, $$P < 0.01$ compared to group receiving the same dose of antidepressant without WAY 100,635, Newman–Keuls test following a significant ANOVA (fluoxetine: $F(4,54) = 15.65$, $P < 0.0001$; befloxatone: $F(4,55) = 4.56$, $P < 0.01$).

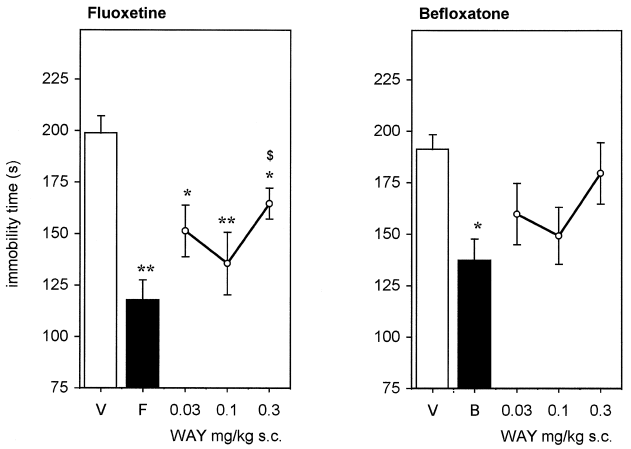

Fig. 5. Antagonism of the anti-immobility effects of fluoxetine (F, 40 mg/kg p.o.) or befloxatone (B, 0.3 mg/kg p.o.) by WAY 100,635. All values are mean ± S.E.M. for $n = 12$. Values for vehicle (V)-treated controls are represented by the white columns, values for animals receiving antidepressant alone are represented by the grey columns. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle-treated control group; $$P < 0.05$, compared to group receiving antidepressant alone, Newman–Keuls test following a significant ANOVA (fluoxetine: $F(4,55) = 7.67$, $P < 0.001$; befloxatone: $F(4,55) = 3.02$, $P < 0.05$).

4. Discussion

In view of the number of clinical reports now providing evidence for a beneficial effect of coadministration of pindolol with antidepressants (see Section 1), there has been much interest in demonstrating this interaction in animal models and in studying the involvement of 5-HT_{1A} receptors. Results from microdialysis and electrophysiological studies have demonstrated that acute administration of various antidepressants, including fluoxetine and befloxatone, decreases the firing rate of raphe neurons and that chronic treatment with antidepressant drugs or acute treatment combined with a 5-HT_{1A} receptor antagonist reverses these effects and leads to greater 5-HT release in terminal regions, the presumed mechanism of action of the antidepressant effects of drugs like fluoxetine (Arborelius et al., 1995; Gardier et al., 1996; Hjorth, 1996; Haddjeri et al., 1998). In contrast, recent preliminary reports of the interaction between fluoxetine and pindolol in the forced swim test have provided varied results, reporting on the one hand an inhibition of the effects of fluoxetine by pindolol (de Vry, 1996) and on the other, a facilitation of its effects by pindolol (Jackson et al., 1996). Another recent abstract reports both effects depending on the dose of fluoxetine used (Detke et al., 1996).

The present results provide no evidence that cotreatment with a 5-HT_{1A} receptor antagonist can facilitate the antidepressant-like activity of fluoxetine or befloxatone in

the forced swim test in rats. This lack of effect was demonstrated for both (±)-pindolol and the selective antagonist WAY 100,635 using a range of doses shown previously to be effective in antagonising both presynaptic and postsynaptic receptors (Gartside et al., 1990; Forster et al., 1995; Remy et al., 1996). In contrast, the effects of both antidepressant compounds appeared to be attenuated by relatively high doses of the selective antagonist WAY 100,635, a finding consistent with previous studies on the role of 5-HT_{1A} receptors in the anti-immobility effects of antidepressant compounds in the forced swim test (Detke et al., 1995; de Vry, 1996). (±)-Pindolol itself had significant anti-immobility effects in the forced swim test. This probably reflects its partial agonist activity at 5-HT_{1A} receptors (Przegalinski et al., 1995; Meltzer and Maes, 1996; Clifford et al., 1998), a suggestion consistent with the tendency for its effect to be antagonised by WAY 100,635. However, the modest effect of pindolol prevented a more detailed analysis of the receptor(s) involved and a role for β-adrenoceptors cannot be ruled out.

Although previous studies have suggested that 5-HT_{1A} receptors mediate the effects of antidepressants in the forced swim test (Detke et al., 1995), the present results provide only weak support for this. Thus, although the effects of fluoxetine and the MAO-A inhibitor befloxatone were both attenuated by WAY 100,635, some caution is needed before concluding that this demonstrates the involvement of 5-HT_{1A} receptors in the effects of befloxatone and fluoxetine. The main reason for this is that a dose of 0.3 mg/kg was required to obtain only partial attenuation of their effects, whereas the doses of WAY 100,635 needed to completely antagonise the effects of 8-OH-DPAT are in general between 0.003 and 0.1 mg/kg (see Section 3 and de Vry, 1996). In the results reported by de Vry (1996), only a single, high dose of WAY 100,635 was used (3 mg/kg) but this was clearly able to antagonise completely the effects of 8-OH-DPAT on the forced swim test but only partially antagonise the effects of fluoxetine. The reasons for this discrepancy are not immediately clear but may relate to differences in the ability of WAY 100,635 to antagonise endogenous, as compared to exogenous, agonists. There are some limited evidence for this, as doses of 0.3 mg/kg of WAY 100,635 seem to be required to increase either 5-HT release or marble-burying behaviour induced by selective 5-HT uptake inhibitors (McNicoll et al., 1995; Invernizzi et al., 1996), both of these effects being presumably mediated by antagonism of endogenously released 5-HT acting at somatodendritic 5-HT_{1A} receptors. In contrast, much lower doses (below 0.1 mg/kg) are reported to block both presynaptic and postsynaptic effects of 8-OH-DPAT (Critchley et al., 1994; Forster et al., 1995). Another reason for questioning the attenuation of befloxatone and fluoxetine by WAY 100,635 is that it was most clearly observed at a dose that had a tendency, albeit nonsignificant, to increase immobility. The possibility that the attenuation by WAY 100,635 represents simply the addition of opposing effects rather than pharmacological antagonism cannot be excluded. In fact, some evidence for the involvement of other 5-HT receptor subtypes in the anti-immobility effects of antidepressants are available. In particular, a role for the 5-HT_{1B} receptor has been suggested (O'Neill et al., 1996; Redrobe et al., 1996).

If postsynaptic 5-HT_{1A} receptors are not involved in the anti-immobility effects of either fluoxetine or befloxatone, it is surprising that antagonism of raphe autoreceptors by either WAY 100,635 or pindolol did not facilitate their effects in this test. There is extensive data available which demonstrate that the effects of 5-HT-uptake inhibitors and monoamine oxidase inhibitors on extracellular 5-HT concentrations are enhanced by 5-HT_{1A} receptor antagonism (Gartside et al., 1995; Hjorth, 1996; Trillat et al., 1998) and that 5-HT_{1A} receptor antagonists can increase serotonergic neuronal activity in rats treated with 5-HT-uptake inhibitors and monoamine oxidase inhibitors (Arborelius et al., 1995; Haddjeri et al., 1998). However, the interaction between fluoxetine and WAY 100,635 is not necessarily uniform throughout the brain. Malagié et al. (1996) found quite marked regional differences for the interaction between a low dose of fluoxetine (1 mg/kg p.o.) and WAY 100,635. Thus, whereas WAY 100,635 in combination with fluoxetine significantly increased extracellular 5-HT levels in the frontal cortex, no effect was seen in the ventral hippocampus. Among several explanations for this difference, one possibility is that the role of 5-HT_{1A} autoreceptors on raphe neurons may be different for different projection areas. In addition to these regional differences in drug interaction, there are also regional differences in the effect of forced swimming on 5-HT neurotransmission differently (Kirkby et al., 1995).

Only two studies appear to have examined antidepressant/pindolol/5-HT_{1A} receptor antagonist combinations using other rat tests of antidepressant activity. These studies used the less widely employed olfactory bulbectomy-induced hyperactivity model or the chronic mild stress model. In contrast to the forced swim test, both these models have been reported to show retarded onset of antidepressant activity. However, rather than accelerate the effect of chronic paroxetine, pindolol abolished its effect on bulbectomy-induced hyperactivity (Cryan et al., 1998). Interestingly, in the same study, pindolol accelerated the desensitization of 8-OH-DPAT-induced hypothermia that occurs after chronic paroxetine treatment (Cryan et al., 1998). It remains unclear whether this represents a presynaptic or postsynaptic effect and what its relevance is to the action of antidepressant. It has also been reported that pindolol can accelerate the onset of antidepressant-like activity of fluvoxamine using the chronic mild stress model in rats (Sluzewska and Szczawinska, 1996).

In addition to the rat studies reported above, there are two studies in mice which have studied the combination of 5-HT-uptake inhibitors with pindolol or a 5-HT_{1A} receptor

antagonist. Redrobe et al. (1996) demonstrated facilitation of subactive doses of three SSRI's with a relatively high dose of (±)-pindolol (32 mg/kg i.p.). However, no effect was obtained with 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine HBr (NAN-190), an antagonist of 5-HT_{1A} and noradrenergic α_1 receptors. In contrast, NAN-190, but not pindolol, antagonised the effects of other antidepressants such as imipramine and desipramine. In contrast, Egawa et al. (1996) reported a marked facilitation of fluvoxamine with NAN-190. Although these two studies present conflicting data concerning the effect of NAN-190, they suggest that additional studies in mice with more selective compounds might be worthwhile.

In conclusion, the present study demonstrates that fluoxetine and befloxatone interact in a similar manner with the selective 5-HT_{1A} receptor antagonist WAY 100,635 but that in neither case was there any evidence for a facilitation of their effects following coadministration with either WAY 100,635 or (±)-pindolol. The clinical benefit of pindolol addition to antidepressant treatment is still under active investigation (McAskill et al., 1998) and, if it indeed proves to be a beneficial adjunctive treatment, tests other than the forced swim test will be required to investigate the mechanisms involved.

Acknowledgements

We would like to thank Eric Descombris for his excellent technical assistance.

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