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Effects of chronic fluoxetine treatment in the presence and absence of (±)pindolol: a microdialysis study

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1 Using *in vivo* microdialysis in the frontal cortex of the freely moving rat we evaluated the effects of chronic treatment with the serotonin specific reuptake inhibitor (SSRI) fluoxetine in the presence and absence of the 5-HT_{1A}/β-adrenergic antagonist (±)pindolol.

2 Chronic vehicle treated animals produced no significant response to a challenge with fluoxetine (10 mg kg^{-1}) on day 8 and 15. Alternatively, a significant ($P<0.05$) decrease in extracellular 5-HT was observed in control animals upon challenge with the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; 0.03 and 0.1 mg kg^{-1}).

3 Conversely, animals treated with fluoxetine (10 mg kg^{-1} o.d.) for 7 and 14 days produced a significant ($P<0.05$) 2 fold increase in extracellular 5-HT when challenged with fluoxetine (10 mg kg^{-1}) on day 8 and 15. Moreover, no significant decrease in extracellular 5-HT was observed upon challenge with either dose of 8-OH-DPAT.

4 Animals chronically treated with (±)pindolol (10 or 20 mg kg^{-1} b.i.d.) produced a significant dose-related increase in extracellular 5-HT upon challenge with fluoxetine on day 15 only. Furthermore, both doses produced a significantly blunted response to the low dose challenge of 8-OH-DPAT (0.03 mg kg^{-1}). In addition, 20 mg kg^{-1} (±)pindolol treated animals also had no response to the higher 0.1 mg kg^{-1} dose of 8-OH-DPAT.

5 Animals treated for 14 days with a combination of (±)pindolol (10 or 20 mg kg^{-1}) and fluoxetine were not significantly different from vehicle treated animals when challenged with fluoxetine or 8-OH-DPAT.

6 Taken together it would therefore appear that although (±)pindolol alone has sufficient intrinsic activity to produce a desensitization of the 5-HT_{1A} receptor, when given in combination with fluoxetine it is able to prevent the desensitization induced by not only fluoxetine but also itself. This may suggest that the clinical augmentation of antidepressant action by pindolol, when co-administered with a SSRI, is *via* antagonism of the 5-HT_{1A} receptor.
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Keywords: 5-Hydroxytryptamine (5-HT, serotonin); microdialysis; frontal cortex; (±)pindolol; fluoxetine; 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)

Abbreviations: AD, antidepressant; β-AR, β-adrenergic receptor; DRN, dorsal raphe neurones; 5-HT, 5-hydroxytryptamine (serotonin); 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; SSRI, serotonin specific reuptake inhibitor

Introduction

The use of serotonin specific reuptake inhibitors (SSRI) in the treatment of depression is now common place and has become a relatively effective pharmacotherapy for many depressed patients. A major draw back of SSRI treatment is that many patients experience a 2–4 week delay in effective therapeutic antidepressant (AD) response (Blier & de Montigny, 1994; Gardier *et al*., 1996). This delay is believed to be due to the time required for adaptive changes in serotonergic neurotransmission to occur. One such adaptation which is thought to play a key role in this mechanism is the desensitization of somatodendritic 5-HT_{1A} autoreceptors. This hypothesis has been supported by preclinical studies using electrophysiology (Chaput *et al*., 1986; Blier *et al*., 1987) and *in vivo* microdialysis techniques (Rutter *et al*., 1994; Invernizzi *et al*., 1996; Hjorth *et al*., 1996; Dawson *et al*., 1998) which demonstrate that the 5-HT_{1A} autoreceptor undergoes adaptation in response to various SSRI treatments.

Clinical investigations, based upon this rationale, have demonstrated an enhancement of SSRI action by the addition of pindolol, a 5-HT_{1A}/β-adrenergic (β-AR) antagonist, to the treatment regime. The initial clinical report from Artigas *et al*. (1994) and later by Blier & Bergeron (1995) demonstrated that the combination of (±)pindolol with a SSRI shortened the onset of antidepressant action to a period of 3–7 days in contrast to 2–4 weeks. These initial observations have been confirmed in double blind placebo-controlled studies (Bordet *et al*., 1998; Zanardi *et al*., 1997, 1998). However, other studies have yielded conflicting results, demonstrating (±)pindolol to produce, at best, only meager enhancements of SSRI-induced AD activity (Berman *et al*., 1997; Perez *et al*., 1997; Tome *et al*., 1997).

Preclinical evidence has also demonstrated pindolol to potentiate SSRI-induced increases in extracellular serotonin (Dreshfield *et al*., 1996; Romero *et al*., 1996a; Hjorth, 1996; Hjorth & Auerbach, 1996; Dawson & Nguyen, 2000) in a comparable manner to more selective 5-HT_{1A} antagonists such as WAY100635 (Romero *et al*., 1996a,b; Hjorth, 1993; Hjorth *et al*., 1997; Dawson & Nguyen, 1998). However, pindolol in addition to its 5-HT_{1A} activity also has significant affinity for the 5-HT_{1B} autoreceptor and 5-$HT_{1B/D}$ antagonists have been demonstrated to produce similar augmentations of SSRIs (Gobert *et al*., 1997; Sharp *et al*., 1997; Dawson & Nguyen, 2000). Moreover, recent data (Assie & Koek, 1996; Bourin, 1998; Dawson & Nguyen, 2000) has suggested that, at least

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acutely, (±)pindolol produces much of its augmentation of a SSRI *via* its action at the 5-HT_{1B} receptor. This is further complicated by the fact (±)pindolol has been shown to be a partial 5-HT_{1A} agonist (Hjorth & Carlsson, 1985, 1986; Clifford *et al*., 1998) and molecules with intrinsic activity do not appear to be as effective at acutely augmenting the increase in extracellular 5-HT induced by a SSRI (Dawson & Nguyen, 1998). Taken together these acute preclinical observations highlight the uncertainty concerning the clinical mechanism of augmentation of a SSRI by (±)pindolol.

The present studies were undertaken to assess the functional neurochemical consequences of prolonged treatment with fluoxetine both alone and in the presence of (±)pindolol using *in vivo* microdialysis in the freely moving rat. Following chronic treatment with various combinations animals were challenged with either fluoxetine or 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) to assess the status of the 5-HT_{1A} receptor and 5-HT transporter.

Methods

Materials

All chemicals used were analytical grade and were purchased from Aldrich & Sigma chemicals (Milwaukee, WI, U.S.A.). (±) Pindolol, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and fluoxetine were purchased from Research Biochemicals International (Natick, MA, U.S.A.).

Animals

Male Sprague-Dawley rats (Charles River; 280–350 g) were used in all experiments. Animals were group housed with food and water available *ad libitum* and maintained on a 12-h light/dark cycle with all work performed during the light phase. Following surgery, the animals were housed separately in plexiglass cages (45 × 45 × 30 cm) with free access to food and water.

Chronic dosing procedure

Rats were administered with either vehicle (water), fluoxetine (10 mg kg^{-1}; ip; od), (±)pindolol (10 or 20 mg kg^{-1}, ip, bid) or (±)pindolol + fluoxetine for 7 and 14 days prior to microdialysis studies. All microdialysis studies were initiated 24 h following the last administered dose.

Surgical procedure

Following induction of anaesthesia with gaseous administration of halothane (2%) (Fluothane, Zeneca, Cheshire, U.K.) the animals were secured in a stereotaxic frame with ear and incisor bars. Anaesthesia was maintained by continuous administration of halothane (1–2%). A microdialysis probe guide cannula (CMA/Microdialysis, Stockholm, Sweden) was implanted into the frontal cortex. Coordinates for the frontal cortex were taken according to Paxinos & Watson (1986): RC +3.2, L −3.5, (reference point taken from bregma), V −1.5 from the skull. A subcutaneous cannula (s.c.) was also implanted at this time between the animal's shoulders. Both cannula were secured to the skull using dental acrylic (Plastics One, Roanoke, VA, U.S.A.). The wound was sutured and the animals left to recover for 24 h in their home cages with free access to food and water.

Microdialysis

A pre-equilibrated (perfused over night in aCSF) microdialysis probe (OD 0.5 mm, membrane length 2 mm; CMA/Microdialysis, Sweden) was implanted, *via* the guide cannula, into the frontal cortex of the unrestrained rat 24 h post surgery. The probe was perfused with artificial cerebrospinal fluid (aCSF) (mM: NaCl 125, KCl 3.0, $MgSO_4$ 0.75 and $CaCl_2$ 1.2 phosphate buffer 1.75, pH 7.4) at a flow rate of 1 μl min^{-1}. A 3 h stabilization period was allowed following probe implantation after which time microdialysis sampling was carried out by a modification of the method of Dawson & Routledge (1996). Four control samples were taken prior to drug injection to achieve a steady baseline. These four samples were averaged and all subsequent values were expressed as a percentage of this preinjection value. Vehicle or fluoxetine (10 mg kg^{-1}) were injected, *via* the s.c. cannula following preinjection baseline determination. 8-OH-DPAT (0.03 mg kg^{-1}) was injected following baseline determination and again, at the higher doses of 0.1 mg kg^{-1}, at t = 180 min or two vehicle injections. A 20 min sampling regime was used throughout the experimental period. At the end of the experiment probe placement was verified histologically and data from animals with incorrect probe placement were discarded.

Analysis of dialysates

5-Hydroxytryptamine (5-HT; serotonin) was separated by reverse phase high performance liquid chromatography (HPLC) (C18 ODS3 column, 150 × 3.0 mm, Metachem, Torrance, CA, U.S.A.) and detected using an ANTEC electrochemical detector (ANTEC, Netherlands) set at a potential of 0.65 V vs a Ag/AgCl reference electrode. Mobile phase was delivered by a Jasco PU980 HPLC pump (Jasco Ltd, Essex, U.K.) at 0.5 ml min^{-1} and contained 0.15 M NaH_2PO_4 buffer at pH 5.2, 0.25 mM EDTA, 315 μM 1-octane sodium sulphonic acid and 10% methanol/2% isopropanol. Data was acquired using the XChrom software package (VG data systems, Altringham, U.K.).

Data analysis

The fmol perfusate values of transmitters for the first four baseline samples were averaged and this value denoted as 100%. Subsequent sample values were expressed as a percentage of this preinjection control value. All results were analysed by 2-way analysis of variance (ANOVA) with repeated measures followed by pairwise comparisons using Bonferroni adjustment for multiple comparisons using the Statview software application (Abacus Concepts Inc., Berkeley, CA 1996) for the PC.

Results

Effects of chronic treatments on basal extracellular concentrations of 5-HT within the rat frontal cortex

Basal extracellular concentrations of 5-HT in animals treated for 7 and 14 days with either fluoxetine (10 mg kg^{-1} o.d.), (±)pindolol (10 mg kg^{-1} b.i.d.) or (±)pindolol + fluoxetine were not significantly different from vehicle treated groups (Table 1). In contrast, extracellular levels of 5-HT were significantly ($P < 0.05$) elevated in animals treated chronically for 14 days with either (±)pindolol (20 mg kg^{-1} b.i.d.) or (±)pindolol (20 mg kg^{-1} b.i.d.) + fluoxetine (Table 1).

Effects of acute fluoxetine (10 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1})

Chronic vehicle treated animals produced no significant change in extracellular 5-HT levels in response to an acute challenge with either fluoxetine (10 mg kg^{-1} s.c.) or vehicle (Figure 1) on day 15. In contrast, a significant increase in extracellular 5-HT was observed in 14 day chronic fluoxetine treated animals when acutely challenged with fluoxetine on day 15, with a maximum value of 197.1±37.3% of preinjection concentrations (Figure 1). Similarly, animals treated chronically with (±)pindolol (10 or 20 mg kg^{-1} b.i.d.) produced a dose-related increase in extracellular 5-HT upon challenged with fluoxetine reaching a maximum value of 204.7±23.5% of preinjection concentrations (Figure 1).

Table 1 Effects of chronic treatments on basal extracellular concentrations of 5-HT within the rat frontal cortex

Chronic treatment	*Basal levels of 5-HT on day 8 or 15* (fmol 20 µl microdialysate^{-1})
(1) 14 days	
Vehicle	6.31±0.98
Fluoxetine (10 mg kg^{-1})	6.14±1.03
(±)pindolol (10 mg kg^{-1})	7.70±2.20
(±)pindolol (20 mg kg^{-1})	10.10±1.30*
(±)pindolol (10 mg kg^{-1})+fluoxetine	6.28±1.33
(±)pindolol (20 mg kg^{-1})+fluoxetine	14.46±2.39*
(2) 7 days	
Vehicle	11.4±1.40
Fluoxetine (10 mg kg^{-1})	10.2±0.78
(±)pindolol (20 mg kg^{-1})	10.5±1.20

*denotes statistical difference from vehicle treated animals $n=8$ per study group.

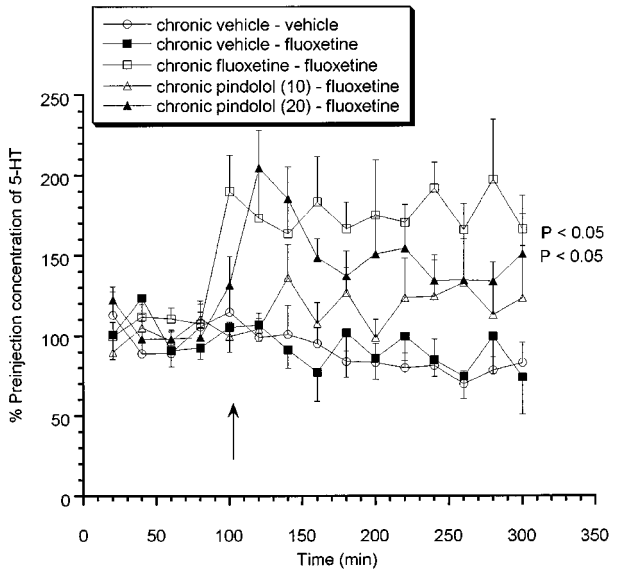

Figure 1 Effects of acute fluoxetine (10 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1}). Data expressed as mean±s.e.mean, $n=8$ per group. Figure legends denote chronic treatment received followed by acute challenge received. Arrow denotes fluoxetine or vehicle injection point. ($P<0.05$) denotes groups which attained statistical significance vs chronic vehicle–fluoxetine treated animals.

Effects of acute 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1})

Chronic vehicle treated animals produced a significant ($P<0.05$) decrease in extracellular 5-HT concentrations in response to an acute challenge with increasing doses of 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on day 15 (Figure 2). Alternatively, neither dose of 8-OH-DPAT produced any effect on extracellular 5-HT levels in those animals that received 14 days fluoxetine treatment (Figure 2). Animals treated with 10 mg kg^{-1} (±)pindolol produced a somewhat reduced response to the low dose of 8-OH-DPAT (0.03 mg kg^{-1}) but produced a significant ($P<0.05$) decrease in response to the higher dose of 0.1 mg kg^{-1} (Figure 2). Furthermore, animals which received chronic 20 mg kg^{-1} (±)pindolol produced no response to either the low or high dose of 8-OH-DPAT (Figure 2).

Effects of acute fluoxetine (10 mg kg^{-1} s.c.) or 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1})+fluoxetine (10 mg kg^{-1})

Animals treated chronically with either dose of (±)pindolol (10 or 20 mg kg^{-1}) together with fluoxetine (10 mg kg^{-1}) produced no response to the acute fluoxetine challenge (Figure 3) on day 15. In contrast, both (±)pindolol (10 mg kg^{-1})+fluoxetine and or (±)pindolol (20 mg kg^{-1})+fluoxetine treated animals produced a significant ($P<0.05$) decrease in extracellular 5-HT in response to 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) and this effect was not significantly different from that observed in vehicle treated groups (Figure 4). A comparative summary of all the 14 day chronic treatments can be seen in Table 2.

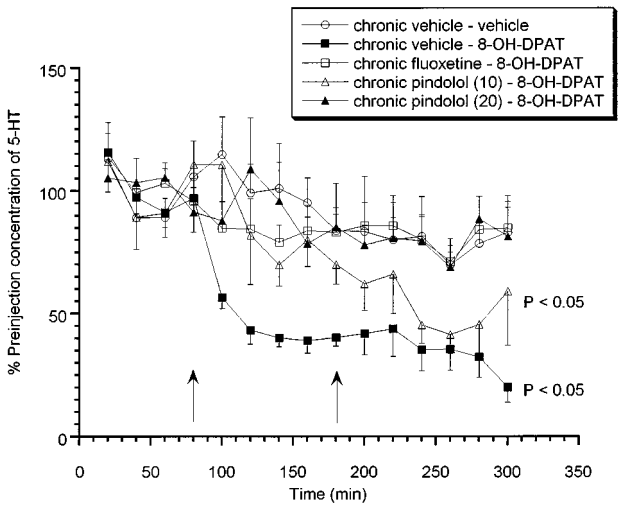

Figure 2 Effects of acute 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1}). Data expressed as mean± s.e.mean, $n=8$ per group. Figure legends denote chronic treatment received followed by acute challenge received (i.e. either low followed by a high dose of 8-OH-DPAT or two vehicle injections). Arrows denote 8-OH-DPAT or vehicle injection point. ($P<0.05$) denotes groups which attained statistical significance vs chronic vehicle–vehicle treated animals.

Effects of acute fluoxetine (10 mg kg^{-1} s.c.) or 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on rats treated chronically for 7 days with (±)pindolol (20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1})

Animals chronically treated for 7 days with vehicle or 20 mg kg^{-1} (±)pindolol produced no significant increase in extracellular 5-HT in response to an acute challenge with fluoxetine (Figure 5) on day 8. However, both vehicle and (±)pindolol treated animals produced a significant ($P<0.05$) 8-OH-DPAT-induced decrease in extracellular 5-HT (Figure 6). Alternatively, 7 days chronic fluoxetine treatment produced a significant ($P<0.05$) increase in extracellular 5-HT in response to the acute fluoxetine challenge, with a maximum value of 205.4±21.1% of preinjection concentrations (Figure 5), but no response to 8-OH-DPAT (Figure 6).

Discussion

The addition of 5-HT$_{1A}$/β-adrenergic antagonist (±)pindolol to a SSRI as an augmentation/acceleration strategy in the treatment of depression has received significant interest. To date, five of seven placebo-controlled studies have demonstrated an acceleration in onset of antidepressant activity and three of six claimed an augmentation in efficacy (for reviews see McAskill *et al*., 1998; Schechter *et al*., 1999). These effects have been ascribed to the 5-HT$_{1A}$ antagonist activities of (±)pindolol preventing the initial reduction in serotonergic firing (Blier *et al*., 1987; Gartside *et al*., 1995) associated with SSRI treatment. This hypothesis has received experimental support from preclinical studies showing that 5-HT$_{1A}$ antagonists can augment a SSRI-mediated increase in extracellular 5-HT (Hjorth, 1993; Hjorth *et al*., 1997; Ivernizzi *et al*., 1996; Romero *et al*., 1996b; Dawson & Nguyen, 1998). However, (±)pindolol has a variety of activities which may be

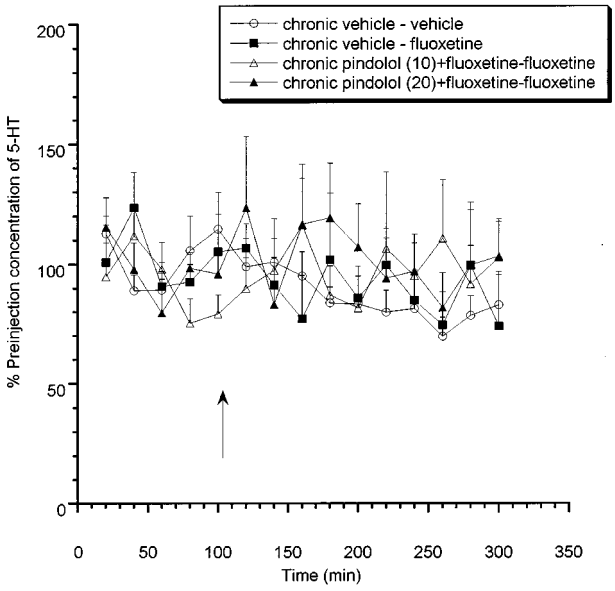

Figure 3 Effects of acute fluoxetine (10 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1})+fluoxetine (10 mg kg^{-1}). Data expressed as mean±s.e.mean, $n=8$ per group. Figure legends denote chronic treatment received followed by acute challenge received. Arrow denotes fluoxetine or vehicle injection point.

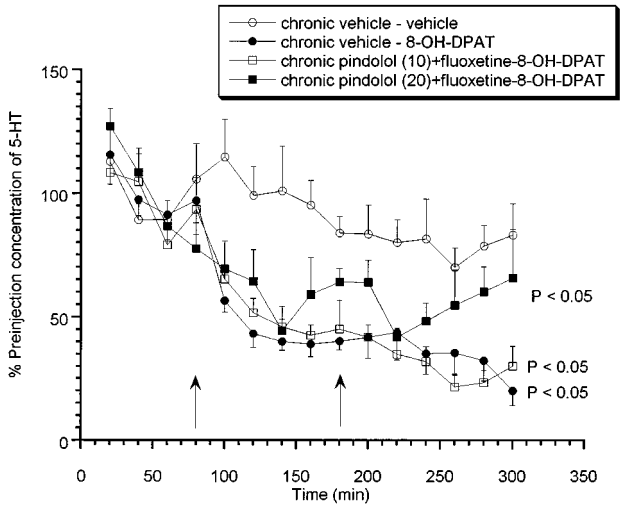

Figure 4 Effects of acute 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on rats treated chronically for 14 days with (±)pindolol (10 or 20 mg kg^{-1})+fluoxetine (10 mg kg^{-1}). Data expressed as mean± s.e.mean, $n=8$ per group. Figure legends denote chronic treatment received followed by acute challenge received (i.e. either low followed by a high dose of 8-OH-DPAT or two vehicle injections). Arrows denote 8-OH-DPAT or vehicle injection point. ($P<0.05$) denotes groups which attained statistical significance vs chronic vehicle–vehicle treated animals.

Table 2 Comparative summary of the effects of acute fluoxetine and 8-OH-DPAT on frontal cortex 5-HT following 7 and 14 day chronic treatment

	Maximal % change from preinjection values in response to acute challenges of		
Chronic treatment	*Fluoxetine* (10 mg kg^{-1})	*8-OH-DPAT* (0.03 mg kg^{-1})	*8-OH-DPAT* (0.1 mg kg^{-1})
(1) 14 days			
Vehicle	102.1±27.7	39.0±5.1*	20.2±6.2*
Fluoxetine (10 mg kg^{-1})	197.1±37.3*	79.2±7.2	71.2±9.5
(±)pindolol (10 mg kg^{-1})	133.1±30.5	70.0±8.8	41.4±7.6*
(±)pindolol (20 mg kg^{-1})	204.7±23.5*	78.7±10.8	69.0±6.1
(±)pindolol (10 mg kg^{-1})+Fluoxetine	116.4±19.6	42.6±4.2*	21.7±4.7*
(±)pindolol (20 mg kg^{-1})+Fluoxetine	119.5±22.8	44.5±4.6*	41.9±7.0*
(2) 7 days			
Vehicle	97.8±10.7	46.4±9.3*	40.3±9.6*
Fluoxetine (10 mg kg^{-1})	205.4±21.1*	94.5±22.0	90.2±13.5
(±)pindolol (20 mg kg^{-1})	120.6±16.5	60.3±9.4*	41.5±5.2*

*denotes statistical difference from vehicle–vehicle treated animals $n=8$ per study group.

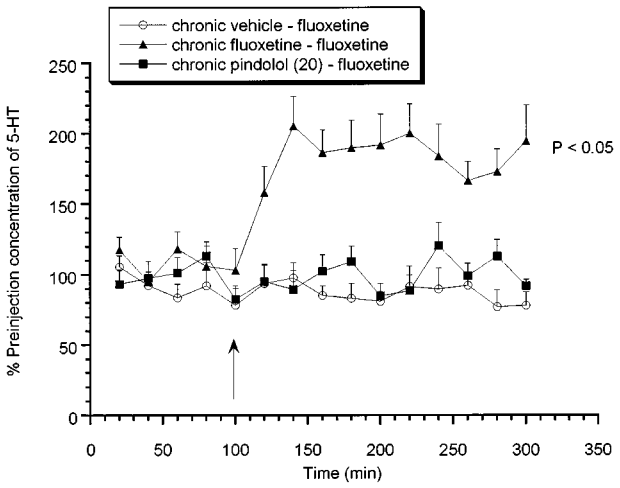

Figure 5 Effects of acute fluoxetine (10 mg kg^{-1} s.c.) on rats treated chronically for 7 days with (±)pindolol (20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1}). Data expressed as mean±s.e.mean, $n=8$ per group. Figure legends denote chronic treatment received followed by acute challenge received. Arrow denotes fluoxetine or vehicle injection point. ($P<0.05$) denotes groups which attained statistical significance vs chronic vehicle–fluoxetine treated animals.

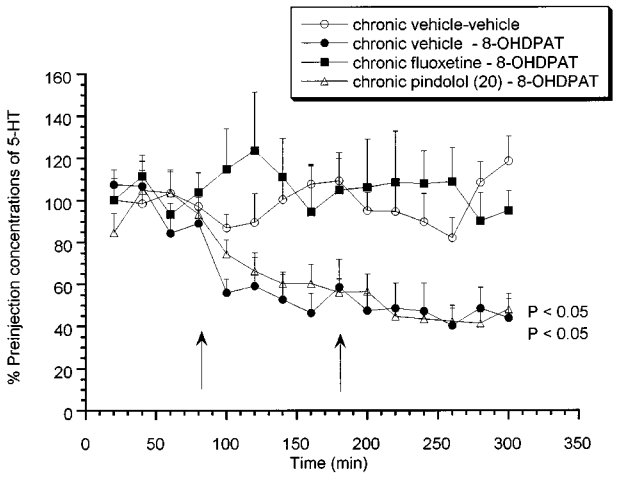

Figure 6 Effects of acute 8-OH-DPAT (0.03 and 0.1 mg kg^{-1} s.c.) on rats treated chronically for 7 days with (±)pindolol (20 mg kg^{-1}) or fluoxetine (10 mg kg^{-1}). Data expressed as mean±s.e.mean, $n=8$ per group. Figure legends denote chronic treatment received followed by acute received (i.e. either low followed by a high dose of 8-OH-DPAT or two vehicle injections). Arrows denote 8-OH-DPAT or vehicle injection point. ($P<0.05$) denotes groups which attained statistical significance vs chronic vehicle–vehicle treated animals.

affecting/contributing to its activity: these include partial 5-HT_{1A} agonism, 5-HT_{1B} antagonism and β-adrenergic antagonism. In addition, much of the preclinical evidence in support of pindolol's potentiation mechanism has come from acute treatment studies. Since the therapeutic utility of this compound, when given with a SSRI, for depression has only been observed with prolonged administration we have evaluated the possible mechanisms of action of (±)pindolol when given chronically and in combination with the SSRI, fluoxetine.

These studies indicate that chronic treatment with vehicle for 7 and 14 days produced no response to a challenge with fluoxetine but produced a decrease in extracellular 5-HT in response to the 5-HT_{1A} agonist 8-OH-DPAT. Acutely treated animals produce similar responses to both fluoxetine (Dawson & Nguyen, 1998) and 8-OH-DPAT (unpublished data) indicating that prolonged vehicle treatment produced no detectable changes. In contrast, animals treated for 7 and 14 days with fluoxetine produced a 2 fold increase in extracellular 5-HT in response to the fluoxetine challenge. Moreover, these animals showed no response to the 8-OH-DPAT challenge. Taken together these data suggest that an adaptive change in serotonergic transmission has occurred that appears to be mediated by a desensitization of the 5-HT_{1A} receptor, illustrated by the blunted response to the agonist. Other groups have reported similar results with fluoxetine (Rutter *et al*., 1994; Le Poul *et al*., 1995; Invernizzi *et al*., 1996) and other SSRI's (Bel & Artigas, 1993; Invernizzi *et al*., 1994), although some investigators have failed to confirm these observations (Bosker *et al*., 1995; Hjorth & Auerbach, 1994; Gundlah *et al*., 1997). It is also interesting to note that no significant difference was observed between basal levels of 5-HT between the vehicle and fluoxetine groups which suggests that even after desensitization of the 5-HT_{1A} receptors, there is little tonic activation of this system under these experimental conditions. Moreover, any SSRI still present following chronic administration (i.e. not washed out) is insufficient to significantly elevate basal extracellular 5-HT concentrations within the frontal cortex.

Animals treated for 14 days with either 10 or 20 mg kg^{-1} of (±)pindolol exhibited a dose-dependent increase in extracellular 5-HT in response to a fluoxetine challenge on day 15. The maximum increase was not significantly different from that observed with 7 and 14 days fluoxetine treatment. Furthermore, both doses produced a significantly blunted response to the low dose challenge of 8-OH-DPAT (0.03 mg kg^{-1}), moreover the 20 mg kg^{-1} (±)pindolol treated animals also had no response to the higher 0.1 mg kg^{-1} dose. Conversely, 7 days chronic treatment produced responses to fluoxetine and 8-OH-DPAT, which were not significantly different from vehicle. Taken together these data demonstrate that (±)pindolol, when administered for 14 days, is able to desensitize the 5-HT_{1A} receptor in a dose-dependent manner which is presumably due to (±)pindolol's partial agonist activity. It has been previously demonstrated by various investigators using both *in vivo* electrophysiology (Clifford *et al*., 1998; Fornal *et al*., 1999) and microdialysis (Clifford *et al*., 1998) that pindolol possesses significant intrinsic activity for the presynaptic 5-HT_{1A} receptor. From these data it would appear that pindolol possesses sufficient activity, when given long term, to produce a similar functional desensitization of the 5-HT_{1A} receptor as that induced by fluoxetine.

In contrast, animals treated with a combination of (±)pindolol (10 or 20 mg kg^{-1}) and fluoxetine produced no response to the day 15 fluoxetine challenge. The decrease induced by 8-OH-DPAT was not significantly different from vehicle for animals receiving chronic 10 mg kg^{-1} (±)pindolol+fluoxetine. The agonist induced response for 20 mg kg^{-1} (±)pindolol+fluoxetine treatment was also of a similar magnitude but the duration of the agonist effect appeared to be somewhat shorter for these animals. It would therefore appear that (±)pindolol has sufficient intrinsic activity to produce a desensitization of the 5-HT_{1A} receptor. However, when given in combination with fluoxetine pindolol is able to prevent the desensitization induced by either agent. The reason for this difference is presumably because of the difference in serotonergic tone in the presence of the SSRI, i.e. under lower tonic conditions (±)pindolol acts as an agonist due to the presynaptic receptor reserve, however under conditions of increased serotonergic tone (as would occur when the 5-HT transporter is blocked) the available receptor reserve is greatly

reduced (due to increased endogenous agonist) so (±)pindolol is able to behave more as an antagonist. Similar results have been observed with the more selective 5-HT_{1A} antagonist WAY100635 (Dawson *et al*., 1998) in that the desensitization induced by chronic fluoxetine treatment can be prevented by co-administration of the antagonist. Concurrently, no change in the sensitivity of the 5-HT_{1A} receptor was detected following chronic treatment with the more selective and silent antagonist alone. One interesting difference is that 20 mg kg^{-1} (±)pindolol ± fluoxetine appears to have produced an increase in basal levels of 5-HT. The reason for this is not totally clear at this time although it may be that high doses of (±)pindolol may have produced some other adaptation response. It is unlikely that this increase is due simply to a desensitization of the 5-HT_{1A} receptor since fluoxetine produced a similar effect but did not increase basal levels. It may also be significant that this occurred at the highest dose of (±)pindolol only, suggesting that this maybe a consequence of some non-selective action. Alternatively, (±)pindolol has significant β-adrenergic activity which has been shown to modulate other transmitter systems (Gobert & Millan, 1999), that may also undergo adaptation upon long term exposure to relatively high doses of pindolol and ultimately lead to alterations in serotonergic transmission within the frontal cortex.

The prolonged administration of both fluoxetine and (±)pindolol has produced a functional adaptation in serotonergic neurotransmission within the frontal cortex. These data demonstrate that this is mediated, at least in part, by the desensitization of the 5-HT_{1A} receptors. The mechanism by which the 5-HT_{1A} desensitization occurs and which sub-population of receptors are involved is not entirely clear. A number of investigators have demonstrated that there is no change in the presynaptic density of 5-HT_{1A} receptors within the dorsal or median raphe neurones (MRN and DRN) following chronic fluoxetine treatment (Le Poul *et al*., 1995; Li *et al*., 1996; Raap *et al*., 1999; Hervas *et al.,* 1999). Therefore, desensitization may be a function of either decreased G-protein coupling or a down regulation of selective G-protein subunits (Li *et al*., 1996; Raap *et al*., 1999). Furthermore, recent data has suggested that postsynaptic receptors are also involved in presynaptic regulation of 5-HT release within the prefrontal cortex (Ceci *et al*., 1994; Casanovas *et al*., 1999; Hajos *et al*., 1999) and postsynaptic alterations in various Gα subunits have been detected upon prolonged exposure to fluoxetine (Li *et al*., 1996). The role of pre vs postsynaptic functional regulation of frontal cortex 5-HT_{1A} receptors cannot be isolated from these experiments and the observations reported may be a net consequence of both auto-regulatory adaptation mechanisms. In addition, (±)pindolol also has significant affinity for the 5-HT_{1B} receptor, an activity which has been shown to contribute to its acute augmentation of SSRI's (Assie & Koek, 1996; Bourin *et al*., 1998; Dawson & Nguyen, 2000) and for β-adrenergic receptors. Clearly the status of the 5-HT_{1B} and β-AR receptors have not been evaluated in this study but alterations in these receptor subtype have been noted following these types of chronic treatment (Gobbi *et al*., 1997) and as such cannot be ruled out.

The time course of the chronic administration and different pharmacokinetic profiles of these drugs is clearly an issue. Fluoxetine has a long half-life and remains in the system for long periods following administration, making it relatively easy to maintain chronic levels. However, the half-life of pindolol is much shorter so systemic levels will be much more phasic (even with multiple dosing of relatively high concentrations). It could therefore be argued that occupancy of the 5-HT_{1A} receptor is not maintained throughout. However, from the data presented it is apparent that even this phasic dosing of (±)pindolol produces sufficient receptor occupancy to firstly, desensitize the 5-HT_{1A} receptor when given alone and moreover, prevent any detectable fluoxetine-induced desensitization when administered with the SSRI. Alternatively, it could be argued that residual pindolol may remain within the system following chronic treatment. Of course the levels of pindolol remaining and the accumulation cannot be fully evaluated from this study. If significant concentrations of pindolol did remain within the brain and occupied the presynaptic 5-HT_{1A} receptor, then administration of fluoxetine would produce an acute increase in extracellular 5-HT and block the 8-OH-DPAT response. Moreover, pindolol and fluoxetine are metabolized by the same pathway which would in theory further increase the circulating or brain bound concentrations of pindolol. Based on this, the animals which received both pindolol (20 mg kg^{-1}) + fluoxetine are likely to have the highest concentration of pindolol following chronic dosing and thus more likely to produce an increase in response fluoxetine and blunted response to 8-OH-DPAT. Animals which receive this combination produced no fluoxetine-induced increase in 5-HT but showed a 8-OH-DPAT induced decrease, indicating that any residual pindolol present at the time of the acute challenges is insufficient to block the 5-HT_{1A} receptor. In addition, 5-HT_{1A} radioligand binding studies performed in parallel with the microdialysis indicated that K_d and B_{max} of [^{3}H]-8-OH-DPAT did not differ among the various treatment groups (unpublished data). This further suggests that at the time of the acute challenges, following chronic pindolol treatment, there was no detectable residual pindolol occupying the 5-HT_{1A} receptor.

In summary, it would appear from these data that (±)pindolol has sufficient intrinsic agonist activity at the 5-HT_{1A} receptor to induce a desensitization of this receptor upon chronic treatment, however, when given in combination with fluoxetine it behaves more like a 5-HT_{1A} antagonist in preventing fluoxetine induced desensitization. This may therefore suggest that the clinical action of pindolol, when co-administered with a SSRI is *via* its antagonism of the 5-HT_{1A} receptor.

References

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

ASSIE, M-B. & KOEK, W. (1996). (−)-Pindolol and (±)-tertatolol affect rat hippocampal 5-HT levels through mechanisms involving not only 5-HT_{1A}, but also 5-HT_{1B} receptors. *Neuropharmacology,* **35,** 213–222.

BEL, N. & ARTIGAS, F. (1993). Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not raphe nuckei. *Synapse,* **15,** 243–245.

BERMAN, R.M., DARNELL, A.M., MILLER, H.L., ANAND, A. & CHARNEY, D.S. (1997). Effect of pindolol in hastening response to fluoxetine in the treatment of major depression: a double-blind, placebo-controlled trial. *Am. J. Psychiatry,* **154,** 37–43.

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

BLIER, P. & DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.,* **15,** 220–226.

BLIER, P., DE MONTIGNY, C. & CHAPUT, Y. (1987). Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J. Clin. Psychopharmacol.,* **7,** 24–35.

BORDET, R., THOMAS, P. & DUPUIS, B. (1998). Effect of pindolol on onset of action of paroxetine in the treatment of major depression: intermediate analysis of a double-blind, placebo-controlled trial. *Am. J. Psychiatry,* **155,** 1346–1351.

BOSKER, F.J., VAN ESSEVELDT. K.E., KLOMPMAKERS, A.A. & WESTENBERG, H.G.M. (1995). Chronic treatment with fluvoxamine by osmotic minipumps fails to induce persistent functional changes in central 5-HT_{1A} and 5-HT_{1B} receptors, as measured by in vivo microdialysis in dorsal hippocampus of conscious rats. *Psychopharmacology,* **117,** 358–363.

BOURIN, M., REDROBE, J.P. & BAKER, G.B. (1998). Pindolol does not act on 5-HT_{1A} receptors in augmenting antidepressant activity in the mouse forced swimming test. *Psychopharmacology,* **136,** 226–234.

CASANOVAS, J.P., HERVAS, I. & ARTIGAS, F. (1999). Postsynaptic 5-HT_{1A} receptors control 5-HT release in the rat medial prefrontal cortex. *Neuroreport,* **10,** 1441–1445.

CECI, A., BASCHIROTTO, A. & BORSINI, F. (1994). The inhibitory effect of 8-OH-DPAT on the firing activity of dorsal raphe serotoninergic neurons in rats is attenuated by lesion of the frontal cortex. *Neuropharmacology,* **33,** 709–713.

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.

CLIFFORD, E.M., GARTSIDE, S.E., UMBERS, V., COWEN, P.J., HAJOS, M. & SHARP, T. (1998). Electrophysiological and neurochemical evidence that pindolol has agonist properties at the 5-HT_{1A} autoreceptor in vivo. *Br. J. Pharmacol.,* **124,** 206–212.

DAWSON, L.A. & NGUYEN, H.Q. (1998). Effects of 5-HT_{1A} receptor antagonists on fluoxetine-induced changes in serotonin in rat frontal cortex. *Eur. J. Pharmacol.,* **345,** 41–46.

DAWSON, L.A. & NGUYEN, H.Q. (2000). The role of 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors on the modulation of acute fluoxetine-induced changes in extracellular 5-HT: the mechanism of action of (±) pindolol. *Neuropharmacology,* **39,** 1044–1052.

DAWSON, L.A. & ROUTLEDGE, C. (1996). Differential effects of potassium channel blockers on extracellular levels of serotonin and dopamine in the striatum of conscious rats. *Br. J. Pharmacol.,* **116,** 3260–3264.

DAWSON, L.A., NGUYEN, H.Q., SMITH, D.L. & SCHECHTER, L.E. (1998). Effects of chronic fluoxetine treatment, in the presence and absence of 5-HT_{1A} receptor blockade, on extracellular concentrations of 5-HT: a microdialysis study. *Soc. Neurosci. Abstr.,* **24,** 438.11.

DRESHFIELD, L.J., WONG, D.T., PERRY, K.W. & ENGLEMAN, E.A. (1996). Enhancement of fluoxetine-dependent serotonin (5-HT) levels by (−) pindolol, an antagonist at 5-HT_{1A} receptors. *Neurochem. Res.,* **21,** 557–562.

FORNAL, C.A., MARTIN, F.J., METZLER, C.W. & JACOBS, B.L. (1999). Pindolol suppresses serotonergic neuronal activity and does not block the inhibition of serotonergic neurons produced by 8-hydroxy-2-(di-n-propylamino)tetralin in awake cats. *J. Pharmacol. Exp. Ther.,* **291,** 229–238.

GARDIER, A.M., MALAGIE, I., TRILLAT, A.C., JACQUOT, C. & ARTIGAS, F. (1996). Role of 5-HT_{1A} autoreceptors in the mechanism of action of serotonergic antidepressant drugs: recent findings from in vivo microdialysis studies. *Fundam. Clin. Pharmacol.,* **10,** 16–27.

GARTSIDE, S.E., UMBERS, V., HAJOS, 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.

GOBBI, M., CRESPI, D., FODDI, M.C., FRACASSO, C., MANCINI, L., PAROTTI, L. & MENNINI, T. (1997). Effects of chronic treatment with fluoxetine and citalopram on 5-HT uptake, 5-HT_{1B} autoreceptors, 5-HT_3 and 5-HT_4 receptors in rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 22–28.

GOBERT, A. & MILLAN, M.J. (1999). Modulation of dialysate levels of dopamine, noradrenaline and serotonin (5-HT) in the frontal cortex of freely moving rats by (−)-pindolol alone and in association with 5-HT reuptake inhibitors: comparative roles of β-adrenergic, 5-HT_{1A} and 5-HT_{1B} receptors. *Neuropsychopharmacology,* **21,** 268–284.

GOBERT, A., RIVET, J.-M., CISTARELLI, L. & MILLAN, M.J. (1997). Potentation of the fluoxetine-induced increase in the frontal cortex of freely moving rats by combined blockade of 5-HT_{1A} and 5-HT_{1B} receptors with WAY100635 and GR127935. *J. Neurochem.,* **68,** 1159–1163.

GUNDLAH, C., HJORTH, S. & AUERBACH, S.B. (1997). Autoreceptor antagonists enhance the effect of the reuptake inhibitor citalopram on extracellular 5-HT: the effect persists after repeated citalopram treatment. *Neuropharmacology,* **36,** 475–482.

HAJOS, M., HAJOS-KORCSOK, E. & SHARP, T. (1999). Role of the medial prefrontal cortex in 5-HT_{1A} receptor-induced inhibition of 5-HT neuronal activity in the rat. *Br. J. Pharmacol.,* **126,** 1741–1750.

HERVAS, I., VILARO, M.T., MENGOD, G. & ARTIGAS, F. (1999). Modulation of the density and sensitivity of 5-HT_{1A} autoreceptors by prolonged treatment with fluoxetine alone or with WAY100635. *Soc. Neurosci. Abstr.,* **24,** 687.12.

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.

HJORTH, S. (1996). (−) Pindolol, but not buspirone, potentiates the citalopram-induced rise in extracellular 5-hydroxytryptamine. *Eur. J. Pharmacol.,* **303,** 183–186.

HJORTH, S. & AUERBACH, S.B. (1994). Lack of 5-HT_{1A} autoreceptor desensitization following chronic citalopram treatment, as determined by in vivo microdialysis. *Neuropharmacology,* **33,** 331–334.

HJORTH, S. & AUERBACH, S.B. (1996). 5-HT_{1A} autoreceptors and the mode of action of selective serotonin reuptake inhibitors (SSRI). *Behav. Brain Res.,* **73,** 281–283.

HJORTH, S., BENGTSSON, H.J. & MILANO, S. (1996). Raphe 5-HT_{1A} autoreceptors, but not postsynaptic 5-HT_{1A} receptors or β-adrenoceptors, restrain the citalopram-induced increase in extracellular 5-hydroxytryptamine in vivo. *Eur. J. Pharmacol.,* **316,** 43–47.

HJORTH, S. & CARLSSON, A. (1985). (−)-Pindolol stereo specifically inhibits rat brain serotonin (5-HT) synthesis. *Neuropharmacology,* **24,** 1143–1146.

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

HJORTH, S. WESTLIN, D. & BENGTSSON, H.J. (1997). WAY100635-induced augmentation of the 5-HT-elevating action of citalopram: relative importance of the dose of the 5-HT_{1A} (auto) receptor blocker versus that of the 5-HT reuptake inhibitor. *Neuropharmacology,* **36,** 461–465.

INVERNIZZI, R., BRAMANTE, M. & SAMANIN, R. (1994). Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: role of presynaptic 5-HT_{1A} receptors. *Eur. J. Pharmacol.,* **260,** 243–246.

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.

LE POUL, E., LAARIS, N., DOUCET, E., LAPORTE, A.M., HAMON, M. & LANFUMEY, L. (1995). Early desensitization of somatodendritic 5-HT_{1A} autoreceptors in rats treated with fluoxetine or paroxetine. *Naunyn-Schmeidebergs Arch. Pharmacol.,* **352,** 141–148.

LI, Q., MUMA, N.A. & VAN DE KAR L.D. (1996). Chronic fluoxetine induces a gradual desensitization of 5-HT_{1A} receptors: reductions in hypothalamic and midbrain Gi and G(o) proteins and in neuroendocrine responses to a 5-HT_{1A} agonist. *J. Pharmacol. Exp. Ther.,* **279,** 1035–1042.

MCASKILL, R., MIR, S. & TAYLOR, D. (1998). Pindolol augmentation of antidepressant therapy. *Br. J. Psychiatry,* **173,** 203–208.

PAXINOS, G. & WATSON, C. (1986). The rat brain in stereotaxic coordinates. Academic Press, New York.

PEREZ, V., GILABERTE, I., FARIES, D., ALVAREZ, E. & ARTIGAS, F. (1997). Randomized, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. *Lancet,* **349,** 1594–1597.

RAAP, D.K., EVANS, S., GARCIA, F., LI, Q., MUMA, N.A., WOLF, W.A., BATTAGLIA, G. & VAN DE KAR, L.D. (1999). Daily injections of fluoxetine induce dose-dependent desensitization of hypothalamic 5-HT_{1A} receptors: reductions in neuroendocrine responses to 8-OH-DPAT and in levels of Gz and Gi proteins. *J. Pharmacol. Exp. Ther.,* **288,** 98 – 106.

ROMERO, L., BEL, N., ARTIGAS, F., DE MONTIGNY, C. & BLIER, P. (1996a). Effects of pindolol on the function of pre- and postsynaptic 5-HT_{1A} receptors: in vivo microdialysis and electrophysiological studies in the rat brain. *Neuropsychopharmacology,* **15,** 349 – 360.

ROMERO, L., ILDEFONSO, H. & ARTIGAS, F. (1996b). The 5-HT_{1A} antagonist WAY100635 selectively potentiates the presynaptic effects of serotonergic antidepressants in rat brain. *Neurosci. Letts.,* **219,** 123 – 126.

RUTTER, J.J., GUNDLAH, C. & AUERBACH, S.B. (1994). Increase in extracellular serotonin produced by uptake inhibitors is enhanced after chronic treatment with fluoxetine. *Neurosci. Letts.,* **171,** 183 – 186.

SCHECHTER, L.E., MCGONIGLE, P. & BARRETT, J.E. (1999). Serotonergic antidepressants: Current and future perspectives. *Curr. Opin. Cent. Periph. Nerv. Syst. Invest. Drugs,* **1,** 432 – 447.

SHARP, T., UMBERS, V. & GARTSIDE, S.E. (1997). 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. *Br. J. Pharmacol.,* **121,** 941 – 946.

TOME, M.B., ISAAC, M.T., HARTE, R. & HOLLAND, C. (1997). Paroxetine and pindolol: a randomized trial of serotonergic autoreceptor blockade in the reduction of antidepressant latency. *Int. Clin. Psychopharmacol.,* **12,** 81 – 89.

ZANARDI, R., ARTIGAS, F., FRANCHINI, L., SFORZINI, L., GASPERINI, M., SMERALDI, E. & PEREZ, J. (1997). How long should pindolol be associated with paroxetine to improve the antidepressant response?. *J. Clin. Psychopharmacol.,* **17,** 446 – 450.

ZANARDI, R., FRANCHINI, L., GASPERINI, M., LUCCA, A., SMERALDI, E. & PEREZ, J. (1998). Faster onset of action of fluvoxamine in combination with pindolol in the treatment of delusional depression: a controlled study. *J. Clin. Psychopharmacol.,* **18,** 441 – 446.

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