Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 516 (2005) 239 – 246

www.elsevier.com/locate/ejphar

Panicolytic-like effect induced by the stimulation of $GABA_A$ and $GABA_B$ receptors in the dorsal periaqueductal grey of rats

Cíntia H. Bueno[a], Hélio Zangrossi Jr.[b], Regina L. Nogueira[c], Vanessa P. Soares[b], Milena B. Viana[d,*]

[a]*Laboratório de Psicofarmacologia, Departamento de Psicologia e Educação, FFCLRP, Universidade de São Paulo, 14040-901, Ribeirão Preto, São Paulo, Brazil*

[b]*Departamento de Farmacologia, FMRP, Universidade de São Paulo, 14049-900, Ribeirão Preto, São Paulo, Brazil*

[c]*Laboratório de Psicologia Comparada, Universidade Estácio de Sá, 22430-060, Rio de Janeiro e Faculdade de Psicologia, Universidade de Ribeirão Preto, 14096-380, Ribeirão Preto, Brazil*

[d]*Departamento de Psicologia do Desenvolvimento, Faculdade de Psicologia, Pontifícia Universidade Católica, 05014-901, São Paulo, Brazil*

Received 6 December 2004; received in revised form 26 April 2005; accepted 29 April 2005

Abstract

Activation of $GABA_A$ and benzodiazepine receptors within the dorsal periaqueductal grey inhibits the escape behaviour evoked by the electrical stimulation of this midbrain area, a defensive reaction that has been related to panic. Nevertheless, there is no evidence indicating whether the same antiaversive effect is also observed in escape responses evoked by species-specific threatening stimuli. In the present study, male Wistar rats were injected intra-dorsal periaqueductal grey with the benzodiazepine receptor agonist midazolam (10, 20 and 40 nmol), the $GABA_A$ receptor agonist muscimol (2, 4 and 8 nmol), the $GABA_B$ receptor agonist baclofen (2, 4 and 8 nmol), or with the benzodiazepine inverse agonist FG 7142 (20, 40 and 80 pmol) and tested in an ethologically-based animal model of anxiety, the elevated T-maze. Besides escape, this test also allows the measurement of inhibitory avoidance which has been related to generalised anxiety disorder. Midazolam, muscimol and baclofen impaired escape, a panicolytic-like effect, without altering inhibitory avoidance. FG 7142, on the other hand, facilitated both avoidance and escape reactions, suggesting an anxiogenic and panicogenic-like effect, respectively. The data suggest that $GABA_A$/benzodiazepine and $GABA_B$ receptors within the dorsal periaqueductal grey are involved in the control of escape behaviour and that a failure in this regulatory mechanism may be of importance in panic disorder.

Keywords: Dorsal periaqueductal grey; GABA; Benzodiazepine; Elevated T-maze; Generalised anxiety; Panic disorder

1. Introduction

The dorsal periaqueductal grey is part of a longitudinally organized neural system responsible for the generation/control of defensive reactions and consequently for the manifestation of aversive emotional states such as fear and anxiety (Graeff, 1990; Bandler and Shipley, 1994; Lovick, 2000; Brandão et al., 2003). Electrical stimulation of the structure has been performed both in human subjects and laboratory animals. In humans, dorsal periaqueductal grey stimulation produces unpleasant and fear-like sensations that resemble the symptomatic expression of a full-blown panic attack (Nashold et al., 1969; Amano et al., 1978). In rats, either electrical or chemical stimulation of the structure induces freezing behaviour alternating with vigorous flight and apparently aimless vertical jumps (Olds and Olds, 1962; Schenberg and Graeff, 1978; Graeff et al., 1986; Sandner et al., 1987; Fanselow, 1991; Jung et al., 2001; Schenberg et al., 2001). This explosive motor behaviour has also been identified as panic-like by several authors (Panksepp, 1982; Graeff et al., 1986; Jenck et al., 1995; Schenberg et al., 2001; Brandão et al., 2003). Based on these findings, it has been proposed that malfunction of the neuronal circuitry

* Corresponding author. Tel./fax: +55 11 38683203.
E-mail address: mviana@pucsp.br (M.B. Viana).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.04.045

within the dorsal periaqueductal grey that regulates flight/escape behaviour may generate panic attacks in humans (Graeff et al., 1986; Deakin and Graeff, 1991; Jenck et al., 1995; Lovick, 2000; Brandão et al., 2003). The successful use of serotonin reuptake inhibitors (e.g. imipramine and fluoxetine) and of high potency benzodiazepines (e.g. alprazolam) in the treatment of panic disorder (Den Boer and Slapp, 1998) is an indicative that changes in serotonin and GABA-mediated mechanisms may be involved in this process.

Using the electrical stimulation of the rat dorsal periaqueductal grey as an experimental model of panic, it was observed that activation of either serotonergic or $GABA_A$/benzodiazepine receptors in this midbrain area inhibits escape behaviour (Bovier et al., 1982; Schenberg et al., 1983; Graeff et al., 1986; Audi and Graeff, 1987; Beckett et al., 1992; Lovick, 1993; Nogueira and Graeff, 1995). Whereas intra-dorsal periaqueductal grey injection of serotonergic antagonists has no effect on this defensive response, microinjection of $GABA_A$ antagonists, by its own, elicits an explosive escape reaction (Schenberg et al., 1983; Graeff et al., 1986; Schutz et al., 1985; Nogueira and Graeff, 1995), in a way similar to the effects of the electrical stimulation of the structure. These results suggest that GABA, but not serotonergic receptors in the dorsal periaqueductal grey are under tonic activation.

In accordance to what has been observed with the dorsal periaqueductal grey electrical stimulation model, it has been recently demonstrated (Zanoveli et al., 2003) that intra-dorsal periaqueductal grey injection of serotonergic receptor agonists also inhibits escape behaviour evoked by species-specific threatening stimuli. In this particular case, animals were submitted to the elevated T-maze, another animal model of anxiety that associates escape responses with panic (Graeff et al., 1993; Viana et al., 1994; Graeff et al., 1998; Teixeira et al., 2000; Sena et al., 2003; Poltronieri et al., 2003).

In the present study, we investigated whether $GABA_A$/benzodiazepine and $GABA_B$ receptors within the dorsal periaqueductal grey are also involved in the regulation of the elevated T-maze escape response. For this purpose, animals were submitted to the test after intra-dorsal periaqueductal grey injection of the benzodiazepine receptor agonist midazolam, the $GABA_A$ receptor agonist muscimol, the $GABA_B$ receptor agonist baclofen or the benzodiazepine inverse agonist FG 7142.

Besides escape, the elevated T-maze model allows the measurement, in the same rat, of another defensive response, inhibitory avoidance. Since this response involves an approach–avoidance conflict to potential threat, it has been related, in terms of psychopathology, to generalised anxiety disorder (Gray and McNaughton, 2001; Graeff and Zangrossi, 2002). In fact, the pharmacological exploitation of inhibitory avoidance has largely supported this assumption (Graeff et al., 1993; Viana et al., 1994; Graeff et al., 1998; Sena et al., 2003). Therefore, by using the elevated T-maze it is possible to verify whether the effects of the agonists used in the present study were selective to a particular defensive response.

After tests in the elevated T-maze, all animals were also submitted to an open field for the evaluation of locomotor activity.

2. Methods

2.1. Subjects

Male Wistar rats, weighing 240–260 g, were housed in groups of five to six per cage until surgery. During the post-surgery period, animals were housed in pairs. Room temperature was maintained at 22±1 °C with lights on from 07:00 to 19:00 h. Food and water were freely available throughout the experiments. All procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behaviour Guidelines for Care and Use of Laboratory Animals, which are in compliance with international laws and policies. All efforts were made to minimize animal suffering.

2.2. Apparatus

2.2.1. The elevated T-maze

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

2.2.2. Open field

The open field used to measure locomotion was a wooden square box (60×60 cm) with walls 30 cm high and the floor divided into nine squares of 20×20 cm.

Luminosity at the level of the maze arms or at the open field centre was 60 lux.

2.3. Drugs

Midazolam, muscimol and baclofen (Sigma, USA) were dissolved in sterile saline 0.9%. FG 7142 (*N*-methyl-β-carboline-3-carboxamine; Sigma, USA) was dissolved in a saline-Tween 80 2% solution. Control animals were administered with either saline or saline-Tween 80 2% solution.

2.4. Procedure

2.4.1. Surgery

Rats were anaesthetised with 2,2,2-tribromoethanol (Aldrich, USA) associated with local anaesthesia (2% lidocaine with a vasoconstrictor; Harvey, Brazil) and placed in a stereotaxic instrument (David-Kopf, USA), with the

incisors bars set at −2.5 mm. Burr holes were drilled in the skull above the dorsomedial periaqueductal grey following the coordinates from the atlas of Paxinos and Watson (1998). A stainless steel 13 mm long guide cannula was inserted into the brain through a hole drilled in the skull, at an angle of 22°. The following coordinates from lambda were used: lateral=+1.9 mm; deep=−3.2 mm. To prevent infections, at the end of the surgery, all animals were injected (IM) with a 0.2 ml of pentabiotic preparation (Pentabiótico Veterinário Pequeno Porte; Forte Dodge, Brazil).

2.4.2. Behavioural tests

On the fifth and sixth days after surgery, animals were gently handled by the experimenter for 5 min. On the sixth day, each animal was pre-exposed for 30 min to one of the open arms of the elevated T-maze. A wood barrier mounted on the border of the maze central area and the open arm's proximal end isolated this arm from the rest of the maze. It has been shown that pre-exposure shortens escape latencies, rendering the escape task more sensitive to the effects of antipanic drugs (Teixeira et al., 2000; Poltronieri et al., 2003).

On the seventh day, animals were microinjected with midazolam (0, 10, 20 and 40 nmol; $n=9–12$), muscimol (0, 2, 4 and 8 nmol; $n=9$), baclofen (0, 2, 4 and 8 nmol; $n=9–12$), or FG 7142 (0, 20, 40 and 80 pmol; $n=8–12$). For drug microinjection, a needle (outside diameter 0.3 mm) was introduced into the guide cannula until its tip was 2 mm below the cannula end. A volume of 0.2 μl was injected over a period of 2 min, using a 10 μl microsyringe (Hamilton, USA) attached to a microinfusion pump (KD Scientific, USA). The displacement of an air bubble inside a polyethylene catheter connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The intracerebral needle was removed 1 min after the injection was finished. Ten minutes after drug administration, animals were tested in the elevated T-maze for inhibitory avoidance measurement. Each rat was placed at the end of the enclosed arm and the time taken to withdraw from this arm with the four paws was recorded (baseline latency). The same measurements were 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), each animal was placed at the end of the same open arm used in the pre-exposure session and the time taken to leave this arm with the four paws was recorded in three consecutive trials (escape 1 to 3), again with 30 s intertrials intervals. A cut off time of 300 s was established for the avoidance and escape latencies. Immediately after being tested in the elevated T-maze, animals were individually placed into the centre of the arena and their behaviour was recorded through the use of a video camera connected to a videocassette recorder, for evaluation of locomotor activity. For 5 min, the total number of squares crossed by the animals and the number of rearing were measured.

2.4.3. Histology

At the end of the experiments, animals were killed under deep anaesthesia with urethane (3 g/kg; Sigma, USA). Brains were perfused through the heart with 10% formalin solution before being removed for histological analysis. Brain slices of 60 μm were made by means of a freezing microtome in order to localize the drug injection sites (Paxinos and Watson, 1998).

2.5. Data analysis

Two-way analysis of variance (ANOVA) with a repeated measure was used to analyse avoidance and escape data in the elevated T-maze, with treatment as the independent and trials (baseline, avoidance 1 and 2, or escape 1 to 3) as the dependent factor. Significant effects of the independent factor or of the interaction between the independent and dependent factors were analysed by one-way ANOVA followed by the Duncan post hoc test. Locomotor activity in the open field was also analysed by one-way ANOVA followed by the Duncan post hoc test.

3. Results

The diagrams of Fig. 1 show microinjection sites of rats treated intra-dorsal periaqueductal grey with midazolam, muscimol,

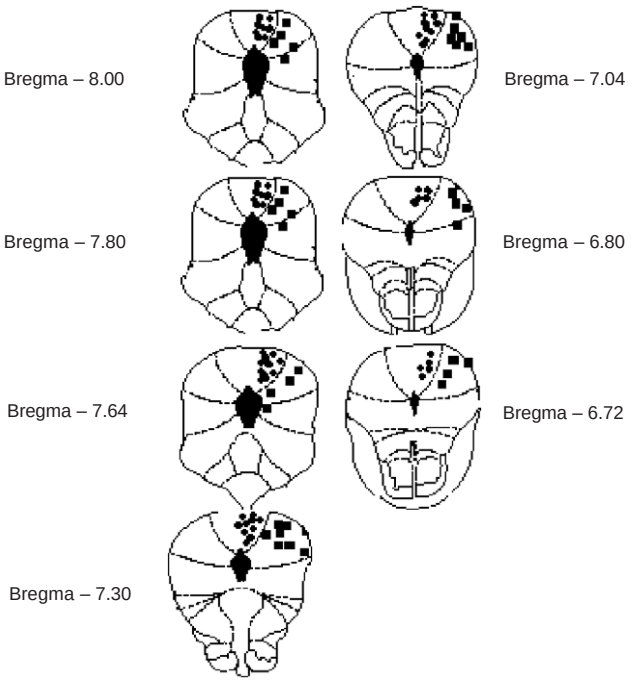

Fig. 1. Localization of injection sites inside (dots) or outside (squares) the dorsomedial periaqueductal grey. Figures represent coordinates from Paxinos and Watson (1998) rat brain atlas, with respect to bregma. The number of points in the figure is less than the total number of rats used ($n=228$) because of several overlaps.

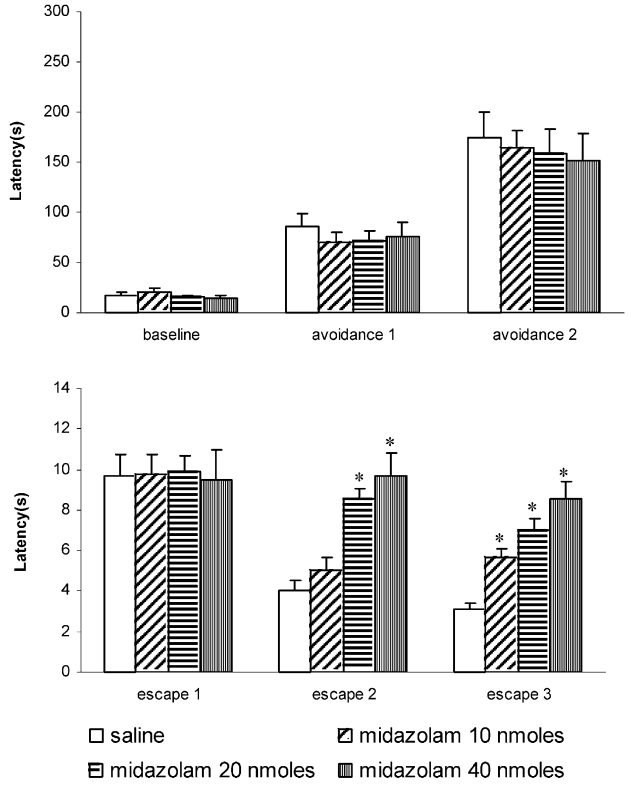

Fig. 2. Effects (mean+S.E.M.) of intra-dorsal periaqueductal grey midazolam (10, 20 and 40 nmol/0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. The latencies to leave the enclosed arm (baseline, avoidance 1 and avoidance 2) or one of the open arms (escape 1–3) were measured sequentially at 30 s intervals beginning 10 min after intra-dorsal periaqueductal grey injection of either drug or control solution. Twenty-four hours before the test, all animals were exposed to one of the open arms for 30 min. *$P<0.05$ with respect to control in the same trial (one-way ANOVA followed by Duncan post hoc test).

baclofen or FG 7142, and their respective control groups. Only animals with injection sites located inside the dorsomedial periaqueductal grey were included in the statistical analysis.

Table 1
Locomotor activity in the open field after dorsal periaqueductal grey drug injection

Drugs	Number	
	Crossings	Rearings
Saline	50.00±4.93	22.58±2.03
Midazolam (10 nmol)	43.25±5.25	20.64±2.31
Midazolam (20 nmol)	47.78±4.65	23.00±2.05
Midazolam (40 nmol)	48.33±2.58	21.11±1.81
Saline	48.22±3.42	23.78±1.78
Muscimol (2 nmol)	44.67±3.48	23.89±1.98
Muscimol (4 nmol)	44.56±3.17	25.78±2.04
Muscimol (8 nmol)	46.22±4.16	24.72±2.12
Saline	52.25±3.78	26.75±1.74
Baclofen (2 nmol)	52.33±4.27	24.33±1.44
Baclofen (4 nmol)	50.67±4.79	23.58±1.76
Baclofen (8 nmol)	48.11±3.89	24.33±1.68
Vehicle	49.58±6.69	25.17±2.66
FG 7142 (20 pmol)	53.58±5.24	22.42±3.18
FG 7142 (40 pmol)	41.89±6.02	22.11±2.00
FG 7142 (80 pmol)	42.75±7.17	20.00±3.78

3.1. Effects of midazolam

As shown in Fig. 2 (upper panel), treatment with midazolam did not interfere with inhibitory avoidance acquisition. Two-way analysis of variance showed a significant effect of trials ($F(2,74)=98.08$; $P<0.01$) but not of treatment ($F(3,37)=0.29$; $P>0.05$) or of treatment versus trials interaction ($F(6,74)=0.17$; $P>0.05$).

On the other hand, one-way escape was impaired by intra-dorsal periaqueductal grey treatment with midazolam (Fig. 2, lower panel). Two-way ANOVA revealed a significant effect of treatment ($F(3,37)=7.99$; $P<0.01$), trials ($F(2,74)=27.71$; $P<0.01$) and treatment versus trials interaction ($F(6,74)=4.62$; $P<0.01$). The Duncan post hoc test showed that escape 2 latency was significantly ($P<0.05$) longer in the groups treated with 20 and 40 nmol of midazolam when compared to control animals. Animals treated with the three doses of midazolam had longer escape 3 latency when compared to the control group.

One-way ANOVA revealed no significant differences between animals treated with midazolam or saline in the open field. Neither the number of crossings ($F(3,37)=0.34$; $P>0.05$), nor the number of rearings ($F(3,37)=0.29$; $P>0.05$) were altered by drug administration (see Table 1).

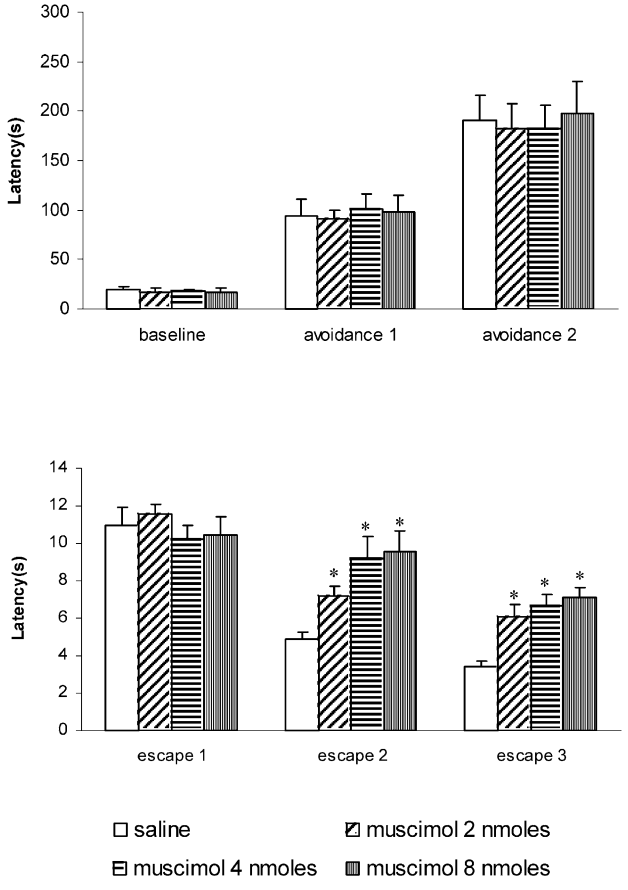

Fig. 3. Effects (mean+S.E.M.) of intra-dorsal periaqueductal grey muscimol (2, 4 and 8 nmol/0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. For further specifications, report to Fig. 2.

3.2. Effects of muscimol

In the same direction as the results with midazolam, treatment with muscimol did not alter inhibitory avoidance acquisition (Fig. 3, upper panel). Two-way analysis of variance showed a significant effect of trials ($F(2,64)=79.78$; $P<0.01$) but not of treatment ($F(3,32)=0.07$; $P>0.05$) or of treatment versus trials interaction ($F(6,64)=0.06$; $P>0.05$).

On the other hand, one-way escape was impaired by intra-dorsal periaqueductal grey treatment with muscimol (Fig. 3, lower panel). Two-way ANOVA revealed a significant effect of treatment ($F(3,32)=5.90$; $P<0.01$), trials ($F(2,64)=38.20$; $P<0.01$) and treatment versus trials ($F(6,64)=2.78$; $P<0.05$). The Duncan post hoc test showed that escape 2 and 3 latencies were significantly longer ($P<0.05$) in rats treated with the three doses of muscimol when compared to the control group.

As indicated in Table 1, one-way ANOVA showed that neither the number of crossings ($F(3,32)=0.04$; $P>0.05$) nor the number of rearings ($F(3,32)=1.04$; $P>0.05$) measured in the open field were affected by treatment with muscimol.

3.3. Effects of baclofen

Fig. 4 (upper panel) shows that treatment with baclofen did not modify inhibitory avoidance acquisition. Two-way analysis of variance showed a significant difference on trials ($F(2,82)=116.71$; $P<0.01$), but not on treatment ($F(3,41)=0.02$; $P>0,05$), or on treatment versus trials interaction ($F(6,82)=0.08$; $P>0.05$).

On the other hand, as with midazolam and muscimol, one-way escape was impaired by intra-dorsal periaqueductal grey treatment with baclofen (Fig. 4, lower panel). Two-way ANOVA revealed a significant effect of treatment ($F(3,41)=3.95$; $P<0.01$), trials ($F(2,82)=24.15$; $P<0.01$) and treatment versus trials interaction ($F(6,82)=5.77$; $P<0.01$). The post hoc test showed that escape 2 and 3 latencies were significantly longer ($P<0.05$) in all groups of rats treated with baclofen when compared to the control group.

As indicated in Table 1, one-way ANOVA showed that neither the number of crossings ($F(3,41)=0.19$; $P>0.05$) nor the number of rearings ($F(3,41)=0.72$; $P>0.05$) measured in the open field were affected by treatment with baclofen.

3.4. Effects of FG 7142

In contrast with the results observed with midazolam, muscimol and baclofen, inhibitory avoidance was facilitated by treatment with FG 7142 (Fig. 5, upper panel). Two-way analysis of variance showed a significant effect of treatment ($F(3,37)=2.92$; $P<0.05$) and trials ($F(2,74)=102.33$; $P<0.01$), but not of treatment versus trials interaction ($F(6,74)=1.08$; $P>0.05$). The Duncan post hoc test showed that avoidance 2 latency was significantly ($P<0.05$) longer in the group treated with 40 pmol of FG 7142 when compared to the control group.

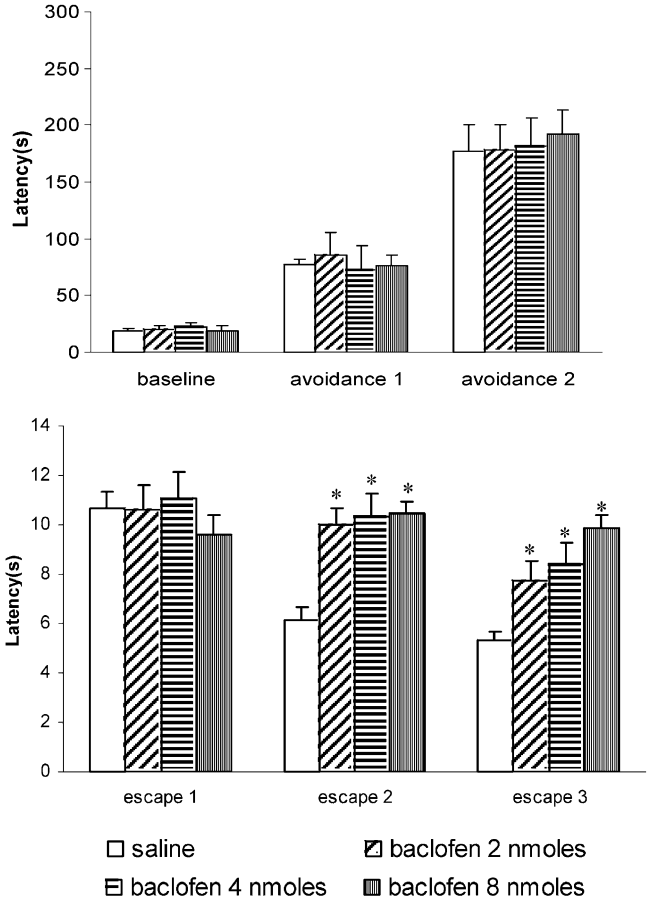

Fig. 4. Effects (mean+S.E.M.) of intra-dorsal periaqueductal grey baclofen (2, 4 and 8 nmol/0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. For further specifications, report to Fig. 2.

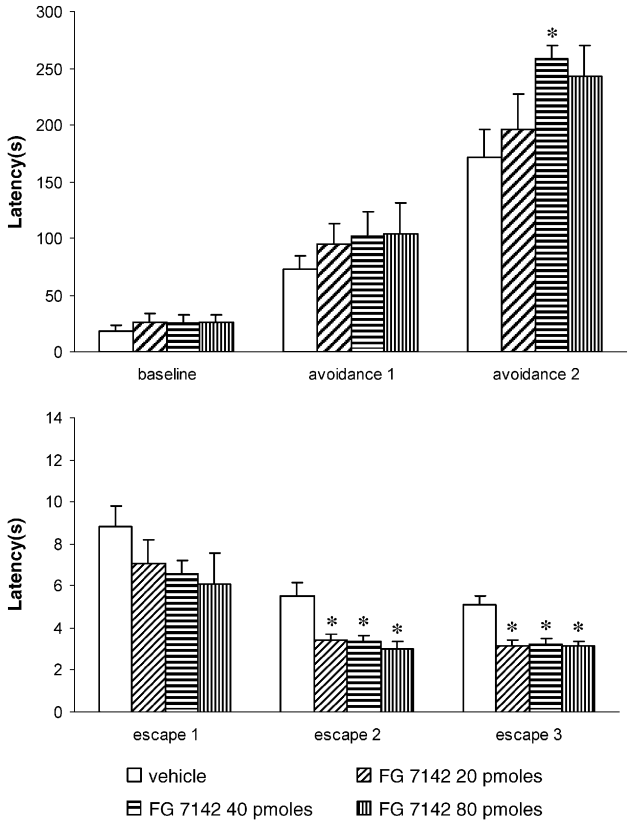

Fig. 5. Effects (mean+S.E.M.) of intra-dorsal periaqueductal grey FG 7142 (20, 40 and 80 pmol/0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. For further specifications, report to Fig. 2.

Again, in contrast to what was observed with the other drugs, one-escape (Fig. 5, lower panel) was facilitated by treatment with FG 7142. Two-way ANOVA revealed a significant effect of treatment ($F(3,37)=9.02$; $P<0.01$), trials ($F(2,74)=28.75$; $P<0.01$) but not of treatment versus trial interaction ($F(6,74)=0.11$; $P>0.05$). The Duncan post hoc test showed that escape 2 and 3 latencies were significantly shorter ($P<0.05$) in rats treated with the different doses of FG 7142 (20, 40 and 80 pmol) when compared to the control group.

As shown in Table 1, neither the number of crossings ($F(3,37)=0.78$; $P>0.05$) nor the number of rearings ($F(3,37)=0.50$; $P>0.05$) measured in the open field were affected by treatment with FG 7142.

4. Discussion

The present results showed that stimulation of $GABA_A$/benzodiazepine and $GABA_B$ receptors in the dorsal periaqueductal grey by local administration of midazolam, muscimol and baclofen impaired one-way escape in the elevated T-maze, a panicolytic-like effect, without altering inhibitory avoidance. On the other hand, intra-dorsal periaqueductal grey administration of the benzodiazepine inverse agonist FG 7142 facilitated one-way escape, a panicogenic-like effect, and improved the acquisition of inhibitory avoidance, an anxiogenic effect. Since no significant effects were observed with any of the drugs tested in the open field, it is reasonable to affirm that the results obtained with the drugs in the elevated T-maze are not due to motor alterations.

The panicolytic-like effect observed with midazolam and muscimol in the elevated T-maze escape task is in agreement with previous reported results obtained with the electrical stimulation of the dorsal periaqueductal grey (Bovier et al., 1982; Graeff et al., 1986; Audi and Graeff, 1987), another animal model that associates escape behaviour with panic disorder. More specifically, in the studies performed by Graeff et al. (1986) and Audi and Graeff (1987) intra-dorsal periaqueductal grey administration of GABA or of the $GABA_A$ receptor agonists muscimol, isovugacine and THIP raised the aversive threshold, defined as the lowest electrical current intensity inducing escape behaviour. In addition, antiaversive effects were observed in the same model after dorsal periaqueductal grey microinjection of the benzodiazepine receptor agonists midazolam and chlordiazepoxide (Bovier et al., 1982; Graeff et al., 1986; Audi and Graeff, 1987).

Similarly to midazolam and muscimol, baclofen impaired elevated T-maze one-way escape, suggesting the involvement of $GABA_B$ receptors within the dorsal periaqueductal grey in the mediation of this defensive response. Unlike the two former drugs, intra-dorsal periaqueductal grey injection of this agonist failed to change escape behaviour induced by the electrical stimulation of the same brain area (Graeff et al., 1986). However, as in the latter study only one dose of baclofen (10 nmol) was evaluated, it is still an open question whether this discrepancy reflects differences between the two tests regarding drug sensitivity or selectivity.

In agreement with the present elevated T-maze findings, the results of a preliminary clinical study have also implicated $GABA_B$ receptors in the physiopathology of panic. In this double-blind controlled analysis, baclofen was reported to significantly reduce the incidence of panic attacks in medication-free panic disorder patients (Breslow et al., 1989). Whether this effect involves the dorsal periaqueductal grey is unknown, but animal studies have demonstrated that this area is rich in $GABA_B$ receptors (Lovick, 2000). In fact, it has been shown that, among the periaqueductal grey anatomical sectors, $GABA_B$ receptors were particularly found in the more dorsal aspects of the structure (Bowery et al., 1987; Lovick, 2000).

Contrarily to the effects observed with midazolam or with the GABAergic agonists muscimol and baclofen, intra-dorsal periaqueductal grey administration of the benzodiazepine inverse agonist FG 7142 facilitated elevated T-maze one-way escape, a panicogenic-like effect. Accordingly, it has been shown that this drug generates intense unrest, intolerable tension and feelings of impending doom in normal human volunteers (Dorow et al., 1983).

In some individuals, this panic-like reaction was so severe that further human experimentation has been abandoned for unethical reasons (Malizia et al., 1995). Although the systemic effect of FG 7142 in the elevated T-maze has not yet been evaluated, the drug increased the rat propensity to escape from the unstable elevated plus-maze, a behavioural response that has also been associated with panic (Jones et al., 2002).

Among all the agonists used in the present study, FG 7142 was the only drug to alter inhibitory avoidance in the elevated T-maze. The drug significantly facilitated the acquisition of this response, a result indicative of an anxiogenic effect. Corroborating this result, anxiogenic effects of intra-dorsal periaqueductal grey administration of FG 7142 had already been shown in the elevated plus-maze (Russo et al., 1993). These evidences indicate that $GABA_A$/benzodiazepine receptors within the dorsal periaqueductal grey may not be exclusively involved in the regulation of defensive behaviours related to panic disorder. In agreement with this proposal, it has been shown that intra-dorsal periaqueductal grey injection of midazolam, at the dose range used in the present study, inhibited avoidance from the open arms in the elevated plus-maze, an anxiolytic effect (Russo et al., 1991). Nevertheless, since the effect of FG 7142 on elevated T-maze avoidance was not pronounced or dose-dependent and taking into account the proposal that the elevated plus-maze is a mixed animal model of anxiety, as it seems to blend both generalised anxiety disorder and panic disorder-associated responses (Handley and McBlane, 1993; Graeff and Zangrossi, 2002), the involvement of dorsal periaqueductal grey $GABA_A$/benzodiazepine receptors in generalised anxiety disorder-like behaviours is yet to be confirmed.

Acknowledgments

This study was supported by CNPq and FAPESP, Brazil. We are grateful to Paulo C. Castrechigni for his technical assistance.

References

Amano, K., Tanikama, T., Iseki, H., Kawabatake, H., Notani, M., Kawamura, H., Kitamura, K., 1978. Single neuron analysis of the human midbrain tegmentum: rostral mesencephalic reliculotomy of pain relief. Appl. Neurophysiol. 41, 66–78.

Audi, E.A., Graeff, F.G., 1987. GABAA receptors in the midbrain central grey mediate the antiaversive action of GABA. Eur. J. Pharmacol. 135, 225–229.

Bandler, R., Shipley, M.T., 1994. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci. 17, 379–389.

Beckett, S.R., Lawrence, A.J., Marsden, C.A., Marshall, P.W., 1992. Attenuation of chemically induced defence response by 5-HT1 receptor agonists administered into the periaqueductal gray. Psychopharmacology (Berl) 108, 110–114.

Bovier, P., Broekkamp, C.L., Lloyd, K.G., 1982. Enhancing GABAergic transmission reverses the aversive state in rats induced by electrical stimulation of the periaqueductal grey region. Brain Res. 248, 313–320.

Bowery, N.G., Hudson, A.L., Price, G.W., 1987. $GABA_A$ e $GABA_B$ receptor distribution in the rat central nervous system. Neuroscience 20, 364–383.

Brandão, M.L., Troncoso, A.C., de Souza Silva, M.A., Huston, J.P., 2003. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: empirical and conceptual considerations. Eur. J. Pharmacol. 463, 225–233.

Breslow, M.F., Fankhauser, M.P., Potter, R.L., Meredith, K.E., Misiaszek, J., .Hope Jr, D.G., 1989. Role of gamma-aminobutyric acid in antipanic drug efficacy. Am. J. Psychiatry 146, 353–356.

Deakin, J.F., Graeff, F.G., 1991. 5-HT and mechanisms of defense. J. Psychopharmacol. (Berl) 5, 305–315.

Den Boer, J.A., Slapp, B.R., 1998. Review of current treatment in panic disorder. Int. Clin. Psychopharmacol. 13, S25–S30.

Dorow, R., Horowski, R., Paschelke, G., Amin, M., Braestrup, C., 1983. Severe anxiety induced by FG 7142 a beta carboline ligand for benzodiazepine receptors. Lancet 8342, 98–99.

Fanselow, M.S., 1991. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis, A., Bandler, R. (Eds.), The Midbrain Periaqueductal Matter: Functional, Anatomical and Immunohistochemical Organization. Plenun Publishing Corporation, New York, pp. 151–173.

Graeff, F.G., 1990. Brain defense systems and anxiety. In: Burrows, G.D., Noyes Jr., R.M. (Eds.), Handbook of Anxiety, The Neurobiology of Anxiety, vol. 3. Elsevier Science Publishers, Amsterdam, pp. 307–354.

Graeff, F.G., Zangrossi Jr., H., 2002. Animal models of anxiety. In: D'Haenen, H., Boer, J.A., Willner, P. (Eds.), Biological Psychiatric. John Wiley and Sons, Southern Gate, pp. 1–15.

Graeff, F.G., Brandão, M.L., Audi, E.A., Schutz, M.T., 1986. Modulation of the brain aversive system by GABAergic and serotonergic mechanisms. Behav. Brain Res. 22, 173–180.

Graeff, F.G., Silveira, M.C., Nogueira, R.L., Audi, E.A., Oliveira, R.M., 1993. Role of the amygdala and periaqueductal gray in anxiety and panic. Behav. Brain Res. 58, 123–131.

Graeff, F.G., Netto, C., Zangrossi Jr., H., 1998. The elevated T-maze as an experimental model of anxiety. Neurosci. Biobehav. Rev. 23, 237–246.

Gray, J.A., McNaughton, N., 2001. The Neuropsychology of Anxiety. Oxford University Press, Oxford.

Handley, S.L., McBlane, J.W., 1993. An assessment of the elevated X-maze for studying anxiety and anxiety-modulating drugs. J. Pharmacol. Toxicol. Methods 29, 129–138.

Jenck, F., Moreau, J.L., Martin, J.R., 1995. Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. Psychiatry Res. 57, 181–191.

Jones, N., King, S.M., Duxon, M.S., 2002. Further evidence for the predictive validity of the unstable elevated exposed plus-maze, a behavioural model of extreme anxiety in rats: differential effects of fluoxetine and chlordiazepoxide. Behav. Pharmacol. 13, 525–535.

Jung, M.E., Depoortere, R., Oglesby, M.W., 2001. Use of negatively reinforcing electrical brain stimulation to detect conventional and nonconventional anxiolytics as well as an anxiogenic drug. Pharmacol. Biochem. Behav. 68, 33–42.

Lovick, T.A., 1993. Serotonergic influence from nucleus raphe obscurus on neurones in the periaqueductal grey matter in the rat. Brain Res. 606, 92–98.

Lovick, T.A., 2000. Panic disorder-A malfunction of multiple transmitter control system within the midbrain periaqueductal gray matter? Neurosci. 6, 48–59.

Malizia, A.L., Coupland, N.J., Nutt, D.J., 1995. Benzodiazepine receptor function in anxiety disorder. In: Biggio, G., Sanna, E., Serra, M., Costa, E. (Eds.), GABAA Receptors and Anxiety: from Neurobiology to Treatment. Raven Press, New York, pp. 115–133.

Nashold Jr., B.S., Wilson, W.P., Slaughter, D.G., 1969. Sensations evoked by stimulation in the midbrain of man. J. Neurosurg. 30, 14–24.

Nogueira, R.L., Graeff, F.G., 1995. Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray generated aversion. Pharmacol. Biochem. Behav. 52, 1–6.

Olds, M.E., Olds, J., 1962. Approach-escape interactions in rat brain. Am. J. Physiol. 203, 803–810.

Panksepp, J., 1982. Toward a general psychobiological theory of emotions. Behav. Brain Sci. 5, 407–467.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, Sidney.

Poltronieri, S.C., Zangrossi Jr., H., Viana, M.B., 2003. Antipanic-like effect of serotonin reuptake inhibitors in the elevated T-maze. Behav. Brain Res. 147, 185–192.

Russo, A.S., Guimaraes, F.S., De Aguiar, J.C., Graeff, F.G., 1991. Anxiolytic effect of midazolam microinjected into the dorsal periaqueductal grey area of rats. Braz. J. Med. Biol. Res. 24, 607–609.

Russo, A.S., Guimaraes, F.S., De Aguiar, J.C., Graeff, F.G., 1993. Role of benzodiazepine receptors located in the dorsal periaqueductal grey of rats in anxiety. Psychopharmacology (Berl) 110, 198–202.

Sandner, G., Schmitt, P., Karli, P., 1987. Mapping of jumping, rearing, squealing and switch-off behaviors elicited by periaqueductal gray stimulation in the rat. Physiol. Behav. 39, 333–339.

Schenberg, L.C., Graeff, F.G., 1978. Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. Pharmacol. Biochem. Behav. 9, 287–295.

Schenberg, L.C., De Aguiar, J.C., Graeff, F.G., 1983. GABA modulation of the defense reaction induced by brain electrical stimulation. Physiol. Behav. 31, 429–437.

Schenberg, L.C., Bittencourt, A.S., Sudre, E.C., Vargas, L.C., 2001. Modeling panic attacks. Neurosci. Biobehav. Rev. 25, 647–659.

Schutz, M.T., de Aguiar, J.C., Graeff, F.G., 1985. Anti-aversive role of serotonin in the dorsal periaqueductal grey matter. Psychopharmacology (Berl) 85, 340–345.

Sena, L.M., Bueno, C., Pobbe, R.L., Andrade, T.G., Zangrossi Jr., H., Viana, M.B., 2003. The dorsal raphe nucleus exerts opposed control on generalized anxiety and panic-related defensive responses in rats. Behav. Brain Res. 142, 125–133.

Teixeira, R.C., Zangrossi Jr., H., Graeff, F.G., 2000. Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. Pharmacol. Biochem. Behav. 65, 571–576.

Viana, M.B., Tomaz, C., Graeff, F.G., 1994. The elevated T-maze: a new animal model of anxiety and memory. Pharmacol. Biochem. Behav. 49, 549–554.

Zanoveli, J.M., Nogueira, R.L., Zangrossi Jr., H., 2003. Serotonin in the dorsal periaqueductal gray modulates inhibitory avoidance and one-way escape behaviors in the elevated T-maze. Eur. J. Pharmacol. 473, 153–161.