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Role of glutamate ionotropic and benzodiazepine receptors in the ventromedial hypothalamic nucleus on anxiety

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Abstract

The dorsomedial part of the ventromedial hypothalamic nuclei (VMHdm) has been related to the modulation of defensive behavior in mammals. The objective of the present study was to test the hypothesis that administration into the VMHdm of midazolam, a benzodiazepine receptor full agonist, or AP7, a glutamate NMDA receptor antagonist, would produce anxiolytic effects in the elevated plus-maze (EPM) or the Vogel's punished licking tests. Male Wistar rats with unilateral cannulae aimed at the VMHdm received intra-cerebral injections of midazolam (15–60 nmol/0.25 μL), AP7 (0.2–2 nmol/0.3μL) or saline and were submitted to the behavioral tests. Midazolam (30 nmol) increased the percentage of time spent in open arms of the EPM. AP7, on the other hand, decreased open and enclosed arm exploration. In the Vogel test, however, both midazolam (30–60 nmol) and AP7 increased the number of punished licks. Histological control experiments found no significant effects when the drugs were injected into the nearby lateral hypothalamic area. These results suggest that facilitation of gabaergic or antagonism of glutamatergic neurotransmission in the VMHdm can produce anxiolytic-like effects.

Keywords: Elevated plus-maze; Vogel; NMDA; Aversion; Midazolam; AP7; Exploratory behavior

1. Introduction

Studies that follow the seminal work by Hess and Brugger (1943), showing that electrical stimulation of the perifornical hypothalamic area in cats produces an "affective defensive response", have related the medial hypothalamus (MH) to defensive behavior (see Canteras, 2002, for review). Flight reactions that resemble behavioral responses exhibited by animals facing natural threats have been widely described in rats after MH electrical or chemical stimulation (Hilton, 1979; Lammers et al., 1988; Silveira and Graeff, 1992; DiMicco et al., 2002). Based on these results, the MH, together with the dorsolateral periaqueductal grey (dlPAG) and amygdala, have been proposed to be part of a brain aversive system that mediates defensive responses (Graeff, 1990).

Among the regions that comprise the MH, the dorsomedial nucleus (DMH) has been the most extensively investigated. Glutamate agonists or GABA antagonism induce flight responses in this region (Silveira and Graeff, 1992; Bailey and DiMicco, 2001; Soltis and DiMicco, 1991; De Novellis et al., 1995). Chronic inhibition of GABA synthesis in the DMH has recently been shown to facilitate panic-like lactate-induced changes in animals, an effect that was prevented by systemic administration of a selective agonist of group II metabotropic glutamate receptor, which inhibits glutamate release (Shekhar and Keim, 2000). These results suggest that glutamate and GABA, the most prevalent excitatory and inhibitory neurotransmitters in the brain, play a major role on modulation of defense reactions in the DMH. To test this hypothesis we have recently showed that intra-DMH administration of midazolam, a benzodiazepine with clinical anxiolytic effects, produces anxiolytic-like effects in the elevated plus-maze (EPM), an animal model of anxiety (Jardim and Guimarães, 2001). Glutamate iono-

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tropic receptor antagonists, however, failed to do so, decreasing general exploratory activity instead (Jardim and Guimarães, 2001, 2004). This contrasts with the anxiolytic effects observed in several animal models after administration of these compounds into the dlPAG (Matheus and Guimarães, 1997; Matheus et al., 1994; Guimarães et al., 1991; Molchanov and Guimarães, 2002).

Another hypothalamic site that has been related to defensive behavior is the dorsomedial part of the ventromedial nucleus (VMHdm). cFos immunohistochemistry studies have revealed that this area is activated in rats during exposure to signs of a predator such as a cat (Canteras et al., 1997; Dilenberg and McGregor, 2001). The VMHdm was proposed, together with the anterior hypothalamic (AHN) and the dorsal premammilary (PMd) nuclei, to be part of a medial hypothalamic defensive system (Canteras, 2002). This system is anatomically connected to several regions related to defensive responses, such as the medial amygdala, lateral septal nucleus, interfascicular nucleus of the bed nuclei of the stria terminalis, infralimbic and prelimbic pre-frontal cortex, dlPAG and the precomissural nucleus (Canteras, 2002).

Despite these pieces of evidence, the effects of anxiolytic drugs directly injected into the VMHdm have not yet been investigated. Therefore, the objective of the present study was to test the hypothesis that administration into the VMHdm of midazolam, a benzodiazepine receptor full agonist, or AP7, a glutamate NMDA receptor antagonist, would produce behavioral changes in the EPM or the Vogel's punished licking test, two largely employed animal models of anxiety.

2. Methods

2.1. Subjects

Male Wistar rats weighing 200–250 g were housed in pairs with free access to food and water in a temperature controlled room (23 ± 1 °C) and a 12 h light, 12 h dark cycle (lights on at 6:00 a.m.). Independent groups of animals were used for each drug or dose tested. Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for care and use of laboratory animals, which are in compliance with international laws and policies.

2.2. Drugs

Midazolam maleate (15–60 nmol, Roche, Brazil) and 2-amino-7-phosphonoheptanoic acid (AP7, 0.2-2 nmol, Ciba-Geigy) were dissolved in sterile saline. The doses were chosen based on previous results obtained after intracerebral injections into the dorsomedial hypothalamus or dorsolateral PAG (Jardim and Guimarães, 2001, 2004; Guimarães et al., 1991; Molchanov and Guimarães, 2002).

2.3. Apparatus

The elevated plus-maze (EPM) consisted of two opposite wooden-made open arms (50 × 10 cm), crossed at a right angle by two arms of the same dimensions enclosed by 40-cm high walls with no roof. The maze was located 50 cm above the floor and a 1-cm high edge made of Plexiglas surrounded the open arms to prevent falls (File, 1992). Animal behavior in the maze was recorded with the help of a video-camera and the Ethovision software (V. 9, Noldus, Netherlands). It detects the position of the animal in the maze and calculates the number of entries and time spent in open and enclosed arms. As described previously (Jardim and Guimarães, 2004), a 6-cm large "exclusion" zone is included between the center of the maze and each arm so that most of the animal's body should be in the open or enclosed arm for an entry to be registered.

The punished licking test was performed in a Plexiglas box (42 × 25 × 20 cm) with a stainless steel grid floor. The metallic spout of a drinking bottle containing water projected into the box. The contact of the animal with the spout and the grid floor closed an electrical circuit controlled by a sensor (Anxio-Meter model 102, Columbus, USA), which produced 7 pulses per second whenever the animal was in contact with both components. Each pulse was considered as a lick, and every 20 licks the animal received a 0.5 mA shock for 2 s. The whole apparatus was located inside a sound-attenuated cage (Jardim and Guimarães, 2004).

2.4. Surgery

Rats were anesthetized with 2.5% 2,2,2-tribromoethanol (10 mL/kg i.p.) and fixed in a stereotaxic frame. The position of the incisor bar was −3.5 mm from the horizontal plane. A unilateral stainless steel guide cannula (0.7 mm OD) aimed at the VMHdm (coordinates: A: −3.1 mm from bregma, L: 0.6 mm, D: −8.0 mm bellow the surface of skull, Paxinos and Watson, 1997) was introduced. The cannula was attached to the bones with stainless steel screws and acrylic cement. An obturator inside the guide cannulae prevented obstruction.

2.5. Procedure

The animals were randomly assigned to one of the treatment groups seven days after the surgery.

Experiment 1. Effect of midazolam injected into the VMHdm in the EPM. The animals received a unilateral intra-VMHdm injection of sterile saline ($n = 5$) or midazolam 15, 30 or 60 nmol ($n = 6$/group) and ten min later were placed in the center of the EPM facing an enclosed arm. The number of entries and time spent in open and enclosed arms were recorded during 5 min. Intra-cerebral injections were performed with a thin dental needle (0.3 mm OD) introduced through the guide cannula until its tip

was 1.5 mm below the cannula end. A volume of 0.25 μL was injected in 30 s using a Microsyringe infusion pump (Kd Scientific, USA). The movement of an air bubble inside the PE-10 polyethylene tubing connecting the microsyringe with the dental needle confirmed drug flow. After each trial, the apparatus was cleaned with an alcohol solution. Saline and drug-treated groups were always run in parallel.

Experiment 2. Effect of midazolam injected into the VMHdm in the punished licking test. Animals were water deprived for 48 h before the test. After the first 24 h of deprivation they were allowed to drink freely for 3 min to find the drinking spout and the number of licks was registered as a pre-test measurement. Twenty-four hours later they received unilateral intra-VMHdm injection of saline (0.25 μL, $n=6$) or midazolam 30 ($n=8$) or 60 ($n=6$) nmol and 10 min later were placed inside the experimental box. The number of punished licks was recorded for 3 min (Molchanov and Guimarães, 2002; Jardim and Guimarães, 2004).

Experiment 3. Effect of midazolam injected into the lateral hypothalamus in the EPM. As a histological control, additional groups of rats received unilateral injections of saline ($n=4$) or midazolam 60 nmol ($n=6$) into the lateral hypothalamus (coordinates: A: −3.0 mm from bregma, L:1.9 mm, D: −7.6 mm bellow the surface of skull, Paxinos and Watson, 1997) and were submitted to the EPM as described above.

Experiment 4. Effect of AP7 injected into the VMHdm in the EPM. Rats received intra-VMHdm injections of saline ($n=.8$) or AP7 0.2 or 2 nmol ($n=9$/group) in 0.3 μL and were submitted to the EPM as described above.

Experiment 5. Effect of AP7 injected into the VMHdm in the punished licking test.

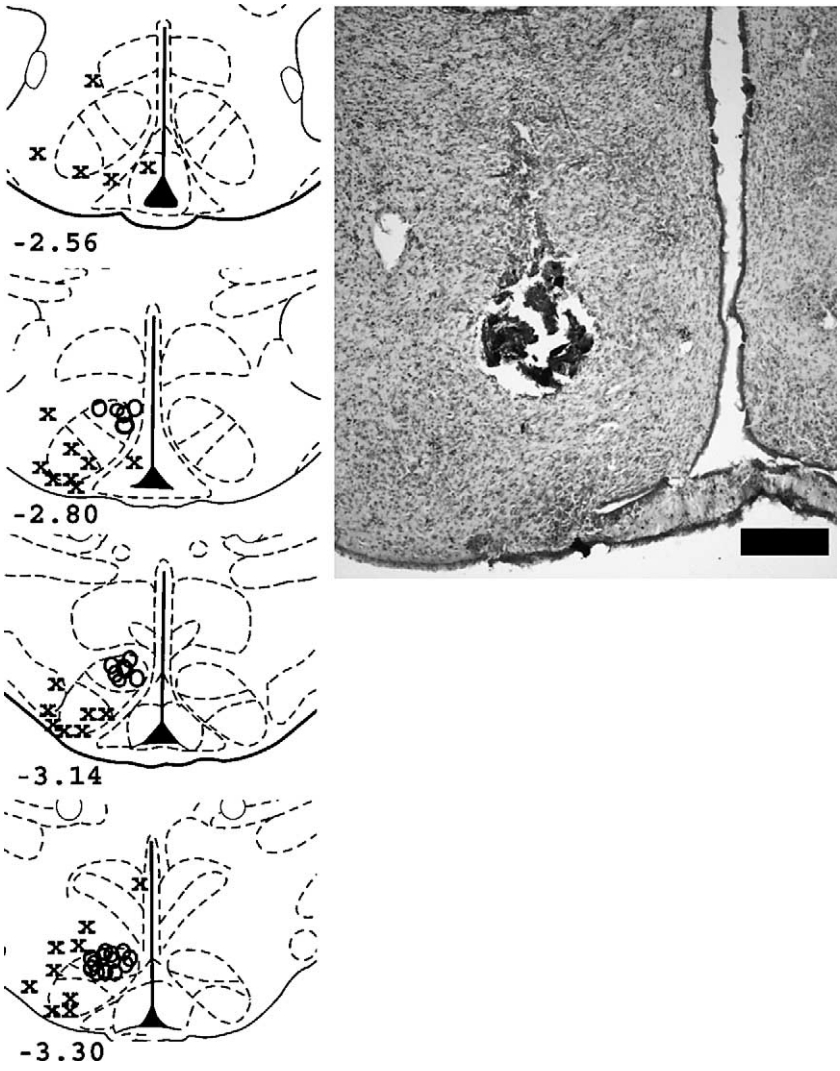

Fig. 1. Left. Histological localization of representative injection sites (circles) in diagrams based on the atlas of Paxinos and Watson (1997). Numbers indicate AP coordinates from bregma. Injection sites outside (x) the ventromedial hypothalamic nucleus, dorsomedial part (VMHdm), were also displayed. Due to overlapping the number of points in the figure is less than the total number of injection sites. Right. Photomicrography of an injection site in the VMHdm (Bar=250 μm).

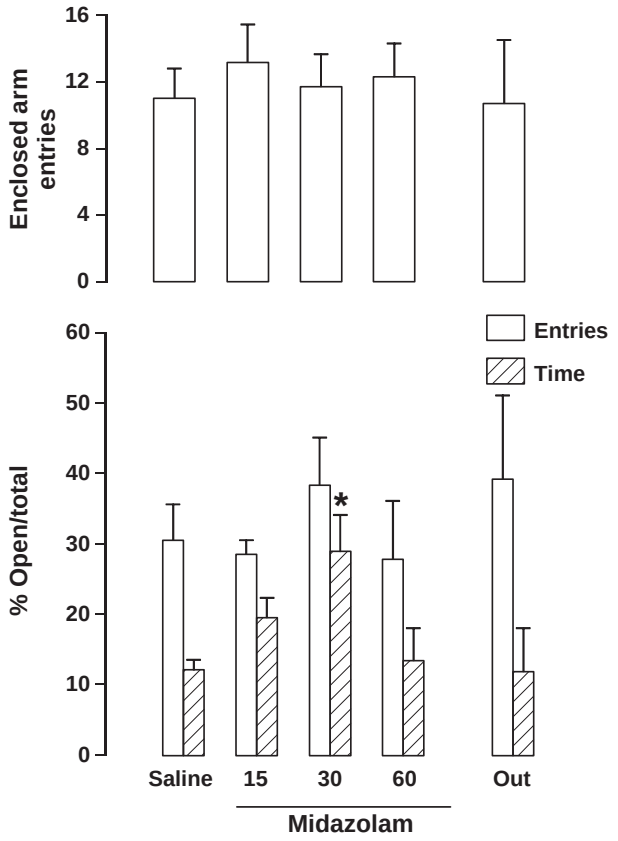

Fig. 2. Effect of midazolam 15–60 nmol ($n=6$/group) or saline (0.25 μL, $n=5$) microinjected into the VMHdm of rats tested in the elevated plus-maze. Columns represent the mean (+S.E.M). In the upper panel, open columns refer to the number of entries made into the enclosed arms. In the lower panel the open columns represent the percentage of entries onto the open arms while the hatched columns refer to the percentage of time spent in the open arms. Out=results from animals ($n=3$) that received midazolam 30 nmol outside the VMHdm. * Indicates significant difference from saline-treated or Out group (ANOVA followed by the Duncan test, $p<0.05$).

Animals received intra-VMHdm injections of saline ($n=7$) or AP7 2 nmol ($n=9$) and were submitted to punished licking test described above.

Experiment 6. Effect of AP7 injected into the lateral hypothalamus in the punished licking test. Additional groups of rats received unilateral injections of saline ($n=4$) or AP7 2 nmol ($n=6$) into the lateral hypothalamus and were submitted to the punished licking test as described above.

2.6. Histology

After the behavioral tests, rats were killed under deep urethane anesthesia and their brains perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. A dental needle was inserted through the guide cannula and a 0.2 μL microinjection of 1% Evans blue was performed. The brains were removed and, after a minimum time period of three days immersed in a 10% formalin solution, frozen sections of 40 μm were obtained in a cryostat (Cryocut, 1800). Injection sites were localized with the help of the Paxinos and Watson's rat brain atlas (1997).

2.7. Statistical analysis

The number of enclosed arm entries, the percentage of open arm entries (100 × open/total entries) and of time spent in the open arms (100 × open/open+enclosed) of the EPM, and the number of licks in the punished licking test were analyzed by Student's t-test or by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparisons, as appropriate. The significance level was set at $p<0.05$.

3. Results

Experiment 1. Representative injection sites into the VMHdm can be seen in Fig. 1.

Animals that received injections outside the VMHdm were discharged from the analysis with the exception of animals receiving 30 nmol of midazolam. Results from these animals were joined together in an additional (out, $n=3$) group. There was no significant difference in the number of enclosed arm entries ($F4,21=0.18$, NS) or in the percentage of open arm entries ($F4,21=0.61$, NS, Fig. 2). Midazolam 30 nmol in the VMHdm, however, caused a significant increase in the percentage of time spent in the open arms as compared to saline and out groups ($F4,21=3.05$, $p=0.039$, Duncan test, $p<0.05$, Fig. 2).

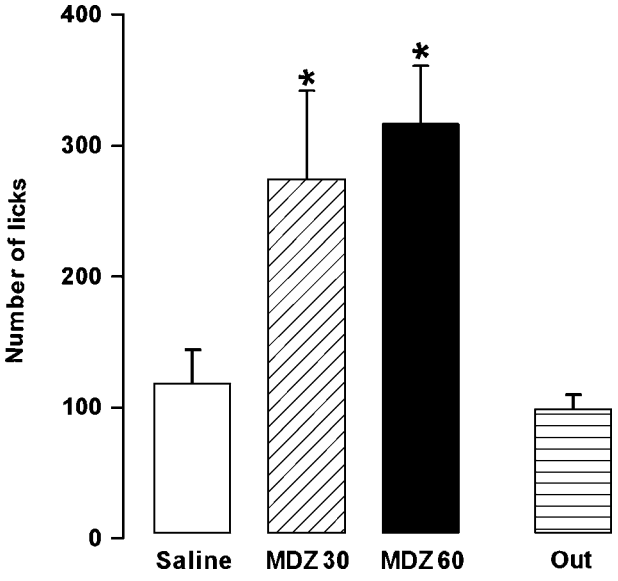

Fig. 3. Effects of midazolam 30 ($n=8$) or 60 ($n=6$) nmol or saline (0.25 μL, $n=6$) administered into the VMHdm on the number of licks in the punished licking test. Results were expressed as mean (+SEM) number of licks in the whole 3 min session. Out=results from animals ($n=6$) that received midazolam 60 nmol outside the VMHdm. * Indicates significant different from saline and Out groups ($p<0.05$, ANOVA followed by the Duncan test).

Experiment 2. Injection sites are also represented in Fig. 1. Animals that received injections outside the VMHdm were discharged from the analysis with the exception of animals receiving 60 nmol of midazolam. Results from these animals were joined together in an additional (out, $n=6$) group. Midazolam (30–60 nmol) significantly increased the number of punished licks as compared to saline or the out group ($F3,26=5.01$, $p<0.01$, Duncan test, $p<0.05$, Fig. 3).

Experiment 3. Midazolam 60 nmol injected into the lateral hypothalamus did not change the percentage of entries and time spent in the open arms of the EPM ($t_8=0.49$ and 1.14, respectively, NS, data not showed). It tended, however, to decrease the number of entries into the enclosed arms (saline=20.2±2.7, midazolam=11.0±2.9, $t_8=2.19$, $p=0.06$).

Experiment 4. AP7 did not change the percentage of entries ($F2,23=3.0$, $p>0.05$) but, at the dose of 0.2 nmol, decreased the percentage of time spent in the open arms ($F2,23=3.26$, $p<0.05$, Duncan test, $p<0.05$). The drug, at the dose of 2 nmol, also significantly decreased the number of enclosed arm entries ($F2,23=5.0$, $p=0.016$, Duncan test, $p<0.05$, Fig. 4).

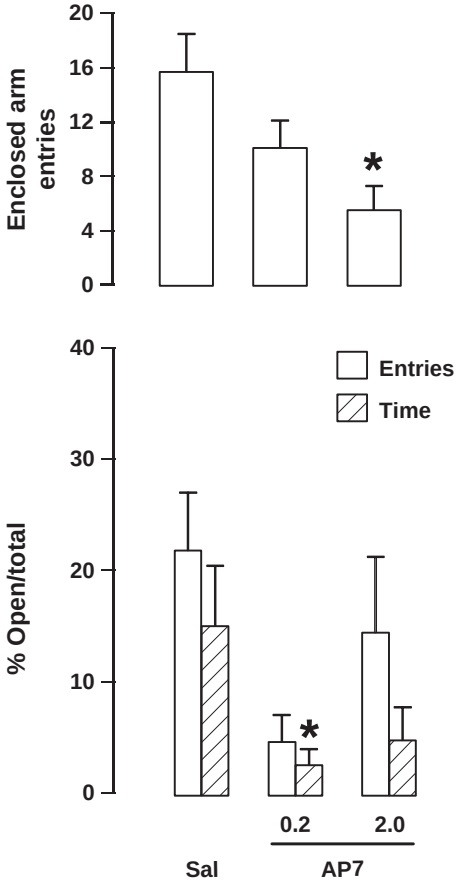

Fig. 4. Effect of AP7 0.2–2 nmol ($n=9$/group) or saline (0.3 μL, $n=8$) microinjected into the VMHdm of rats tested on the elevated plus-maze. * Indicates significant difference from saline ($p<0.05$, ANOVA followed by the Duncan test). Further specifications as in Fig. 2.

Experiments 5 and 6. AP7 (2 nmol), when injected into the ventromedial hypothalamus, significantly increased the number of punished licks as compared to saline or out groups ($F2,16=4.86$, $p=0.022$, Duncan, $p<0.05$, Fig. 5). No effect was found when the drug was injected into the lateral hypothalamus (Fig. 5). There was no difference among treatments in the pre-test measurements (data not shown).

4. Discussion

Midazolam, a benzodiazepine receptor full agonist, increased the percentage of time spent in the open arms of the EPM without changing the number of entries into the enclosed arms, suggesting an anxiolytic effect (File, 1992). This effect seems to be mediated by the VMHdm, since animals that receive the active dose (30 nmol) of midazolam outside this region were not different from control. In a previous study we have showed anxiolytic effects of this drug when administered into the DMH (Jardim and Guimarães, 2001). To control for possible drug diffusion from the VMHdm to the latter region, we injected the anxiolytic dose (60 nmol) of midazolam observed in the DMH into the lateral hypothalamic area, at a similar distance from both sites. Most of these injections reach the medial tuberal nucleus and no drug difference was found in open arms exploration of the EPM. The effect of midazolam in the VMHdm, however, was of small magnitude, and was only detected in the percentage of time spent in the open arms. Moreover, it was not dose-dependent, disappearing at the higher dose. This contrasts with the effects observed with the same drug injected into the DMH (Jardim and Guimarães, 2001). We have no explanation for the lack of dose-response relationship. Although no decrease in the number of enclosed arm entries was detected with the higher (60 nmol) dose in the VMHdm, the same dose tend to decrease the number of enclosed arm entries when injected into the lateral hypothalamic area. This effect is proposed to reflect a general decrease in exploratory activity (File, 1992). Therefore, it cannot be ruled out that drug diffusion to this area could have interfered with midazolam effects after VMHdm administration. In a recent study aimed at investigating the effects of systemically injected midazolam in rats exposed to cat odor McGregor et al. (2004) found that the benzodiazepine was able to decrease cFos expression in most hypothalamic areas, including the DMH, but failed to do so in the ventromedial and paraventricular nuclei. The results suggest that these areas are less sensitive to benzodiazepine action, what could also help to explain the small effect observed in the present study in the EPM. Another possibility is that benzodiazepine sensitivity in the VMHdm depends on the anxiety model employed. Corroborating this possibility, anxiolytic effects of midazolam in this region was found in another widely employed model of

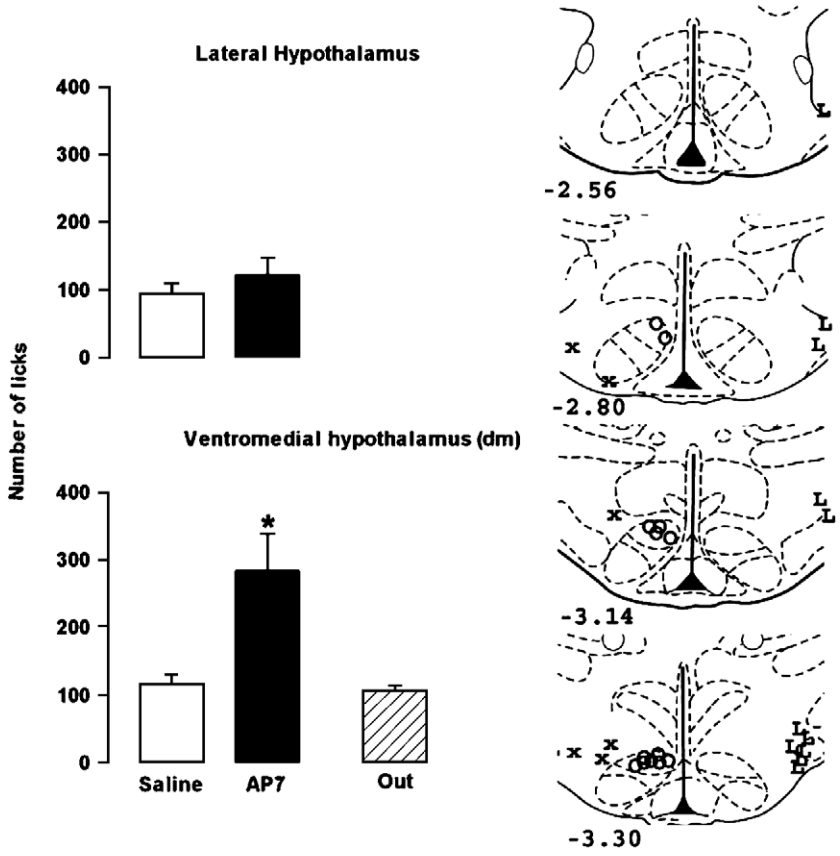

Fig. 5. Left. Effects of AP7 2 nmol ($n=6-9$/group) or saline (0.3 μL, $n=4-7$/group) administered into the lateral hypothalamic area (upper panel) or the VMHdm (lower panel) on the number of licks in the punished licking test. Further specifications as in Fig. 3. * Indicates significant difference ($p<0.05$, t-test). Right. Histological localization of representative injection sites into the VMHdm (circles) or the lateral hypothalamic area (L) in diagrams based on the atlas of Paxinos and Watson (1997). Numbers indicate AP coordinates from bregma. Injection sites outside (x) the VMHdm were also showed.

anxiety, the Vogel punished licking test (Vogel et al., 1971). This model does not depend on exploratory activity to the same extend as the EPM, what could help to explain the different results obtained in the two tests. Moreover, the EPM and Vogel tests are based on different aspects of defensive behaviors such as innate versus learned fear-inducing stimuli and natural open arm avoidance versus artificial electrical shocks, respectively. Accordingly, variable sensitivity to detect effects of non-benzodiazepine anxiolytics, such as buspirone and antidepressants, have been described for these two tests (Graeff and Zangrossi, 2002).

Administration of AP7 into the VMHdm decreased the percentage of time spent in the open arms of the EPM. This effect, however, was not dose-dependent, being observed with the lower dose (0.2 nmol) employed. Moreover, the drug induced a dose-dependent decrease in the number of enclosed arm entries. No effect was seen when AP7 was injected into the lateral hypothalamic area. Although a decrease in open arm exploration of the EPM is usually interpreted as an anxiogenic effect, this result can be distorted by concomitant interference with general exploratory activity, reflected by a decrease in the number of enclosed arm entries (File, 1992). The present results were very similar to those found in two previous studies (Jardim and Guimarães, 2001, 2004) showing that injections of glutamate ionotropic antagonists into a nearby region, the DMH, decrease exploratory activity of both enclosed and open arms of the EPM. These effects do not seem to depend on gross motor impairment detectable by the catalepsy or rota-rod tests (Jardim and Guimarães, 2004) but may involve interference with other motivational drives mediated by the medial hypothalamus (Bernardis and Bellinger, 1998). Moreover, temporary inactivation of the DMH and adjacent perifornical region, in addition to decrease fear responses, can produce a quiescent state that may be related to arousal reduction (Carrive, 2002). Together, these results indicate that glutamatergic neurotransmission in the medial hypothalamus may be involved in the control of general exploratory activity. Therefore, animal models based on evaluation of this activity are probably not the most appropriate to investigate the role of glutamate-mediated neurotransmission in this region in anxiety. Corroborating this suggestion, AP7, at a dose that produce anxiolytic effects in the dlPAG, did increase the number of punished licks when tested in the Vogel's test, a model that depends less on exploratory activity than the EPM and uses distinct fear-inducing stimuli (Vogel et al.,

1971). No effect was found when the drug was injected into the lateral hypothalamic area.

The result obtained with the punished licking test agrees with the anxiolytic effects of NMDA-receptor antagonists detected after either systemic (Stephens et al., 1986) or intra-PAG or amygdala administration (Guimarães et al., 1991; Matheus et al., 1994; Maren et al., 1996; Molchanov and Guimarães, 2002). AP7, however, failed to change anxiety in this test when injected into the DMH (Jardim and Guimarães, 2004). This latter region plays an important role in behavioral, autonomic and neuroendocrine responses to threatening stimuli (Bailey and DiMicco, 2001). It receives a moderate projection from the medial hypothalamic defensive system with the exception of the PMd (Canteras, 2002). However, it does not project back to these sites and, in addition, sends only sparse projections to the PAG (Canteras, 2002; Thompson et al., 1996). The VMHdm, on the contrary, sends considerable projections to the PAG (Canteras, 2002, Parry et al., 2002). It has been recently showed that neurons from the former structure that project to the lateral or dlPAG are activated by nociceptive somatic stimuli (Parry et al., 2002). Although a decrease in nociceptive threshold by AP7 cannot be ruled out, NMDA receptor antagonists failed to do so in the dlPAG (Molchanov and Guimarães, 2002; Vaccarino et al., 1997) despite showing anxiolytic effects in the Vogel test (Molchanov and Guimarães, 2002). Moreover, lesions of the VMH decrease, rather than increase, response threshold for phasic noxious stimuli (Mukherjee et al., 2002).

In conclusion, the present results suggest that facilitation of gabaergic or antagonism of glutamatergic neurotransmission in the VMHdm can produce anxiolytic-like effects. They also suggest, together with anatomical and functional data from the literature, that the VMHdm and the DMH could contribute differently to the modulation of defensive responses. More studies are needed to elucidate the extension of this contribution.

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References

Bailey TW, DiMicco JA. Chemical stimulation of the dorsomedial hypothalamus elevates plasma ACTH in conscious rats. Am J Physiol Regul Integr Comp Physiol 2001;280:R8–15.

Bernardis LL, Bellinger LL. The dorsomedial hypothalamic nucleus revisited: 1998 update. Proc Soc Exp Biol Med 1998;218:284–306.

Canteras NS. The medial hypothalamic defensive system: hodological organization and functional implications. Pharmacol Biochem Behav 2002;71:481–91.

Canteras NS, Chiavegatto S, Ribeiro do Valle LE, Swanson LW. Severe reduction of defensive behavior to a predator by discrete hypothalamic chemical lesions. Brain Res Bull 1997;44:297–305.

Carrive P. Role of periventricular structures in the expression of the behavioural and cardiovascular components of conditioned fear. First ISBRA (IBRO School in Brazil) Abstract book; 2002. p. 26–7.

De Novellis V, Stotz-Potter EH, Morin SM, Rossi F, DiMicco JA. Hypothalamic sites mediating cardiovascular effects of microinjected biccuculine and EAAs in rats. Am J Physiol Regul Integr Comp Physiol 1995;269:R131–40.

Dilenberg RA, McGregor IS. Defensive behavior in rats toward predatory odors: a review. Neurosci Biobehav Rev 2001;25:597–609.

DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV. The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. Pharmacol Biochem Behav 2002;71:469–80.

File SE. Behavioural detection of anxiolytic action. In: Elliott JM, Heal DJ, Marsden CA, editors. Experimental approaches to anxiety and depression. New York: Wiley; 1992. p. 25–44.

Graeff FG. Brain defence systems and anxiety. In: Roth M, Burrows GD, Noyes R, editor. Handbook of anxiety, vol. 3. Amsterdan: Elsevier Science Publishers; 1990. p. 307–54.

Graeff FG, Zangrossi Jr H. Animal models of anxiety disorders. In: D'haenen H, den Boer JA, Willner P, editors. Biological psychiatry. New York: John Wiley & Sons; 2002. p. 879–93.

Guimarães FS, Carobrez AP, De Aguiar JC, Graeff FG. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology 1991; 103:91–4.

Hess WR, Brugger M. Das subkortikale zentrum der affectiven adwehrreaktion. Helv Physiol Pharmacol Acta 1943;1:33–52.

Hilton SM. The defense reaction as a paradigm for cardiovascular control. In: Brooks CMcC, Koizumi K, Sato A, editors. Integrative functions of the autonomic nervous system. Tokyo: University of Tokyo Press and Elsevier Japan; 1979. pp. 444–9.

Jardim MC, Guimarães FS. GABAergic and glutamatergic modulation of exploratory in the dorsomedial hypothalamus. Pharmacol Biochem Behav 2001;69:579–84.

Jardim MC, Guimarães FS. Role of glutamate ionotropic receptors in the dorsomedial hypothalamic nucleus on anxiety and locomotor behavior. Pharmacol Biochem Behav 2004;79:541–6.

Lammers JH, Kruk MR, Meelis W, Van Der Poel AM. Hypothalamic substrates for brain stimulation-induced patterns of locomotion and escape jumps in the rat. Brain Res 1988;449:294–310.

Maren S, Aharonov G, Stote DL, Fanselow MS. *N*-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditioned fear in rats. Behav Neurosci 1996;110: 1365–74.

Matheus MG, Guimarães FS. Antagonism of non-NMDA receptors in the dorsal periaqueductal grey induces anxiolytic effect in the elevated plus maze. Psychopharmacology 1997;132:14–8.

Matheus MG, Nogueira RL, Carobrez AP, Graeff FG, Guimarães FS. Anxiolytic effect of glycine antagonists microinjected into the dorsal periaqueductal grey. Psychopharmacology 1994;113:565–9.

McGregor IS, Hargreaves GA, Apfelbach R, Hunt GE. Neural correlates of cat-odor induced anxiety in rats: region-specific effects of the benzodiazepine midazolam. J Neurosci 2004;24:4134–44.

Molchanov ML, Guimarães FS. Anxiolytic-like effects of AP-7 injected into the dorsolateral or ventrolateral columns of the periaqueductal gray of rats. Psychopharmacology 2002;160:30–8.

Mukherjee K, Mathur R, Nayar U. Hyperalgesic response in rats fed sucrose from weaning to adulthood: role of VMH. Pharmacol Biochem Behav 2002;73:601–10.

Parry DM, Semeneko FM, Conley RK, Lumb BM. Noxious somatic inputs to hypothalamic–midbrain projection neurons: a comparison of the columnar organization of somatic and visceral inputs to the periaqueductal grey in the rat. Exp Physiol 2002;87.2:117–22.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 3rd ed. San Diego: Academic Press; 1997.

Shekhar A, Keim SR. LY354740, a potent group II metabotropic glutamate receptor agonist prevents lactate-induced panic-like response in panic-prone rats. Neuropharmacology 2000;39:1139–46.

Silveira MCL, Graeff FG. Defense reaction elicited by microinjection of kainic acid into the medial hypothalamus of the rat: antagonism by a GABAA receptor agonist. Behav Neural Biol 1992;57:226–32.

Soltis RP, DiMicco JA. GABAA and excitatory amino acid receptors in the dorsomedial hypothalamus and heart rate in rats. Am J Physiol 1991; 260:R13–20.

Stephens DN, Meldrum BS, Weidmann R, Schneider C, Grutzner M. Does the excitatory amino-acid receptor antagonist 2-APH exhibit anxiolytic activity? Psychopharmacology 1986;90:166–9.

Thompson RH, Canteras NS, Swanson LW. Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol 1996;376:143–73.

Vaccarino AL, Clemmons HR, Mader Jr GJ, Magnusson JE. A role of periaqueductal grey NMDA receptors in mediating formalin-induced pain in the rat. Neurosci Lett 1997;236:117–9.

Vogel JR, Beer B, Clody DE. A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 1971;21:1–7.