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Diazepam, but not buspirone, induces similar anxiolytic-like actions in lactating and ovariectomized Wistar rats

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Abstract

Previous reports indicate that the behavioural effects (including anxiolytic-like actions, hypothermia, "serotonergic syndrome," maternal behaviour and aggression and reduction in ambulation) of the 5-HT_{1A} agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), are completely blocked in lactating rats. The present study compares the behavioural effects of buspirone (1.25, 2.5 and 5.0 mg/kg) and diazepam (0.5, 1.0, 2.0 and 4.0 mg/kg) between ovariectomized and mid-lactating rats. The study was carried out on Wistar female rats under inverted light/dark cycle conditions, by using the burying behaviour paradigm, the elevated plus maze and a general activity test. In both ovariectomized and lactating rats, diazepam produced a dose-dependent reduction in burying behaviour and an increase in the time spent in open arms, responses interpreted as anxiolytic. Buspirone at all doses (1.25, 2.5 and 5.0 mg/kg) produced clear motor impairments in lactating, but not in ovariectomized animals, indicating that the effects of this drug on the anxiety paradigms are unspecific. Diazepam, by contrast, at the highest dose (4.0 mg/kg) similarly inhibited ambulation in both conditions. In the elevated plus maze, control lactating subjects spent more time in the open arms compared with saline-treated ovariectomized subjects, suggesting an anxiolytic-like effect of lactation per se. The present results support the idea that some behavioural actions of drugs acting at the serotonergic system vary between ovariectomized and lactating rats.

Keywords: Buspirone; Diazepam; Elevated plus maze; Burying behaviour; Lactating and ovariectomized rats

1. Introduction

It has been demonstrated that the anxiety-like effects of both benzodiazepines and serotonergic agonists vary depending upon sex differences (Fernández-Guasti and Picazo, 1990; Uphouse et al., 1991) and the endocrine stage of the female (Bitran and Dowd, 1996; Bitran et al., 1991; Fernández-Guasti and Picazo, 1990, 1997; Uphouse et al., 1991). Recently, we reported on the absolute blockade of the behavioural effects of the serotonergic agonist: 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in lactating females. The responses blocked were: (a) anxiolytic-like actions in two paradigms: the burying behaviour and the freezing tests (Fernández-Guasti et al., 1998), (b) the hypothermia and serotonergic syndrome (Ferreira et al., 2000), (c) the maternal behaviour and aggression (Ferreira et al., 2000) and (d) the reduction in ambulatory behaviour (Fernández-Guasti et al., 1998).

Buspirone and diazepam share anxiolytic properties in the clinics (Goldberg and Finnerty, 1979; Rickels et al., 1982) and in some animal tests such as the burying behaviour (Fernández-Guasti and Picazo, 1990, 1997; Treit, 1985; Treit et al., 1981). However, in the elevated plus maze test, buspirone has been found to have inconsistent actions with some researchers finding anxiogenic-like actions and others reporting either no effect or anxiolytic-like responses (Cole and Rodgers, 1994; Griebel et al., 1997, 1998; Hendrie et al., 1997; Klint, 1991; Sanger, 1991), while diazepam consistently produces anxiolytic-like effects in this test (Cole and Rodgers, 1995; Griebel et al., 1998; Hendrie et al., 1997). Diazepam and buspirone differ in their mechanism of action; thus, while the former stimulates the benzodiazepine (BZD) site within the $GABA_A$–BZD receptor complex (Haefely,

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1990), the latter, as 8-OH-DPAT, acts primarily as a 5-HT_{1A} agonist although it also possesses, within others, dopaminergic antagonistic activity (Dourish, 1987).

The purpose of the present study was to analyse whether buspirone and diazepam produce differential actions in lactating and ovariectomized rats. The animal models selected for testing the putative modification in anxiety-like actions produced by these compounds were the burying behaviour and the elevated plus maze tests. A large body of evidence supports these tests for analysing experimental anxiety (Broekkamp et al., 1989; Sanger, 1991). Additionally, an ambulatory behaviour test was performed to assess potential effects of drugs on motor activity that mask anxioselective drug actions.

It has been demonstrated that lactating rats show less fear-like responses (Ferreira et al., 1989; Fleming and Luebke, 1981; Hansen et al., 1985; Hard and Hansen, 1985) than virgin females. In particular, in the elevated plus maze test, Lonstein et al. (1998) recently reported that lactating female rats display anxiolytic-like responses. On these bases, the second objective of the present study consisted in analysing the putative anxiolytic-like action of lactation in the two animal models of anxiety used here.

2. Methods

2.1. Animals

A total of 226 female Wistar rats weighing 250–300 g were used in this study. All animals were housed in a room under inverted light/dark cycle conditions (lights on at

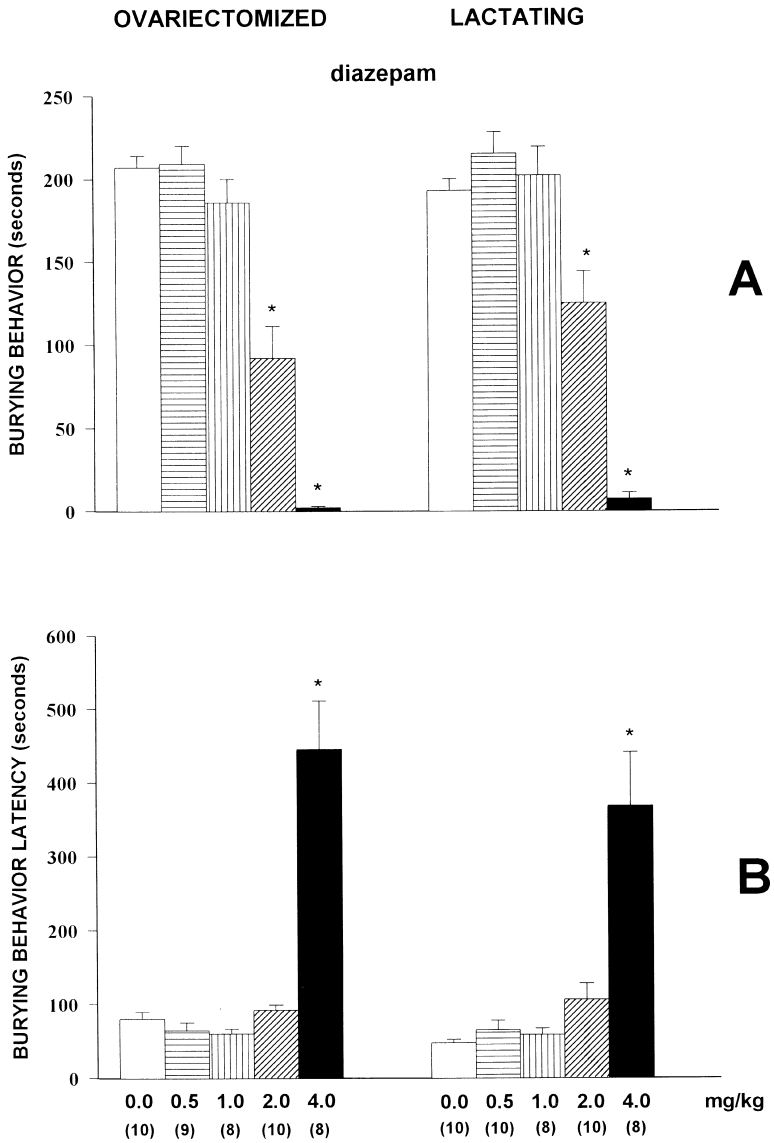

Fig. 1. Effect of various doses of diazepam on the cumulative burying behaviour (panel A) and the burying behaviour latency (panel B) in ovariectomized and lactating rats. Figure shows the mean ± S.E. Number of rats per group is shown in parentheses below each bar. Data were analysed by two-way ANOVA for each parameter (results in text) followed by Newman–Keuls post hoc test, * $P < .05$.

2200 h) with *ad libitum* access to water and Purina Rat Chow throughout the experiments. Females were kept five to six per cage from weaning onwards and isolated in individual cages before the anxiety tests. Rats were divided into two main groups: ovariectomized and lactating. Ovariectomy was performed through a ventral incision under pentobarbital (40 mg/kg ip) anaesthesia. After ovariectomy, animals were individually caged and kept for recovery at least for 15 days. The other group of females was mated with sexually active studs and individually caged when pregnant. These animals were checked daily for delivery and that day numbered as Day 1. Thereafter, the animals were maintained with their pups until Days 7–9 of lactation when they were separated from the litter 3 h before the beginning of the observations.

The pharmacological treatments, common to both conditions — ovariectomy and lactation — were: buspirone (0.0, 1.25, 2.5 and 5.0 mg/kg, Sigma, St. Louis, MO, −30 min, ip) and diazepam (0.0, 0.5, 1.0, 2.0 and 4.0 mg/kg, Hoffman La-Roche, Basel, Switzerland, −20 min, ip). Buspirone was dissolved in physiological saline, while diazepam was dissolved in propylene glycol 40%. All doses of drugs and vehicles were injected in 2.0 ml/kg. Doses and latencies were selected according to previous reports (Fernández-Guasti and Picazo, 1990, 1997; Fernández-Guasti et al., 1998). For all tests, observers were unaware of the drug treatments.

The general principles of laboratory animal care were followed (NIH publication 85-23, 1985). For these series of experiments, the local ethical committee approved the protocol for animal use.

2.2. Anxiety tests

2.2.1. Burying behaviour

The burying behaviour test was conducted in a red, dimly lit room. For this test, a cage measuring 27 × 16 × 23 cm (identical to the animal home cage), with an electrified prod (7 cm long) emerging from one of its walls 2 cm above the bedding material (fine sawdust), was used. Thus, when the rat touched the prod, it received an electric shock of 0.3 mA (the electric source was a constant current shocker, model 5806, LaFayette Instruments Co, USA). After the animal was placed in the test cage, its behaviour was observed for 10 min. Once the animal received a shock, it displayed a phylogenetic learned behaviour characterised by pushing the sawdust ahead with rapid alternating movements of the forepaws oriented to cover the electrified prod. The parameters scored in this test were: burying behaviour latency, i.e., time from the first shock to the burying behaviour display and cumulative burying behaviour, i.e., cumulative time that the rat spends burying the prod during a 10-min period. The cumulative burying behaviour has been directly related with the experimental anxiety levels (Pinel and Treit, 1978; Treit, 1985), while the burying behaviour latency may inversely reflect the animal's reactivity (Picazo and Fernández-Guasti, 1995).

2.3. Elevated plus maze test

Although we have shown in a previous study that certain drug actions do not vary in the burying behaviour and the elevated plus maze paradigms when sequentially or independently tested (Fernández-Guasti et al., 1999), other results have demonstrated an interaction between the two anxiety tests (Griebel et al., 1993; Hogg, 1996). Based on these last results, in the present series of experiments, independent groups of animals were tested in the elevated plus maze paradigm after similar treatments. The elevated plus maze has been described in detail elsewhere (Pellow et al., 1985). Briefly, the experimental device consisted of an elevated (40 cm above the floor), plus-shaped maze, with four arms that were 50 cm long and 10 cm wide, in a red, dimly lit room. The opposing arms were surrounded by

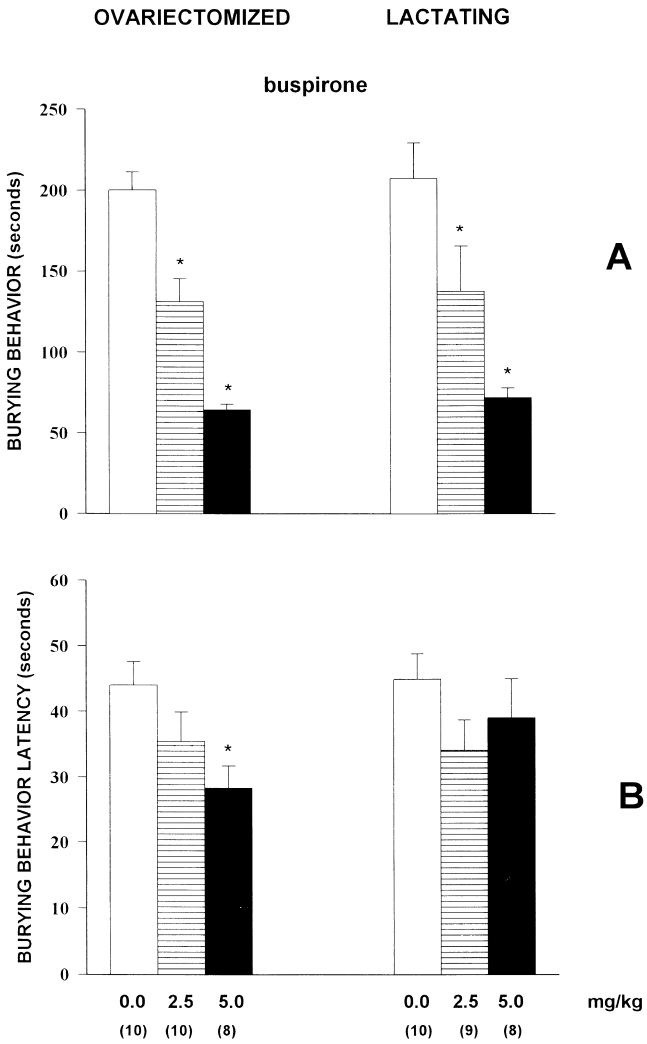

Fig. 2. Effect of various doses of buspirone on the cumulative burying behaviour (panel A) and the burying behaviour latency (panel B) in ovariectomized and lactating rats. Figure shows the mean ± S.E. Number of rats per group is shown in parentheses below each bar. Data were analysed by two-way ANOVA for each parameter (results in text) followed by Newman–Keuls post hoc test, * $P < .05$.

white 40-cm-high opaque Plexiglas walls (closed arms), while the other arms lacked walls (open arms). Each animal was removed from its individual cage and placed in the centre of the maze facing a closed arm. One observer was situated 2 m from the centre of the maze. Entry into an arm was defined as the animal placing all four paws into it. The cumulative time spent in open arms, open arm entries and total arm entries were recorded over a 5-min session. Data are expressed as percentage number of entries into the open arms/total number of entries and as percentage of time spent in the open arms/total time test. The number of rearings and bolus defecated were also registered although no drug effects were found. When a rat fell down on the elevated plus maze, it was excluded from the study. After each rat was tested, the maze was wiped cleaned with a humid fabric.

Anxiolytic (fear-reducing) drugs are expected to increase the number of entries and time spent in the open arms as compared to the closed arms. The total number of arm entries seems to reveal locomotor activity (Pellow et al., 1985) although this parameter cannot be considered independent of the anxiety state.

2.4. Activity test

To assess potential effects of the drugs on motor activity, ovariectomized and lactating rats were tested in an actimeter measuring $43 \times 36 \times 19$ cm placed over a

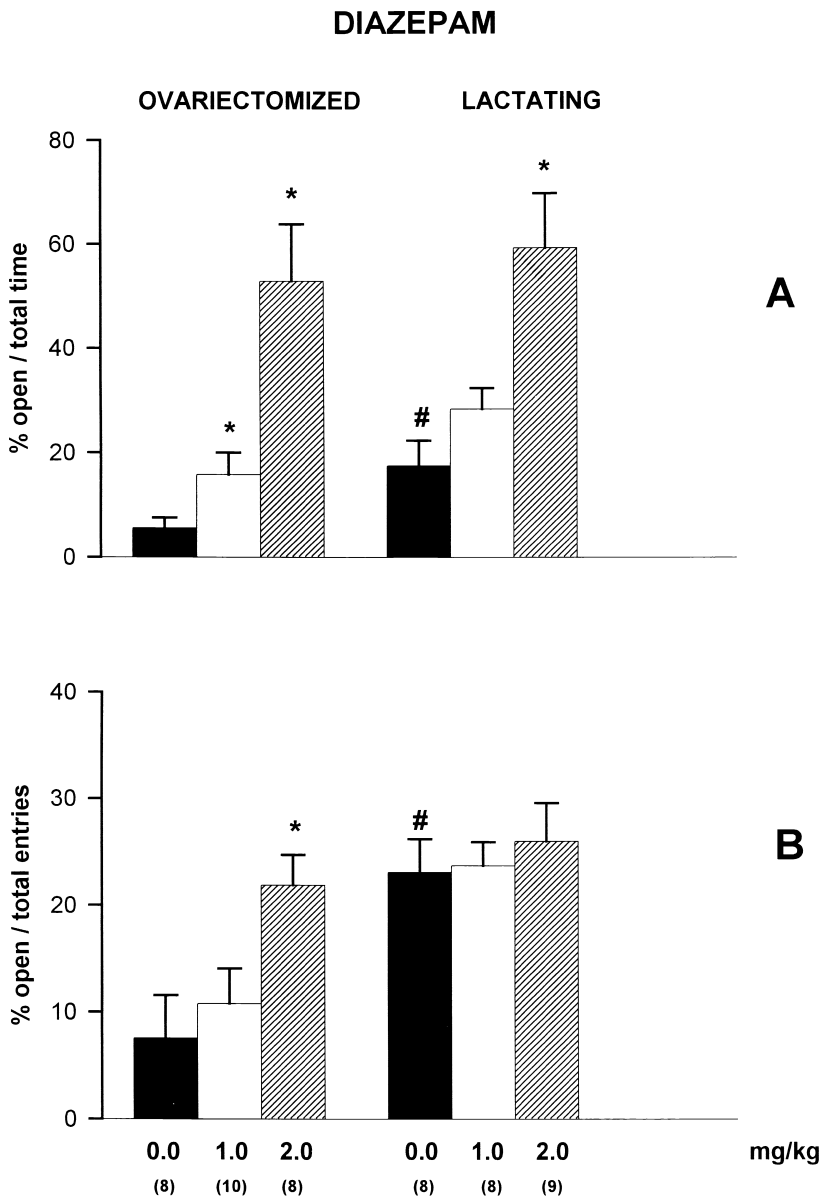

Fig. 3. Effect of various doses of diazepam on the time spent in open arms (panel A) and total arm entries (panel B) in ovariectomized and lactating rats. Figure shows the mean ± S.E. Number of rats per group is shown in parentheses below each bar. Data were compared by two-way ANOVA for each parameter (results in text) followed by Newman–Keuls post hoc test, * $P < .05$ vs. the respective control group. Comparisons between both control groups, # $P < .05$.

sensitive plaque 38 × 40 cm (Stoelting, Chicago, IL). The chamber used to perform this test was similar to that used to keep the animals before being isolated in individual cages. The number of counts over a 10-min session was recorded. The activity test was performed immediately after the burying behaviour test. A new testing cage was used for each rat.

2.5. Statistical analysis

All data were statistically analysed using a two-way ANOVA considering (a) drug dose-treatment, (b) condition: ovariectomized or lactating and (c) the possible interaction between these two factors. Post hoc comparisons were made by the Newman–Keuls test for a $P < .05$. For comparisons of specific groups in ambulatory behaviour, Student's t test was used (Steel and Torrie, 1985).

3. Results

The cumulative burying behaviour of ovariectomized and lactating mother rats after diazepam treatment is shown in panel A of Fig. 1. The results showed a dose-dependent decrease in this behaviour [two-way ANOVA $F(4,90) = 94.35$, $P < .001$ for drug treatment] that was independent of the condition [$F(1,90) = 1.34$, n.s.] and lacked an interaction between these factors [$F(4,90) = 1.09$, n.s.]. These data indicated that the effect of diazepam was similar between ovariectomized and lactating rats. Diazepam sig-

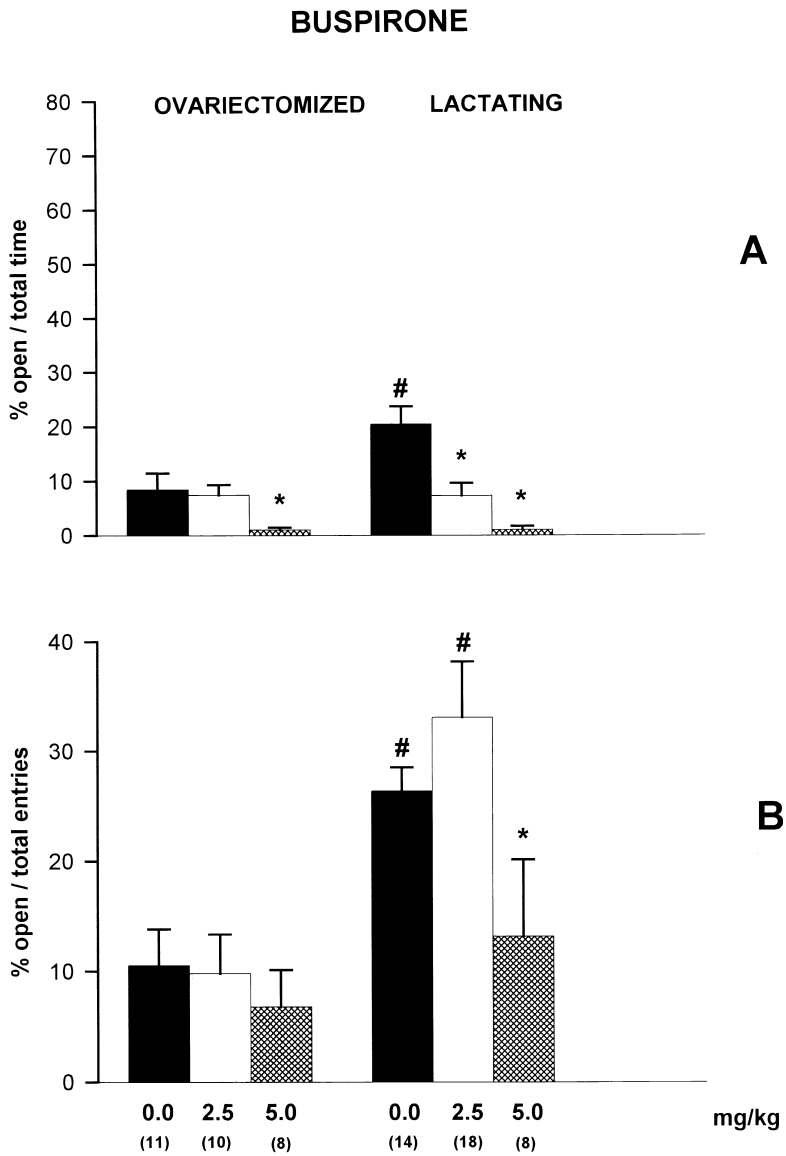

Fig. 4. Effect of various doses of buspirone on the time spent in open arms (panel A) and total arm entries (panel B) in ovariectomized and lactating rats. Figure shows the mean ± S.E. Number of rats per group is shown in parentheses below each bar. Data were compared by two-way ANOVA for each parameter (results in text) followed by Newman–Keuls post hoc test, * $P < .05$ vs. the respective control group. Comparisons between both control groups, # $P < .05$.

nificantly increased the burying behaviour latency at the highest dose of 4.0 mg/kg. Thus, the two-way ANOVA revealed statistically significant differences for drug treatment [$F(4,90)=41.51$, $P<.001$] but not for condition [$F(1,90)=0.87$, n.s.] or for Condition × Drug interaction [$F(4,90)=0.60$, n.s] (Fig. 1, panel B). The effect of diazepam in this parameter was also similar between ovariectomized and lactating rats.

Fig. 2 shows the cumulative burying behaviour (panel A) and burying behaviour latency (panel B) of ovariectomized and lactating rats after buspirone treatment. Concerning burying behaviour, the results of the two-way ANOVA revealed a statistically significant difference only for the effect of drug treatment [$F(2,54)=33.78$, $P<.001$]. Neither the condition (ovariectomized or lactating) nor the interaction between these factors was statistically significant [$F(1,54)=0.25$, n.s. and $F(2,54)=0.001$, n.s., respectively]. Clearly, a similar dose–response reduction in burying behaviour was observed after buspirone injection in both ovariectomized and lactating rats. Such reduction, however, in lactating rats is most likely due to motor alterations (vide infra). Fig. 2, panel B summarises the burying behaviour latencies after buspirone administration. Buspirone treatment with the higher dose (5.0 mg/kg) reduced this parameter in ovariectomized rats. However, no statistically significant differences between both conditions were observed [two-way ANOVA $F(2,54)=3.62$, $P<.05$, $F(1,54)=0.91$, n.s and $F(2,54)=0.97$, n.s, for drug treatment, condition and interaction, respectively].

The results for diazepam in the elevated plus maze test are shown in Fig. 3. Interestingly, a clear difference between both control groups was observed in the percentage of time spent in open arms (panel A) and in the percentage of entries into open arms (panel B) (for both parameters, $P<.05$, Newman–Keuls post hoc test). In both parameters, lactating females showed higher values as compared with the group of ovariectomized subjects. Diazepam produced both a dose- [$F(2,50)=21.54$, $P<.001$] and a condition- [$F(1,50)=3.2$, $P<.05$] dependent difference in the time spent in open arms (panel A) without a statistically significant interaction [$F(2,50)=0.11$, n.s.]. The percentage of open arm entries/total arm entries (panel B) was also significantly augmented in the control lactating rats as compared with the saline-treated ovariectomized subjects. After diazepam treatment, the percentage of open arm entries was increased in ovariectomized rats (panel B). In lactating rats, no further increase in open arm entries was found after diazepam treatment probably due to a "ceiling effect" caused by the increase in control values (vide supra). The two-way ANOVA revealed statistical differences both for dose [$F(2,50)=3.78$, $P<.01$] and for condition [$F(1,50)=16.5$, $P<.001$], but not for interaction [$F(2,50)=1.65$, n.s.].

Fig. 4 shows the effect of buspirone on the elevated plus maze test in ovariectomized and lactating females. Regarding the control lactating rats, a statistically significant increase in the percentage of time spent in open arms (panel A) (Newman–Keuls post hoc test, $P<.05$) and in the percentage of open arm entries (panel B) (Newman–Keuls post hoc test, $P<.05$) was found. Treatment with buspirone produced a decrease in the percentage of time spent in open arms [panel A, two-way ANOVA $F(2,68)=11.45$, $P<.001$, $F(1,68)=3.17$, n.s. and $F(2,68)=3.57$, $P<.05$, for drug treatment, condition and interaction, respectively] accompanied by a reduction after the highest dose in the percentage of open arm entries/total entries [panel B, two-way ANOVA $F(2,68)=2.87$, $P=$ns, $F(1,68)=15.81$, $P<.001$ and $F(2,68)=1.53$, n.s., for drug treatment, condition and interaction, respectively]. Such reductions, however, are most likely due to motor effects.

Fig. 5 shows the ambulatory activity of the rats, expressed as total number of counts after diazepam (panel A) and buspirone (panel B) injection. A reduction in this parameter was found after the highest dose of diazepam (4.0 mg/kg) both in lactating and ovariectomized rats [drug treatment, $F(4,90)=5.64$, $P<.01$]. Although lactating rats seem more sensitive to this treatment [condition, $F(1,90)=9.07$, $P<.01$], no statistically significant interaction between

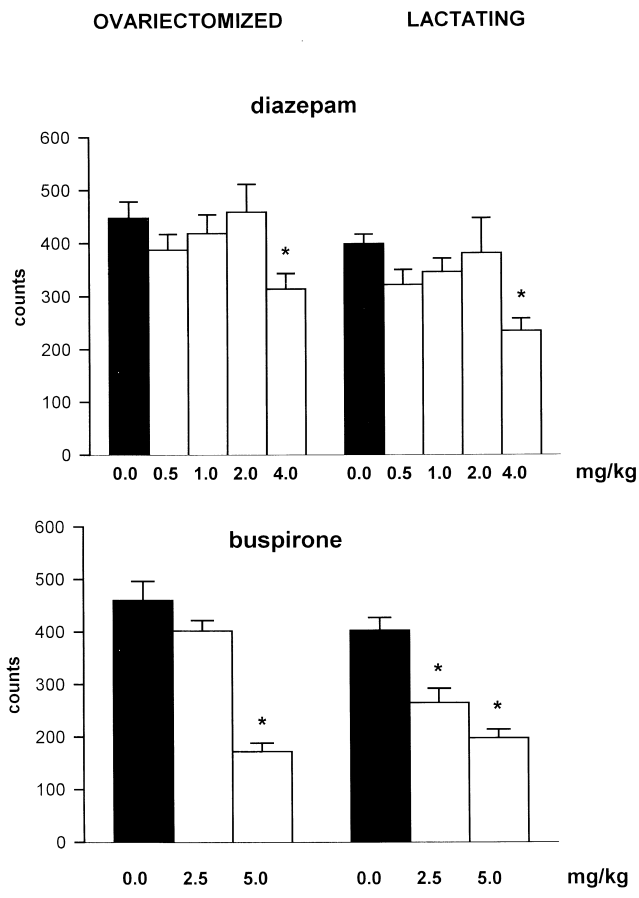

Fig. 5. Effect of various doses of diazepam (upper panel) and of buspirone (lower panel) on ambulatory behaviour of ovariectomized and lactating rats. Figure shows the mean ± S.E. The number of subjects for these groups corresponds to those shown in Figs. 1 and 2. Data were compared by two-way ANOVA (results in text) followed by Newman–Keuls post hoc test, * $P<.05$.

these factors was found [$F(4,90) = 0.06$, n.s.]. Interestingly, buspirone treatment produced a clear dose-dependent reduction in ambulatory behaviour in both ovariectomized and lactating rats [drug treatment, $F(2,54) = 53.26$, $P < .001$]. This motor impairment, was more pronounced in lactating rats since the dose of 2.5 mg/kg reduced ambulation in this group without modifying it in ovariectomized animals. Indeed, the two-way ANOVA revealed statistically significant differences for condition [$F(1,54) = 8.15$, $P < .01$] and, importantly, for the interaction between the main factors [$F(2,54) = 5.71$, $P < .01$]. A lower dose of buspirone (1.25 mg/kg) also reduced the motor activity in lactating rats (mean ± S.E. of control versus experimental, 393 ± 26 vs. 289 ± 43, $P < .05$, Student's t test) without affecting the burying behaviour values.

4. Discussion

Concerning diazepam, the results of the present investigation show that this drug at certain doses produced a reduction in burying behaviour and an increase in the time spent in open arms in the elevated plus maze test that are not mediated by motor actions. The actions of this drug did not differ between ovariectomized and lactating females. Additionally, buspirone motor effects were more pronounced in lactating rats when compared with ovariectomized, veiling the possible anxioselective effects of this drug. Another interesting result of the present investigation is that in the elevated plus maze, but not in the burying behaviour paradigm, lactating control dams showed a decreased anxiety-like behaviour (spending more time in the open arms and entering more frequently these arms), in comparison with the ovariectomized control group.

It is interesting to note that diazepam, by contrast with 8-OH-DPAT, displays similar anxiolytic-like actions in ovariectomized and lactating rats. During lactation, there is a basal low secretion of steroid hormones accompanied by an important prolactin and oxytocin suckling-dependent release. We have recently shown that diazepam anxiolytic-like effects vary depending upon the estrous cycle phase and, in general, the endocrine stages related with changes in progestin secretion (Fernández-Guasti and Picazo, 1990). On these bases, it may be proposed that benzodiazepines' actions may be influenced by changes in steroid hormones, most likely progestins, possibly by acting at the same receptor complex (Bitran and Dowd, 1996; Bitran et al., 1991; Fernández-Guasti and Picazo, 1995, 1997). The anxiolytic-like action of diazepam in lactating rats contrast with that obtained after 8-OH-DPAT (Fernández-Guasti et al., 1998; Picazo et al., 2000). These results suggest that the blockade of the behavioural actions of 8-OH-DPAT rather than being a generalised resistance of lactating rats to anxiolytics, is a phenomenon restricted to this drug. The possible causes underlying such blockade are unknown (Fernández-Guasti et al., 1998; Ferreira et al., 2000; Picazo et al., 2000) although several interpretations have been posed: within others, a 5-HT_{1A} receptor's long-term desensitization (Picazo et al., 2000). Against this idea is the present finding showing anxiolytic-like actions of the 5-HT_{1A} agent, buspirone; however, these actions are accompanied by a reduced ambulation possibly mediated by a dopaminergic antagonism (vide infra). A possible change in the number or affinity of 5-HT_{1A} receptors in the brain of lactating females is under study in our laboratory. Another ideas to explain the lack of behavioural actions of 8-OH-DPAT in lactating rats involve a possible interference of maternal behaviour and/or the endocrine changes associated with lactation. Recently, we have demonstrated that neither the endocrine process that accompanies lactation nor the sensory cues produced by the young is the underlying cause of the lack of effects of 8-OH-DPAT (Picazo et al., 2000).

Most of the animal models used to test anxiety involve motor coordination. Therefore, it is generally accepted that when a drug produces both an impaired ambulation and an anxiolytic- or anxiogenic-like response, the latter cannot be interpreted as a specific drug action (Dawson et al., 1995). Thus, the only result that can be discussed in terms of anxiety-like behaviour after buspirone injection is that in ovariectomized rats after the dose of 2.5 mg/kg. In lactating rats, in contrast, all doses of buspirone reduced motor activity and thereby no conclusions about reactivity- or anxiety-like effects can be proposed. A possible interpretation for this difference includes a putative summatory effect of a dopaminergic antagonistic buspirone action (Hjorth and Carlsson, 1982) together with a low dopaminergic tone during lactation (Hoffman et al., 1994). In a recent study, Collinson and Dawson (1997) reported that buspirone dose-dependently decreased the time spent in the open arms of the maze suggesting an anxiogenic-like effect, that, however, was masked by a decreased locomotor activity. The selective 5-HT_{1A} receptor antagonist, WAY 100635, failed to reverse the effects induced by buspirone in the plus maze. Furthermore, the inhibition of motor activity produced by 8-OH-DPAT, which lacks a dopaminergic antagonistic effect, in ovariectomized rats, was completely blocked in lactating subjects (Fernández-Guasti et al., 1998). These findings sustain possible dopaminergic mediation in the action of buspirone in the plus maze, as has been previously suggested (Cole and Rodgers, 1994). In this line, we have recently shown that buspirone, but not 8-OH-DPAT, inhibits the most active components of maternal behaviour (Ferreira et al., 2000) and that this inhibition is most likely mediated by its dopaminergic antagonistic activity. Summarizing, these data indicate that lactating rats are more sensitive to the motor actions of buspirone than ovariectomized subjects and suggest that some behavioural effects of serotonergic compounds, but not of diazepam and possibly other benzodiazepines, differ between these conditions.

In the present study, lactating control females displayed anxiolytic-like actions in the elevated plus maze, but not in the burying behaviour paradigm as compared with the

ovariectomized group. Such a difference may be interpreted based on the characteristics of the animal model. In various animal models of anxiety, it has been demonstrated that lactating rats showed less fear (Ferreira et al., 1989; Fleming and Luebke, 1981; Hansen et al., 1985; Hard and Hansen, 1985). Thus, in the freezing and conflict tests, mother females showed anxiolytic-like behaviour when compared with virgins. In the elevated plus maze test, an animal model that exploits the natural aversion of rodents to height and open spaces, Lonstein et al. (1998) showed that lactating female rats display anxiolytic-like responses evidenced as an increase in the time spent in the open arms. These results are in line with present data and in contrast with those of Silva et al. (1997) who reported opposite findings. The nature of this difference most likely relies on testing the animals sequentially in the different paradigms. Interestingly, others have established that the elevated plus maze test is very sensitive to the aftereffects of other behavioural manipulations (Griebel et al., 1993; Hogg, 1996; Lapin, 1995). Thus, the initial exposure of the females to the open field test influences the results observed in the elevated plus maze paradigm (Silva et al., 1997). In this line, it is interesting to mention that recently, we (Fernández-Guasti et al., 1999) have shown that the anxiolytic-like action of diazepam is not modified in animals tested in the plus maze after being exposed to the burying behaviour. These data suggest that the pharmacologically induced anxiolysis is more pronounced than that induced by physiological processes and, thereby, may be reflected in various anxiety paradigms after sequential testing.

The difference in the anxiolytic-like behaviour reduction observed in lactating rats between the two animal models, could involve the nature of the stimulus that triggers anxiety as well as the nature of the response (Handley, 1991). Thus, in the burying behaviour test, the animal is confronted with an electrified prod that is recognized as an aversive stimulus and in which the expression of an active behaviour, such as burying, denotes the anxiety-like state. In the elevated plus maze test, curiosity and caution are evoked by a novel situation and under normal circumstances, the animal chooses to explore the nonaversive area (closed arms). In analyzing the changes in emotionality during lactation, it is interesting to note that an anxiolytic-like action is consistently observed in classic conflict tests (Ferreira et al., 1989), such as punished drinking or food intake in a novel environment (it is worth clarifying that in these tests, the natural increase in food and water intake in lactating rats was properly controlled). However, in other paradigms such as the shock probe (Ferreira et al., 1989) and the burying behaviour (present data and those of Fernández-Guasti et al., 1998), no anxiety-like reduction is found. The shock probe test (modified from Meert and Colpaert, 1986) is usually performed in the same cage as the punished drinking test, except that the animal is not water-deprived and no water is available, but the probe is electrified. Thus, the rat receives an electric shock each time it contacts the probe. This paradigm, as the burying behaviour test, shares the same nonrewarding aversive stimulus that evokes anxiety towards a specific incentive and thereby cannot be considered as a conflict test (Handley, 1991). Interestingly, Rodgers and Cole (1994) proposed the plus maze test as a conflict paradigm in which the rat has to decide upon exploring a new environment and taking the risk of being in a heightened open space. Accordingly, during lactation, an anxiety-like behaviour is found in this test. These data taken together further suggest that during lactation, certain behaviours, normally not observed in virgin or ovariectomized animals, are disinhibited. Nevertheless, females during this particular period are equally able to respond to other stimuli. In conclusion, lactation seems to selectively modify the expression of certain behaviours and affects the behavioural responses of specific drugs acting at the central nervous system.

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