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Behavioural sensitization of mesolimbic dopamine D_2 receptors in chronic fluoxetine-treated rats

Maria Collu [b,*], Alessandra S. Poggiu [b], Paola Devoto [b], Gino Serra [a]

[a] *Department of Pharmacological Sciences, University of Sassari, Sassari, Italy*
[b] *'B.B. Brodie' Department of Neuroscience, University of Cagliari, Via Porcell 4, I-09124 Cagliari, Italy*

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Abstract

A common action of chronic antidepressant treatments is the potentiation of dopaminergic transmission in the limbic system. We now report that chronic, but not acute, treatment with fluoxetine (2.5 mg/kg by intragastric gavage once a day for 21 days) potentiates the locomotor stimulant effect of quinpirole, a selective dopamine D_2/D_3 receptor agonist. However, neither quinpirole-induced stereotypies nor the sedative effects elicited by low doses of this dopamine receptor agonist are influenced by chronic fluoxetine. These results suggest that fluoxetine, as well as classical antidepressants, sensitize postsynaptic dopamine D_2/D_3 receptors in the mesolimbic system.

Keywords: Dopamine receptor; Mesolimbic dopamine system; Antidepressant drug; Fluoxetine; Chronic treatment

1. Introduction

A delay (2–3 weeks) in the onset of clinical effects is the main characteristic of all antidepressant drugs. This observation has led several authors to question the hypothesis that their therapeutic action is due to the acute effects on monoaminergic neuronal transmission (i.e., blockade of serotonin and/or noradrenaline reuptake or monoaminoxidase inhibition). However, it is now generally believed that the therapeutic actions of these drugs may be attributed to the biological effects induced by long-term treatment. In the last two decades a great number of experimental data have accumulated on chronic antidepressant drug-induced adaptive changes, mainly at the monoaminergic receptors, which are considered relevant for the clinical effect (Serra et al., 1992; Caldecott-Hazard et al., 1991).

Among these effects of long-term treatment with antidepressant drugs is the potentiation of dopamine transmission, particularly in the limbic system (Serra et al., 1992). Indeed, while anhedonia and lack of motivation are among the core symptoms of depression, some evidence indicates that the mesolimbic dopaminergic system plays a crucial role in incentive motivation and reward (Fibiger, 1993). Thus, it is likely that potentiation of dopaminergic transmission in the limbic system plays a role in the improvement of depression, or at least of anhedonia and lack of motivation. Moreover, as activation of dopaminergic transmission seems to be associated with mania, it may be suggested that the increased dopaminergic transmission induced by chronic antidepressants could be responsible for the switches from depression to mania observed in susceptible patients (Serra et al., 1992; Gessa et al., 1995).

As to the mechanism by which antidepressant drugs potentiate dopamine transmission, we have shown that chronic treatment with different antidepressant drugs, or repeated electroconvulsive shocks, potentiated the behavioural responses mediated by selective stimulation of postsynaptic dopamine D_2/D_3 receptors in the mesolimbic system. This suggested that the behavioural sensitization might be due to enhanced neurotransmission at the dopamine D_1 receptors (which have a permissive role in the expression of dopamine D_2/D_3-mediated behavioural responses) (Serra et al., 1990). Accordingly, in spite of the behavioural evidence for supersensitivity of postsynaptic dopamine D_2 receptors, chronic antidepressant drugs cause no changes in dopamine D_2 receptor density or affinity (Klimek and Nielsen, 1987). On the contrary, they decrease both dopamine D_1 receptor number and the sensitivity of adenylate cyclase to dopamine, whereas they in-

* Corresponding author. Fax: (39-70) 657-237; e-mail: mcollu@unica.it

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crease the V_{max} of this enzyme (positively coupled to the dopamine D_1 receptor) in the limbic system (Klimek and Nielsen, 1987; De Montis et al., 1989). These effects could be an adaptive change secondary to the activation of dopamine D_1 receptors downstream of the recognition site induced by chronic antidepressant drugs (Serra et al., 1992). Finally, in support of the hypothesis for a role of the enhancement of dopamine transmission at dopamine D_1 receptors in the mechanism of action of antidepressant drugs, we have recently shown that selective dopamine D_1 receptor agonists exert an antidepressant-like effect in two animal models of depression (D'Aquila et al., 1994).

However, more recently an alternative interpretation has been proposed by Maj et al. (1996). They have observed that chronic treatment with different antidepressants changes the binding of the dopamine agonist [^{3}H]N-0437 to dopamine D_2 receptors in the limbic system. These results led to the suggestion that the enhanced behavioural responsiveness to dopamine receptor agonists could be due to an increased number and/or affinity of dopamine D_2 receptors (Maj et al., 1996).

The potentiation of dopaminergic transmission seems to be a feature shared by various antidepressant treatments (tricyclic antidepressant drugs, monoaminoxidase inhibitors, atypical antidepressant drugs such as mianserin and iprindole, repeated electroconvulsive shocks and rapid eye movement-sleep deprivation) (Serra et al., 1992). On the other hand, conflicting results have been reported concerning the effect of chronic selective serotonin reuptake inhibitors, the most commonly prescribed antidepressant drugs, on the sensitivity of dopaminergic receptors (Spyraki and Fibiger, 1981; Arnt et al., 1984; Gambarana et al., 1995).

The purpose of the present study was to further investigate the effects of chronic fluoxetine on dopamine receptor sensitivity by analysing behavioural responses to quinpirole, a dopamine D_2/D_3 receptor agonist.

2. Materials and methods

2.1. Animals

Naive, male Sprague-Dawley rats (Charles River, Italy), weighing 100–125 g at the beginning of the experiments, were group-housed (3 or 4 per cage) and maintained with a 12-h light-dark cycle (lights on at 8:00 a.m.) under standard temperature and humidity with food and water available ad libitum.

2.2. Behavioural experiments

2.2.1. Locomotor activity

Motor activity was measured by placing the animals individually in motility cages (Omnitech Digiscan Animal Activity Monitor, Columbus, OH, USA). Each cage had 2 sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart and 2 cm above the cage floor. Motor activity was defined as the horizontal activity counts.

2.2.1.1. Quinpirole-induced motor activity. Thirty-six hours following the last treatment, the rats were allowed 2 h to adapt to the motility cages, then removed, injected with 0.15 mg/kg quinpirole or saline (1 ml/kg) and were immediately returned for an additional 90-min test.

2.2.1.2. Quinpirole-induced hypomotility. Thirty-six hours after the last chronic treatment the rats were given either 0.012 mg/kg quinpirole or saline. Ten minutes after injection they were individually put into the motility cages and horizontal activity was scored for 45 min.

All data were collected every 10 min and processed with the Digiscan Analyser.

2.2.2. Stereotypies

Stereotyped behaviour was measured by individually placing the animals into Perspex cages with a wire grid floor. After 60 min for adaptation the rats were injected with quinpirole (2 mg/kg). Stereotyped behaviour was quantified for 90 min according to the Creese and Iversen scale (Creese and Iversen, 1975) by an observer who was not aware of the drug treatment. Each animal was observed for 15 s at 10-min intervals, starting 10 min after quinpirole injection.

All experiments were performed between 09:00 and 18:00 h.

2.3. Drugs and treatments

Fluoxetine HCl (as the commercially available oral solution, Prozac, Ely-Lilly) was diluted in distilled water; vehicle (0.3125 mg benzoic acid, 375 mg saccharose, 125 mg glycerine, 1.31 mg mint flavour) was dissolved in 5 ml distilled water.

Both treatments were given at the volume of 5 ml/kg by intragastric gavage (pediatric feeding tube, Medico Plast, Germany). Acute treatment consisted of a single administration of 2.5 mg/kg fluoxetine; chronic treatment consisted of the administration of 2.5 mg/kg fluoxetine once a day (7:30 p.m.) for 21 days. Control rats were treated acutely or chronically with vehicle (5 ml/kg). Quinpirole HCl (RBI/Amersham Life Science, Italy) was dissolved in saline and administered s.c.

2.4. Statistics

All data were evaluated for statistical significance by multiple analysis of variance (MANOVA), followed by post-hoc testing (Newman-Keuls test) when required.

3. Results

3.1. Effect of acute and chronic fluoxetine on locomotor activity induced by quinpirole

As shown in Fig. 1, the administration of 0.15 mg/kg of quinpirole produced a slight, but not statistically significant, stimulation of locomotor activity in rats chronically treated with vehicle and previously adapted to the motility cage. This behavioural response to quinpirole was markedly potentiated in chronic fluoxetine-treated animals ($F(3,38) = 4.51$, $P < 0.01$). The behavioural stimulatory response to quinpirole observed in acute fluoxetine-treated rats did not differ from that observed in control animals ($F(3,27) = 0.93$, $P > 0.05$) (Fig. 2).

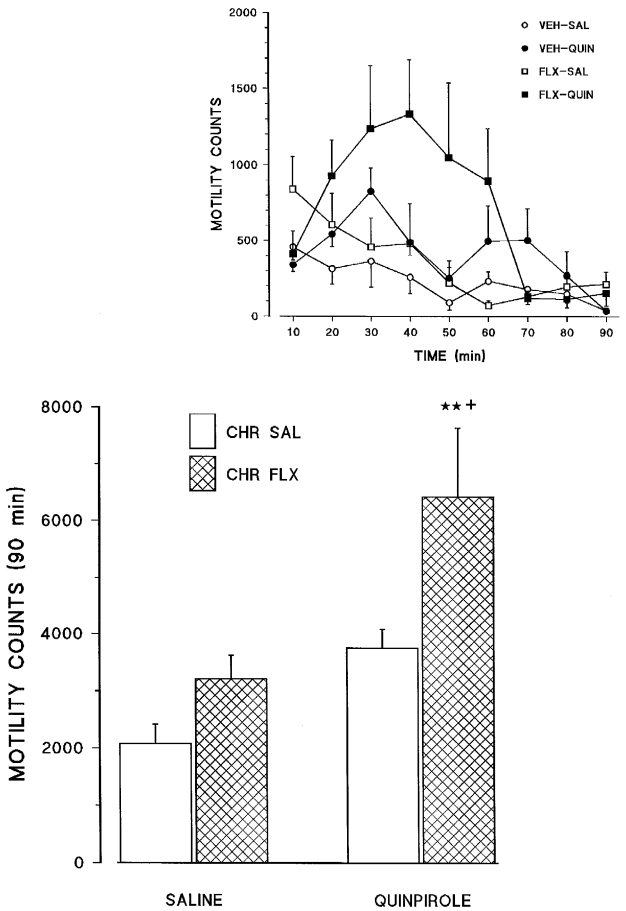

Fig. 1. Effect of chronic fluoxetine on quinpirole-induced locomotor activity in rats adapted to testing conditions. Each value represents the mean ± S.E.M. for 10–12 animals. Fluoxetine (FLX) (2.5 mg/kg) was administered intragastrically once a day (7:30 p.m.) for 21 days; control rats (VEH) were treated the same way but received vehicle (5 ml/kg). All experiments were preformed 36 h after the last treatment. After 2-h adaptation to the motility cages, the rats were given quinpirole (0.15 mg/kg s.c.) or saline (1 ml/kg s.c.). Motor activity was then measured for the 90 min following. The insert depicts the time course of the quinpirole effect on locomotor activity. During the adaptation period no differences were observed between chronic FLX- and VEH-treated animals. $^{**}P < 0.01$ vs. the corresponding control group (Newman-Keuls test). $^{+}P < 0.01$ vs. control rats receiving quinpirole (Newman-Keuls test).

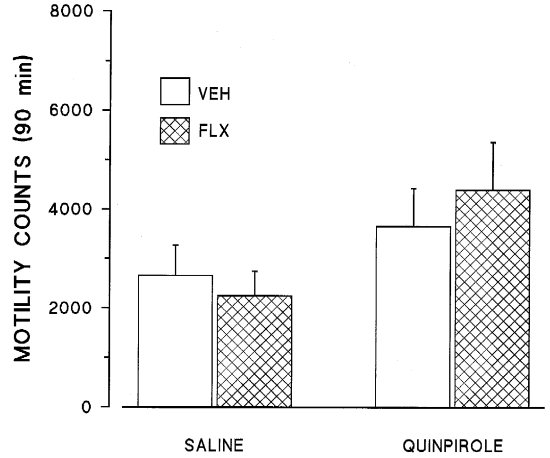

Fig. 2. Effect of acute fluoxetine on quinpirole-induced locomotor activity in rats adapted to testing conditions. Each value represents the mean ± S.E.M. for 7–8 animals. Rats received a single injection of either fluoxetine or vehicle at 7:30 p.m., and experiments were carried out 36 h after injection as described for Fig. 1.

3.2. Effect of chronic fluoxetine on stereotypy induced by quinpirole

The administration of 2 mg/kg quinpirole induced a stereotyped behaviour characterized by intense sniffing and/or rearing in control rats. The behavioural effects of quinpirole began within 10 min, reached their peak at 20 min and lasted for at least 90 min (Fig. 3). The stereotyped responses to quinpirole observed in chronic fluoxetine-treated rats did not significantly differ from those observed in control rats.

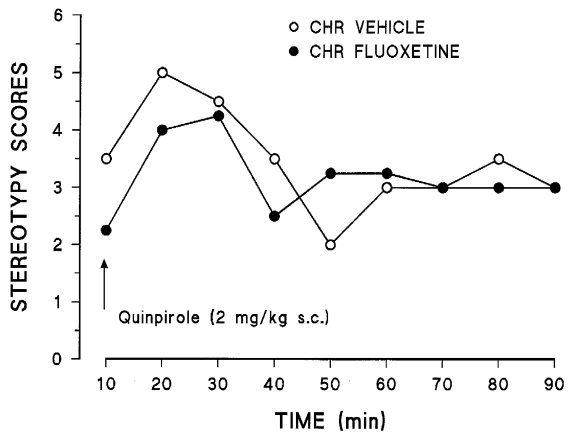

Fig. 3. Effect of chronic fluoxetine on quinpirole-induced stereotypies. Each value represents the median from 8 animals. Treatments were performed as described for Fig. 1. 36 h after last treatment and 1-h adaptation to the observation cage, quinpirole (2 mg/kg) was administered s.c. and stereotypies were scored for 90 min according to Creese and Iversen (1975).

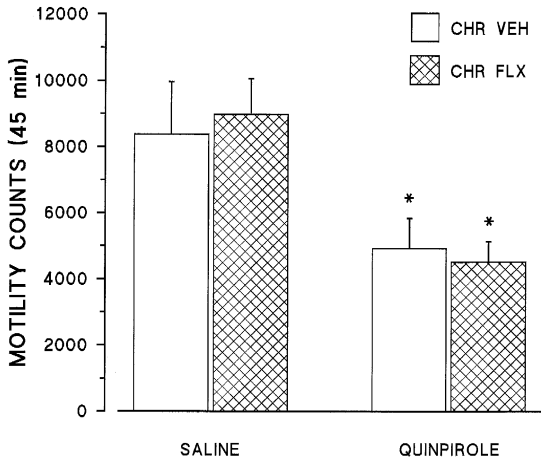

Fig. 4. Effect of chronic fluoxetine on quinpirole-induced hypomotility in rats. Each value represents the mean ± S.E.M. for 6 animals. Treatments were given as described for Fig. 1. After the last treatment (36 h before), the rats were injected s.c. with 0.012 mg/kg quinpirole or saline (1 ml/kg). Ten minutes later the animals were individually put into the motility cages and motility was scored for 45 min. * $P < 0.05$ vs. the corresponding control group (Newman-Keuls test).

3.3. Effect of chronic fluoxetine on hypomotility induced by a low dose of quinpirole

Administration of 0.012 mg/kg quinpirole to vehicle-treated rats decreased locomotor activity by 45–50%. The same behavioural inhibitory response to quinpirole was observed in chronic fluoxetine-treated rats ($F(3,20) = 2.98$, $P > 0.05$) (Fig. 4).

4. Discussion

The present study showed that chronic but not acute fluoxetine potentiates the locomotor stimulant effect of quinpirole, a dopamine D_2/D_3 receptor agonist. However, neither quinpirole-induced stereotypy, nor the sedative effect produced by low doses of this compound, were influenced by chronic fluoxetine. Since quinpirole-induced stimulation of locomotor activity is considered to be mediated by postsynaptic dopamine D_2/D_3 receptors in the limbic system, these findings suggest that chronic fluoxetine sensitizes such receptors selectively in this dopaminergic system.

Our results are in contrast with recent findings by Gambarana et al. (1995), showing that fluoxetine (10 mg/kg i.p.), administered daily for 2 weeks, failed to potentiate quinpirole-induced hypermotility. The discrepancy could be explained by different schedules and dosages of treatment, as, in the present study, fluoxetine was administered i.g. once a day for 3 weeks at the dose of 2.5 mg/kg. However, although no convincing interpretation is available at the moment, it is interesting that the effect of fluoxetine on amphetamine-induced hypermotility seems to depend also upon the route of administration. Indeed, Arnt et al. (1984) found that chronic fluoxetine, administered for 14 days i.g. at the dose of 20 mg/kg, potentiates amphetamine-induced hypermotility. However, no changes in this behavioural response were observed following i.p. injection of 8 mg/kg fluoxetine twice daily for 14 days by Spyraki and Fibiger (1981) and Martin-Iverson et al. (1983).

The ability of fluoxetine to sensitize dopamine receptors in the mesolimbic system is further supported by recent results from our laboratory showing that, as observed after chronic imipramine (Collu et al., 1994), chronic treatment with this selective serotonin reuptake inhibitor potentiates cocaine-induced conditioned place preference in rats (Collu et al., 1996). Moreover, Simon and Appel (1995) have demonstrated that fluoxetine potentiates the discriminability of 2.5 mg/kg cocaine in drug-discrimination experiments, further suggesting that this drug increases the sensitivity of dopamine receptors. Finally, Willner (1995) reported that the effect of fluoxetine to reverse the 'anahedonia' induced by chronic mild stress is antagonized by the block of dopamine D_2 receptors with raclopride.

Behavioural, biochemical and electrophysiological evidence from several laboratories including our own suggests that different mechanisms underlie the sensitization of postsynaptic dopamine D_2/D_3 receptors induced by classical antidepressants and fluoxetine in the limbic system. Accordingly, we have recently demonstrated that, while both chronic imipramine and fluoxetine increased the effectiveness of cocaine to induce conditioned place preference, only the latter drug itself elicits this behavioural response, considered an expression of the reinforcing properties of a drug and mediated by activation of the mesolimbic dopamine system (Collu et al., 1996). Moreover, Tanda et al. (1995) have observed that the blockade of 5-HT_3 receptors potently inhibits fluoxetine- but not desipramine-induced dopamine release in the prefrontal cortex. Finally, Ashby et al. (1995) have recently reported that chronic fluoxetine increases the number of spontaneously active dopamine neurons in the ventral tegmental area. This observation suggests that fluoxetine may activate the mesolimbic dopamine system, not only by increasing the sensitivity of postsynaptic dopamine receptors, but also by stimulating the activity of dopaminergic neurons. More recently, Maj et al. (1996) have shown that chronic fluoxetine, mianserin, imipramine and amitriptiline caused different changes in the parameters of [^{3}H]N-0437 binding to dopamine D_2 receptors in the synaptosomal membranes of the limbic areas. While chronic fluoxetine increases both B_{max} and K_d, chronic treatment with the other antidepressants elicits a decrease in K_d and increases (2 h after washout) or causes no changes (72 h after washout) in the B_{max} of dopamine D_2 receptors. As in the present work, the binding study was performed in animals given fluoxetine per os. The similarity of the treatment regimens used in the two studies supports the hypothesis that the enhanced behavioural responses to quinpirole with

chronic fluoxetine may be correlated with the increased density of dopamine D_2 receptors in the limbic forebrain.

In conclusion, the present results suggest that fluoxetine shares with classical antidepressant drugs the ability to potentiate mesolimbic dopamine transmission. This hypothesis is in line with the clinical observation that selective serotonin reuptake inhibitors share with other antidepressant drugs not only the antidepressive efficacy, but also the ability to produce switching from depression to mania (Gram, 1994). However, the hypothesis that the mechanism by which fluoxetine activates dopamine transmission differs from that of imipramine could account for some of their clinical differences. For instance, in contrast to what is observed with classical tricyclic antidepressant drugs, fluoxetine appears to be effective in the treatment of mild to moderate but not severe major depression (Gram, 1994).

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