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Acute and Chronic Effects of Gepirone and Fluoxetine in Rats Tested in the Elevated Plus-maze: An Ethological Analysis

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SILVA, R. C. B. AND M. L. BRANDÃO. *Acute and chronic effects on gepirone and fluoxetine in rats tested in the elevated plus-maze: An ethological analysis.* PHARMACOL BIOCHEM BEHAV **65**(2) 209–216, 2000.—The potential role of 5-hydroxytryptamine (5-HT) in anxiety has been the subject of much research, most of it addressed to the hypothesis that 5-HT promotes anxiety and, therefore, that drugs that reduce 5-HT functions will be effective anxiolytic agents in human anxiety disorders. However, the effects of serotoninergic drugs in different behavioral paradigms have been inconsistent. These inconsistencies have been particularly well illustrated in the elevated plus-maze. In the present study we provided an ethopharmacological analysis (in addition to conventional measures) of the behavior of rats in the elevated plus-maze with transparent walls after acute and chronic treatments with gepirone, an agonist of 5-HT_{1A} receptors, and fluoxetine, a selective inhibitor of serotonin reuptake. Although gepirone has been used to treat anxiety, fluoxetine is a mainstay in the treatment of depression. Acute treatment with gepirone (1, 3, 5.6, and 10 mg/kg, IP) produced an anxiogenic profile with increased risk assessment behaviors (e.g., flat-back approach) and decreased behavioral measures that are inversely related to "anxiety" (e.g., head dipping and end-arm activity). In contrast, chronic gepirone (10 mg/kg day, PO) produced an opposite effect showing an anxiolytic profile that is consistent with the clinical use of this drug, which shows efficacy after 2–4 weeks of treatment. Acute fluoxetine (5.6 and 10 mg/kg, IP) also produced an anxiogenic profile with reduced head dipping and end-arm activity. On the other hand, chronic fluoxetine (10 mg/kg day, PO) had no effect on any of the behavioral measures. These data demonstrate: (a) the anxiogenic and anxiolytic effects of acute and chronic gepirone, respectively, corroborate with the observed effects of these treatments in the clinic; (b) similarly, the anxiogenic effects of acute fluoxetine observed here have also been reported in clinical studies with 5-HT reuptake blockers. This class of compounds has not been systematically used as anxiolytic; (c) the elevated plus-maze with transparent walls shows good sensitivity for evaluating serotonergic drugs with anxiogenic and anxiolytic profile. © 2000 Elsevier Science Inc.

Elevated plus-maze Ethological analysis Gepirone Fluoxetine Rat Thigmotaxis

ANIMAL models of anxiety are used as screening tools in the search for compounds with therapeutic potential and as stimulations for research on mechanisms underlying emotional behavior (34). The elevated plus-maze is one of the most widely used animals models in contemporary preclinical research on anxiety (16,19,32). This model is based on the innate fear rodents have for open and elevated spaces (23). Rats on the elevated plus-maze tend to avoid the open arms and prefer to stay in the enclosed arms. When confined to the open arms, rats show behavioral and physiological manifestations of fear, such as freezing, defecation, and increases in plasma corticosteroids (28,38). The avoidance of the open arms occurs primarily because they prevent the rat from engaging in thigmotaxic behavior (38). Thigmotaxis is a natural defensive response that keeps the rat in contact with a vertical surface, thereby avoiding predators (36,38).

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Anxiolytic drugs increase the number of entries into and the time spent in the open arms, whereas anxiogenic agents do the opposite (14,28,29,40). The ratio (14) and the percentage (28) of open-arm to total arm entries have been used as indices of anxiety. Often the percentage of time spent on the open arms is also reported (28). These indices relate negatively with anxiety, because they are typically increased by anxiolytic and decreased by anxiogenic drugs (14,28,29).

While on the elevated plus-maze rats display a variety of behaviors that are amenable to ethological analysis (3). In potentially dangerous situations such as the elevated plus-maze rats engage in a cluster of behaviors collectively referred to as risk assessment. These measures are generally more sensitive to drug action than are the traditional indices of anxiety in this test (32). Thus, the combined use of ethologically derived behavioral measures with the traditional spatiotemporal indices greately enhances the utility and the sensitivity of the plus-maze model of anxiety (32).

Manipulations of 5-HT neurotrasmission produce highly inconsistent effects on anxiety. The elevated plus-maze has presented the widest variety of effects (15). Clearly, one methodological issue that may contribute to this inconsistency is the tendency for research to employ acute treatment, whereas both 5-HT_{1A} anxiolytics and 5-HT reuptake inhibitors are clinically effective only following a period of chronic treatment (32).

The elevated plus-maze modified with transparent walls, recently validated in our laboratory (1), allows more accurate recording of behavior, specially in the enclosed compartments and for the assessment of new ethologically defined categories of behavior to facilitate the study of different facets of anxiety and the mode of action of anxiolytic drugs (2).

The purpose of the present study was to examine the effects of 5-HT drugs, gepirone, an agonist of 5-HT_{1A} receptors, and fluoxetine, a selective inhibitor of serotonin reuptake, in acute and chronic treatments, using an ethopharmacological analysis (expressed by traditional and ethological measures) of behavior in the elevated plus-maze with transparent walls.

METHOD

Subjects

Male Wistar rats weighing 230–300 g, from the animal house of the Campus of Ribeirão Preto of the University of São Paulo, were used. These animals were transported to a room adjacent to the laboratory 72 h before the test, where they were housed in groups of six per cage under a 12:12-h light–dark cycle (lights on at 0700 h) at 23 ± 1°C, and given free access to food and water. The animals were taken to the test room at least 1 h before testing.

Apparatus

The plus-maze consisted of two open arms, 50 × 10 cm (length × width), and two enclosed arms 50 × 10 × 50cm (length × width × height), arranged such that the two arms of each type were opposite to each other. The maze was elevated to a height of 50 cm. The walls of the closed arms were made of sheets of transparent Plexiglas. The level of illumination was 100 lx on the floor level of the walled arms.

Procecure

The rats were placed individually in the center of the modified maze facing a closed arm and allowed 5 min of free exploration. The behavior of the animals was recorded by a video-camera positioned above the maze, allowing for the discrimination of all behaviors, with the signal relayed to a monitor in another room via a closed-circuit TV camera. The maze was throughly cleaned after each test with a solution of 20% ethanol and then dried. Each rat was tested only once.

All the experiments were carried out between 0800 and 1000 h. Videotapes were subsequentely scored by an observer using ethological analysis software (The Observer) developed by Noldus (The Netherlands). Using separate location and behavior keys, this software allows the real-time scoring of the videotapes of any behavior by direct keyboard entry to a PC. Behaviors scored from videotape included traditional and novel plus-maze parameters. Samples of animal behavior were correlated, and the coefficient rate was equal to 0.9.

The behavior of each animal in the maze was analyzed, taking into account the standard measures recorded in each section of the maze (closed and open arms, central platform), comprising the frequency of open and closed-arm entries (arm entry defined as all four paws into an arm), total arm entries, and the amount of time spent by the animals in each section of the maze. These data were additionally used to calculate the percentage of time in the center platform.

The ethological items recorded were grooming, rearing, peeping out, stretched-attend posture, flat-back approach, scanning, head dipping, end-arm activity, and immobility. These categories were defined following work in rats (3,8) and in mice (33): (a) grooming: species-typical sequences beginning with the snout, progressing to the ears and ending with whole-body groom; (b) rearing: partial or total rising onto the hind limbs; (c) scanning: scrutinizing in any direction, including sniffing (olfactory exploration of maze floor and walls); (d) head dipping (unprotected): exploratory movement of head/shoulders over sides of the open arms and down towards the floor; (e) end-arm activity: number of times the rat reached the end of an open arm; (f) peeping out: stretching the head/shoulders from the closed arms to the central platform; (g) stretched-attend posture (SAP): when the animal stretches to its full lenght and turns back to the anterior position; (h) flat-back approach (FBA): locomotion when the animal stretches to its full length and cautiously moves forward; and (i) immobility: animal still, without any moviment over 6 s.

Drugs

Groups of animals (n = 12) were tested, in the modified maze only, following pretreatment with saline, gepirone HCl (Bristol-Myers, Wallingford, CT, USA) or fluoxetine (Lilly Research Laboratories, Indianapolis, IN, USA). Gepirone and fluoxetine were dissolved in saline (0,9%) shortly before use. Saline also served as the vehicle control. Compounds were administered (1 ml/kg, IP) 30 min before testing.

Acute experiments. Rats were randomly allocated to the following groups: (a) vehicle control and gepirone (1, 3, 5.6, and 10 mg/kg); (b) vehicle control and fluoxetine (5.6 and 10 mg/kg). Drugs and vehicles were injected intraperitoneaily (IP), 30 min before testing. Each rat received only one injection. The dose of 10 mg/kg was chosen on the basis of our preliminary studies with doses ranging from (1–10 mg/kg).

Chronic experiments. Rats were housed in groups of six per cage for 2 weeks in Plexiglas cages and randomly allocated to three groups: (a) control, without any drug treatment; (b) submitted to 10 mg/kg gepirone daily, PO; and (c) submitted to 10 mg/kg fluoxetine daily, PO. As to this latter two treatments, preliminary experiments were carried out to

determine the average daily water intake of the animals. Gepirone and fluoxetine were added to the water recipients of the second and third groups, respectively, so that nearly 10 mg/kg was given each day for 2 weeks. The main reason for grouping six animals per cage was due to previous study from this laboratory (22) showing that the stress caused by isolation sum up to the anxiogenic effects of drugs. However, rats treated with chronic gepirone and fluoxetine had a significant reduction in water intake (manuscript in preparation). To control for this reduction in water intake the following precautions were taken: 1) drug solutions were prepared every day, and concentrations were recalculated on the basis of water intake in the previous day, increasing concentrations so as to compensate for intake decreases and keep as constant as possible the 10 mg/kg/rat daily drug intake; 2) the water recipient always contained more drug solutions than the real necessity of the group of animals, so as to eliminate the possibility of an animal to drink more water at the expenses of another animal; 3) the experiments began early in the morning (0800 h) when dosing of the drug is higher, because rats drink more during the night when they are more active.

Statistical Analysis

Data are reported as means ± SEM. Results of experiments were analyzed by one-way analyses of variance (ANOVA). Dunnett post hoc comparisons were carried out if significant overall *F*-values ($p < 0.05$) were obtained ($n = 12$ for each group).

RESULTS

Gepirone

Acute treatment. ANOVA did not reveal a significant effect of gepirone (*df* 4,91) on the percentage of open-arm entries ($F = 0.35$, $p > 0.05$), or center platform ($F = 1.97$, $p >$

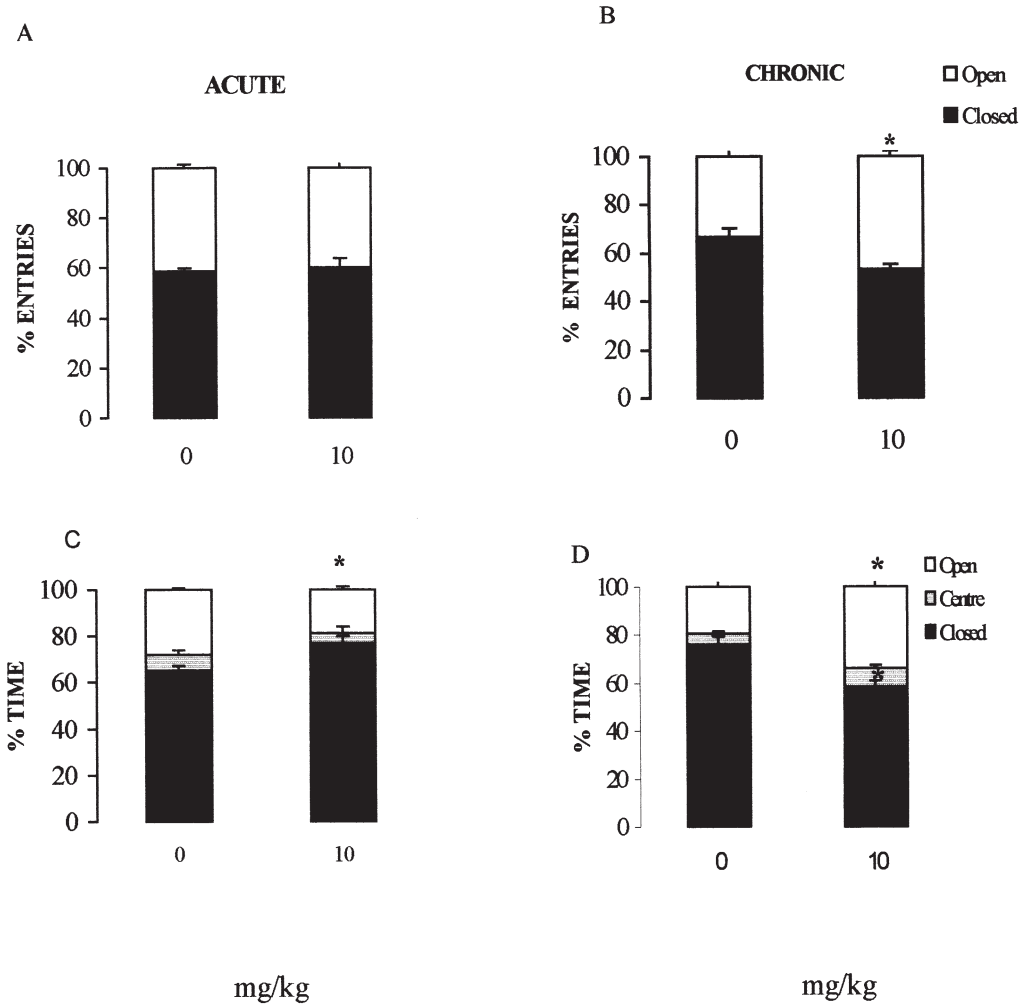

FIG. 1. On the top, mean (±SEM) percentage of entries in open and closed arms of the modified elevated plus-maze by rats tested with acute (30 min before the test, A) and chronic (B) gepirone (10 mg/kg, PO) daily for 2 weeks. On the bottom, effects of acute (C) and chronic (D) gepirone on the percentage of time spent in open and closed arms and center platform of the modified elevated plus-maze. *$p < 0.05$, Dunnett's test. Control groups $n = 48$ for acute and $n = 12$ for chronic treatments. Drug group $n = 12$ for acute and chronic treatments.

0.05), but decreased the percentage of time spent in the open arms of the modified maze ($F = 2.66$, $p < 0.05$) (Fig. 1A and C). Acute gepirone did not change the number of entries into the enclosed arms ($F = 1.42$, $p > 0.05$).

Regarding the ethological measures (Fig. 2A), gepirone significantly (*df* 4,91) decreased the following items: rearing ($F = 11.72$, $p < 0.001$); scanning ($F = 3.17$, $p < 0.05$); head dipping ($F = 5.60$, $p < 0.001$), and end-arm activity ($F = 5.52$, $p < 0.001$). Post hoc analyses revealed that these effects were due to the 3-mg/kg dose for scanning, and all doses for the others categories, except for 1 mg/kg, which did not significantly change rearings. Significant increases were seen in: grooming ($F = 3.13$, $p < 0.05$); flat-back approach ($F = 6.05$, $p < 0.001$), and immobility ($F = 5.80$, $p < 0.001$). Post hoc analysis showed that these effects were due to the dose of 1 mg/kg for grooming, 5.6 and 10 mg/kg for flat-back approach, and 3, 5.6, and 10 mg/kg for immobility. No significant effect were seen in: peeping out ($F = 1.30$, $p > 0.05$); and sap ($F = 1.37$, $p > 0.0\,5$).

Chronic treatment. Gepirone produced statistically significant increases (*df* 1,22) on the percentage of entries in the open arms ($F = 10.15$, $p < 0.01$), on the percentage of time spent in the open arms ($F = 47.80$, $p < 0.001$), and closed arm of the modified maze ($F = 4.03$, $p < 0.05$) (Fig. 1B and D).

Regarding ethological measures (Fig. 2B) significant decreases (*df* 1,22) occurred in the following categories: flat-back approach ($F = 8.66$, $p < 0.001$) and immobility ($F = 7.16$, $p < 0.005$). Significant increases were seen in head dipping ($F = 23.04$, $p < 0.001$) and end-arm activity ($F = 50.81$, $p < 0.001$). No significant effects were seen in: peeping out ($F = 2.20$, $p > 0.05$); grooming ($F = 0.61$, $p > 0.05$); rearing ($F = 1.14$, $p > 0.05$); scanning ($F = 0.28$, $p > 0.05$), and sap ($F = 1.79$, $p > 0.005$).

Postural elements characteristic of the serotonergic sindrome such as forepaw treading, hind limb abduction, and flat body posture were not seen following acute or chronic gepirone administration.

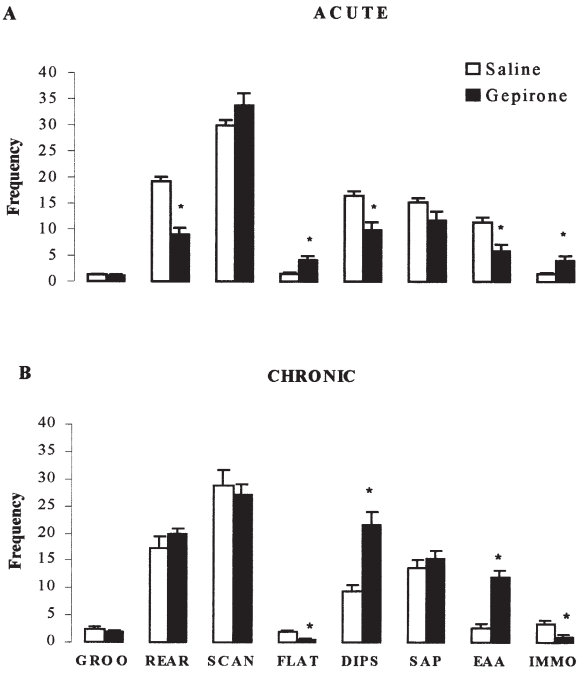

FIG. 2. Effects of acute (10 mg/kg, IP) and chronic gepirone (10 mg/kg, PO) on the ethological measures of the behavior of rats in the elevated plus-maze with transparent walls. Data are presented as means (±SEM). *$p < 0.05$, Dunnett's test. GROO, grooming; REAR, rearing; SCAN, scanning; FLAT, flat-back approach; DIPS, head dipping; SAP, stretched-attend posture; EAA, end-arm activity; IMMO, immobility. *N* for acute treatment: control group = 48, gepirone = 12. *N* for chronic treatment: control and gepirone groups = 12.

Fluoxetine

Acute treatment. Acute fluoxetine decreased the percentage (*df* 2, 45) of open arms entries ($F = 4.57$, $p < 0.05$), percentage of time spent in the open arms ($F = 6.29$, $p < 0.05$), and center platform ($F = 7.72$, $p < 0.01$). Post hoc analysis revealed that these effects were due to the dose of 10 mg/kg (Fig. 3A and C). Because acute fluoxetine increased the number of entries into the enclosed arms ($F = 4.70$, $p > 0.05$) the anxiogenic-like effects of this drug cannot be attributed to a motor deficit.

Regarding ethological measures (Fig. 4A), fluoxetine significantly decreased (*df* 2, 45) the following categories: head dipping ($F = 5.19$, $p < 0.01$) and end-arm activity ($F = 5.41$, $p < 0.01$). Post hoc analysis showed that for both categories the effects were due to the dose of 10 mg/kg. No significant effect could be detected in: peeping out ($F = 1.00$, $p > 0.05$); grooming ($F = 0.62$, $p > 0.05$); rearing ($F = 0.39$, $p > 0.05$); scanning ($F = 0.86$, $p > 0.05$); flat-back approach ($F = 0.32$, $p > 0.05$); stretched-attend posture ($F = 1.39$, $p > 0.05$); and immobility ($F = 2.45$, $p > 0.05$).

Chronic treatment. Chronic treatment with fluoxetine had no significant effect on the percentage (*df* 1, 22) of open-arm entries ($F = 0.60$, $p > 0.05$), percentage of time spent in the open arms ($F = 0.44$, $p > 0.05$, and center platform of the modified maze ($F = 3.30$, $p > 0.05$) (Fig. 3B and D).

Regarding ethological measures (Fig. 4B), no significant effect (*df* 1,22) were seen in any of the categories: peeping out ($F = 1.00$, $p > 0.05$); grooming ($F = 0.72$, $p > 0.05$); rearing ($F = 2.20$, $p > 0.05$); scanning ($F = 2.40$, $p > 0.05$); flat-back approach ($F = 1.94$, $p > 0.05$); head dipping ($F = 0.06$, $p > 0.05$); stretched-attend posture ($F = 3.21$, $p > 0.05$); end-arm activity ($F = 0.19$, $p > 0.05$) and immobility ($F = 0.27$, $p > 0.05$).

DISCUSSION

Animals exposed to the elevated plus-maze show a sensitization of fear of the open arms (23). This aversion to open arms is enhanced significantly in rats isolated from periods varying from 1 h to 2 weeks (22). Recently, it has been shown that rats still display open-arm avoidance when tested in an elevated plus-maze with transparent walls, and that it allows more accurate recording of behavior, especially in the enclosed compartments (1,2). The utility of this modified maze for serotoninergic drugs could be related to a better dissociation of fear components of the test, i.e., the openness and height are much more prominent than in the traditional elevated plus-maze test (2).

The 5-HT_{1A} receptor subtype is particularly important in psychiatry, as it is supposed to be the primary site of action of anxiolytic/antidepressant drugs of the azapirones class (buspirone, gepirone, ipsapirone, tandospirone). 5-HT_{1A} receptors are localized presynaptically on cell bodies and dendrites of raphe neurons (somatodendritic autoreceptors), and postsynaptically on target sites such as the hippocampus, sep-

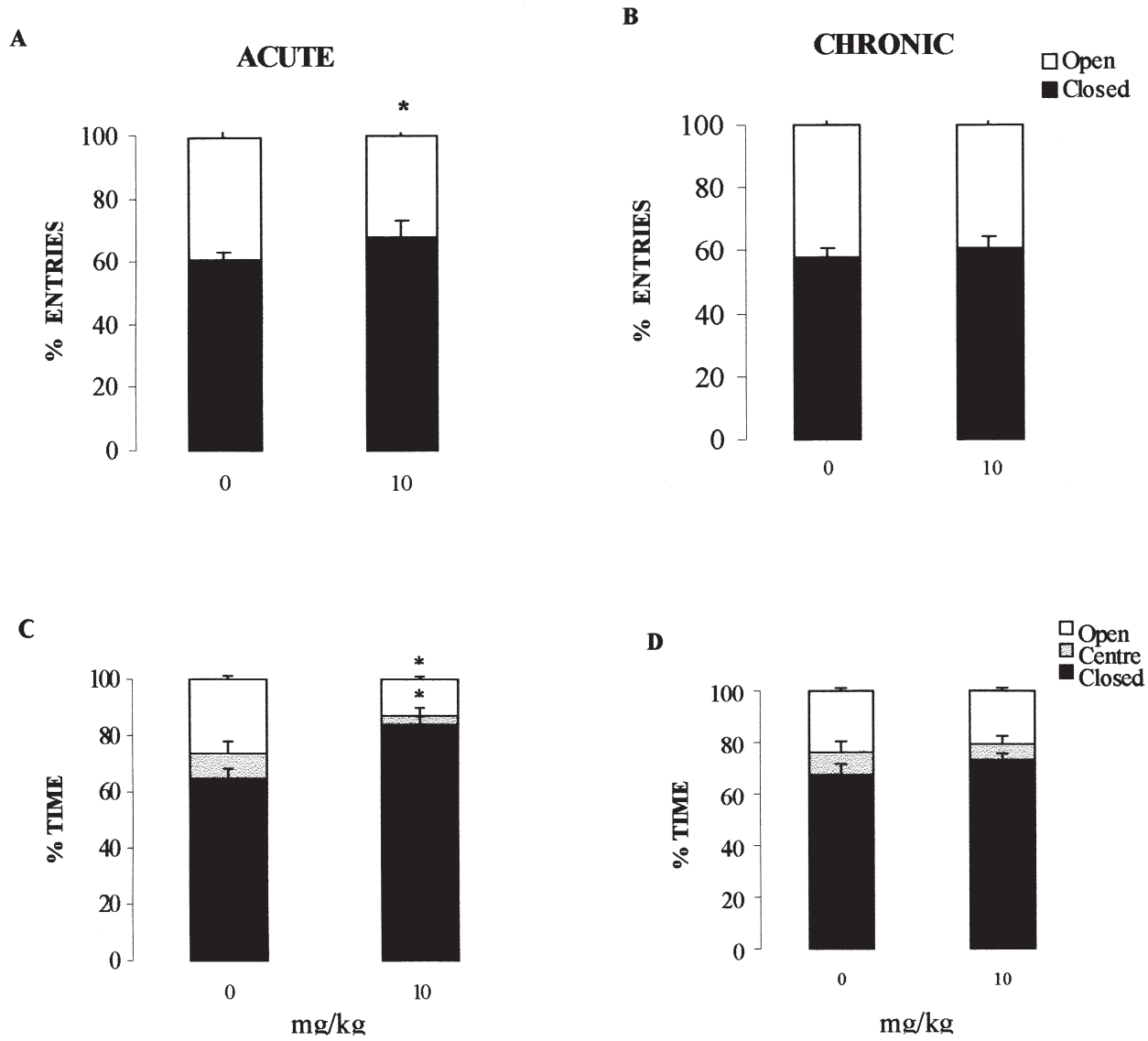

FIG. 3. On the top, mean (±SEM) percentage of entries in open and closed arms of the modified elevated plus-maze by rats tested with acute (30 min before the test, A) and chronic (B) fluoxetine (10 mg/kg, PO) daily for 2 weeks. On the bottom, effects of acute (C) and chronic (D) fluoxetine on the percentage of time in open and closed arms and center platform of the modified elevated plus-maze. *$p < 0.05$, Dunnett's test. Control groups $n = 48$ for acute and $n = 12$ for chronic treatments. Drug group $n = 12$ for acute and chronic treatments.

tum, neocortex, certain nuclei of the amygdala, and hypothalamus (10).

Our results show that acute treatment with gepirone (10 mg/kg, IP) produced an anxiogenic profile characterized by decreased time spent in the open arms and center platform of the modified plus-maze. The effects of acute gepirone are not the result of changes in motor activity, because this 5-HT agonist did not affect the number of entries into the enclosed arms. In contrast, chronic gepirone (10 mg/kg, PO) produced anxiolytic effects with increased open-arm entries and more time spent in the open arms and center platform of the maze.

The delayed anxiolytic effect of gepirone has been explained by a progressive desensitization of the somatodendritic 5-HT_{1A} autoreceptors, combined with the tonic activation by gepirone of postsynaptic 5-HT_{1A} receptors (4,5,25). The double pre- and postsynaptic location of 5-HT_{1A} receptors has important physiological correlates. The activation of presynaptic 5-HT_{1A} autoreceptors by a selective agonist produces an inhibitory effect on serotonin neurotransmission, while the activation of postsynaptic 5-HT_{1A} receptors produces an excitatory effect (24). Therefore, the present results support previous suggestion that anxiolytic effects mediated by 5-HT_{1A} receptors take place only when some kind of adaptation of the 5-HT neurotransmission is induced by the sustained presence of the 5-HT_{1A} agonist (4,5,25).

Assessment of the ethological measures revealed that acute gepirone (10 mg/kg, IP), also produced an anxiogenic profile with increased risk assessment behavior (flat-back approach) and decreased head-dipping and end-arm activity. These results are consistent with reports showing that anxio-

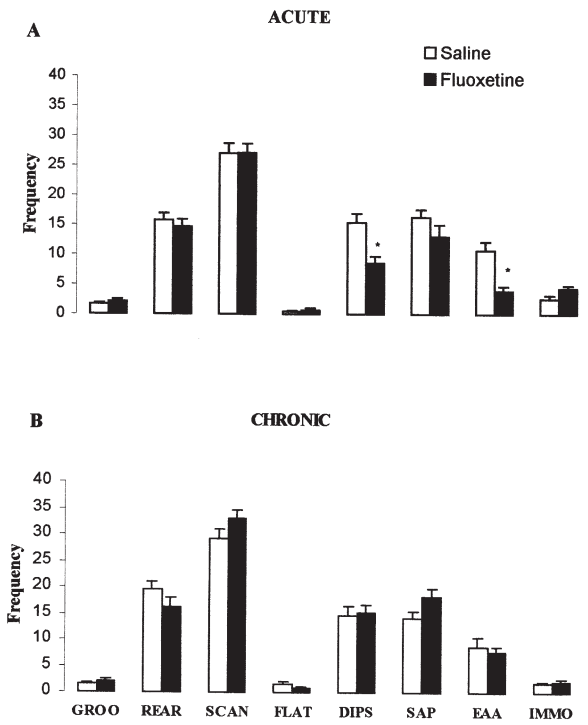

FIG. 4. Effects of acute (10 mg/kg, IP) and chronic fluoxetine (10 mg/kg, PO) on the ethological measures of the behavior of rats in the elevated plus-maze with transparent walls. Data are presented as means (±SEM). *$p < 0.05$, Dunnett's test. GROO, grooming; REAR, rearing; SCAN, scanning; FLAT, flat-back approach; DIPS, head dipping; SAP, stretched-attend posture; EAA, end-arm activity; IMMO, immobility. *N* for acute treatment: control group = 24, fluoxetine = 12. *N* for chronic treatment: control and fluoxetine groups = 12.

genic effects can be revealed through increases in risk assessment measures because, in general, they are more sensitive to drug action than are the traditional indices of anxiety in this test (6,7,13,30,31). In contrast, chronic gepirone (10 mg/kg, PO), produced an opposite effect showing an anxiolytic profile with a reduced flat-back approach (an effect that may be due to a reduced fear of leaving safe areas of the maze) and increased head dippings and end-arm exploration, indicating an enhanced tendency to actively explore the potentially dangerous areas (6). Our results indicate that acute gepirone increased immobility and reduced total rearings, while chronic gepirone produced an opposite effect. In support to this data it has been shown that the horizontal exploratory behavior activity and rearing activity were decreased by systemically and intracerebral administration into the dorsal raphe nuclei of the 8-OH-DPAT, an agonist of 5-HT_{1A} receptors (17). The supression of locomotor activity by systemic 8-OH-DPAT administration could be due to predominat effects in the dorsal raphe reducing serotoninergic transmission in the forebrain (17). Once more, the effects of chronic gepirone could be due to desensitization of somatodendritic receptors at raphe nuclei and activation of postsynaptic 5-HT receptors in the brain structers.

Stretched-attend posture (SAP) was not affected by both treatments used in the present study. Two recent studies on the effects of BZ receptor ligands in rats also failed to confirm that SAP is superior to traditional indices of anxiety (8,12).

Scanning, in the way it is operationally defined here, was not affected by either acute or chronic gepirone, suggesting that this response may not be directly linked to anxiety. It has been suggested that although scanning, including sniffing, appears to be a function of defense-related factors, it mainly reflects foraging/searching for consumatory objects (2,3). Peeping out was increased by acute gepirone, but not in a significant way, and no effect was found after chronic treatment. Grooming was not affected by either acute and chronic treatments.

Postural elements characteristic of the serotonergic sindrome produced by 5-HT_{1A} agonists such as 8-OH-DPAT, such as forepaw treading, hind limb abduction, and flat body posture were not seen following acute or chronic gepirone administration. It has been shown that these behavioral elements are induced via postsynaptic 5-HT_{1A} receptor stimulation (39). One probable reason for these discrepancies may be due to the fact that, whereas 8-OH-DPAT shows full agonistic properties in all models of 5-HT_{1A} receptors, gepirone, ipsipapirone, and other pyrimidinyl piperazines are partial agonists in the postsynaptic receptors (9).

Evidence derived from clinical studies (27) suggests that antidepressant drugs can effectively treat anxiety disorders, especially those in which panic attacks are major symptom. More recent research has suggested that serotonin selective reuptake inhibtors (SSRIs), are also effective in this regard, and may actually have therapeutic advantage over conventional tricyclics (10). However, preclinical investigations with SSRIs in animal models of anxiety disorders reveal highly variable effects of these drugs [for review, see (11)]. Thus far, there is no single animal model of anxiety that may be said to strictly correspond to one type of anxiety disorder (21,37). Specifically, the evidence for an anxiolytic effect of fluoxetine on animal models of anxiety is controversial, and studies on its chronic effects are few.

Fluoxetine increase extracellular 5-HT levels around cell bodies, which in turn, activate somatodendritic 5-HT_{1A} autoreceptors highly abundant in the raphe nuclei. This leads to an inhibition of the firing of 5-HT neurons, and to the subsequent decrease in 5-HT release in nerve terminal forebrain regions. When the treatment is continued for 2–3 weeks there is a gradual recovery to normal firing activity of 5-HT neurons due to a desensitization of the 5-HT_{1A} autoreceptors (18). Also, fluoxetine has a potent blocking effect on 5-HT_2 receptors (26). 5-HT_2 receptors are widely expressed in the brain, and appear to mediate many important effects of serotonin. Thus, some therapeutic effects of fluoxetine may be a consequence of blocking 5-HT transporters and 5-HT_2 receptors (26). Our results show that acute treatment with fluoxetine (10 mg/kg, IP) decreased the percentage of open-arm entries and of time spent in the open arms and center platform of the maze, suggesting an anxiogenic profile. The effects of fluoxetine are not the result of changes in motor activity, because this drug did not affect the number of entries into the enclosed arms. Regarding ethological measures, acute fluoxetine reduced head-dipping and end-arm activity, but had no effect on any other behavioral categories. Chronic fluoxetine (10 mg/kg, PO) showed no effect on any of the spatiotemporal measures, and behavioral items. Thus, fluoxetine is no longer anxiogenic after chronic administration. Similar results have also been reported by other authors (15,20). These differences observed for the effects of fluoxetine in relation to those reported for gepirone and probably due to the distinct pharmacological profile of these drugs, particularly the blockade of 5-HT_2 receptors with fluoxetine. In this regard, in our

hands systemic ketanserin also showed anxiogenic effects in rats exposed to the elevated plus-maze (25).

In conclusion, our results demonstrate that the anxiogenic and anxiolytic effects of acute and chronic gepirone, respectively, are similar to those reported by many other studies using animal models of anxiety providing evidence for the mode of action of 5-HT_{1A} agonists useful in clinics. The lack of anxiolytic effects of chronic fluoxetine also conforms with the poor efficacy of this drug in generalized anxiety dissorder, although the SSRI have proven useful for panic disorders and are widely used in the treatment of depression. The use of this class of compounds in the latter clinical conditions is usually accompained by anxiety in the initial phase of the treatment, as observed with acute administration of fluoxetine in the present study. Thus, the elevated plus-maze with transparent walls shows good sensitivity for evaluating serotoninergic drugs with an anxiogenic and anxiolytic profile. Finally, the present study confirms previous evidence that the so-called end-arm exploration (end-arm activity) and head dipping, taken together with the spatiotemporal measures, are particularly useful for detecting anxiolytic and anxiogenic effects of serotonergic drugs (35).

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