Anxiogenic-like effect of acute and chronic fluoxetine on rats tested on the elevated plus-maze

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Abstract

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The selective serotonin reuptake inhibitor fluoxetine (FLX) is widely prescribed for depression and anxiety-related disorders. On the other hand, enhanced serotonergic transmission is known to be classically related to anxiety. In this study, the effects of acute (5.0 mg/kg) and chronic (5.0 mg/kg, 22 days) FLX were investigated in both food-deprived and non-deprived rats tested in the elevated plus-maze. Significant main effects of the three factors (drug, food condition and administration regimen) were observed, but no interaction between them. The administration of either acute or chronic FLX resulted in an anxiogenic effect, as detected by a significant reduction in the percentage of time spent in the open arms and in the percentage of open arm entries. Food deprivation yielded an anxiolytic-like profile, probably related to changes in locomotor activity. The administration regimen resulted in an anxiolytic profile in chronically treated rats, as would be expected after 22 days of regular handling. The anxiogenic action of acute FLX is consistent with both its neurochemical and clinical profile. The discrepancy between the anxiogenic profile of chronic FLX and its therapeutic uses is discussed in terms of possible differences between the type of anxiety that is measured in the plus-maze and the types of human anxiety that are alleviated by fluoxetine.

Introduction

Besides being one of the most widely prescribed drugs for depression, fluoxetine (FLX) is also used for anxiety-related disorders such as obsessive-compulsive disorder, panic, social phobia and bulimia nervosa (1-5). However, the classic hypothesis relating increased serotonergic function to anxiety (6,7) predicts that fluoxetine, a selective serotonin reuptake inhibitor (SSRI), would pro-

voke an anxiogenic effect when tested on animal models. After acute administration, there is an increase in extracellular serotonin in several subcortical brain regions due to reuptake blockade (8,9). Although this extracellular 5-HT seems to inhibit the firing of raphe neurons and thus to reduce 5-HT release from nerve terminals (9), it is often reported that the initial effect of fluoxetine administration in humans is an exacerbation of anxiety (10,11), and this effect has been

related to the sharp increase in synaptic serotonin concentrations after acute administration of the drug (7,12). During chronic administration there is an increase of extracellular concentrations of 5-HT at cortical and subcortical levels, and the long-term 5-HT reuptake blockade provokes desensitization of somatodendritic 5-HT$_{1A}$ and terminal 5-HT$_{1B}$ autoreceptors, respectively leading to a disinhibitory effect on raphe neurons firing and to reduced feedback inhibition of 5-HT release (13-15). Both of these neuroadaptive changes lead to enhanced serotonergic release and transmission (16,17).

However, the data obtained in animal studies using SSRI antidepressants are contradictory (12,18,19). Specifically, the evidence of the effect of fluoxetine on animal models of anxiety is controversial, and studies on its chronic effects are scarce. Acute fluoxetine has been shown to promote anxiogenic-like effects in rats tested in the elevated plus-maze (20-22), in the hole-board (20), and in a light-aversion test (20). The same effects are observed in the novelty-suppressed feeding model of anxiety (23) and in the antipredator defensive behavior of mice (24). Vogel's conflict test (22), however, yielded anxiolytic-like effects in rats, and so did another light aversion test (25). Effects of the same nature, albeit weak, were reported for the shock-induced ultrasonic vocalization test in rats (19,26). Chronic administration of fluoxetine also suggests an anxiolytic effect of the drug, since it has been reported to "dramatically" decrease flight reactions and defensive attack towards a predator (24) and to reduce latency to begin eating in a novel environment (23). On the other hand, some studies have reported the absence of either an anxiogenic or an anxiolytic effect of the drug, administered acutely or chronically. For example, acute fluoxetine was inactive in a light aversion test (19,27) and in the plus-maze (28,29). When administered chronically at the dose of 10 mg/kg, it had no effect on a conditioned-fear stress test (30).

The elevated plus-maze is a widely used animal model of anxiety involving unconditioned responses based on exploration. Although an anxiogenic effect or a lack of effect of acute fluoxetine has been found in some of the studies using the elevated plus-maze, the chronic effect of the drug on this model has not been investigated. The present experiment was conducted in order to examine the effect of both acute and chronic fluoxetine on the elevated plus-maze. A possible interaction with food deprivation was also investigated, since a differential effect of acute versus chronic fluoxetine has been reported in a model of anxiety that relies on food deprivation (23).

Material and Methods

Subjects

The subjects were 94 experimentally naive male Wistar rats weighing approximately 300 g at the beginning of the experiment. They were singly housed in 24.0 x 17.5 x 19.0-cm metal cages and were maintained on a 12-h dark-light cycle (lights on at 7:00 h) in a temperature- (22 ± 2ºC) and humidity- (55% RH) controlled environment. Half of the subjects were maintained at 80% of their free-feeding body weight, whereas the other half had free access to standard rodent lab food. Water was always available. Animals were weighed every day except on weekends.

Apparatus

A standard wooden plus-maze apparatus consisting of 50 x 10 x 40-cm opposite closed arms and 50 x 10-cm open arms that radiated from a central 10 x 10-cm space was used. The apparatus was elevated to a height of 50 cm above floor level by a single support. The open arms were surrounded by a 1-cm translucid Plexiglas ledge, and a dim white

light (15 W) placed 90 cm above the central area was the only source of illumination.

Procedure

Experiments were carried out between 9:00 and 17:00 h. Animals were randomly assigned to two groups, fluoxetine (FLX, N = 46) and vehicle (VEH, N = 48). The drugs were administered either acutely or chronically. In each of these conditions animals were either food deprived or fed *ad libitum*. Thus, FLX and VEH subjects were divided into 4 subgroups (N = 11-13 each): food deprived under acute treatment, food deprived under chronic treatment, satiated under acute treatment and satiated under chronic treatment. The eight groups were submitted to the behavioral procedure in alternate order, so as to counterbalance possible sequence effects.

Each subject was placed in the central area of the maze facing one of the closed arms, either 60 min after the first injection (acute treatment) or 60 min after the 22nd injection (chronic treatment) of fluoxetine or vehicle, in alternate order. Animals were observed for 5 min by a trained observer who sat quietly 1.5 m from the center of the maze and recorded the time spent in and the number of entries into each arm. An entry was recorded when the animal's four limbs had entered an arm. The observer was "blind" to the animal's condition.

Drugs

Fluoxetine hydrochloride (Lilly, São Paulo, SP, Brazil) was dissolved in Tween-80 plus 0.9% NaCl. Daily *ip* injections of 5 mg/kg were administered for 22 days, at the same time each day, in the chronic treatment subjects. Rats in the acute treatment groups received the same dose but only once. The corresponding treatment was given to the animals in the control group, which received vehicle injections (0.9% NaCl and Tween-80). All injections were performed in a different room from that of the plus-maze testing. The drug dosage was selected on the basis of its effectiveness on chronic behavioral procedures such as polydipsia (31) and stress-induced anhedonia (32).

Data analysis

For each animal, the percent of time spent in the open arms (100 x open/(open + closed)), the percent of open arm entries (100 x open/total), the number of entries into the closed arms and the total number of entries (open + closed arms) were computed. Data were analyzed by a three-factor (drug, treatment regimen, and food condition) analysis of variance, with two levels for each factor (FLX x VEH; chronic x acute; satiated x deprived).

Results

Analysis of variance showed that there were no 2-way or 3-way significant interactions between factors. Since 3-way and 2-way interactions were not significant, main effects were analyzed. The results are summarized in Figure 1.

There was a significant main effect of drug condition. Fluoxetine decreased the percentage of time spent in the open arms $(F(1,86) = 9.825, P<0.01)$, the percentage of open arm entries $(F(1,86) = 6.884, P<0.01)$, total arm entries $(F(1,86) = 18.112, P<0.001)$ and number of entries into the closed arms $(F(1,86) = 11.085, P<0.001)$. This main effect is shown in Figure 1, where it can be seen that the FLX group means are lower than the VEH group means in all comparisons. This effect occurred independently of alimentary condition or treatment regimen.

The ANOVA also revealed a significant main effect of food condition: food deprivation increased the percentage of time spent in the open arms $(F(1,86) = 76.593, P<0.001)$, the proportion of open arm entries $(F(1,86) =$

41.293, P<0.001), total arm entries (F(1,86) = 39.316, P<0.001) and the number of entries into the closed arms (F(1,86) = 12.432, P<0.001). This effect occurred whether the animals received fluoxetine or vehicle, either acutely or chronically.

Furthermore, ANOVA revealed a sig-

Figure 1 - Effect of fluoxetine on rats tested on the elevated plus-maze. Percent time spent in the open arms, percent entries into the open arms, total number of entries and number of entries into the closed arms are given for the eight groups in the experiment. "Administration regimen" (acute x chronic) and "food condition" (food deprived x satiated) are plotted as a function of the "drug" factor (fluoxetine x vehicle). Significance levels as revealed by a 3-factor ANOVA are shown in the text. Data are reported as means ± SEM. FLX = Fluoxetine, VEH = vehicle, Ac = acute, Ch = chronic, Dep = food deprived, Sat = satiated.
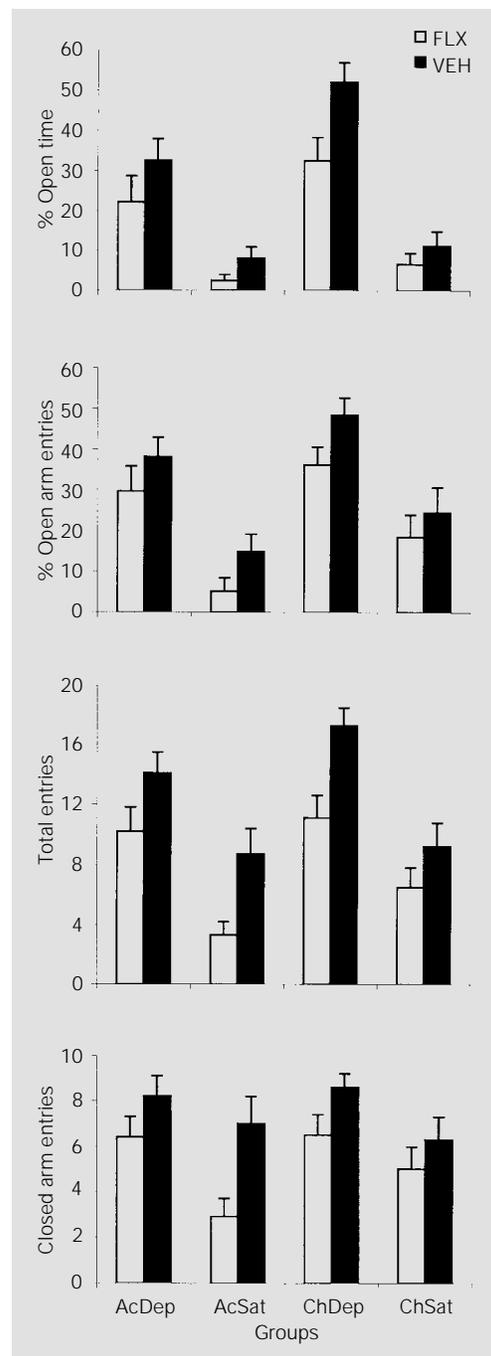
nificant main effect of treatment regimen. The percent of time spent in the open arms was significantly higher in rats submitted to a chronic regimen of either FLX or VEH injections (F(1,86) = 8.896, P<0.01), as also was the percent of open arm entries (F(1,86) = 7.362, P<0.01). The treatment regimen did not affect total number of entries (F(1,86) = 2.871, P>0.05) or number of entries into the closed arms (F(1,86) = 0.286, P>0.05). This effect was not related to alimentary condition or treatment regimen.

Changes in average body weight after chronic treatment were not significantly different between FLX and VEH in the satiated food condition. Average (± SEM) body weights for the VEH and FLX groups increased from 309.2 ± 14.0 and 306.3 ± 17.1 g at the beginning of the experiment to 357.1 ± 11.4 and 360.0 ± 13.3 g at the end of the experiment, respectively ($t = 0.10$ for initial weight; $t = 0.16$ for final weight).

Discussion

Fluoxetine induced an anxiogenic-like effect in rats tested in the elevated plus-maze: the drug decreased the proportion of open arm time and entries, regardless of whether it was being administered acutely or chronically, and independently of the subjects being food deprived or satiated. Simultaneously it decreased locomotor activity, as indicated by a reduction in the total number of arm entries and the number of closed arm entries. This motor activity reduction by fluoxetine has been observed before (20,33). The anxiogenic-like effect in the plus-maze has also been reported (20-22), but only after acute administration of the drug. In the present experiment both the chronic and the acute regimen of administration resulted in an anxiogenic profile.

The observed anxiogenic effect after both acute and chronic administration can be seen in the light of the classic hypothesis relating 5-HT and anxiety. The anxiogenic effect

after acute fluoxetine administration could be related to the increased extracellular 5-HT around subcortical structures, observed after acute administration of SSRIs. The anxiogenic effect after chronic treatment would be consistent with the desensitization of 5-HT$_{1A}$ autoreceptors and the consequent reduction in their inhibitory effects on raphe activity. It could also be related to the desensitization of 5-HT$_{1B}$ autoreceptors and the resulting decrease in feedback inhibition of serotonin release observed in the chronic condition (9,14,17).

Still, the differential clinical effect of acute and chronic fluoxetine administration would lead to the expectation of a differential effect on the elevated plus-maze indices of anxiety. In fact, an anxiogenic effect of fluoxetine would be compatible with clinical reports of increased anxiety during the first days of drug administration (34), but would not be consistent with its growing use in the chronic treatment of anxiety disorders (4). As the symptoms of anxiety disorders are heterogeneous and multidetermined, it is possible that the elevated plus-maze model is not sensitive to the specific anxiety symptoms that show clinical improvement with chronic fluoxetine. The main criterion for the use of the elevated plus-maze as an animal anxiety model is its sensitivity to the anxiolytic action of benzodiazepines, but it is known that several anxiety disorders, such as phobias and panic, are resistant to benzodiazepines (35). It is possible that the cat odor test for phobia (36), or the polydipsia model for the obsessive compulsive disorder (31), for example, would be able to detect an anxiolytic action of chronic fluoxetine in the rat.

The possibility that the plus-maze results reported here reflect motor effects of the drug cannot be discarded. As noted, motor activity reduction after fluoxetine administration has been reported. The effect of fluoxetine in the present experiment may have been confounded by changes in motor behavior, as there was a decrease in the total number of arm entries and in the number of entries into the closed arms, a reliable index of locomotor activity in the elevated plus-maze (37). A model that evaluated both passive and active avoidance, such as the elevated T-maze, would help in dismissing this possibility (38).

Although fluoxetine has been reported to produce decreased appetite and food intake both in humans (39,40) and in rats (41), subjects in the present study did not show any changes in body weight after 22 days of fluoxetine administration. The loss of appetite resulting from fluoxetine treatment would suggest caution in dealing with anorexic patients, since although the target of the prescription are emotional effects, the drug can cause concomitant body weight loss (39). However, there was no evidence of any interaction between fluoxetine and food deprivation on the plus-maze effects within the parameters of drug administration and food restriction used. There was, however, a marked effect of food deprivation on the indices of the plus-maze: food-deprived animals showed a marked increase in proportional open arm entries and time, and in the number of total entries and the number of entries into the closed arms. The elevated plus-maze, as an exploratory model of anxiety, relies on the conflict between the tendency to explore a novel environment and the tendency to avoid open spaces (42,43). Food deprivation increases the probability of foraging responses, and this factor, rather than anxiety reduction, could account for the high locomotor activity and the increased tendency of food-deprived rats to enter and remain in the open spaces in this experiment. On the other hand, the chronic treatment regimen resulted in an anxiolytic profile that was not confounded by locomotor activity. In fact, daily injections of either drug or vehicle resulted in subjects scoring proportionally more time and more entries into the open arms when compared to acutely in-

jected subjects. Their total number of entries and number of entries into the closed arms, however, were not significantly different, lending support to the hypothesis of an anxiolytic effect. This effect probably resulted from the routine handling necessarily involved in the injection procedure, a factor known to lead to reduced anxiety as measured in the elevated plus-maze and other anxiety paradigms (44,45).

The present results show the anxiolytic action of handling and suggest an anxiogenic action of both acute and chronic fluoxetine on animals tested in the elevated plus-maze. The anxiogenic action of fluoxetine is consistent with the neurochemical effects of the drug on the serotonergic system. These findings, however, should be extended to other anxiety models and to other doses of the drug, especially regarding the chronic procedure, since lower doses might have effects on anxiety-related measures but not on activity parameters.

Acknowledgments

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