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Effects of acute and chronic treatment with *Hypericum perforatum* L. (LI 160) on different anxiety-related responses in rats

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Abstract

The study investigates the effects of acute and chronic oral treatment with *Hypericum perforatum* L. (HP LI 160, 62.5–500 mg/kg) in rats submitted to different anxiety models: the elevated T-maze (for inhibitory avoidance and escape measurements), the light/dark transition, and the cat odor test. These models were selected for their presumed capacity of evidencing specific subtypes of anxiety disorders as recognized in clinical practice. The results showed that acute HP (125 mg/kg) impaired elevated T-maze inhibitory avoidance, an anxiolytic effect, without altering escape performance. Chronic HP (250 mg/kg) enhanced avoidance latencies only in animals that were preexposed to the open arms of the maze. Preexposure shortens escape latency, improving it as an escape index. Differently from the reference drug imipramine (IMP, 15 mg/kg), chronic HP did not impair escape from the open arms of the maze. On the other hand, similarly to IMP, the extract increased the number of transitions between the two compartments in the light/dark transition model. Treatment regimens with HP and IMP did not alter behavioral responses of rats to a cloth impregnated with cat odor. These observations suggest that HP LI 160 exerts anxiolytic-like effects in a specific subset of defensive behaviors, particularly those related to generalized anxiety.

Keywords: *Hypericum perforatum* L.; Anxiety; Elevated T-maze; Light/dark transition model; Cat odor test

1. Introduction

Hypericum perforatum L. (HP), popular name Saint John's wort, has a long history of use in folk and traditional medicine for the treatment of different clinical conditions, including psychological disorders such as depression and anxiety (The United States Pharmacopeial Convention, 1998). The compound is probably one of the most well studied herbal agents. Its profile in depression has attracted a great amount of interest as attested by the increasing number of controlled clinical trials and laboratory studies published during the last years (Butterweck et al., 1997; Gambarana et al., 1999; Kim et al., 1999; Linde et al., 1996; Özturk, 1997). Important clinical results were reported in an article containing a meta-analysis of 23 clinical trials conducted with 1757 outpatients suffering from mild to moderate depression (Linde et al., 1996). HP was significantly superior to placebo and comparably effective with standard antidepressants while producing fewer side effects. Additionally, the therapeutic profile of HP in depression has also been attested by experiments conducted with different animal models (Butterweck et al., 1997; Gambarana et al., 1999; Kim et al., 1999; Özturk, 1997). It has been proposed that the antidepressant effect of HP may be due to its pharmacological action on monoaminergic systems, i.e., inhibition of serotonin (5-HT), norepinephrine, and dopamine reuptake (Müller et al., 1997; Neary and Bu, 1999; Perovic and Müller, 1995) and alteration of both 5-HT_2 and β-receptors (Müller et al., 1997).

Far less investigated, however, is HP therapeutic profile in anxiety. Nevertheless, the great suffering reported by individuals with anxiety disorders together with high medical–social costs should be a factor of encouragement for undergoing research all over the world to seek for altern-

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ative, less expensive forms of treatment. In doing that, a first thing that one has to keep in mind is that clinical anxiety is a heterogeneous syndrome, comprising distinctive pathological conditions, such as panic, phobias, posttraumatic stress, and generalized anxiety disorder (GAD) (American Psychiatric Association, 1994). It has also been evidenced that these different conditions may be unequally affected by pharmacological therapy. Thus, while GAD is successfully treated with benzodiazepines and with serotonergic compounds such as buspirone, panic disorder and phobias do not, in general, respond favorably to these same pharmacological agents (Nutt, 1991).

At a preclinical level of investigation, there is also a growing body of evidence suggesting that anxiety, as operationally defined in a given animal model, may differ from that generated in other models depending on its nature (if innate or learned), its response to the effects of drugs and environmental manipulations, and its underlying neural substrate (File, 1993; Handley and McBlane, 1993; Zangrossi and Graeff, 1997). Based on these evidences, efforts have been made in order to validate experimental models specifically related to certain kinds of anxiety disorders (Graeff, 1991; Graeff et al., 1993; Griebel et al., 1995; Hendrie and Weiss, 1994; Johnston and File, 1998; Zangrossi and File, 1992a,b, 1994).

Taking the above evidences into account, the purpose of the present work was to explore the therapeutic potential of HP in anxiety through the use of three different animal models: the elevated T-maze (Graeff et al., 1993; Viana et al., 1994), the light/dark transition model (Crawley, 1981; Crawley and Davis, 1982; Crawley and Goodwin, 1980), and the cat odor test (Zangrossi and File, 1992a,b, 1994).

The elevated T-maze (Graeff et al., 1993; Viana et al., 1994), derived from the elevated plus-maze (Pellow et al., 1995), was developed to allow the measurement, in the same animal, of two subtypes of anxiety-related responses, a conditioned (inhibitory avoidance from the open arms) and an unconditioned type (escape). Compounds representative of three classes of anxiolytics — namely the agonist of benzodiazepine receptors diazepam, the 5-HT_{1A} agonist buspirone, and the nonselective 5-HT_2 antagonist ritanserin—have been shown to selectively impair inhibitory avoidance while leaving one-way escape unchanged (Graeff et al., 1998). These results are compatible with the view that inhibitory avoidance relates to GAD. On the other hand, impairment of open arm escape has been described with chronic administration of the antipanic compound, imipramine (IMP) (Custódio Teixeira et al., 2000). This last result was obtained after the inclusion of a new parameter in the test procedure: a 30-min prior forced exposure to one of the open arms of the maze. The modification resulted in a decrease in the latency to leave this arm on a later trial, assuring that during measurements of escape animals were really escaping from aversion and not simply ambling. The findings obtained with IMP after this procedural modification are in agreement with the interpretation that latency to leave the open arm may be correlated to panic (Graeff et al., 1997, 1998).

The light/dark transition model (Crawley, 1981; Crawley and Davis, 1982; Crawley and Goodwin, 1980) uses the aversion of rodents for brightly lit spaces when animals are placed in a two-compartment box, one light and the other dark. The number of transitions between the two compartments and the time spent in the illuminated compartment are parameters largely used to assess the anxiolytic profile of different pharmacological compounds. In a way similar to their therapeutic profile in GAD, benzodiazepines increase both the number of transitions and the time spent in the lighted compartment (Crawley, 1981; Crawley and Davis, 1982; Crawley and Goodwin, 1980). Finally, the exposure to cat odor (Zangrossi and File, 1992a,b, 1994) investigates behavioral responses of rats during exposure to a cloth impregnated with the predator's odor. These responses, as originally measured, are resistant to different classes of anxiolytic drugs, which has led to the suggestion of a relationship between the test and symptomatic aspects of human phobias (Zangrossi and File, 1992a,b, 1994).

For the present experiment, investigation of the acute effects of a commercially available HP extract (LI 160) was performed in the elevated T-maze and in the cat odor test (Experiment 1). Chronic effects of HP LI 160 (Experiment 2) were evaluated in the elevated T-maze, light/dark transition, and cat odor test and compared to that of the tricyclic antidepressant IMP. Taking into account that escape responses in the elevated T-maze seem to be improved after preexposure to one of the open arms of the maze (Custódio Teixeira et al., 2000), in Experiment 2 tests in the model were conducted both in animals not exposed and preexposed to the open arms. The light/dark transition model was included to further extend the investigation of HP potential effect in GAD-related responses, as suggested by results obtained in Experiment 1. In order to avoid confusing results due to treatment effects on locomotor activity, in both experiments the animals were also tested in the arena.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) bred in the animal house of the University of São Paulo in Ribeirão Preto, Brazil, were housed five to six per cage for 5 days until the experiments in the elevated T-maze were carried out. After that, each animal was individually housed for five extra days when behavioral responses to the light/dark transition model (Experiment 2) and to cat odor exposure (Experiments 1 and 2) were evaluated. Room temperature was controlled (23 ± 1 °C) and the lights were on from 07:00

to 19:00 h. Food and water were available ad libitum. Experimental procedures were in agreement with the National Institutes of Health Guide for care and use of laboratory animals.

2.2. Extract, drugs, and vehicles

HP LI 160 (Lichtwer Pharma, Germany; Extraction solvent: 80% methanol (v/v); herb-extract ratio 4–7:1; naphtodiantrones 0.28%; hyperforin 3.2%) was suspended in saline and sonicated for a period of 30 min for later oral administration (po; volume of 10 ml/kg). Imipramine hydrochloride (IMP, Sigma, USA) was dissolved in saline and also administered orally (po; volume of 10 ml/kg). Both compounds were prepared on the same day of the experiments.

2.3. Apparatus

2.3.1. Elevated T-maze

The elevated T-maze was made of wood and had three arms of equal dimensions (50 × 12 cm). One arm was enclosed by 40-cm high walls and was disposed perpendicularly to two opposite open arms. The entire apparatus was elevated 50 cm above the floor. To avoid falls, the open arms were surrounded by a Plexiglas rim 1 cm high.

2.3.2. Arena

The arena used to measure locomotion was a wooden square box (60 × 60 cm) with walls 30 cm high, and had the floor divided into nine smaller squares of equal dimensions (20 × 20 cm).

2.3.3. Light/dark transition model

The apparatus used was a box made of wood with overall dimensions of 48 × 24 × 27 cm (length × width × height) and a grid floor composed of bars 5 cm apart. The box was further divided by a barrier possessing a doorway (10 × 10 cm) through which rats could traverse into two chambers of equal dimensions (24 × 24 × 27 cm): one painted black, not illuminated, and one painted white and brightly illuminated with a 300 lx light source.

2.3.4. Cat odor test

Animals were exposed to cat odor while in their single home cages. The cages were polypropylene boxes measuring 56 × 25 cm and covered by a raised wire top that stood 16 cm high from the cage floor. Food and water compartments were localized at one extremity of the cages and had 15 cm in extension. During tests, these compartments were furnished with a water bottle and regular food pellets. The cat odor was obtained by rubbing a damp cloth (20 × 20 cm) against the fur of male laboratory-housed domestic cats for 5 min. This procedure was carried out 1 h before each test session. Cloths with cat odor were kept in sealed plastic bags. Each cloth was used for four exposures only.

2.4. Procedure

2.4.1. Experiment 1—acute administration

On the third day after their arrival in the laboratory, animals ($N = 67$) were gently handled by the experimenter for 5 min during two consecutive days. On the fifth day, they were randomly allocated to different treatment groups and injected per os with one of the doses of HP LI 160 (62.5, 125, 250, or 500 mg/kg) or saline. One hour after treatment with HP LI 160 or saline, each animal was placed at the distal end of the enclosed arm of the elevated T-maze facing the intersection of the arms. The time taken by the rat to leave this arm with the four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (Avoidance 1 and 2) at 30-s intervals, during which animals were placed in a Plexiglas cage where they had been previously habituated. Following avoidance training (30 s), rats were placed at the end of the right open arm of the maze and the latency to leave this arm with the four paws was recorded for three consecutive times (Escape 1, 2, and 3) with 30-s intertrial intervals. Immediately after being tested in the elevated T-maze, each animal was placed for 5 min in the arena for the evaluation of locomotor activity. During this time, the total number of lines crossed and frequency of rearings was measured.

After tests in the arena, animals were individually housed (see cage specification in Section 2.3.4) for five consecutive days. On the fifth day, rats were again administered with the same dose of HP LI 160 or saline and submitted to the cat odor test. For that, two rats at a time were taken to the experimental room in their individual home cages. The two single home-cages were then placed side by side and one odor cloth was placed on the top of each cage, in the extremity opposite to the food and water compartments. The odor exposure lasted for 5 min and was video-recorded for later scoring. For each rat, number of contacts with the cloth (distance inferior or equal to 5 cm from the cloth), as well as the time spent sheltering under the food and water compartment were quantified.

2.4.2. Experiment 2—chronic administration

A second group of animals ($N = 54$) was injected per os with HP LI 160 (62.5, 125, or 250 mg/kg), IMP (15 mg/kg), or saline for 14 consecutive days. On Day 14, they were tested in the elevated T-maze 1 h after injections. Immediately after the T-maze session, animals were tested in the arena for 5 min, as described above.

After tests in the arena, these animals were, as in Experiment 1, individually housed for five consecutive days. During this period, they continued receiving the same pharmacological treatment. On the fifth day, rats were again administered with HP LI 160, IMP, or saline and 1 h later submitted to the light/dark transition model. For that, each rat was placed in the middle of the lighted compartment facing the doorway separating the two compartments. After the first transition from the lighted to the dark compartment,

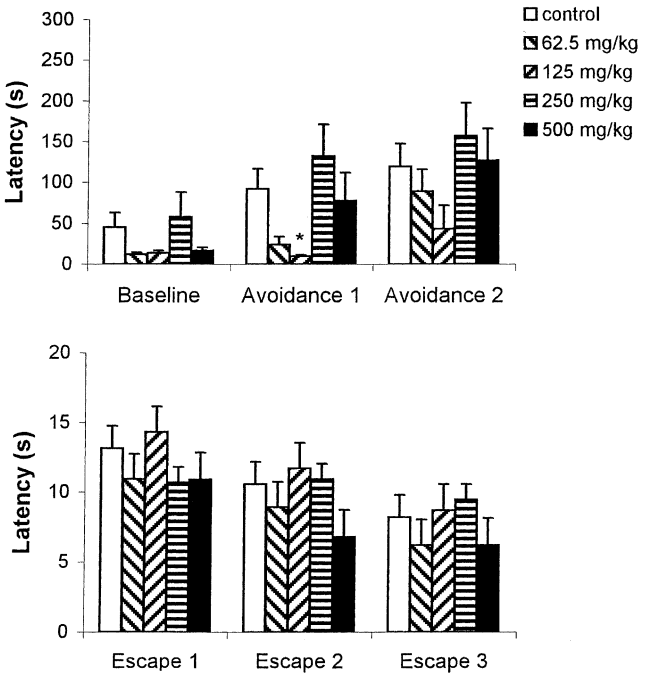

Fig. 1. Effects (mean ± S.E.M.) of acute per os administration of HP LI 160 on the behavior of rats submitted to the avoidance (upper panel) and escape (lower panel) tasks of the elevated T-maze. $n = 19$ (control), 15 (HP 62.5 mg/kg), 10 (HP 125 mg/kg), 13 (HP 250 mg/kg), and 10 (HP 500 mg/kg). * $P < .05$ with respect to control (Duncan test).

the behavior of the animals was recorded for an additional 5-min period, through the use of a video camera connected to a VHS recorder, for measurements of two parameters, total number of transitions between the two compartments, and time spent in the lighted compartment.

One hour after being tested in the light/dark transition model, animals were submitted to the cat odor test as described above.

To test for the effects of a prior forced exposure on the behavior of animals in the elevated T-maze, an additional group of rats ($N = 61$) was treated per os with HP LI 160 (62.5, 125, or 250 mg/kg), IMP (15 mg/kg), or saline for 13 consecutive days and, on Day 13, preexposed for 30 min to one of the open arms of the elevated T-maze. On Day 14, these animals were tested in the elevated T-maze and in the arena as described above.

2.4.3. Statistical analysis

Avoidance and escape measurements from the open arms of the elevated T-maze were submitted to split-plot analysis of variance (ANOVA) with treatment as the independent and trials as the dependent factor. In case of significant effect of treatment or of treatment versus trials interaction, data were analyzed by one-way ANOVA followed by the Duncan post hoc test. Behavioral data from the arena, the light/dark transition model, and from the cat odor test were submitted to one-way ANOVA followed by the Duncan post hoc test. In all cases, a value of $P \leq .05$ was considered significant.

3. Results

3.1. Experiment 1—acute administration

As illustrated in the upper panel of Fig. 1, treatment with HP LI 160 impaired inhibitory avoidance performance in the elevated T-maze. Split-plot ANOVA showed a significant effect of treatment [$F(4,64) = 2.80$; $P = .033$] and trials [$F(2,130) = 21.51$; $P < .001$], but not of treatment versus trials interaction. The Duncan post hoc test showed that on Avoidance 1 the dose of 125 mg/kg of HP decreased the latency to leave the enclosed arm, when compared to the control group ($P < .05$). Escape performance (Fig. 1, bottom), locomotor activity in the arena, and behavioral responses to a cat odor were not affected by HP LI 160 (data not shown).

3.2. Experiment 2—chronic administration

3.2.1. Elevated T-maze

As illustrated in the upper panel of Fig. 2, avoidance measurements of animals not exposed to one of the open arms of the maze were not affected by treatment. Split-plot

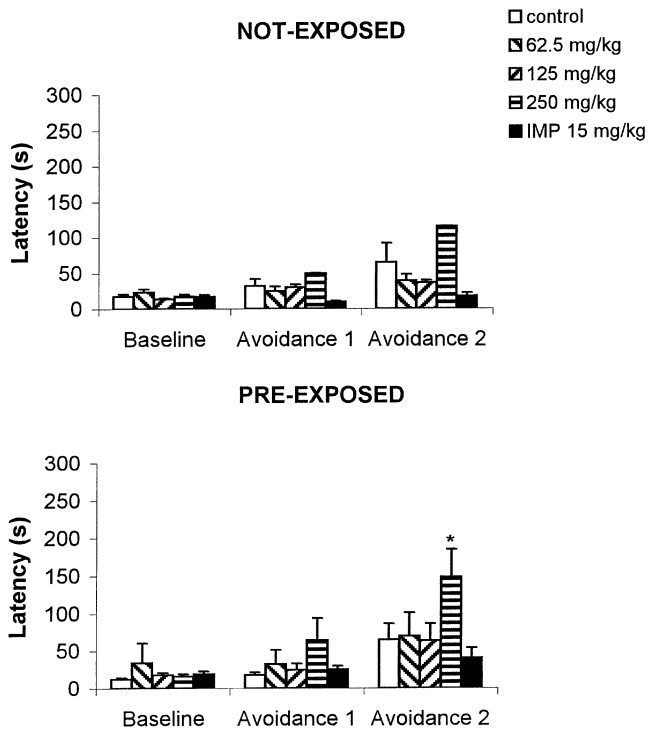

Fig. 2. Effects (mean ± S.E.M.) of chronic per os administration of HP LI 160 and IMP on the behavior of rats submitted to the avoidance task of the elevated T-maze. The upper panel shows results for animals not exposed previously to one of the open arms of the maze: $n = 12$ (control), 12 (HP 62.5 mg/kg), 10 (HP 125 mg/kg), 10 (HP 250 mg/kg), and 10 (IMP 15 mg/kg). The lower panel shows results for animals preexposed to one of the open arms of the maze: $n = 12$ (control), 11 (HP 62.5 mg/kg), 12 (HP 125 mg/kg), 13 (HP 250 mg/kg), and 13 (IMP 15 mg/kg). * $P < .05$ with respect to control (Duncan test).

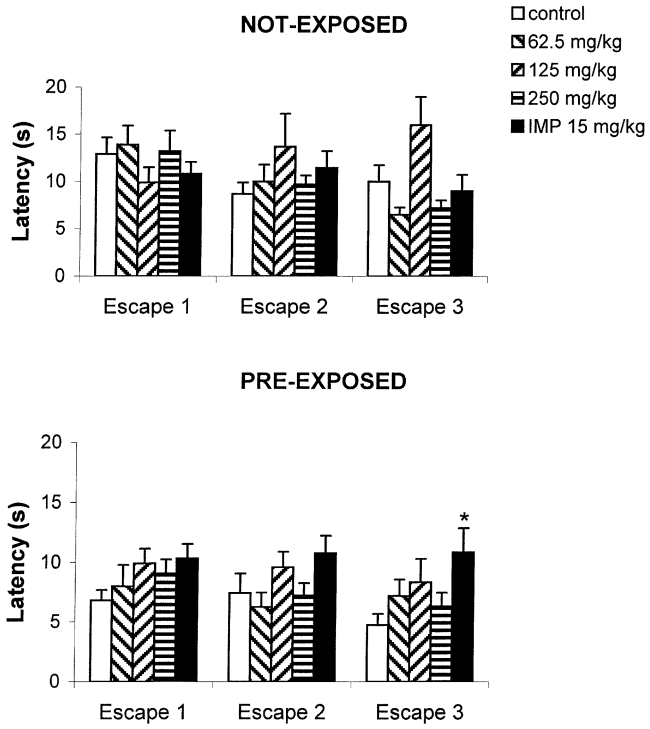

Fig. 3. Effects (mean ± S.E.M.) of chronic per os administration of HP LI 160 and IMP on the behavior of rats submitted to the escape task of the elevated T-maze. The upper panel shows results for animals not exposed previously to one of the open arms of the maze: $n = 12$ (control), 12 (HP 62.5 mg/kg), 10 (HP 125 mg/kg), 10 (HP 250 mg/kg), and 10 (IMP 15 mg/kg). The lower panel shows results for animals preexposed to one of the open arms of the maze: $n = 12$ (control), 11 (HP 62.5 mg/kg), 12 (HP 125 mg/kg), 13 (HP 250 mg/kg), and 13 (IMP 15 mg/kg). * $P < .05$ with respect to control (Duncan test).

ANOVA showed a significant effect of trials [$F(2,98) = 9.46$; $P < .001$], but no effect of treatment or of treatment versus trials interaction. For animals preexposed to one of the open arms of the model (see lower panel of Fig. 2), split-plot ANOVA showed a significant effect of trials [$F(2,112) = 21.12$; $P < .001$] and of treatment *versus* trials interaction [$F(8,112) = 2.38$; $P = .021$], but no effect of treatment. The Duncan post hoc test showed that on Avoidance 2 the dose of 250 mg/kg of HP increased the latency to leave the enclosed arm when compared to the control group ($P < .05$).

Similarly to Experiment 1, escape measurements of animals not exposed to one of the open arms of the maze were not affected by treatment (upper panel of Fig. 3). Split-plot ANOVA showed a significant effect of trials [$F(2,98) = 7.17$; $P = .001$], but no significant effects of treatment or of treatment versus trials interaction. For animals preexposed (see lower panel of Fig. 3), however, split-plot ANOVA showed a significant effect of treatment [$F(4,56) = 2.79$; $P = .035$], but not of trials or of treatment *versus* trials interaction. The Duncan post hoc test showed that on Avoidance 3 IMP increased the latency to leave the open arm when compared to the control group ($P < .05$).

3.2.2. Light/dark transition model

The upper panel of Fig. 4 illustrates the effects of treatment on measurements of number of transitions between the two compartments of the light/dark transition model. One-way ANOVA showed a significant effect of treatment [$F(4,53) = 2.74$; $P = .039$]. The Duncan post hoc test showed that this effect was due to an increase in the number of transitions presented both by the group treated with 250 mg/kg of HP LI 160 and the one treated with IMP when compared to the control group ($P < .05$).

Measurements of time spent in the lighted compartment were also altered by treatment (see lower panel of Fig. 4). One-way ANOVA showed a significant effect between groups [$F(4,53) = 6.05$; $P = .001$]. The Duncan post hoc test showed that this effect was due to an increase in the time spent in the lighted compartment presented by the group treated with IMP when compared to the control group ($P < .05$).

As in Experiment 1, neither locomotor activity in the arena nor behavioral responses to a cat odor were affected by treatments (data not shown).

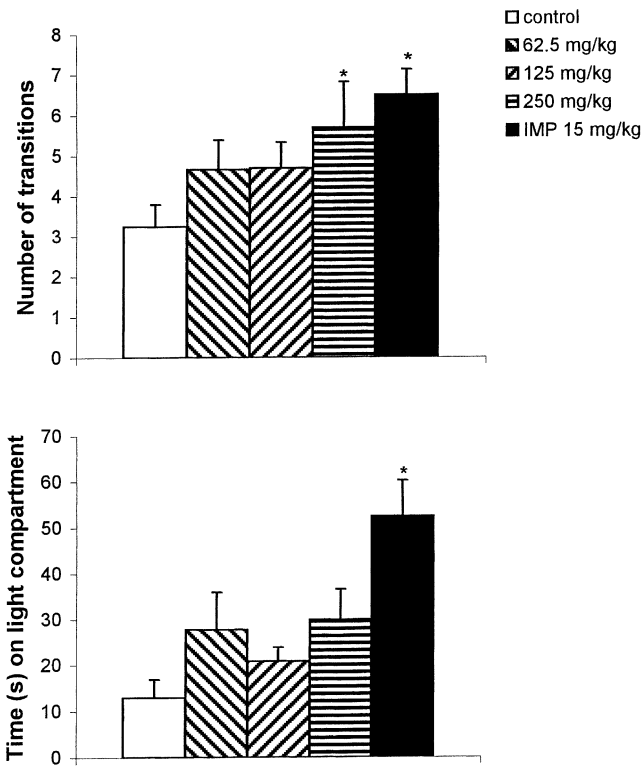

Fig. 4. Effects (mean ± S.E.M.) of chronic per os administration of HP LI 160 and IMP on the behavior of rats submitted to the light/dark transition model. The upper panel shows measurements of number of transitions between the two compartments and the lower panel shows the time spent by the animals under the lighted compartment. $n = 12$ (control), 12 (HP 62.5 mg/kg), 10 (HP 125 mg/kg), 10 (HP 250 mg/kg), and 10 (IMP 15 mg/kg). * $P < .05$ with respect to control (Duncan test).

4. Discussion

The results from Experiment 1 showed an anxiolytic effect of HP LI 160 in one of the tasks — inhibitory avoidance — of a new animal model of anxiety, the elevated T-maze. In this particular task, acute per os administration of 125 mg/kg of HP LI 160 impaired inhibitory avoidance from the open arms, without altering measurements of one-way escape, in a way similar to compounds used in clinical practice to treat GAD, i.e., the benzodiazepine diazepam and the 5-HT1A agonist buspirone (Graeff et al., 1993, 1998; Viana et al., 1994). In agreement with these results, anxiolytic effects of HP have recently been described by two other studies (Kumar et al., 2000; Vandenbogaerde et al., 2000). One of these studies was performed with the light/dark transition model (Vandenbogaerde et al., 2000). The second one tested the effects of subchronic administration of an Indian variety of HP in traditional anxiety models, such as the elevated plus-maze and the social interaction test (Kumar et al., 2000). In the study performed with the light/dark transition model, effects of HP were comparable to those elicited by treatment with lorazepam and were blocked by the administration of the benzodiazepine antagonist flumazenil, suggesting that at least the acute anxiolytic effect of HP is mediated by the GABA/benzodiazepine receptor system.

Contrarily to Experiment 1, in Experiment 2 no significant anxiolytic effects were observed with HP LI 160 on inhibitory avoidance of the elevated T-maze. Nevertheless, a similar pattern of the drug effect may be observed in the two experiments, with lower doses either not altering or showing a tendency to decrease avoidance latencies and higher doses acting in the opposite direction. In Experiment 2, the tendency to increase inhibitory avoidance latencies, observed with the dose of 250 mg/kg, became a true anxiogenic effect in animals that where preexposed to one of the open arms of the maze. This last result does not relate to an impairment of locomotor activity since no changes on number of crossings or rearings were observed with HP in the arena. Interestingly, in the study performed by Vandenbogaerde et al. (2000), acute treatment with a high dose of HP also evoked a rebound effect, pointing to an inverted U-shaped activity of the extract. Nevertheless, for the present experiment it remains to be explored if a longer period of treatment with higher doses of HP generates anxiolytic effects in the elevated T-maze. In this sense, it is interesting to note that Experiment 2 showed an anxiolytic effect of HP in the light/dark transition model when animals were treated for five extra days with the dose of 250 mg/kg. This effect, attested by an increase in the number of transitions between the two compartments, was similar to the one obtained with the reference drug IMP.

Additionally, in Experiment 2 escape latencies were not, as in Experiment 1, modified by HP treatment, indicating that the extract is not effective in altering panic-like defensive responses. This conclusion is strengthened by results obtained with IMP. In the present study, chronic administration of this antipanic compound impaired escape performance in animals preexposed to one of the open arms of the maze, thus an anxiolytic effect. These results confirm previous data (Custódio Teixeira et al., 2000) and suggest that preexposure provides a better index of escape.

Furthermore, neither IMP nor any one of the doses of HP LI 160 administered in both experiments did alter behavioral responses of rats to a cloth impregnated with cat odor. One more time, these results agreed with previous ones (Johnston and File, 1998; Zangrossi and File, 1992a,b, 1994) showing that this condition is resistant to a wealth of pharmacological treatments.

In conclusion, the present results show that HP LI 160 exerts anxiolytic-like effects in a specific subset of defensive behaviors, particularly those that have been related to GAD.

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