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Effects of 5-HT_{1A} receptor antagonists on fluoxetine-induced changes in extracellular serotonin concentrations in rat frontal cortex

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Abstract

Clinical studies in which serotonin specific reuptake inhibitors have been co-administered with pindolol have demonstrated a shortened time to onset of antidepressant activity. This effect has been attributed to the antagonist effects of pindolol at the presynaptic 5-HT_{1A} receptor which augments the action of the serotonin specific reuptake inhibitors. In the present study, we demonstrate that acute fluoxetine-induced increases in extracellular serotonin concentrations, as measured by microdialysis in the frontal cortex, can be potentiated by 5-HT_{1A} receptor blockade using *N*-[2-[4-(2-methoxyphenyl)-1- piperazinyl]ethyl]-*N*-(pyridinyl)cyclohexanecarboxamide (WAY100635), the silent and selective 5-HT_{1A} receptor antagonist. WAY100635 at doses as low as 0.03 mg/kg s.c. maintained this potentiation effect across a range of fluoxetine doses. In addition, using antagonists with different intrinsic agonist activities for the 5-HT_{1A} receptor, we have determined that only compounds with very low intrinsic agonist activity can produce a potentiation of the acute fluoxetine-induced increases in extracellular serotonin. © 1998 Elsevier Science B.V.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Microdialysis, 5-HT_{1A} receptor; Fluoxetine, 5-HT_{1A} receptor antagonist; 5-HT_{1A} receptor agonist

1. Introduction

The involvement of serotonin (5-hydroxytryptamine, 5-HT) in the pathogenesis of depression and its mechanistic role in the action of antidepressant drugs has been well established (Delgado et al., 1990; Briley and Moret, 1993). The most direct evidence for this has come from the clinical efficacy of the serotonin specific reuptake inhibitors in the treatment of depression. These drugs exert their action by increasing the concentration of serotonin within the synaptic cleft by blocking its transport/reuptake and, hence enhancing serotonergic transmission (Hyttel, 1994). However, the onset of antidepressant activity requires a period of chronic treatment before this activity can be observed, usually 2–4 weeks (Blier and De Montigny, 1994; Gardier et al., 1996). Electrophysiological data has demonstrated the desensitization of presynaptic serotonin receptors during chronic treatment with serotonin specific reuptake inhibitors (Blier et al., 1987) and microdialysis studies have also shown an enhancement in extracellular serotonin following chronic treatment (Rutter et al., 1994; Invernizzi et al., 1996). This would suggest that the delayed onset of action of serotonin specific reuptake inhibitors is due to the action of the inhibitory somatodendritic serotonin receptors in response to the serotonin specific reuptake inhibitors-induced increases in extracellular serotonin. Clinical data using co-administration of a serotonin specific reuptake inhibitors with the β-adrenoceptor/5-HT_{1A} receptor antagonist pindolol, in small open trial studies, has demonstrated a shortened time to onset of antidepressant activity (Artigas et al., 1994; Blier and Bergeron, 1995) which has been attributed to the antagonist effects of pindolol at the presynaptic 5-HT_{1A} receptor, thus preventing desensitization. Conversely, more recent data using double blind clinical trials (Berman et al., 1997; Perez et al., 1997) has demonstrated little or no improvement in antidepressant activity. It is therefore clear that this is still an area of controversy.

The present study demonstrates how blockade of the somatodendritic 5-HT_{1A} receptor with the selective and silent antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl)cyclohexanecarboxamide (WAY-100635) (Forster et al., 1995) can potentiate acute fluoxetine-induced changes in extracellular serotonin concentrations in the frontal cortex of the rat. The relative degrees of

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blockade of both the 5-HT_{1A} receptor versus the serotonin transporter have also been analyzed. A range of antagonists/agonists were chosen due to their differing degrees of intrinsic agonist activity for the 5-HT_{1A} receptor. These compounds were: *N*-tert-butyl 3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropananamide dihydrochloride ((+)-WAY100135), a partial agonist (Escandon et al., 1994; Assie and Koek, 1996) with low detectable intrinsic activity (Routledge et al., 1993), 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]-8-azaspiro[4.5]decane-7,9-dione (buspirone), a known partial agonist (Sprouse and Aghajanian, 1987), and (*R*)-*N*-[2-[4[(1H-indol-4-yl)-1-piperazinyl]-1-methylethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide (WAY101363), a partial agonist with high antagonist affinity (Sukoff and Rosenzweig-Lipson, 1997) but detectable agonism. Using these compounds we have determined that only compounds with very low intrinsic agonist activity can produce a potentiation of these fluoxetine-induced increases in serotonin in the frontal cortex.

2. Materials and methods

All chemicals used were analytical grade and were purchased from Aldrich and Sigma chemicals (Milwaukee, WI). Buspirone was purchased from Research Biochemicals International (Natick, MA). WAY100635, WAY101363 and (+)-WAY100135 were synthesized by Chemical Sciences, Wyeth Ayerst Research (Princeton, NJ).

2.1. Surgical procedure

Male Sprague–Dawley rats (280–350 g Charles River) were used in all experiments. Animals were group housed in cages with food and water available ad libitum. Following surgery, the animals were singly housed in Plexiglass cages (45 cm^2) with food and water available ad libitum.

Following induction of anaesthesia with gaseous administration of halothane (2%) (Fluothane, Zeneca, Cheshire, UK) 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 and 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 animals shoulders. Both cannula were secured to the skull using dental acrylic (Plastics One, Roanoke, VA). The wound was sutured and the animals left to recover for 24 h in their home cages with free access to food and water.

2.2. 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 postsurgery. The probe was perfused with artificial cerebrospinal fluid (aCSF) (NaCl, 125 mM; KCl, 3.0 mM; $MgSO_4$, 0.75 mM and $CaCl_2$, 1.2 mM, pH 7.4) at a flow rate of 1 μl/min. 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 and 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. 5-HT_{1A} compounds or vehicle were injected, via the s.c. cannula followed 20 min later by the serotonin specific reuptake inhibitor or vehicle. A 20 min sampling regime was used throughout the experimental period. At the end of the experiment the probe placement was verified histologically and data from animals with incorrect probe placement was discarded.

2.3. Analysis of dialysates

Serotonin (5-HT) was separated by reverse phase high performance liquid chromatography (HPLC) (C18 ODS2 column, 100 × 3.0 mm, Metachem, Torrance, CA) and detected using an ANTEC electrochemical detector (ANTEC, Netherlands) set at a potential of 0.65 V versus a Ag/AgCl reference electrode. The mobile phase was delivered by a Jasco PU980 HPLC pump (Jasco, Essex, UK) at 0.6 ml/min and contained 0.15 M NaH_2PO_4 buffer at pH 4.3, 0.25 mM EDTA, 1.5 mM 1-octane sodium sulphonic acid and 5% isopropanol. Data was acquired using the XChrom software package (VG data systems, Altringham, UK).

2.4. 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. Results were analyzed by analysis of variance (ANOVA) with repeated measures followed by post hoc Duncans using the Super ANOVA software application (Abacus Concepts, Berkeley, CA, 1989) on an Apple Macintosh computer.

3. Results

3.1. Effects of fluoxetine concentration on WAY100635-induced potentiation of extracellular serotonin concentrations

In order to determine the effects of increasing serotonin transporter blockade on extracellular serotonin concentra-

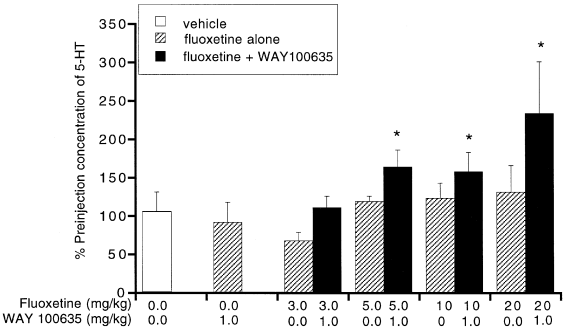

Fig. 1. Maximum effects of increasing dose of fluoxetine on extracellular serotonin concentrations in the presence and absence of 1 mg/kg WAY100635. * Denotes statistical difference $P < 0.05$ (fluoxetine versus fluoxetine + WAY100635). Mean ± S.E.M., $n = 6$ unless otherwise stated.

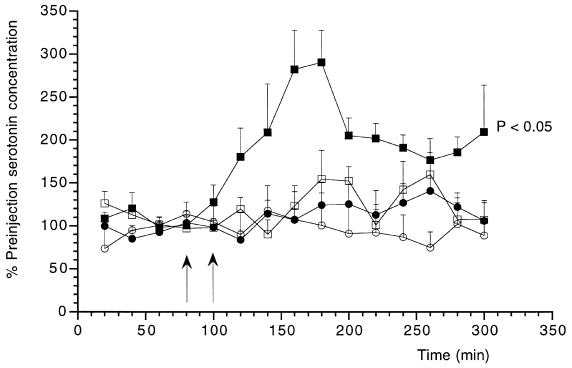

Fig. 2. Effects of different doses of WAY100635 on extracellular serotonin concentrations when given in combination with fluoxetine (20 mg/kg). (○) 1 mg/kg WAY100635/vehicle treated animals ($n = 6$), (●) vehicle/fluoxetine treated animals ($n = 10$), (□) 0.01 mg/kg WAY100635/fluoxetine treated animals ($n = 7$), (■) 0.03 mg/kg WAY100635/fluoxetine treated animals ($n = 6$). Data expressed as mean ± S.E.M., n numbers per study group are denoted in parentheses. Arrows denote drug or vehicle injection points.

tions following antagonism of the 5-HT_{1A} receptor, animals were administered with WAY100635 (1 mg/kg, s.c.) followed by the serotonin specific reuptake inhibitor fluoxetine (3–20 mg/kg, s.c.) (Fig. 1). WAY100635 alone had no effect on extracellular serotonin concentrations. The lowest dose of fluoxetine (3 mg/kg) alone and in the presence of WAY100635, produced no significant increases in extracellular serotonin concentrations. Again, fluoxetine alone at the slightly higher dose of 5 mg/kg produced no significant increase in extracellular serotonin concentrations. However, after administration of WAY100635 (1 mg/kg) a significant ($P < 0.05$) increase was observed reaching a maximum value of 185 ± 41%. Fluoxetine at 10 and 20 mg/kg produced small, but non-significant, increases in extracellular serotonin with maximum values of 123 ± 20 and 126 ± 23%, respectively (Fig. 1). However, these increases were significantly ($P < 0.05$) potentiated by the prior administration of 1 mg/kg WAY100635 with the maximum effect being observed with 20 mg/kg (234 ± 67%) (Fig. 1).

3.2. Effects of WAY100635 concentration on fluoxetine-induced changes in extracellular serotonin concentrations

The dose of fluoxetine which produced the greatest observable potentiation effect with 1 mg/kg WAY100635 (i.e., 20 mg/kg) was then used to determine an effective dose response to 5-HT_{1A} receptor blockade. WAY100635 (0.01–0.3 mg/kg s.c.) was administered prior to 20 mg/kg of fluoxetine. Doses of 0.03, 0.1 and 0.3 mg/kg produced significant ($P < 0.05$) increases in extracellular serotonin concentrations when administered prior to fluoxetine, with maximum values of 291 ± 37, 236 ± 43 and 249 ± 74%, respectively. The greatest potentiation was observed for 0.03 mg/kg (Fig. 2), however, this was not statistically different from that produced by higher doses. The lowest dose of WAY100635 (0.01 mg/kg) produced no significant increase in extracellular serotonin concentrations (Fig. 2). To determine whether the lowest effective dose of WAY100635 (0.03 mg/kg) could maintain this effect at lower serotonin specific reuptake inhibitors concentrations, this experiment was repeated using 5 mg/kg of fluoxetine. Potentiation was observed comparable to that seen previously with 1 mg/kg WAY100635 and 5 mg/kg fluoxetine with a maximum value of 191 ± 37%.

3.3. Effects of 5-HT_{1A} agonism on fluoxetine-induced changes in extracellular serotonin concentrations

A number of 5-HT_{1A} receptor agonist were chosen with varying degrees of intrinsic activity and were administered at doses known to produce postsynaptic antagonism when tested against the 5-HT_{1A} receptor agonist 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) in vivo (Sukoff and Rosenzweig-Lipson, 1997). Buspirone (0.1 and 1 mg/kg, s.c.), when administered alone, produced a reduction in

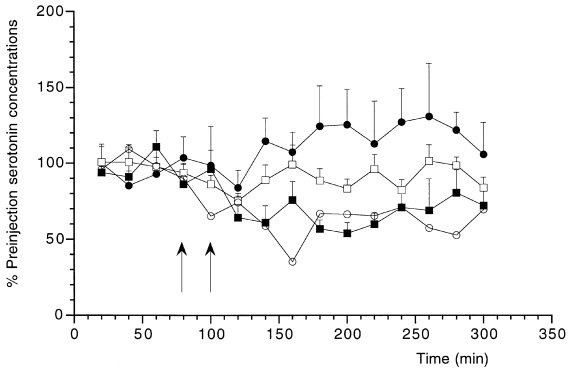

Fig. 3. Effects of buspirone on extracellular serotonin concentrations when given in combination with fluoxetine (20 mg/kg). (○) 1 mg/kg buspirone/vehicle treated animals ($n = 6$), (●) vehicle/fluoxetine treated animals ($n = 10$), (□) 0.1 mg/kg buspirone/fluoxetine treated animals ($n = 5$), (■) 1 mg/kg buspirone/fluoxetine treated animals ($n = 6$). Data expressed as mean ± S.E.M., n numbers per study group are denoted in parentheses. Arrows denote drug or vehicle injection points.

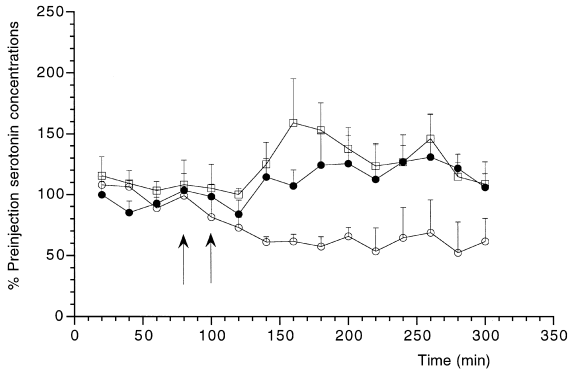

Fig. 4. Effects of WAY101363 on extracellular serotonin concentrations when given in combination with fluoxetine (20 mg/kg). (○) 1 mg/kg WAY101363/vehicle treated animals ($n = 6$), (●) vehicle/fluoxetine treated animals ($n = 10$), (□) 1 mg/kg WAY101363/fluoxetine treated animals ($n = 6$). Data expressed as mean ± S.E.M., n numbers per study group are denoted in parentheses. Arrows denote drug or vehicle injection points.

basal serotonin release reaching a maximum value of 53 ± 13% of preinjection concentrations at 1 mg/kg. Similarly, both doses of buspirone (0.1 and 1 mg/kg) still produced a decrease in extracellular serotonin when given in combination with 20 mg/kg fluoxetine (Fig. 3) with maximum values of 83 ± 6 and 53 ± 7%, respectively. WAY 101363 (1 mg/kg) produced a decrease in basal serotonin concentration to 52 ± 25% of preinjection controls. However, when given in conjunction with 20 mg/kg of fluoxetine the same non significant increase induced by fluoxetine alone was maintained (Fig. 4). (+)WAY100135 administered alone did not affect basal serotonin concentration values at either 1 or 10 mg/kg. When administered prior to fluoxetine (20 mg/kg) 1 mg/kg of WAY100135 produced an increase in serotonin concentration to a maximum value of 178 ± 18%. Similarly, 10 mg/kg produced a further significant ($P < 0.05$) augmentation to a maximum of 227 ± 18% (Fig. 5).

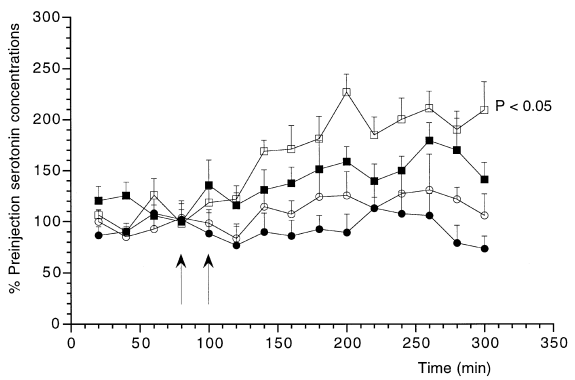

Fig. 5. Effects of (+)WAY 100135 on extracellular serotonin concentrations when given in combination with fluoxetine (20 mg/kg). (●) 10 mg/kg WAY100135/vehicle treated animals ($n = 5$), (○) vehicle/fluoxetine treated animals ($n = 10$), (■) 1 mg/kg WAY 100135/fluoxetine treated animals ($n = 8$), (□) 10 mg/kg WAY 100135/fluoxetine treated animals ($n = 6$). Data expressed as mean ± S.E.M., n numbers per study group are denoted in parentheses. Arrows denote drug or vehicle injection points.

4. Discussion

Administration of single doses of the serotonin specific reuptake inhibitor, fluoxetine (5–20 mg/kg) did not produce significant increases in extracellular serotonin levels in the frontal cortex. The effects of fluoxetine were not dose-related and reached a maximum value of only 125% after 20 mg/kg; however, it should be noted that as the dose increased the response became more variable indicating some evidence of a dose-relationship. These observations are consistent with acute systemic administration of various serotonin specific reuptake inhibitors (Adell and Artigas, 1991; Hjorth, 1993; Invernizzi et al., 1996). It is known that systemic treatment with fluoxetine can produce variable increases in extracellular serotonin in terminal regions which is paralleled by increases of greater magnitude in the somatodendritic cell body regions of the dorsal raphe (Malagie et al., 1995). Again, this is an effect seen with other serotonin specific reuptake inhibitors (Adell and Artigas, 1991; Invernizzi et al., 1992; Gartside et al., 1995). It is well known that elevated extracellular serotonin concentrations within the somatodendritic regions will suppress serotonergic neuronal cell firing via its action at the inhibitory presynaptic 5-HT_{1A} receptor. Blockade of these receptors, by prior administration of the silent and selective 5-HT_{1A} receptor antagonist WAY100635 (1 mg/kg, s.c.), prevented this inhibition. Thus, a much larger and significant dose-related augmentation of terminal extracellular serotonin concentration was observed in the presence of the 5-HT_{1A} receptor antagonist. The maximum increase/potentiation was observed at 20 mg/kg of fluoxetine, therefore this dose was used to determine the degree of 5-HT_{1A} receptor antagonism required to maintain this presynaptic block and hence potentiation of serotonin levels.

WAY100635, when administered alone, produced no change in extracellular serotonin concentrations, indicating that under these conditions, the somatodendritic 5-HT_{1A} receptors are not tonically activated by endogenous neurotransmitter, as previously reported (Fletcher et al., 1994). WAY100635 at doses of 0.3, 0.1 and 0.03 mg/kg were all similarly effective at producing an augmentation of the 20 mg/kg fluoxetine-induced effect; in fact, 0.03 mg/kg of WAY100635 appeared to produce a more robust increase than higher doses, however, this was not statistically different. The lowest dose failed to produce any significant effect suggesting that presynaptic 5-HT_{1A} receptor blockade by WAY100635 falls below a critical level somewhere between these two drug concentrations, which brings about this loss of effect. By means of a comparison, the lowest effective dose of WAY100635 (0.03 mg/kg) was tested against the lowest effective dose of fluoxetine to produce a combination effect (i.e. 5 mg/kg) and again a potentiation,

comparable to that seen previously with the higher dose of the 5-HT_{1A} receptor antagonist, was observed. These data are in agreement with those presented by Hjorth et al. (1996) using the serotonin specific reuptake inhibitor, citalopram, which demonstrated a comparable dose response to WAY100635. It would appear, from this data, that an adequate blockade of the serotonin transporter is a prerequisite for this interaction between serotonin specific reuptake inhibitor and 5-HT_{1A} receptor antagonists to occur. Moreover, the degree to which uptake is inhibited may be all important in determining the extent to which extracellular serotonin concentrations are elevated, at least when administered acutely. Concurrently, presynaptic 5-HT_{1A} receptor antagonism does not affect the magnitude of the augmentation, but the degree of receptor blockade appears to be critical in determining whether the effect actually occurs at all. Also, clinically it may be advantageous to keep 5-HT_{1A} receptor antagonism to the minimum level if the therapeutic site in the antidepressant action is the postsynaptic 5-HT_{1A} receptor, as has been suggested (Stahl, 1994).

A variety of compounds, with a range of antagonist/agonist activity profiles for the 5-HT_{1A} receptor, were chosen to determine whether the effect observed with WAY100635 could be reproduced. Buspirone posses relatively high affinity for the 5-HT_{1A} receptor and is a known partial agonist (Sprouse and Aghajanian, 1987). When administered alone, buspirone produced a decrease in extracellular serotonin concentrations which is consistent with this compound's demonstrated agonist activity at the presynaptic 5-HT_{1A} receptor (Sharp et al., 1989; Routledge et al., 1993). When administered prior to fluoxetine, buspirone produced no evidence of a potentiation of extracellular serotonin concentrations at either dose tested, again suggesting that it is acting as an agonist at the 5-HT_{1A} autoreceptor. It should be noted that buspirone shows action at α-adrenoceptors and dopamine D_2 receptors (Hjorth and Carlsson, 1982), however at these low doses this is not likely to be significant. Interestingly, buspirone has been shown to enhance the efficacy of serotonin specific reuptake inhibitors in the clinic (Jacobsen, 1991; Joffe and Schuller, 1993; Blier and Bergeron, 1995). However, this data would indicate that buspirone's action is mediated by a mechanism other than potentiation of serotonin specific reuptake inhibitor-induced serotonin release in the forebrain, as suggested by Gobert et al. (1997). WAY101363 is a high affinity 5-HT_{1A} receptor antagonist (Sukoff and Rosenzweig-Lipson, 1997) which when administered alone, produces a decrease in extracellular serotonin which can be reversed by prior treatment with WAY100635 (data not shown), thus demonstrating this molecule to have agonist activity at the presynaptic 5-HT_{1A} receptor and hence to be a partial agonist. When this compound was given in combination with fluoxetine no significant increases in serotonin were observed. As with buspirone this lack of augmentation can be attributed to WAY101363's agonist characteristics. (+)WAY100135 has been shown to be a silent and selective 5-HT_{1A} receptor antagonist (Routledge et al., 1993). However, more recent studies (Escandon et al., 1994; Assie and Koek, 1996) have shown this compound to be a partial agonist. When this molecule was administered alone there was no effect on extracellular serotonin at either dose tested, demonstrating this molecule to have no detectable intrinsic agonist activity within this assay system. When given in combination with fluoxetine an augmentation was observed and this increased in a dose-dependent manner. These data demonstrate that molecules which exhibit agonist-like activity at the presynaptic 5-HT_{1A} receptor are unable to produce any augmentation of extracellular serotonin concentrations within the frontal cortex when given in combination with a serotonin specific reuptake inhibitor, such as fluoxetine. Indeed, it would appear from this study that only molecules which posses little or no intrinsic agonist activity can produce any improvement on serotonin concentrations above that produced acutely by fluoxetine.

The postulated role of 5-HT_{1A} receptor antagonists combined with a serotonin specific reuptake inhibitor in the therapeutic treatment of depression is unclear. Pindolol, in combination with a serotonin specific reuptake inhibitor, has been demonstrated to induce a faster rate of onset of antidepressant action in the clinic (Artigas et al., 1994; Blier and Bergeron, 1995; Perez et al., 1997) and has also been shown to produce augmentation of forebrain serotonin concentrations when acutely administered in combination with various serotonin specific reuptake inhibitors (Dreshfield et al., 1996; Hjorth, 1996; Hjorth and Auerbach, 1996; Romero et al., 1996). If pindolol is inducing its therapeutic effects via its antagonistic action at the presynaptic 5-HT_{1A} receptor, alleviating the need for desensitization, then molecules which are silent antagonists, such as WAY100635, should produce a similar more rapid onset of antidepressant activity when given in combination with an serotonin specific reuptake inhibitor. Clearly, the theoretical role of the 5-HT_{1A} receptor in the mechanism of therapeutic enhancement of serotonin specific reuptake inhibitor-induced antidepressant action, as supported by the acute neurochemical data presented here, will remain unproven until more selective compounds can be tested clinically.

In summary, the present study demonstrates how blockade of the somatodendritic 5-HT_{1A} receptor, with a selective antagonist, can potentiate extracellular serotonin concentrations in the frontal cortex of the rat when acutely administered in combination with fluoxetine. It appears that serotonin transporter blockade is a prerequisite for this effect to occur. The relative degrees of blockade of both the presynaptic 5-HT_{1A} receptor versus the serotonin transporter suggest that sufficient antagonism of the 5-HT_{1A} receptor is critical, but once this level has been attained the magnitude of the potentiation effect depends solely on the degree of transport blockade. In addition, using antagonists

with a variety of intrinsic agonist/antagonist activities for the 5-HT_{1A} receptor, we have determined that only compounds with very low intrinsic agonist activity can produce an augmentation of fluoxetine-induced changes in extracellular serotonin.

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