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European Journal of Pharmacology 486 (2004) 25–30
www.elsevier.com/locate/ejphar

Chronic fluoxetine-induced desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors: regional differences and effects of WAY-100635

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Received 11 September 2003; received in revised form 26 November 2003; accepted 5 December 2003

Abstract

Desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors is thought to be the mechanism underlying the therapeutic effects of fluoxetine and other selective serotonin reuptake inhibitors when these are administered chronically. The blockade of 5-HT_{1A} autoreceptors occurring on administration of a selective serotonin reuptake inhibitor together with a 5-HT_{1A} autoreceptor antagonist is responsible for the acute increase in 5-hydroxytryptamine (serotonin, 5-HT) levels observed under these circumstances. The effects of repeated administration of selective serotonin reuptake inhibitors together with 5-HT_{1A} receptor antagonists have not been widely studied. In this work, we found that the effects of fluoxetine (5 mg/kg, i.p., daily for 12 days) to desensitize 5-HT_{1B} autoreceptors in the frontal cortex, as measured by the effect of the locally administered 5-HT_{1B} receptor agonist, 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one (CP 93129), and to desensitize 5-HT_{1A} autoreceptors as measured by the action of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT; 50 μg/kg, s.c.) to reduce 5-HT levels in cortex, were prevented by concomitant administration of the 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY-100635; 0.3 mg/kg, s.c.). 5-HT_{1B} receptor activity in the hypothalamus, as measured by the effects of locally administered CP 93129, and 5-HT_{1A} autoreceptor activity, as determined by the effects of subcutaneous 8-OH-DPAT to reduce 5-HT levels in hypothalamus, were not altered either by fluoxetine alone or by fluoxetine in the presence of WAY-100635. The data suggest that the regulation of extracellular levels of 5-HT in the cortex and hypothalamus is subject to different autoregulatory mechanisms.
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Keywords: 5-HT (5-hydroxytryptamine, serotonin); Antidepressant; Fluoxetine; 5-HT_{1A} receptor; 5-HT_{1B} receptor; Microdialysis

1. Introduction

Selective serotonin (5-HT) reuptake inhibitors have become very popular because of their effectiveness in treating several mood disorders, including depression, anxiety, and eating disorders. However, the delay after onset of administration until a therapeutic effect is observed remains one of the major problems associated with the use of antidepressant drugs in the treatment of depression. A further problem is that many patients do not respond to any of the drugs in clinical use. Several strategies have been proposed to overcome these problems, notably the use of potentiating agents, which themselves may not have therapeutic effects, to augment or accelerate the effects of established antidepressants. The observation made in many laboratories—that the effect of acute administration of a selective serotonin reuptake inhibitor such as fluoxetine to increase synaptic 5-HT levels is potentiated by simultaneous administration of an antagonist of 5-HT_{1A} or 5-HT_{1B} autoreceptors—resulted in the development of pindolol as an augmenting or accelerating agent. It was reasoned (Artigas et al., 1994) that antagonist administration mimicked the effect of chronic administration of a selective serotonin reuptake inhibitor, which induces desensitization of both types of autoreceptor (Blier and de Montigny, 1994, 1998; Pineyro and Blier, 1999). However, clinical trials with pindolol were equivocal (for reviews, see McAskill et al., 1998; Artigas et al., 2001). In addition, the mechanism proposed for the action of this agent (Artigas et al., 1994) was based on the results of acute animal experiments only, and did not take into account the fact that pindolol and the selective serotonin reuptake inhibitor must be taken repeatedly before a therapeutic

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doi:10.1016/j.ejphar.2003.12.008

action is observed. More recently, three studies have investigated the effects of chronic (2 week) administration of fluoxetine in combination with either pindolol, which is in fact a partial agonist at presynaptic 5-HT_{1A} autoreceptors and an antagonist at 5-HT_{1B} autoreceptors, or the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY-100635). Dawson et al. (2000) observed that while either pindolol or fluoxetine given alone desensitized 5-HT_{1A} autoreceptors, in the presence of fluoxetine, pindolol prevented the fluoxetine-induced desensitization. Similar results were obtained by Hervas et al. (2001) and Dawson et al. (2002) using WAY-100635. In all these experiments, however, only 5-HT_{1A} autoreceptor activity, and not 5-HT_{1B} autoreceptor activity, was determined. Recent results from our laboratory as well as others (Sayer et al., 1999; Newman et al., 2000; Gur et al., 2000, 2002) have shown that desensitization of 5-HT_{1B} autoreceptors, particularly in the hypothalamus, occurs after long-term administration of a variety of antidepressant drugs and treatments such as the tricyclics desimipramine and clomipramine, the serotonin and noradrenaline reuptake inhibitor venlafaxine, and transcranial magnetic stimulation, as well as after chronic administration of selective serotonin reuptake inhibitors.

In the present work, we have determined the effects of 12-day combined fluoxetine and WAY-100635 administration on basal 5-HT levels and 5-HT_{1A} and 5-HT_{1B} autoreceptor activity in the frontal cortex and hypothalamus. Although it is now established that activation of postsynaptic 5-HT_{1A} receptors on cortical—probably glutamatergic—neurons may also inhibit firing of serotonergic neurons and thus exert a negative effect on 5-HT release in nerve terminal areas (Casanovas et al., 1999; Hajos et al., 1999), the term "5-HT_{1A} autoreceptors" will be used in this paper to denote both the 5-HT_{1A} autoreceptors located somatodendritically on serotonergic neurons in the raphe nuclei and the subset of postsynaptic 5-HT_{1A} receptors in the cortex that influences 5-HT release.

2. Materials and methods

2.1. Treatment of animals

Male Albino rats (Sabra strain, derived from Wistar strain) were used in all experiments. The rats were housed by treatment group in a temperature-controlled environment (24 °C) with a regular 12 h light/dark cycle. Food and water were freely available. Fluoxetine was administered at 5 mg/kg by intraperitoneal injection on each treatment day, and WAY-100635 at 0.3 mg/kg was administered 1 h later by subcutaneous injection. This procedure was adopted instead of simultaneous injections since Taber et al. (2000) showed in acute experiments that the increase in cortical 5-HT levels obtained when WAY-100635 was injected 80 min after fluoxetine was more than twice that observed when the two compounds were coadministered. Injections were begun in a staggered manner so that a 3-day experimental period (1 day for implantation of guides and probes and 2 days for collection of fractions) was available for each pair of animals.

2.2. Implantation and perfusion of microdialysis probes

The microdialysis experiments were performed simultaneously on three animals, using a Bioanalytical Systems (BAS) "Raturn" interactive awake animal system. One day after the last injection, animals were anaesthesised with a 17:3 mixture of ketamine (100 mg/ml) and xylazine (2%) and mounted in a stereotaxic apparatus. Guides for dialysis probes (CMA/12) were implanted into the frontal cortex at anterior 3.2 mm from bregma, 2.5 mm lateral, and 2.0 mm vertical, and into anterior hypothalamus at anterior 1.5 mm from bregma, 1.3 mm lateral, and 7.0 mm vertical. A subcutaneous cannula was also implanted at the back of the neck and secured to the skull with screws and dental acrylic. The rats were maintained under anesthesia for approximately 1 h, after which they were free-moving and had unlimited access to food and water. Dialysis probes (4 mm for cortex and 2 mm for hypothalamus) were inserted into the guides towards the end of the period of anesthesia. The inlets of the probe were connected, through plastic tubing with an internal volume of 12 μl/m, to 1-ml gas-tight syringes mounted on a microinfusion pump. The inlet and outlet tubing of the probe were mounted to a steel wire running from the head of the rat to a balanced arm. Movement of the turntable of the "Raturn" allowed the animal to rotate and rear without entangling the fluid tubing. The probes were perfused with Ringer's solution containing 2.25 mM $CaCl_2$, 4 mM KCl, 147 mM NaCl, and 10 μM citalopram, pH 6.5, at 0.2 μl/min overnight. The following morning, the flow rate was increased to 0.5 μl/min, and 30-min fractions were collected. After each experiment, the dialysis probes were removed under anesthesia, sterilised in alcohol, and, if still intact, reinserted into new animals. The animal procedures outlined above received the approval of the Institutional Animal Care and Use Committee of the Hebrew University Faculty of Medicine and Dental Medicine and Hadassah Medical Organization.

2.3. 5-HT receptor challenges

On the second experimental day for each animal, fractions were injected into the high-performance liquid chromatography (HPLC) apparatus immediately after collection for measurement of 5-HT. Once stable baseline 5-HT levels had been obtained, usually after collecting four or five experimental samples, the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT; 50 μg/kg), was injected via the subcutaneous cannula. A further six fractions were then collected. On the following day, once stable baseline 5-HT levels had been obtained, the 5-$HT_{1B/1D}$

Table 1
Basal levels of 5-HT in microdialysates from rats administered fluoxetine and WAY-100635

Treatment	Cortex	Hypothalamus
Saline	12.4 ± 1.9 (13)	16.8 ± 4.6 (14)
Fluoxetine (5 mg/kg daily for 12 days)	27.5 ± 7.1 (17)	11.0 ± 1.6 (14)
Fluoxetine + WAY-100635 (0.3 mg/kg daily for 12 days)	33.4 ± 9.3 (14)	14.5 ± 2.7 (14)

Results are expressed as femtomoles per 5 μl of dialysate, and are mean ± S.E.M. of the number of observations in parentheses.

receptor agonist, 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo [3,2-*b*]pyrid-5-one (CP 93129), at a concentration of 10 μM was infused into the cortex and into the hypothalamus via the microdialysis probes during two fractions (i.e., for 60 min) and a further four or five fractions were collected.

2.4. Determination of 5-HT levels

Concentrations of 5-HT were determined by a BAS HPLC system. Samples were injected immediately after collection using a Rheodyne 9125 injector with a 5-μl injection loop. The mobile phase was made up of 90 mM sodium dihydrogen phosphate, 10 mM NaCl, 0.5 mM EDTA, 0.15g/l sodium octyl sulphate, and 10.5% acetonitrile, pH 5, and was delivered by the HPLC pump at 1.0 ml/min. The mobile phase was passed through a flow splitter and pumped through a 10 cm C-18 5-mm reversed-phase column at 0.1 ml/min. 5-HT content was analysed with a LC-4C electrochemical detector (BAS) with a glassy carbon working electrode set at 550 mV vs. an Ag/AgCl reference electrode. Concentrations of 5-HT were calculated by comparing peak levels from the microdialysis samples with those of external standards of known concentrations of 5-HT. The detection limit was 0.5–1 fmol. The average of the first four or five baseline samples was taken as 100%.

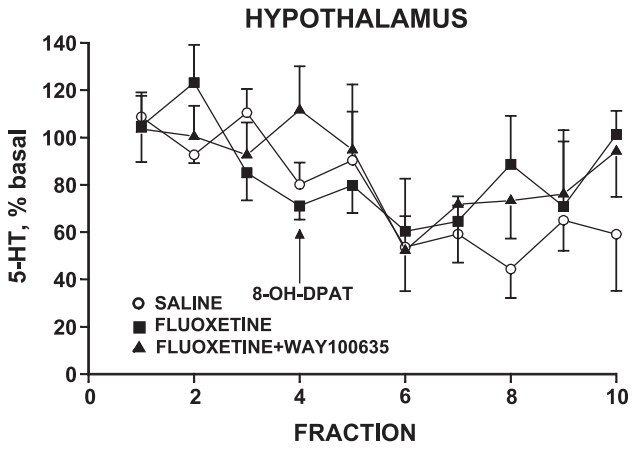

Fig. 2. Effects of fluoxetine and a combination of fluoxetine and WAY-100635 on the action of 8-OH-DPAT to reduce 5-HT levels in hypothalamus. Results are mean of data from five to six animals in each.

2.5. Data analysis

5-HT levels expressed as percentages of the initial levels for each animal were analysed over the time course for each challenge by two-way analysis of variance (ANOVA), with treatment as a ''between-groups'' variable and time (fraction number) as a ''within-groups'' variable (i.e., as a repeated measure), followed by the use of planned comparisons.

3. Results

Basal 5-HT levels were elevated in the frontal cortex of rats, which received fluoxetine either alone or together with WAY-100635, compared to the levels in rats that received saline (Table 1). A planned comparison ANOVA showed a significant overall effect of fluoxetine ($F[1,41] = 4.08$, $P = 0.05$). In hypothalamus, basal 5-HT levels did not differ significantly among the three groups of rats.

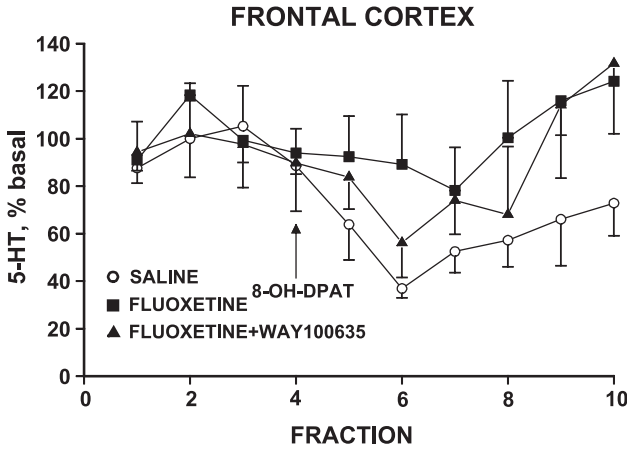

Fig. 1. Effects of fluoxetine and a combination of fluoxetine and WAY-100635 on the action of 8-OH-DPAT to reduce 5-HT levels in frontal cortex. Results are mean of data from five to six animals in each group.

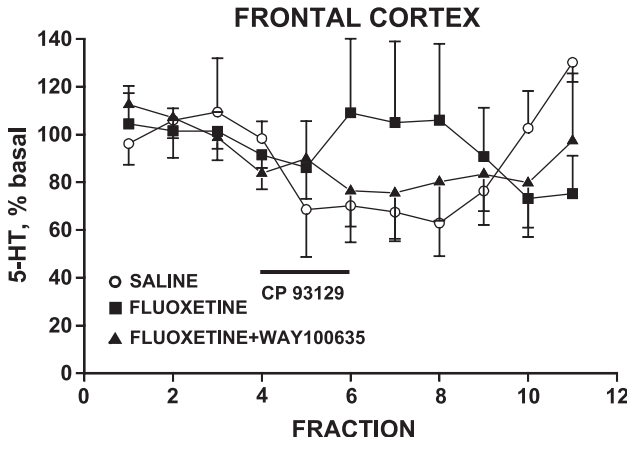

Fig. 3. Effects of fluoxetine and a combination of fluoxetine and WAY-100635 on 5-HT-1B autoreceptor activity in frontal cortex. Results are mean of data from five to eight animals in each group.

5-HT_{1A} receptor activity as measured by the effect of 8-OH-DPAT in the frontal cortex was reduced after fluoxetine administration but not after the combination of fluoxetine and WAY-100635. Overall analysis of the data for the action of 8-OH-DPAT in frontal cortex (Fig. 1) showed a significant effect of treatment ($F[2,14]=3.55$, $P=0.05$) and a significant effect of time after the administration of 8-OH-DPAT ($F[6,84]=6.01$, $P=0.00003$). Planned comparisons for the effect of treatment on the response to 8-OH-DPAT showed a significant difference between animals that received fluoxetine and animals that received saline ($F[1,14]=6.71$, $P=0.021$), while the other paired comparisons were not significant.

5-HT_{1A} receptor activity as measured by the effect of 8-OH-DPAT in the hypothalamus (Fig. 2) showed a significant effect of time after administration of 8-OH-DPAT only ($F[6,72]=2.59$, $P=0.024$). There was no significant effect of treatment, indicating that there were no differences between the groups of animals.

Analysis of the data for 5-HT_{1B} receptor activity in the frontal cortex (Fig. 3), as measured by the effect of the 5-HT_{1B} agonist CP-93129 at 10 μM to reduce 5-HT levels, showed a significant interaction between treatment and time after administration of CP-93129 ($F[14,126]=1.83$, $P=0.041$). The response to the 5-HT_{1B} agonist was reduced in fluoxetine-treated animals compared to that in animals that had received saline, while in animals that had received fluoxetine and WAY-100635, an intermediate response was observed, indicating that WAY-100635 had partially prevented the fluoxetine-induced desensitization of 5-HT_{1B} receptors.

Analysis of the data for 5-HT_{1B} receptor activity in the hypothalamus (Fig. 4) showed a significant effect of time after administration of CP-93129 only ($F[5,70]=4.37$, $P=0.001$) but no effect of treatment, indicating that neither fluoxetine nor the combination of fluoxetine and WAY-100635 affected 5-HT_{1B} receptor activity in this brain area.

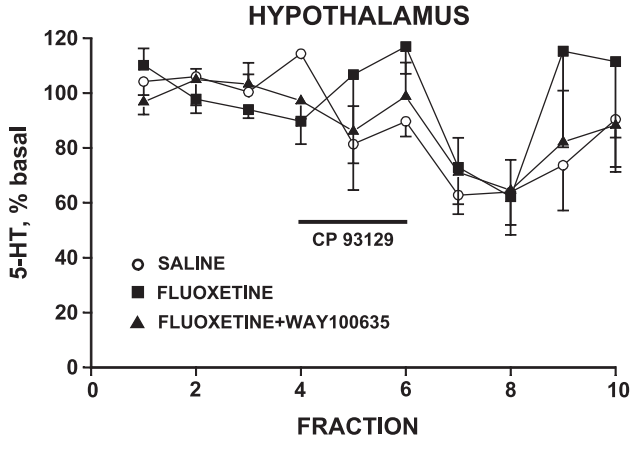

Fig. 4. Effects of fluoxetine and a combination of fluoxetine and WAY-100635 on 5-HT-1B autoreceptor activity in hypothalamus. Results are mean of data from five to six animals in each group.

4. Discussion

Chronic administration of fluoxetine has been shown in a large number of studies, using both electrophysiological (LePoul et al., 1995, 2000) and in vivo microdialysis methods (Rutter et al., 1994; Kreiss and Lucki, 1995; Invernizzi et al., 1996; Dawson et al., 2000, 2002) to induce subsensitivity of 5-HT_{1A} autoreceptors in the rat brain. In the majority of these studies, measurements were performed in the frontal cortex, and chronic fluoxetine administration led to an increase in 5-HT release. The present results show that fluoxetine, both in the presence and absence of WAY-100635, increased basal 5-HT release in the frontal cortex but not in the hypothalamus. Extracellular 5-HT levels are controlled by the 5-HT transporter, somatodendritic 5-HT_{1A} receptors, 5-HT_{1B} autoreceptors, and also postsynaptic 5-HT_{1A} receptors, and differential regulation of any of these by fluoxetine in different brain regions may contribute to the regional differences in basal 5-HT release. Indeed, our results show that 5-HT_{1A} autoreceptor activity, as measured by the effect of 8-OH-DPAT in the frontal cortex, and 5-HT_{1B} autoreceptor activity in the frontal cortex were decreased by fluoxetine while the respective parameters in the hypothalamus were unaffected. Previous work using citalopram (Invernizzi et al., 1994, 1995) has shown that chronic administration of SSRI led to desensitization of 5-HT_{1A} autoreceptors as shown by the effect of 8-OH-DPAT to reduce 5-HT levels in the cortex but not in the hippocampus. This regional difference may be related to the higher density of 5-HT_{1A} receptors in the dorsal raphe, which innervates the frontal cortex, as opposed to the median raphe, which innervates the dorsal hippocampus. The anterior hypothalamus, like the dorsal hippocampus, is chiefly innervated by the median raphe (Van de Kar and Lorens, 1979), and 5-HT release in this area may thus not be under the stringent control of 5-HT_{1A} autoreceptors. Although 10 μM citalopram was present in the perfusion fluid used in the present experiments, this concentration partially inhibits 5-HT uptake in a small area surrounding the probe only, and causes little activation of 5-HT autoreceptors (Celada et al., 2002). Concentrations in the region of 50 μM are required to significantly activate 5-HT_{1A} receptors and reduce 5-HT release (Romero and Artigas, 1997). In any case, citalopram was present during the microdialysis procedure in both saline-treated and fluoxetine-treated rats in the present experiment, so that any effect of citalopram would be manifested to an equal extent in all animals.

The present results showed that in addition to preventing fluoxetine-induced desensitization of 5-HT_{1A} autoreceptors as measured by the effect of subcutaneous 8-OH-DPAT on 5-HT levels in the frontal cortex, WAY-100635 also prevented fluoxetine-induced desensitization of 5-HT_{1B} autoreceptors in the frontal cortex. Such "heterologous" desensitization is a novel finding that has not been reported before, and appears to represent a form of "cross-talk" between 5-HT_{1A} and 5-HT_{1B} receptors. "Cross-talk" effects have been

reported in knockout mice lacking either 5-HT_{1A} or 5-HT_{1B} receptors. In 5-HT_{1A} receptor knockout mice, Knobelman et al. (2001), using in vivo microdialysis, found that the effect of the 5-HT_{1B} receptor agonist, CP 94253, to reduce 5-HT levels in striatum was actually increased, suggesting a compensatory supersensitivity of 5-HT_{1B} receptors. A similar effect was observed by Gardier et al. (2001) in 5-HT_{1B} knockout mice. In these mice, the efficacy of 8-OH-DPAT to induce hypothermia was increased, suggesting an increase in activity of the somatodendritic 5-HT_{1A} receptors. However, the reverse situation has also been described. Knobelman et al. (2001) found that in 5-HT_{1B} knockout mice, the action of 8-OH-DPAT to reduce 5-HT levels in the ventral hippocampus was reduced, suggesting desensitization of the somatodendritic 5-HT_{1A} receptors situated in the median raphe. Similarly, Ase et al. (2002) reported decreased G-protein coupling of 5-HT_{1A} receptors in the brains of 5-HT_{1B} knockout mice. Clearly, the compensatory responses that occur in knockout mice, which are deficient in a particular receptor from birth, could not be expected to occur during a 12-day period of administration of a 5-HT_{1A} receptor antagonist. However, the lack of desensitization of 5-HT_{1B} receptors in rats administered the combination of fluoxetine and WAY-100635 suggests that a compensatory change may have taken place at these receptors and that such a change is only evident upon increased levels of 5-HT in the synaptic gap during prolonged treatment with fluoxetine.

Our finding of a reduction in cortical 5-HT_{1B} autoreceptor activity after chronic administration of fluoxetine provides a functional correlate for the data of Neumaier et al. (1996), who found a reduction in mRNA levels coding for the 5-HT_{1B} receptor in the dorsal raphe nucleus after 7 days of daily fluoxetine administration at 3 mg/kg. Since no reductions were found in other brain areas, it was inferred that only 5-HT_{1B} autoreceptors and not heteroreceptors were desensitized at this time point. The present observation that no desensitization of 5-HT_{1B} autoreceptors was seen in the hypothalamus complements our earlier findings that fluoxetine, administered for 7 days but at a dose of 10 mg/kg, induced desensitization of 5-HT_{1B} autoreceptors, as measured by the effect of the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935, to increase 5-HT levels in the ventral hippocampus but not in the hypothalamus (Dremencov et al., 2000). Previous studies have shown that high doses and prolonged periods of administration are required for fluoxetine to induce the desensitization of 5-HT_{1B} receptors in the hypothalamus. Thus, using superfused slices from guinea pig hypothalamus, Bergqvist et al. (1999) showed desensitization of 5-HT_{1B} autoreceptors after 10 mg/kg fluoxetine given daily for 8 weeks. Similarly with paroxetine, desensitization of hypothalamic 5-HT_{1B} receptors was observed after a 3-week administration at 10 mg/kg both with superfused slices from guinea pigs (Blier and Bouchard, 1994) and with in vivo microdialysis in rats (Sayer et al., 1999).

Since the majority of studies show no change in 5-HT_{1A} receptor binding at either presynaptic or postsynaptic sites after prolonged selective serotonin reuptake inhibitor administration, it has been suggested that functional desensitization may be due to changes at the G-protein level. Li et al. (1996) showed a reduction in levels of Gi-2 and Go in the midbrain after only 3 days of fluoxetine administration at 10 mg/kg, and in levels of Gi-1 and Go in the hypothalamus after 7 days, and suggested that these effects may be responsible for the desensitization of somatodendritic and hypothalamic postsynaptic 5-HT_{1A} receptors, respectively. Gi and Go levels in the frontal cortex, however, were unchanged at any of the time points examined. However, in a recent abstract, Vadizan et al. (2002) found that Gi-2 levels in rat cortical membranes were significantly decreased after administration of fluoxetine at 10 mg/kg daily for 14 days. Gi-2 proteins have a higher coupling affinity for 5-HT_{1B} receptors than for 5-HT_{1A} receptors (Lin et al., 2002). This finding could explain the decrease in 5-HT_{1B} autoreceptor activity in the frontal cortex observed after 12 days of fluoxetine administration.

The present results, as well as those of Hervas et al. (2001) and Dawson et al. (2002), imply that concurrent treatment of patients with an SSRI and a 5-HT_{1A} receptor antagonist such as WAY-100635 would not be beneficial, since prolonged administration of the antagonist reduces or prevents the action of the selective serotonin reuptake inhibitor to desensitize 5-HT_{1A} autoreceptors rather than potentiates the effect of the selective serotonin reuptake inhibitor to increase basal 5-HT levels, as happens on acute administration. The clinical action of pindolol as an augmenting or accelerating agent in the treatment of depression must therefore have a different basis, and this view has indeed been put forward (Artigas et al., 2001; Cremers et al., 2001). The only clinically relevant effect of antagonist administration appears to be its ability to induce immediate autoreceptor blockade, and an antagonist could thus be administered for a short time until the desensitizing action of the SSRI had set in, at which time antagonist treatment should be discontinued.

Acknowledgements

This work was supported by grant no. MH 60580 from the National Institutes of Health to M.E. Newman and a Fogarty International Research Collaboration Award from the National Institutes of Health (TW 01496) to L.D. Van de Kar and M.E. Newman. We thank Tatiana Dobroborski for excellent technical assistance.

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