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Influence of subcutaneously administered lysine-vasopressin, chlorodiazepoxide in behavioral tests

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Abstract

The aim of the study presented here was to determine the influence of subcutaneously administered lysine-vasopressin (LVP, 1 U/kg, s.c.), chlorodiazepoxide (BDZ, 20 mg/kg, i.p.), and vehicle (veh, chlorobutanol + saline (0.85%) + Tween 80, 0.1 mL/100 g) administered through the peritoneum on anxiety-related- behavior using the Vogel conflict test, the elevated plus-maze test (EPM) and the marble-burying test. The results of the Vogel test referring to the number of shocks received by rats after administration of vehicle + BDZ was highly significant ($p < 0.01$), that is, the animals did not show any inhibition during the phase of shock. However, when LVP + BDZ were used the data obtained showed that there was a significant inhibition of BDZ action on the number of shocks received ($p > 0.05$). In the second phase of the test the veh + BDZ group received a significant number of shocks, benzodiazepine effect and the group receiving LVP + BDZ showed the same result as the vehicle + LVP group ($p > 0.05$). In the elevated plus-maze (EPM), the group of mice treated with veh + BDZ showed no significant change in their behavior, that is, number of entries and time spent on the open arm was not inhibited ($p < 0.01$). Already the veh + LVP group has shown inhibition in the number of entries and the time spent on the open arm ($p > 0.05$). The same result was obtained when the LVP + BDZ group was used in the EPM. In the marble-burying test, the number of marbles hidden was significantly higher in mice treated with the veh + BDZ ($p < 0.01$). The group treated with veh + LVP presented a small number of hidden spheres ($p > 0.05$). The data obtained in this study show that LVP in behavioral tests related to anxieties presents an inhibitory action on the BDZ, and the LVP alone does not present any significant effect when compared with the veh ($p > 0.05$). Veh is the shortened form of vehicle which is the chemical element (solvent) used for dilution of the compound test.

Keyword: lysine-vasopressin, chlorodiazepoxide, behavioral tests, rodents.

INTRODUCTION

The arginine-vasopressin is present in humans and Lysine-vasopressin in some species of animals, such as pigs, Syrian hamsters. Its synthesis is pavocellular and magnocellular neurons of hypothalamic nuclei (Argiolas, 1999). The axons conduct these neurons to the neurohy-pophysis (hypothalamo- neurohypophysial system) and to the external zone of the median eminence (hypothalamic-pituitary-adrenal axis) (Aguilera and Rabadan-Diehl, 2000). Although vasopressin is released from these sys-tems into the bloodstream and involved in maintaining water balance and blood pressure, it is also released intracerebrally, for example into limbic brain structures (Castelli et al., 1987). In the latter, it may act as a neuro-transmitter and/or a neuromodulator and is, thus, involved in various functions, including behavioral perfor-mance (Kosekova et al., 1993). Moreover, it has been suggested that such an involvement is mediated predo-minantly via V1 receptor functions, including behavioral performance. Moreover, it has been suggested that such an involvement is mediated predominantly via V1 recep-tor subtype (Shewey and Dorsa, 1988). The septal area that receives projections from vasopressin-containing neurons from the nucleus bed of the stria terminalis seems to be an important neuroanatomical s tructure in

this respect (Danzer et al., 1988; De Vries and Buijs, 1983). Several findings were obtained on the main action of vasopressin principally on learning, memory and behavior (Engelmann et al, 1996, 2004). Other observations have suggested that vasopressin applied peripherally may influence learning and memory (Bohus and De Wied,). Landgraf and Neumann (2004) reported that vasopressin released in the brain or administered peripherally can cross the blood-brain barrier and induce behavioral changes. According to Deyo et al. (1986), Ebenezer (1994) and Ermisch et al. (1993), peripheral administration of vasopressin is involved directly in the process of memory and causes changes in behavior, an unresolved issue that has been discussed controversially. There have been initial findings suggesting participation of limbic brain structures (Treit et al., 1993). However no study has shown the participation of LVP in anxiety-related behavior. In addition, AVP is involved in several behavioral models, including anxiety (Welt et al., 2006) describing an increased AVP released within the hypothalamic paraventricular nucleus in the rat in response to benzodiazepine treatment in the rat. Furthermore, there are different lines of evidence for an interaction between vasopresinergic and GABAergic signaling in the brain (Jakab and Leranth, 1990) . This finding suggests that there might be indeed an interaction between benzodiazepine action and that of vasopressin at brain level. In the present study LVP (1 U / kg, s.c.) was used in three behavioral anxiety-related models, to assess the participation of LVP in emotional states.

MATERIAL AND METHODS

Subjects

For the Vogel conflict test, 15 Male Wistar rats, with starting weights of 250 – 350 g, were housed in a temperature-controlled room (23 ± 2^{O}C) under standard laboratory conditions with free access to food and with deprivation of water for 48 h, a 12 h light/12 h dark cycle (lights on at 06 am). Two groups of 15 mice, with starting weights of 25 – 35 g, were used for the elevated plus maze test and marble-burying test respectively. The animals (rats and mice) were divided into 5 groups (each group with 15 animals) thus divided: veh; veh + BDZ (20 mg / kg, i.p.); veh + LVP (1 U/kg, s.c.); veh + BDZ and LVP + BDZ. The mice were kept in the same conditions as above but with free access to water. All experiments were conducted between 10 am and 16 pm. Procedures conformed to the guidelines for the use of laboratory animals of the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethical Committee of the Federal University of Pernambuco (UFPE) protocol number 008198/2005-30.

Drugs

One batch of LVP was obtained from the Federal University of São Paulo (Brazil) in blisters, and a second batch was purchased locally (Recife PE by Sigma). The LVP was stored at −20°C and preserved in chlorobutanol at pH 4. BDZ (20 mg/kg) and was obtained from the Farmasa® laboratory (Sao Paulo, Brazil) with blisters of Psicosedin® and Sigma®, suspended in Tween 80. For the rats, LVP was administered subcutaneously (0.2 U/100 g) and BDZ was administered through the peritoneum (0.1 mL /100 g). For mice, a dose of LVP of 0.2 U/10 g was administered subcutaneously, and BDZ administered by intraperitoneal route at a dose of 20 mg/10g. The vehicle consisted of chlorobutanol + saline (0.85%) + Tween 80.

Water consumption evaluation

The apparatus was the same as used in the Vogel conflict test. However, each rat was without water 48 h before the beginning of the Vogel conflict test. The mice were given food and water *ad libitum*.

Vogel conflict test

The Vogel conflict test was performed in a Plexiglas box (42 X 50 X 25 cm) with a stainless steel grid floor. The metallic nozzle of a bottle containing drinking water was built into the box. The contact of the animal with the beak and with the floor grating produced an electrical circuit controlled by a sensor (Anxio-Meter Model 102, Columbus, USA). Each pulse was considered after the animal tried to drink water and, after 20 licks, the animal received a shock of 0.5 mA for two seconds. The sensor recorded the total number of licks, and a shock was produced during the test period. The whole apparatus was located inside a sound-attenuated cage (Vogel et al., 1971; Petersen and Lassen., 1979; Ford et al., 1979).

Elevated plus-maze test

The elevated plus- maze (EPM) validated for the mouse (Lister, 1987) consisted of two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) and a common central platform (5 x 5 cm). The whole apparatus was raised to a height of 40 cm from the floor. At the beginning of the session, a mouse was placed in the center of the maze, and was allowed to explore both arms for 5 min. The parameters observed were: time spent and the number of entries in each type of arm. The mice were divided into two groups of 15 animals each. Diazepam (2.5 mg/kg, i.p.) was used as positive control and LVP (1 U/Kg, s.c.) as a drug test. All experiments were conducted between 10:00 am and 4:00 pm. After each test, the unit was cleaned with a 70% ethanol solution.

Marble-burying test

Twenty-five glass spheres (20 mm in diameter) were used for each test. Opaque cages (30 x 36 x 13 cm) had a 5 cm layer of sawdust. Mice were placed individually in plastic cage with sawdust for 15 min (habituation time), and then returned to their original cage. Twenty-five spheres of glass were placed at random on the layer of sawdust. Mice were reintroduced into the cage (a mouse was placed in the same cage that it had been accustomed to). The test group received the LVP 1 U / kg, via subcutaneous, 15 min before administration of BDZ (20 mg/kg). After 15 min the test was terminated and the number of hidden spheres counted. Two-thirds or more of spheres hidden shows anti-anxiety effect of the drug. After each test, the sawdust was replaced; the spheres of glass.

Statistical analysis

A review of data obtained from the Vogel conflict tests, the total

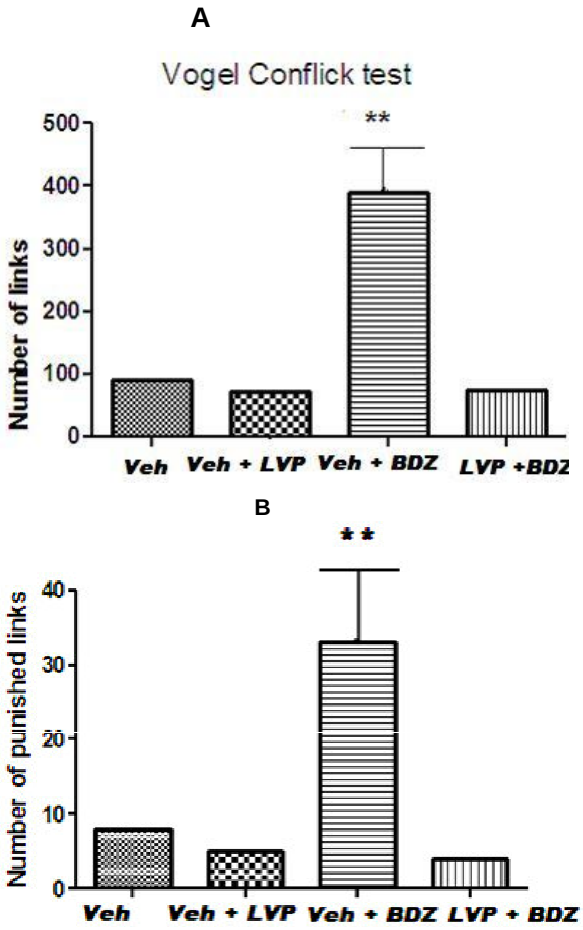

Figure 1. Vogel conflict test: **A:** Effects of vehicle (veh), veh + BDZ (20 mg/ kg,i.p.), veh + LVP, veh + BDZ and LVP + BDZ (n = 15 for each group), and LVP(1 U/kg,s.c.). The BDZ compared with LVP showed to be statistically insignificant (P > 0.05) for a session of 3 were washed with distilled water and 70% alcohol (Broekkamp et al., 1986; Njung´e and Handley., 1991),
B: Effects of veh, veh + LVP, veh + BDZ, LVP + BDZ on the number of licks. BDZ compared with LVP also was not significant (P > 0.05) during the period of punishment. Each session was 3 min, and each bar is the mean ± SEM (Mann-Whitney U test). using non-parametric Kruskal–Wallis tests followed by Mann-Whitney *U* test. The data from the Elevated plus-maze and Marble-burying tests were analyzed using one-way variance analysis (ANOVA) with post-hoc Dunnett's test ($P < 0.05$ and was considered significant).

number of licks and the number of punished licks were analyzed min. using non- parametric Kruskal–Wallis tests followed by Mann-Whitney U test. The data from the Elevated plus-maze and Marble-burying tests were analyzed using one-way variance analysis (ANOVA) with post-hoc Dunnett's test (P 0.05 and was considered significant). All data are expressed as mean ± S.E.M.

RESULTS

Vogel conflict test

After four weeks of testing, the animals reached the previously determined base line. The maximum number of shocks was 10 - 40 per animal during the punishment period. The high level of fear produced by shock during licking of drinking water (determined by the amperage shock) was maintained and measured for the changes in behavioral punishment induced by the administration of BDZ, compared with the control group, and induced by previous administration of LVP could be observed before and after the administration of the LVP. As expected, the administration of just the BDZ alone resulted in the release of behavioral punishment (shock to the tongue) [F (1.7) = 16, 9; p < 0.004]. Furthermore, the prior administration of LVP inhibited the anxiolytic effect of BDZ (release of behavioral punishment) [F (17) = 2.2; p > 0.18] and the administration of LVP alone did not produce the release of behavioral punishment [F (18) = 2.4; p >0.05. Demonstrating that the LVP blocks the anxiolitic effect produced by BDZ. (Figure 1). The effect of BDZ on behavioral punishment was observed [F (1.14) = 5.9; *P* < 0.02] (Figure 1).

Elevated plus maze

Previous administration of LVP presented an anxiolitic effect in animals treated with BDZ, the number of entries and the time spent on the open arm was not statistically significant (p > 0.05). The mice administered with LVP had lower number of entries and time spent on the open arm (p > 0.05) compared with mice treated only with BDZ (p < 0.05). Conversely, the number of entries and time spent on the closed arm was significantly higher when the mice were treated only with LVP (*P* <0.05) (Figure 2).

Marble-burying test

The effect on the behavior of the LVP in Marble-burying test was investigated. Treatment with the veh + LVP resulted in a significant decrease in the ball burying behavior. However, the administration of the veh + BDZ promoted a significant increase in the number of balls buried p < 0.05. Moreover, the treatment of animals with veh + LVP did not promote an increase in the number of balls buried (p > 0.05). And the treatment of LVP + BDZ was similar to the result of the veh + LVP, that is, LVP promoted an inhibiting action of BDZ (p > 0.05). These data were evaluated using ANOVA followed by post- hoc Kruskal–Wallis and the Mann-Whitney *U* tests (Figure 3).

DISCUSSION

The effect of vasopressin has often been studied in behavioral models. However, the effect of the presence of the amino acid lysine in position 8 of the molecule of vasopressin is present in small variety of animals and is little studied (Griebel et al., 2002). It has been shown that

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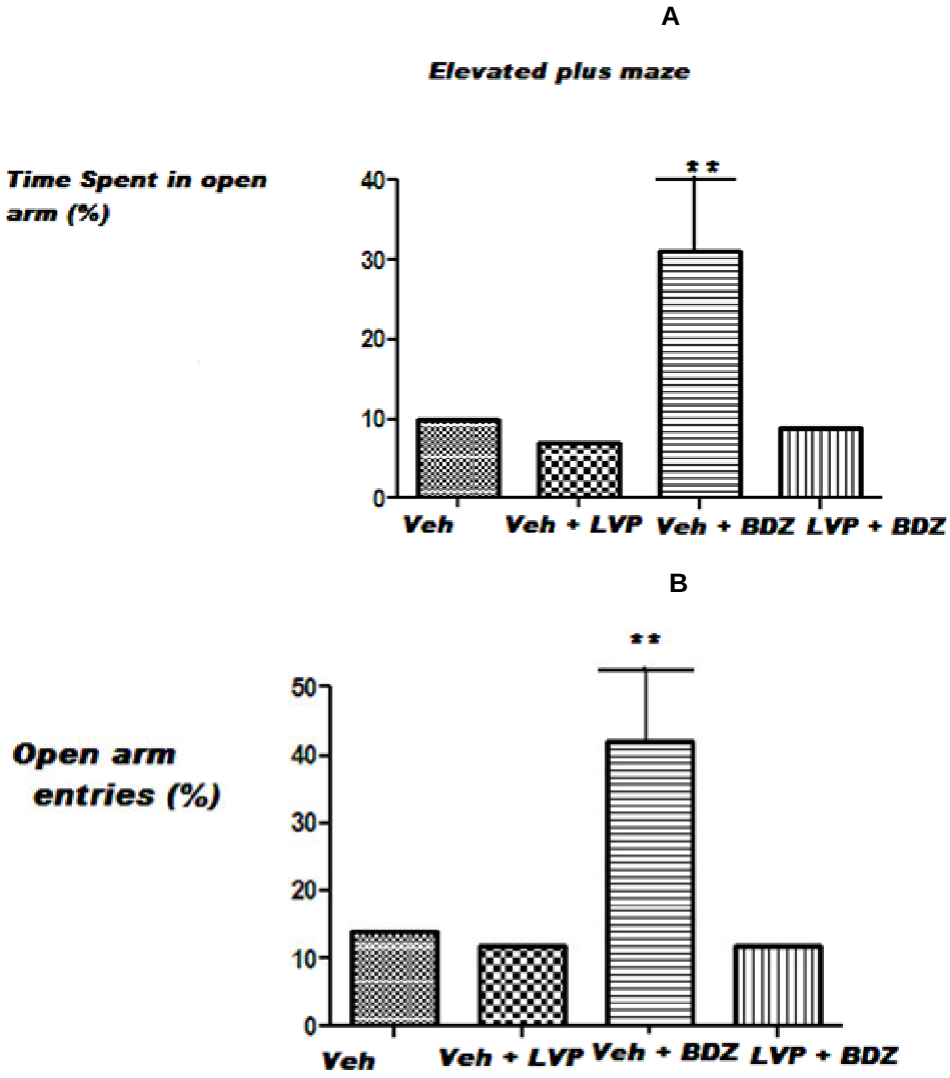

Figure 2. Elevated plus maze. **A:** The Effect of BDZ + LVP in time of stay in the open arm was statistically insignificant ($p > 0.05$). **B:** The number of entries in the same arm during a period of 15 min of duration was also statistically insignificant ($p > 0.05$). Each bar is mean ± S.E.M. (Kruskal-Wallis followed by Mann-Whitney U test). (n = 15 for each group).

vasopressin administered peripherally modifies the behavior of rats and mice (Hayes and Chambers, 2005; Gohlke et al., 2001). The studies of Welt et al. (2006), Gohlke et al. (2001) showed that peripheral administration of vasopressin promotes behavioral changes in rodents, as did studies on the intracerebrally application. In fact anxiety is listed as one of the behavioral disturbances that most affects the quality of life of human beings (Schmidt et al., 2006). The conflict test showed that the subcutaneous administration of just LVP, at a dose of 1 U / kg, caused inhibition of behavior.

Just the BDZ alone, unlike LVP, causes the initiation of conflict (anxiolitic effect). However, previous administration (15 min prior), of LVP inhibits the anxiolitic action of BDZ (initiation of behavioral punishment). The results show that the LVP blocks the BDZ action with its anxiolitic mechanism. The results of the Vogel conflict test show that subcutaneous administration of LVP at a dose of 1 U/kg LVP inhibits BDZ action, leading to a significant increase in behavioral punishment [$F(1.7) = 2.2$; $P > 0.18$] (Figure 1). Moreover, BDZ alone significantly reduces the behavioral punishment to the animal (Vogel et al., 1971; Petersen et al., 1979) . In the EPM experiment, LVP inhibits the action of the BDZ, causing a significant increase in the number of animals at the time of entry and time spent in the closed arm. The BDZ presented its anxiolitic effect, by increasing the permanence of rodents in the open arm (Lister, 1987). Data obtained in the marble-burying test showed that the peripheral administration of LVP produced the same effects as

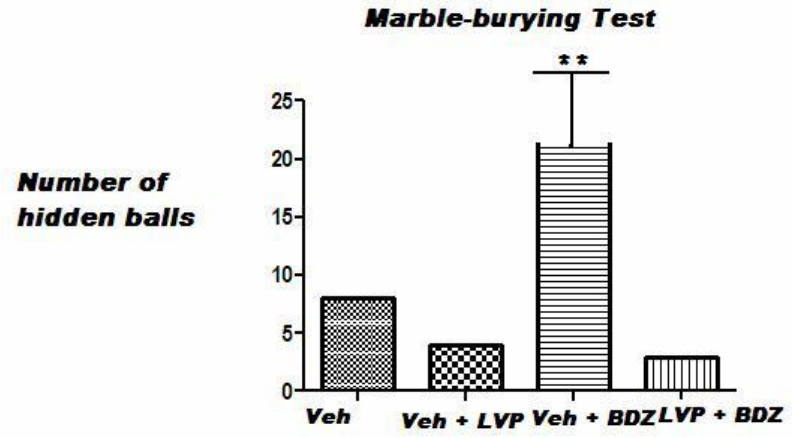

Figure 3. Effects of BDZ and LVP on marble burying by mice. The marbles were hidden accounting for 5 min duration, beginning immediately after application of the drug. Compared with veh, veh + LVP, veh + BDZ and LVP + BDZ. P > 0.05 (n = 15 for each group). Each bar is mean ± S.E.M. (Kruskal-Wallis followed by Mann-Whitney U test.

obtained in behavioral models carried out before. Demonstrating once again that the AVP vs LVP promotes inhibittion of the anxiolitic effect of BDZ (Broekkamp et al., 1986; Njung'e and Handley, 1991). These data show that AVP + LVP present in the periphery can cross the blood-brain barrier and promote behavioral change in animals. Probably the site of action of the vasopressin is at the $GABA_A$ receptor as neurotransmission/neuromodulator or through their vasopresinergic receptor V1 (Bielsky and Young, 2004).

The data presented in this study indicate a blockade of the effects of BDZ, probably acting in the GABA receptor. Another possibility is through action neuromodulator, acting directly on the neural substrate of anxiety. The results obtained with the vasopressin leads to a blocking the action of the BDZ, not an action anxiogenic.

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