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European Journal of Pharmacology 369 (1999) 267–270

ejp

Short communication

Anxiolytic effect of intra-amygdala injection of midazolam and 8-hydroxy-2-(di-*n*-propylamino)tetralin in the elevated T-maze

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Received 29 December 1998; revised 2 February 1999; accepted 5 February 1999

Abstract

The effects of intra-amygdala injection of midazolam (20 nmol) and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; 8 and 16 nmol) were investigated in rats submitted to the elevated T-maze, a new animal model of anxiety. This test allows the measurement, in the same rat, of conditioned and unconditioned fear/anxiety responses. Both drugs impaired inhibitory avoidance of the open arms of the T-maze (task representing conditioned fear), indicating an anxiolytic effect, but did not change escape performance from one of the open arms (representing unconditioned fear). The results further implicate γ-aminobutyric acid (GABA)/benzodiazepine and serotonergic systems within the basolateral/lateral amygdala in the modulation of conditioned anxiety responses.

Keywords: Anxiety; Amygdala; Midazolam; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); 5-HT (5-hydroxytryptamine, serotonin); T-maze

1. Introduction

The role of the amygdala in the mediation of fear and anxiety has been extensively investigated in recent years (Davis, 1992; Graeff et al., 1993). The clinical effectiveness of benzodiazepine and buspirone-like drugs in treating generalised anxiety states has motivated efforts for the elucidation of the involvement of the amygdaloid γ-aminobutyric acid (GABA)/benzodiazepine and serotonergic systems in the modulation of emotional processes.

The amygdala is particularly rich in benzodiazepine receptors (Young and Kuhar, 1980). Many studies have demonstrated that the administration of benzodiazepine compounds directly into this brain area can reduce the anxiety generated in different animal models (Hodges et al., 1987; Zangrossi and Graeff, 1994; Gonzalez et al., 1996). Nevertheless, the amygdala loci responsible for anxiolytic effects are still controversial, with some studies pointing to the central subdivision and others to the basolateral area. Thus, in conflict tests, an anxiolytic-like effect of benzodiazepines has been shown both in central (Kataoka et al., 1987) and in basolateral subdivisions (Hodges et al., 1987). In the more 'natural' model of the elevated plus-maze, the basolateral nucleus seems to be more important than the central area for the anxiolytic effects of benzodiazepine drugs (Green and Vale, 1992; Zangrossi and Graeff, 1994; Pesold and Treit, 1995; but see Gonzalez et al., 1996).

The intra-amygdala effects of buspirone or other 5-HT_{1A} receptor agonist drugs have been less explored. It has been suggested that the effects of both systemic and intra-amygdala administered 5-HT receptor interacting drugs may be dependent on the type of animal model used (Handley and McBlane, 1993a; Zangrossi and Graeff, 1994; Gonzalez et al., 1996). In this respect, the full 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) injected into the basolateral complex has anxiogenic effects in a modified Geller–Seifter paradigm (Hodges et al., 1987) and in the social interaction test (Gonzalez et al., 1996), but does not affect the anxiety measured in the elevated plus-maze (Zangrossi and Graeff, 1994; Gonzalez et al., 1996).

In the present study we evaluated the effects of the benzodiazepine receptor agonist midazolam and the 5-HT_{1A} receptor agonist 8-OH-DPAT injected into the basolat-

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00075-8

eral/lateral amygdala of rats submitted to a new animal model of anxiety, the elevated T-maze (Viana et al., 1994; Zangrossi and Graeff, 1997). This test is derived from the elevated plus-maze (Pellow et al., 1985) and has been developed to allow the measurement, in the same rat, of conditioned and unconditioned fear responses. These two tasks supposedly relate to generalised anxiety and panic disorders, respectively (Graeff et al., 1998). In the elevated T-maze, conditioned fear is represented by inhibitory avoidance of the open arms and unconditioned fear by escape responses from one of the open arms. It has been proposed (Graeff et al., 1998) that these two types of fear/anxiety responses may explain the inconsistent effects obtained with 5-HT-interacting drugs in the elevated plus-maze (Rodgers and Cole, 1994), which has been defined by some authors as a 'mixed' test of anxiety (File et al., 1993; Handley and McBlane, 1993b).

2. Materials and methods

2.1. Subjects and surgery

Male Wistar rats (University of São Paulo, Campus of Ribeirão Preto, Brazil), weighing 280–310 g, were stereotaxically implanted, under tribromethanol (2-2-2-tribromethanol, Aldrich, WI, USA) anaesthesia, with bilateral cannulae aimed at the basolateral/lateral amygdaloid complex. The two stainless steel guide cannulae were inserted according to the coordinates: AP = 2.5 mm, L = 4.8 mm, V = 4.8 mm below the dura. Following surgery, animals were allowed to recover for 6–7 days.

2.2. Apparatus

The elevated T-maze was made of wood and had three arms of equal dimensions (50 cm × 12 cm). One of the arms, enclosed by 40-cm high walls, was perpendicular to two opposed open arms. In order 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. Illumination was provided by a 60-W white light bulb fixed to the ceiling (50 lx on maze central area).

2.3. Experimental procedure

Prior to the experimental sessions, animals were gently handled for 5 min for two consecutive days. On the following day, they were randomly assigned to different treatment groups ($n = 9–16$) and bilaterally injected (drugs dissolved in saline; total volume 0.2 μl/2 min), into the basolateral/lateral amygdaloid complex, with midazolam maleate (20 nmol; Roche, Brazil), 8-OH-DPAT (8 or 16 nmol; Sigma, USA) or saline. During this procedure, animals were gently held by the experimenter. The needles were introduced 2 mm below the cannula end. Ten minutes after injections, animals were tested in the elevated T-maze. Each animal was initially placed at the end of the enclosed arm of the T-maze and the time taken to withdraw from this arm with the four paws was recorded (baseline latency). Next, the same measurement was repeated in two subsequent trials (avoidance 1 and avoidance 2) at 30-s intervals. Following avoidance training (30 s), each animal was placed at the end of the right open arm and the time taken to withdraw from this arm with the four paws was recorded (escape). Immediately after the T-maze session, animals were individually placed into the centre of a wooden square arena (60 cm × 60 cm), with walls 30 cm high, in order to evaluate locomotor activity. The total distance travelled during 5 min was recorded with a video tracking system (Ethovision; Noldus, The Netherlands).

After the experiment, the injection sites were histologically verified. Only data from animals with cannula placement in the basolateral/lateral nuclei were used. Avoidance data were analysed by two-way ANOVA (analysis of variance), with treatment as 'between subjects' and trials as 'within subjects' factors, respectively. Escape and locomotor activity in the arena were analysed either using unpaired *t*-test (midazolam) or one-way ANOVA (8-OH-DPAT).

3. Results

As illustrated in the upper panel of Fig. 1, midazolam decreased the inhibitory avoidance latency. Two-way

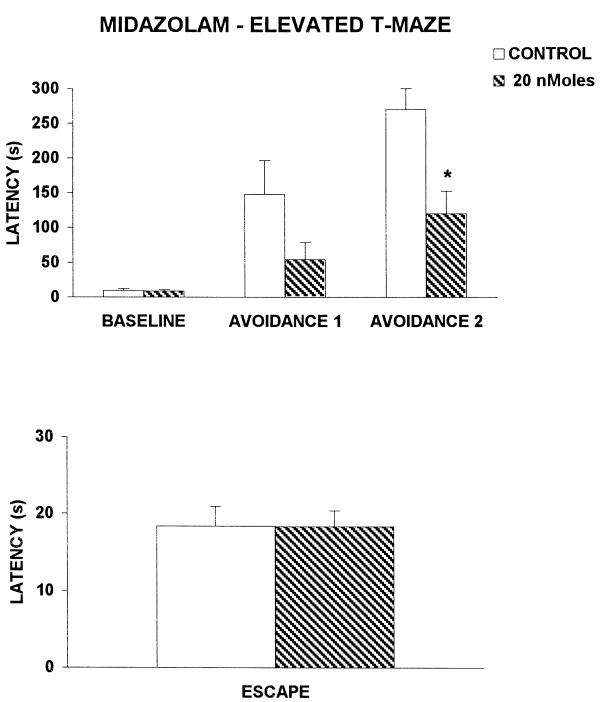

Fig. 1. Effect (mean + S.E.M.) of midazolam microinjected into the basolateral/lateral amygdaloid complex on the behaviour of rats exposed to the elevated T-maze. $N = 9$ (control) and 16 (midazolam). $^{*}P < 0.05$, compared to the control group in a same trial.

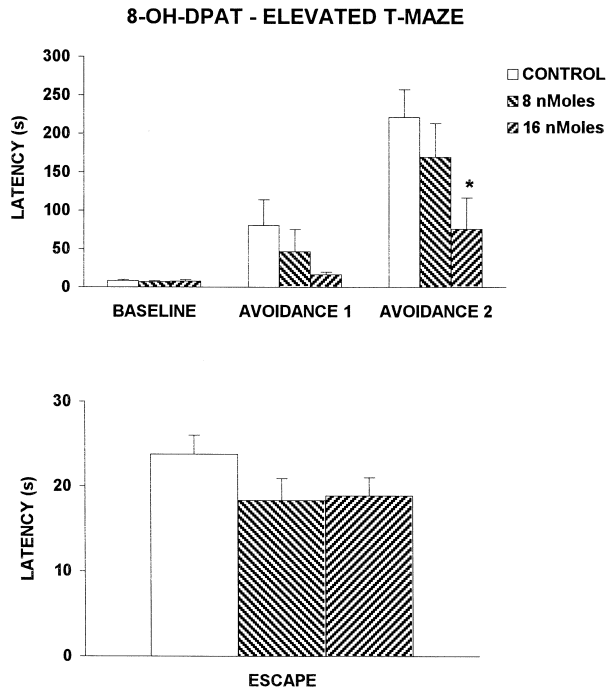

Fig. 2. Effect (mean + S.E.M.) of 8-OH-DPAT microinjected into the basolateral/lateral amygdaloid complex on the behaviour of rats exposed to the elevated T-maze. $N = 12$ (control) and 10 (8 and 16 nmol). $^{*}P < 0.05$, compared to the control group in a same trial.

ANOVA showed a significant effect of treatment ($F_{1,23} = 8.44$; $P < 0.01$) and trials ($F_{2,46} = 29.44$; $P < 0.001$), as well as a significant treatment × trial interaction ($F_{2,46} = 4.86$; $P < 0.05$). Unpaired *t*-test showed that the effect of the drug was significantly different from control on avoidance 2 [$T(21.73) = 3.42$; $P < 0.01$]. Escape performance was not affected by the drug treatment (Fig. 1, bottom).

The upper panel of Fig. 2 illustrates the dose-dependent, anxiolytic effect of 8-OH-DPAT on inhibitory avoidance. Like midazolam, 8-OH-DPAT significantly decreased the avoidance latency. Two-way ANOVA showed a significant effect of treatment ($F_{2,29} = 3{,}86$; $P < 0.05$) and trials ($F_{2,58} = 31{,}80$; $P < 0.001$), but no significant treatment × trial interaction. One-way ANOVA showed a significant difference among groups on avoidance 2 ($F_{2,29} = 3{,}45$; $P < 0.05$). At that time point, the Student–Newman–Keuls post hoc test indicated a significant difference between the control group and the group treated with 16 nmol of 8-OH-DPAT ($P < 0.05$). Again, escape performance was not affected by the drug treatment (Fig. 2, bottom).

Intra-amygdala injection of midazolam or 8-OH-DPAT did not affect the total distance travelled by the animals in the arena (data not shown).

4. Discussion

The results of the present study showed that the benzodiazepine midazolam and the 5-HT_{1A} receptor agonist 8-OH-DPAT injected into the basolateral/lateral nuclei of the amygdala impaired inhibitory avoidance but did not change the escape response measured in the elevated T-maze test of anxiety. These findings are in agreement with the effects of systemically injected benzodiazepines and 5-HT_{1A} receptor agonists in the same animal model (Viana et al., 1994). In terms of psychopathology, inhibitory avoidance in the T-maze has been related to generalised anxiety and escape from the open arm to panic disorder (Graeff et al., 1998). Thus, the effect of both drugs in decreasing avoidance of the open arms correlates with their clinical profile (Nutt, 1990), suggesting the involvement of the GABA/benzodiazepine and the serotonergic system within the amygdaloid nuclei in the mediation of anticipatory or generalised anxiety states. The importance of these neurotransmitter systems in this brain area is less evident in a situation supposedly mimicking panic.

Similar to the effects obtained for inhibitory avoidance in the T-maze, anxiety engendered by exposure to the open arms of the elevated plus-maze is also decreased by intra-basolateral injection of midazolam (Green and Vale, 1992; Zangrossi and Graeff, 1994; Pesold and Treit, 1995). Additionally, the drug is effective in ameliorating anxiety in a Geller–Seifter test (Hodges et al., 1987). In contrast to midazolam, intra-basolateral injection of 8-OH-DPAT failed to change anxiety in the elevated plus-maze (Zangrossi and Graeff, 1994; Gonzalez et al., 1996). These results suggest that the conditioned anxiety generated by the elevated T-maze is more susceptible to the effects of 5-HT_{1A}-acting drugs than the supposedly 'mixed' anxiety of the elevated plus-maze. However, the discrepancy between the results of the two tests may lie in the dose of the drug used. In the study of Gonzalez et al. (1996), 8-OH-DPAT was injected in a much lower dose range (50–200 ng, approximately 0.15–0.6 nmol) than in the present experiment (8–16 nmol). In our previous study (Zangrossi and Graeff, 1994), even though intra-amygdala injection of 8-OH-DPAT (2, 4 and 8 nmol) had no significant overall effect in the elevated plus-maze, the analysis of the data showed that, when individually compared to the control group, the dose of 8 nmol significantly increased ($P = 0.049$) the percentage of time spent in the open arms [(mean ± S.E.): control group = 10.63 ± 2.24; 8-OH-DPAT = 19.46 ± 3.82], indicating an anxiolytic effect. Thus, inhibition of open arm avoidance may occur in both elevated mazes. It is noteworthy that the anxiety generated by open spaces seem to be different from the anxiety generated by other aversive stimuli such as social contact or punishment with electric shocks. In the latter cases, low doses of 8-OH-DPAT (50–500 ng) cause clear anxiogenic effects both in the social interaction test (Gonzalez et al., 1996) and in a modified Geller–Seifter paradigm (Hodges et al., 1987).

The differential effects of intra-amygdala injection of midazolam and 8-OH-DPAT on inhibitory avoidance and escape behaviours in the elevated T-maze further indicates that different kinds of fear are generated by this model.

Acknowledgements

The authors thank Mr. José Roberto Stella for technical assistance. This study was supported by FAPESP, Brazil (grant no. 95/9712-3).

References

Davis, M., 1992. The amygdala and conditioned fear. In: Aggleton, J.P (Ed.), The Amygdala. Neurobiological Aspects of Emotion, Memory and Mental Dysfunction. Wiley-Liss, pp. 255–305.

File, S.E., Zangrossi, H. Jr., Viana, M., Graeff, F.G., 1993. Trial 2 in the elevated plus-maze: a different form of fear?. Psychopharmacology 111, 491–494.

Gonzalez, L.E., Andrews, N., File, S.E., 1996. 5-HT1A and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. Brain Res. 732, 145–153.

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

Graeff, F.G., Ferreira Neto, C., Zangrossi Jr., H., 1998. The elevated T-maze as an experimental model of anxiety. Neurosci. Biobehav. Rev. (in press).

Green, S., Vale, A.L., 1992. Role of amygdaloid nuclei in the anxiolytic effects of benzodiazepines in rats. Behav. Pharmacol. 3, 261–264.

Handley, S.L., McBlane, J.W., 1993a. 5-HT drugs in animal models of anxiety. Psychopharmacology 112, 13–20.

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

Hodges, H., Green, S., Glenn, B., 1987. Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not discrimination. Psychopharmacology 92, 491–504.

Kataoka, Y., Shibata, Z., Yamashita, K., Ueki, S., 1987. Differential mechanisms involved in the anticonflict action of benzodiazepines injected into the central amygdala and mammillary body. Brain Res. 416, 243–247.

Nutt, D.J., 1990. The pharmacology of human anxiety. Pharmacol. Ther. 47, 233–266.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Pesold, C., Treit, D., 1995. The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines. Brain Res. 671, 213–221.

Rodgers, R.J., Cole, J.C., 1994. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper, S.J., Hendrie, C.A. (Eds.), Ethology and Psychopharmacology. Wiley, Chichester, UK, pp. 9–44.

Young, W.S., Kuhar, M.J., 1980. Radiohistochemical localization of benzodiazepine receptors in rat brain. J. Pharmacol. Exp. Ther. 212, 337–346.

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.

Zangrossi, H. Jr., Graeff, F.G., 1994. Behavioral effects of intra-amygdala injections of GABA and 5-HT acting drugs in the elevated plus-maze. Braz. J. Med. Biol. Res. 27, 2453–2456.

Zangrossi, H. Jr., Graeff, F.G., 1997. Behavioral validation of the elevated T-maze, a new animal model of anxiety. Brain Res. Bull. 44, 1–5.