



Short communication

Fluoxetine-induced anxiety and nervousness

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Abstract:

The aim of this study was to model fluoxetine-induced increase in anxiety appearing in the initial phase of the treatment with this antidepressant drug. The effects of acute administration of fluoxetine given alone and co-administered with a subthreshold dose of pentetrazole (PTZ), a proconvulsant agent with well recognized anxiogenic properties, were examined in the open field test of neophobia in rats. It was found that a single injection of fluoxetine at the dose of 5 and 10 mg/kg did not change motor and exploratory behavior of rats. Furthermore, fluoxetine (10.0 mg/kg) co-administered with a subthreshold dose of PTZ (10.0 mg/kg) had a strong and selective inhibitory influence on rat exploratory behavior. Pharmacokinetic study did not show any changes in brain concentration of PTZ in fluoxetine-pretreated animals. The central mechanism of the reported effects might involve stimulation of 5-HT_{2C} receptors by fluoxetine in animals with PTZ-induced decrease in the threshold for emotional arousal. The present data describe a new animal model to study the central action of antidepressants reflecting dysphoric-like effects observed in the initial phase of the treatment.

Key words:

fluoxetine, pentetrazole, anxiety, open field, pharmacokinetics, rat

Abbreviations: Flx – fluoxetine, PTZ – pentetrazole, SSRIs – selective serotonin reuptake inhibitors

Introduction

The clinical profile of Selective Serotonin Reuptake Inhibitors (SSRIs) includes the pharmacotherapy of mood and anxiety disorders. However, it is well recognized that some patients treated with a prototypical SSRI, fluoxetine (Flx), may become akathic while

others may show an increase in anxiety, insomnia, and anorexia in the initial phase of the treatment [2, 6, 17]. Moreover, it has recently become clear, that all antidepressants including SSRIs may increase risk of suicidal attempts during the first few days of treatment (suicidal thinking and behavior), in children and adolescents treated with these agents [5, 8]. There is not an adequate and easy to apply model of the aforementioned dysphoric and anxiety-like changes appearing at the beginning of treatment with Flx and other SSRIs. Two papers on a similar topic reported that Flx and m-chlorophenylpiperazine (m-CPP, a 5-HT_{2C} receptor agonist) decreased the time of social interac-

tion and increased self-grooming in rats [1, 3]. The reason why some effects of antidepressants remained unnoticed might be the fact that conventional tests for antidepressant drugs had been invented before introduction of SSRIs to clinical practice, and as such these tests are more sensitive to the pharmacological profiles of older drugs. It is apparent, therefore, that there is still a space for new methods and tests which could selectively detect the different spectra of psychotropic effects of SSRIs. To that end, in this paper we describe an animal model which has appeared to be sensitive to some important clinical aspects of an early treatment with Flx, i.e. the increase in anxiety. The effects of acute treatment of rats with Flx given alone and co-administered with a subthreshold dose of pentetrazole (PTZ), a proconvulsant agent with well recognized anxiogenic properties [9], were examined in the open field. Open field test belongs to the behavioral test battery evaluating innate emotional reactions of rodents, e.g. neophobia, fear of new, open, brightly lit places. It is also used to assess total locomotor activity of animals [13, 14].

Materials and Methods

Animals

Male Wistar rats ($n = 31$ for acute treatment, $n = 44$ in Flx vs. PTZ study), weighting 180–200 g upon arrival, were housed four per a polycarbonate cage measuring $60 \times 30 \times 25$ cm. They were maintained on a 12/12 h light/dark cycle (lights on 07:00–19:00 h) in a room with ambient temperature (19–22°C). Rats were allowed free access to food and water for the duration of the experiment and were given 7–9 days to acclimate to laboratory conditions before the tests were performed. The Committee for Animal Care and Use at the Medical University in Warsaw approved all experiments. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC).

Fluoxetine treatment

The animals were injected with fluoxetine hydrochloride (Fluoksetyna, Anpharm, Poland) at the doses of 5 and 10 mg/kg intraperitoneally, in a volume of 2 ml/kg (0.9% NaCl solution) 1 h prior to the open field test.

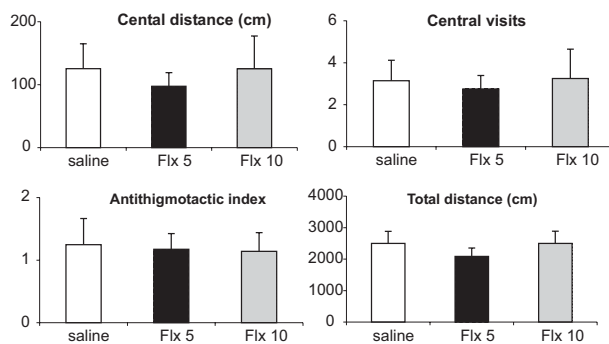


Fig. 1. Effects of acute *ip* administration of fluoxetine (Flx) in the open field test. Data are shown as the means \pm SEM. Open bars – saline, solid bars – Flx 5 mg/kg, gray bars – Flx 10 mg/kg

The drug was dissolved in vehicle and prepared immediately before the injection. The control group was given vehicle in a volume of 2 ml/kg.

Fluoxetine vs. PTZ interaction study

On the experimental day rats were divided into 4 groups treated either with fluoxetine hydrochloride at the dose of 10 mg/kg (2 ml/kg) or vehicle (0.9% NaCl solution in a volume of 2 ml/kg). One hour later rats were given either pentetrazole (PTZ, Sigma-Aldrich, Poland) at the dose of 10 mg/kg or vehicle (0.9% NaCl solution, 2 ml/kg) according to the following paradigm: group 1 – saline (saline/saline), group 2 – PTZ (saline/PTZ), group 3 – Flx (fluoxetine/saline), group 4 – Flx/PTZ (fluoxetine/PTZ).

Open-field test

The test was performed in a soundproof chamber under dim light and continuous white noise (65dB) without previous habituation to testing conditions. The apparatus used in the experiment consisted of two arenas (80 cm in diameter with 30-cm high walls, each). During the 15-min experimental session, the following parameters were measured (with the use of a computerized Video-Mot system, Bad Homburg, Germany): the distance and number of entries into the central area (50 cm in diameter), the total distance – determined as a sum of central and border area distance. Moreover, the antithigmotactic index was calculated as a ratio of the number of entries into the central area to total distance multiplied by 1000. The apparatus was cleaned thoroughly with 95% ethanol

after each animal was tested. All tests were conducted between 9:00 and 17:00 h [13].

In the Flx vs. PTZ interaction study, 15 min. After the last injection, the animals were placed in the open field apparatus, and the test was performed according to the previously described procedure.

Pharmacokinetic study

Concentration of PTZ in the brain tissue was examined according to the procedure described previously [19]. Briefly, 1 h after Flx and 15 min after PTZ administration rats were placed in the open field apparatus. Immediately after the test, the animal were sacrificed, their brains were rapidly removed and tissue homogenate was prepared by sonification. The brain tissue samples were placed in plastic 1.7 ml Eppendorf tubes containing the solution used for homogenate preparation (2% HClO₄ with 10 µg/ml of ¹H-benzotriazole as an internal standard), sonificated for 30 min, and centrifuged at 20,000 rpm for 7 min at 4°C. Then the clear supernatant was collected, filtered through PVDF Durapore 45 µm filter and injected onto the HPLC column.

Analysis of PTZ concentration was performed using a modified high-performance liquid chromatographic (HPLC) method reported previously by Ramzan and Levy [12]. The HPLC system consisted of Shimadzu LC-6A pump, Shimadzu CTO-6A oven, Shimadzu SPO-6A spectrophotometric detector (wavelength of 214 nm), Phenomenex Luna C₁₈ 150 mm, 5 µm column equipped with Phenomenex KJO-4282 precolumn. The sample was injected through Rheodyne 7132 injection valve with 20 µl sample loop. The column temperature was 26–27°C. The mobile phase consisted of 5 mM NaH₂PO₄ solution with 12% acetonitrile. During the whole procedure the mobile phase was degassed with helium. The flow rate was 0.3 ml/min. Chromatogram registration and analysis, and construction of calibration curve for PTZ was performed using ChromaX 2000 software. The concentration of PTZ was calculated as µg/g of brain tissue.

Statistical methods

The data are shown as the means ± SEM. The open field test results were analyzed by one-way ANOVA followed by *post-hoc* LSD test (Statistica for Windows, Release 6, Stat-Soft Inc., USA). The pharmacokinetic data were assessed by Student's *t*-test for in-

dependent variables. The probability value of *p* < 0.05 was considered significant.

Results

Fluoxetine treatment

Flx administered at the dose of 5 and 10 mg/kg *ip* did not affect in a significant way rat behavior in the open field test: time spent in the central area [F(2, 20) = 0.62; *p* > 0.05], central visits [F(2, 20) = 0.06; *p* > 0.05], antithigmotactic index [F(2, 20) = 0.02; *p* > 0.05], total distance [F(2, 20) = 0.47; *p* > 0.05] (Fig. 1).

Fluoxetine vs. PTZ interaction study

One-way ANOVA revealed significant differences between groups in most of the behavioral parameters studied: central distance [F(3, 39) = 3.78; *p* < 0.05], number of central visits [F(3, 39) = 4.75; *p* < 0.01], and antithigmotactic index [F(3, 39) = 6.33; *p* < 0.01]. The total distance travelled in the open field test was not changed [F(3, 39) = 1.43; *p* > 0.05]. *Post-hoc* test showed that animals given PTZ after the pretreatment with Flx were affected the most profoundly, in comparison with saline group (central distance, *p* < 0.05; central visits, *p* < 0.05; antithigmotactic index, *p* < 0.01), with Flx group (central distance, *p* < 0.01; central visits, *p* < 0.01; antithigmotactic index, *p* < 0.01), and PTZ group (central distance, *p* < 0.05; central visits,

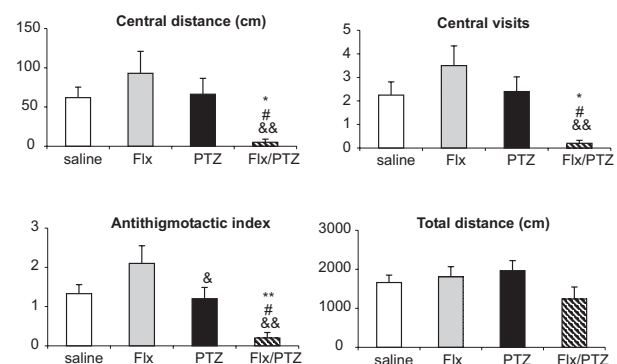


Fig. 2. Interaction between *ip* administered fluoxetine (Flx) and pentetrazole (PTZ) in the open field test. Data are shown as the means ± SEM. Open bars – saline; gray bars – Flx 10 mg/kg; solid bars – PTZ 10 mg/kg; diagonally striped bars – PTZ after pretreatment with Flx 10 mg/kg. * differs from control, # differs from PTZ, & differs from Flx. *,#,& *p* < 0.05; **, && *p* < 0.01

$p < 0.05$; antithigmotactic index, $p < 0.05$). Moreover, there was a significant decrease in antithigmotactic index in PTZ group as compared to Flx group ($p < 0.05$) (Fig. 2).

Pharmacokinetics study

Student's *t*-test did not reveal any significant changes in the concentration ($\mu\text{g/g}$) of PTZ at the dose of 10 mg/kg in rats pretreated with Flx at the dose of 10 mg/kg, in the whole brain tissue samples (PTZ group 8.35 ± 0.43 , Flx/PTZ group 8.29 ± 0.33) ($t = 0.21$, $df = 16$; $p > 0.05$).

Discussion

It was found that a single injection of Flx at the dose of 5 and 10 mg/kg did not cause a decrease in exploratory behavior of rats in the open field test (a decrease in the antithigmotactic index). Furthermore, Flx (10.0 mg/kg) co-administered with a subthreshold dose of PTZ had a strong and selective inhibitory influence on rat exploration, and the antidepressant significantly potentiated the anxiogenic-like effects of this drug. Since the acute Flx/PTZ administration decreased exploratory parameters such as central distance, central visits and antithigmotactic index, whereas it did not affect total locomotor activity (total distance), the observed effects can be considered selective for increased animal neophobia [14, 15]. Moreover, the changes were not related to the increase in the brain concentration of PTZ. These data indicate that the animal test applied in this study modeled some important features of early effects of the treatment with Flx in the clinical setting, i.e. the increase in anxiety. Thus, this opens new possibility to study the intrinsic central mechanisms responsible for this clinically important phenomenon.

The central mechanism of the reported behavioral effects of Flx is not clear. Pharmacological profile and pharmacokinetics of Flx differ from other antidepressants. It is generally recognized that Flx exerts its effects by inhibition of serotonin uptake into neurons with an ensuing increase in the extracellular concentration of serotonin. However, there are studies showing that Flx has a direct action on the 5-HT_{2C} receptors (in original terminology the 5-HT_{1C} receptor) [4, 18]. Importantly, the main metabolite of Flx, R-Flx,

unlike other selective serotonin reuptake inhibitors, also possesses moderate affinity ($K_i = 64 \text{ nmol/l}$) for the serotonin 2C receptor [11]. The 5-HT_{2C} receptor agonism can account for the anxiogenic effect of the drug, since it has been shown earlier that an increased anxiety in rodents, and possibly, also in humans, e.g. agitation or jitteriness after SSRIs and panic attacks after mCPP, is mediated by activation of 5-HT_{2C} receptors [1, 3]. Moreover, there is also evidence for an increase in the density of 5-HT₂ receptors in the brains of suicide victims [10], and molecular studies locate these changes in the altered coding sequence of the serotonin 5-HT_{2C} receptor pre-mRNA [7]. Altogether, these data strongly suggest the contribution of 5-HT_{2C} receptors to the central processes related to the mood-regulating effects of Flx. The intrinsic mechanism of interaction between PTZ, a non-competitive GABA-A receptor antagonist, and Flx remains to be elucidated. However, it can be tentatively suggested, given the fundamental role of GABA in the control of emotions, that PTZ-induced decrease in the threshold for emotional arousal revealed the potency of a single dose of Flx to increase anxiety *via* 5-HT_{2C} receptors.

Despite the fact that Flx is also a strong inhibitor of hepatic CYP 2D6 metabolic enzymes [16], the pharmacokinetic interpretation of the effects of a joint administration of Flx with PTZ is not likely, since it was shown that pretreatment of rats with Flx, in the same dose-range, did not change the concentration of peripherally administered PTZ in the brain tissue.

In summary, the present data describe a new animal model in which rats are "sensitized" to the anxiogenic-like effects of fluoxetine thus reflecting dysphoric-like effects of antidepressant drugs observed in the initial phase of the treatment.

Acknowledgment:

The study was supported by statutory grant from the Institute of Psychiatry and Neurology in Warszawa (no. 55/2004).

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Received:

July 29, 2005; in revised form: December 20, 2005.