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Serotonin in the dorsal periaqueductal gray modulates inhibitory avoidance and one-way escape behaviors in the elevated T-maze

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Abstract

The dorsal periaqueductal gray has been implicated in the modulation of escape behavior, a defensive behavior that has been related to panic disorder. Intra-dorsal periaqueductal gray injection of serotonin or drugs that mimic its effects inhibits escape induced by electrical or chemical stimulation of this brainstem area. In this study, we investigate whether intra-dorsal periaqueductal gray injection of 5-HT receptor agonists attenuates escape generated by an ethologically based model of anxiety, the elevated T-maze. This test also allows the measurement of inhibitory avoidance, which has been related to generalized anxiety disorder. The effects of the 5-HT receptor agonists were compared in animals with or without a previous exposure to the open arms of the elevated T-maze. In these two test conditions, intra-dorsal periaqueductal gray injection of the endogenous agonist serotonin or the 5-$HT_{2B/2C}$ receptor agonist *m*-chlorophenylpiperazine (mCPP) enhanced inhibitory avoidance, suggesting an anxiogenic effect. The 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) impaired this response, suggesting an anxiolytic effect, and the preferential 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) was ineffective. All these agonists inhibited escape behavior. Apart from mCPP, the effect on escape was detected only in animals pre-exposed to the open arm. None of the drugs tested affected locomotion in the open-field test. Taken altogether, our findings suggest that 5-HT_{1A} and 5-HT_{2c} receptors in the dorsal periaqueductal gray exert opposed control on inhibitory avoidance, implicating these receptors in anxiety conditioning. As previously observed in tests employing the aversive stimulation of the dorsal periaqueductal gray, 5-HT_{1A} and 5-HT_{2A} receptors in this brain area are involved in escape inhibition. Therefore, in different animal models, the activation of these two subtypes of receptors in the dorsal periaqueductal gray consistently attenuates the expression of a panic-related behavior.

Keywords: Periaqueductal gray, dorsal; Panic; Anxiety; 5-HT (5-hydroxytryptamine, serotonin); T-maze, elevated

1. Introduction

A wealth of evidence indicates that the dorsal periaqueductal gray plays an important role in fear and anxiety (Bandler and Shipley, 1994; Graeff, 1990, 1994; Lovick, 2000). Electrical or chemical stimulation of this brain region induces defensive behaviors such as fight and flight responses that are suggestive that the experimental animal is undergoing a markedly aversive experience (Olds and Olds, 1962; Graeff et al., 1993). In human patients, electrical stimulation of this structure, during the course of stereotaxic neurosurgery, evokes strong feelings of fear, impending death or non-localized pain and marked autonomic changes, as in a full blown panic attack (Nashold et al., 1969; Amano et al., 1978). Given the striking similarities between the autonomic and behavioral effects of the dorsal periaqueductal gray stimulation and the symptoms of panic attacks, suggestion has been made that the dorsal periaqueductal gray is involved in the genesis of panic disorder in human and that the dorsal periaqueductal gray stimulation in animals can model panic attacks (Graeff, 1990; Jenck et al., 1995; Lovick, 2000; Schenberg et al., 2001).

Evidence from experiments using aversive electrical or chemical stimulation of the dorsal periaqueductal gray of the rat led to the view that 5-HT receptors located in this brain area (Pazos and Palacios, 1985; Pazos et al., 1985; Brandão et

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al., 1991; Griffiths and Lovick, 2002) regulate escape behavior, the most frequently assumed index of panic (Schütz et al., 1985; Nogueira and Graeff, 1991, 1995; Beckett et al., 1992a; Mongeau and Marsden, 1997). For instance, intradorsal periaqueductal gray injection of the endogenous agonist 5-HT, the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) or the preferential 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) raised the threshold of dorsal periaqueductal gray electrical stimulation for inducing escape (Nogueira and Graeff, 1995; Jacob et al., 2002). More recently, Jacob et al. (2002) have shown that the inhibitory effect of DOI and 8-OH-DPAT on escape was significantly increased in rats chronically treated with imipramine, implicating the 5-HT_{1A} and 5-HT_{2A} receptors of the dorsal periaqueductal gray in the antipanic action of antidepressants.

In the last years, other animal models have been developed based on the assumption that escape behavior is related to panic disorder (for a review see Blanchard et al., 2001; Graeff and Zangrossi, 2002). Different from the invasive and rather artificial way of inducing escape by means of intracerebral stimulation, these tests rely on species' specific threatening stimuli such as predators or exposure to open and elevated spaces. One of these models is the elevated T-maze (Graeff et al., 1993; Viana et al., 1994; Zangrossi and Graeff, 1997).

This test was developed based on Deakin and Graeff (1991) hypothesis of the opposed role of 5-HT in generalized anxiety disorder and panic disorder. Briefly stated, the authors proposed that 5-HT, whereas inhibiting the expression of panic-like responses (e.g. escape) by acting in the dorsal periaqueductal gray, facilitates more flexible and coordinated defensive reactions (e.g. inhibitory avoidance) by acting in structures such as the amygdala and frontal cortex. Since the latter behavior strategies usually involve learning and memory, they represent conditioned anxiety and may be related, in clinical terms, to generalized anxiety disorder. As a consequence, the elevated T-maze was intended to generate, in the same rat, inhibitory avoidance and one-way escape behaviors. So far, reported drug effects on these tasks have largely supported the proposed relationship of these responses to generalized anxiety and panic disorder, respectively (Graeff et al., 1993, 1998; Viana et al., 1994; Teixeira et al., 2000; Poltronieri et al., in press).

In the present study, we evaluated the effects of the intradorsal periaqueductal gray injection of the endogenous agonist 5-HT, the 5-HT_{1A} receptor agonist 8-OH-DPAT, the preferential 5-HT_{2A} receptor agonist DOI and the 5-$HT_{2B/2C}$ receptor agonist *m*-chlorophenylpiperazine (mCPP) in the modulation of the defensive tasks generated by the elevated T-maze. The main objective was to verify whether conclusions from experiments using electrical or chemical aversive stimulation of the dorsal periaqueductal gray also apply to an ethologically based model of inducing escape response. Additionally, it was of interest to investigate whether 5-HT receptors in the dorsal periaqueductal gray are also involved in the modulation of inhibitory avoidance.

In this study, the effects of the 5-HT receptor agonists were evaluated both in animals with or without a previous 30-min exposure to one of the open arms of the elevated T-maze. It has been shown that this pre-exposure, by shortening latencies to withdrawal from the open arm during the test, renders the escape task more sensitive to the effects of antipanic drugs (Teixeira et al., 2000; Poltronieri et al., in press). In order to avoid confounding results due to potential drug effects on locomotor activity, immediately after being tested in the elevated T-maze, animals' behavior was also evaluated in an open field.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 220–230 g, housed in pairs in Plexiglas-walled cages under 12:12 dark/light cycle (lights on at 07:00) at 22±1 °C, and given free access to food and water throughout the experiment. The experiments reported in this article were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior) which are based on the guide for care and use of laboratory animals of The United Sates National Institutes of Health.

2.2. Apparatus

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 Plexiglass rim. The whole apparatus was elevated 50 cm above the floor.

The open field test was performed in a wooden square arena (60×60 cm), with 30-cm-high walls.

2.3. Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulfate (5-HT; Sigma, USA), (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT; Sigma), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI; RBI, USA) and *m*-chlorophenylpiperazine hydrochloride (mCPP; RBI). All drugs were dissolved in sterile saline.

2.4. Surgery

The animals were anaesthetized with 2,2,2 tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame (David Kopf, USA). A guide cannula made of stainless steel (outer diameter 0.6 mm, inner diameter 0.4 mm) was implanted in the midbrain aimed at the dorsal periaqueductal gray.

Holding the incisor bar 2.5-mm below the interaural line, the cannula was introduced 1.9 mm lateral to lambda, at an angle of 22° with the sagital plane, until the cannula was 3.2 mm below the surface of the skull. The guide cannula was fixed to the skull by means of acrylic resin and two stainless steel screws. At the end of the surgery, the cannula was sealed with a stainless wire to protect it from congestion.

2.5. Procedure

2.5.1. Animals without a previous exposure to one of the open arms

On the fifth and sixth days after the surgery, animals were gently handled by the experimenter for 5 min. On the seventh day, they were randomly assigned to different treatment groups, and injected into the dorsal periaqueductal gray with 5-HT (0, 5, 10 or 20 nmol/0.5 μl; n=11–12), 8-OH-DPAT (0, 0.8, 1.6 or 3.2 nmol/0.2 μl; n=8–9), DOI (0, 4, 8 or 16 nmol/0.2 μl; n=9–10) or mCPP (0, 10, 20 or 40 nmol/0.2 μl; n=8). For drug injection, a needle (outside diameter 0.3 mm) was introduced through the guide cannula until its tip was 2 mm below the cannula end. The drugs were injected during 120 s using a 5-μl microsyringe (Hamilton 701-RN, USA) attached to a microinfusion pump (KD Scientific, USA). The displacement of an air bubble inside the polyethylene catheter connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The intracerebral needle was removed 60 s after the injection was finished. The doses of the drugs were chosen on the basis of previous literature results (Jenck et al., 1989; Nogueira and Graeff, 1991, 1995; Tilakaratne and Friedman, 1996; Mongeau and Marsden, 1997) and pilot studies conducted in our laboratory.

Ten or twenty (DOI) minutes after injection, animals were tested in the elevated T-maze. For that, each animal was placed at the distal end of the enclosed arm of the elevated T-maze facing the intersection of the arms. The time taken by the rat to leave this arm with the four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30 s intertrial intervals, during which animals were placed in a Plexiglas cage where they had been previously habituated. Following avoidance training (30 s), rats were placed at the end of one of the open arms and the latency to leave this arm with the four paws was recorded for three consecutive times (escape 1, 2 and 3) with 30-s intertrial intervals. A cutoff time of 300 s was established for the avoidance and escape latencies. Immediately after being tested in the elevated T-maze, each animal was placed for 5 min in the open field for the evaluation of locomotor activity. The total distance traveled was analyzed by a video tracking system (Ethovision, Noldus, Holland).

2.5.2. Animals previously exposed to one of the open arms

Animals were handled as described above. On the sixth day after the surgery, rats were exposed to one of the open arms of the T-maze for 30 min. 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.

On the next day, animals were injected into the dorsal periaqueductal gray with 5-HT (0 or 20 nmol/0.5 μl; n=11–12), 8-OH-DPAT (0, 1.6 or 3.2 nmol/0.2 μl; n=14–16), DOI (0, 8 or 16 nmol/0.2 μl; n=11–12) or mCPP (0 or 40 nmol/0.2 μl; n=9). These doses were selected according to the results obtained in animals without a previous exposure to the open arm. Ten or twenty (DOI) minutes after injection, animals were tested in the elevated T-maze and in the open field as described above, with exception that escape latencies were always evaluated in the same previously experienced open arm.

2.6. Histology

After the experiments, animals were sacrificed under deep urethane anesthesia. The brain was perfused intracardially with saline solution (0.9%) followed by 10% formalin solution, before being removed and fixed in 10% formalin. Frozen sections of 55 μm were cut using a microtome in order to localize the site of the drug injection, according to Paxinos and Watson's atlas (1986). Incorrect cannula placement was identified in 21.5% of the animals tested. Only animals with injection sites located inside the dorsal periaqueductal gray were included in the statistical analysis.

2.7. Statistical analysis

Split-plot analysis of variance (*split-plot* ANOVA) was used to analyze both avoidance and escape data, with drug treatment as the independent factor and trials as the repeated measure. In case of significant differences with the independent factor or with the interaction between the independent and repeated factors, one-way ANOVA, followed by the Duncan post-hoc test, was performed. Locomotor activity data were submitted to one-way ANOVA, followed by the Duncan post-hoc test.

3. Results

3.1. Animals without a previous exposure to the open arm

Drug effects on both behavioral tasks measured by the elevated T-maze are summarized in Table 1.

3.1.1. 5-HT

Intra-dorsal periaqueductal gray administration of 5-HT facilitated inhibitory avoidance [treatment effect—$F(3,43)$= 4.91, P<0.01]. Split-plot ANOVA revealed a significant effect of trial [$F(2,86)$=29.73, P<0.001], but not a trial by treatment interaction [$F(6,86)$=1.89, NS]. The post hoc test showed that 20 nmol of 5-HT increased (P<0.01) baseline and avoidance 1 latencies.

Table 1
Latencies (mean±S.E.M.) to withdrawal from the enclosed arm (inhibitory avoidance) or from an open arm (escape) of the elevated T-maze after intra-dorsal periaqueductal gray injection of different 5-HT receptors agonists

Drug (nmol)	*n*	Baseline	Avoidance 1	Avoidance 2	Escape 1	Escape 2	Escape 3
5-HT							
0	12	78.8±26.9	93.2±26.9	167.2±32.3	14.4±1.1	11.1±1.4	9.9±2.3
5	12	66.4±22.3	114.7±31.3	132.7±35.1	17.2±2.1	14.0±3.2	14.6±2.6
10	12	25.2±4.3	89.0±23.8	180.4±33.7	13.7±2.0	11.2±1.6	9.6±2.2
20	11	169.1±34.8[a]	234.0±32.6[a]	249.9±26.0	18.9±5.2	14.0±1.6	16.4±3.9
8-OH-DPAT							
0	8	108.5±36.9	171.2±40.4	229.1±30.3	13.5±1.7	10.4±1.5	8.5±1.8
0.8	8	37.9±9.1[b]	69.5±16.8[a]	89.2±25.7[a]	9.0±1.0	11.4±4.6	10.7±3.6
1.6	8	26.1±3.4[b]	35.1±10.7[a]	47.1±20.03[a]	15.5±3.9	15.0±3.8	11.5±2.6
3.2	9	27.4±5.4[b]	50.4±9.8[a]	96.9±26.4[a]	15.8±2.2	13.4±2.2	10.9±2.4
DOI							
0	10	51.5±17.7	156.5±39.6	219.3±31.5	13.3±1.6	10.7±1.5	10.3±0.5
4	9	53.7±17.0	144.0±37.7	183.6±38.6	14.4±2.6	11.4±2.2	10.9±2.9
8	9	80.88±33.0	146.5±38.1	205.7±46.2	19.2±4.0	15.5±3.6	11.1±2.1
16	10	71.9±30.8	115.1±37.9	166.4±39.3	18.4±2.4	12.9±1.4	8.9±1.5
mCPP							
0	8	27.8±4.2	78.1±26.3	101.2±23.9	10.6±0.8	7.6±1.1	5.8±0.7
10	8	20.4±2.6	59.4±14.4	142.3±36.6	11.0±1.6	10.5±1.6	8.5±2.0
20	8	34.2±8.6	107.8±33.1	174.6±34.4	14.4±2.4	11.8±1.6	10.7±1.6
40	8	81.8±33.1	190.3±34.5[b]	257.4±28.9[a]	17.9±2.1[b]	15.5±2.6[b]	14.7±3.1[b]

All animals were näive to the open arms of the elevated T-maze before testing.
[a] ($P<0.01$) compared to the control group in a same trial.
[b] ($P<0.05$) compared to the control group in a same trial.

5-HT had no effect on one-way escape performance [treatment effect—$F(3,43)=1.80$, NS]. There is no trial effect [$F(2,86)=2.81$, NS] nor a trial by treatment interaction [$F(6,86)=0.15$, NS].

As shown in Table 2, 5-HT did not change the total distance traveled in the open field.

3.1.2. 8-OH-DPAT

Contrarily to 5-HT, intra-dorsal periaqueductal gray injection of 8-OH-DPAT impaired inhibitory avoidance [treatment effect—$F(3,29)=8.72$, $P<0.001$]. Split-plot ANOVA revealed significant effect of trial [$F(2,58)=21.32$, $P<0.001$] and a trial by treatment interaction [$F(6,58)=2.29$, $P<0.05$]. The Duncan test showed that the three doses of the drug significantly decreased baseline ($P<0.05$), avoidance 1 ($P<0.01$) and avoidance 2 ($P<0.001$) latencies.

8-OH-DPAT did not change one-way escape [treatment effect—$F(3,29)=1.80$, NS]. There is no trial effect [$F(2,58)=2.27$, NS] or a trial by treatment interaction [$F(6,58)=0.70$, NS].

The total distance traveled in the open field was not changed by 8-OH-DPAT (see Table 2).

3.1.3. DOI

Intra-dorsal periaqueductal gray injection of DOI did not affect inhibitory avoidance performance [treatment effect—$F(3,35)=0.83$, NS]. There is a significant effect of trial [$F(2,70)=36.15$, $P<0.001$], but not a trial by treatment interaction [$F(6,70)=0.89$, $P=0.51$].

DOI did not affect one-way escape [treatment effect—$F(3,35)=1.84$, NS]. There is also a trial effect [$F(2,70)=11.15$, $P<0.001$], but not a trial by treatment interaction [$F(6,70)=0.81$, NS].

Table 2
Effect (mean±S.E.M.) of intra-dorsal periaqueductal gray injection of different 5-HT receptor agonists on the distance traveled in the open field by rats with or without a previous exposure to one of the open arms

Animals without pre-exposure		Animals with pre-exposure	
Drug (nmol)	Distance traveled (m)	Drug (nmol)	Distance traveled (m)
5-HT		5-HT	
0	13.0±1.0	0	15.8±1.1
5	13.6±1.3	20	13.5±1.1
10	16.9±3.9		
20	11.5±0.9		
8-OH-DPAT		8-OH-DPAT	
0	13.0±1.0	0	14.2±0.9
0.8	15.0±1.4	1.6	13.4±1.0
1.6	16.1±1.3	3.2	15.6±1.0
3.2	14.5±1.9		
DOI		DOI	
0	16.4±1.8	0	14.2±1.5
4	15.5±1.7	8	12.2±1.8
8	11.7±1.0	16	15.2±1.6
16	13.1±1.6		
mCPP		mCPP	
0	14.2±1.2	0	17.9±2.0
10	14.3±0.9	40	13.6±2.4
20	14.0±0.8		
40	15.8±2.1		

Table 2 shows that locomotion in the open field was not changed by intra-dorsal periaqueductal gray injection of DOI.

3.1.4. mCPP

Intra-dorsal periaqueductal gray injection of mCPP facilitated inhibitory avoidance [treatment effect—$F(3,37)=4.97$, $P<0.01$]. Split-plot ANOVA showed a significant effect of trial [$F(2,74)=40.54$, $P<0.001$], but not a significant trial by treatment interaction [$F(6,74)=0.63$, NS]. The Duncan test showed that the dose of 40 nmol significantly increased avoidance 1 ($P<0.05$) and avoidance 2 ($P<0.001$) latencies.

One-way escape was impaired by mCPP [treatment effect—$F(3,37)=4.65$, $P<0.01$]. There is a significant effect of trial [$F(2,74)=7.55$, $P<0.01$], but not a trial by treatment interaction [$F(6,74)=0.24$, NS]. The post hoc test showed that mCPP 40 nmol increased ($P<0.05$) escape latencies along the three trials.

The distance traveled in the open field was not altered by the drug (see Table 2).

3.2. Animals previously exposed to one of the open arms

3.2.1. 5-HT

Fig. 1 (upper panel) shows that, as in open arm naïve rats, 5-HT facilitated inhibitory avoidance [treatment effect—$F(1,22)=6.00$, $P<0.05$]. Split-plot ANOVA revealed a trial effect [$F(2,44)=26.99$, $P<0.001$], but not a trial by treatment interaction [$F(2,44)=2.90$, NS]. The Student's t-test showed that 5-HT significantly increased ($P<0.05$) avoidance 1 and avoidance 2 latencies.

As shown at the lower panel of Fig. 1, differently from animals naïve to the open arm, 5-HT impaired one-way escape performance [treatment effect—$F(1,22)=16.26$, $P=0.001$]. Split-plot ANOVA also showed significant effect of trial [$F(2,44)=5.23$, $P<0.01$], but not a trial by treatment interaction [$F(2,44)=0.38$, NS]. The Student's t-test showed that 5-HT significantly ($P<0.01$) increased escape 1, 2 and 3 latencies.

Locomotor activity in the arena was not changed by 5-HT (see Table 2).

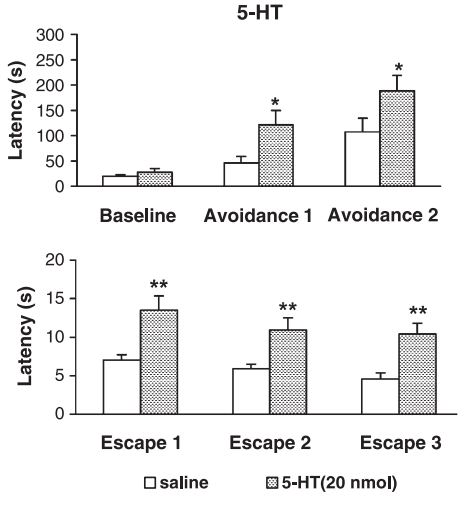

Fig. 1. Effects (mean±S.E.M.) of intra-dorsal periaqueductal gray injection of 5-HT or saline on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured by the elevated T-maze. Twenty-four hours before the test, all animals were exposed to one of the open arms for 30 min. (n=11–12 rats); *$P<0.05$, **$P<0.01$ with respect to control in a same trial.

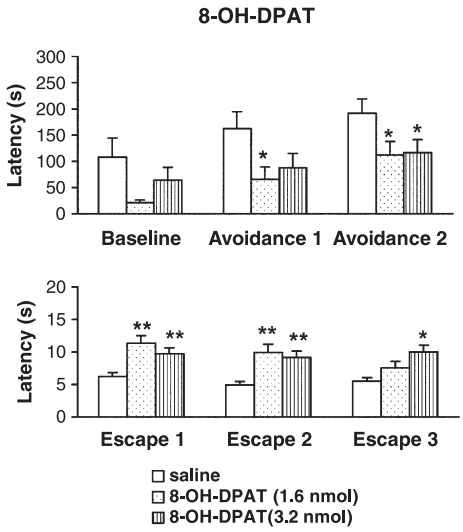

Fig. 2. Effects (mean±S.E.M.) of intra-dorsal periaqueductal gray injection of 8-OH-DPAT or saline on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured by the elevated T-maze. For further specification, see legend of Fig. 1. (n=14–16 rats); *$P<0.05$, **$P<0.01$ with respect to control in a same trial.

3.2.2. 8-OH-DPAT

Fig. 2 (upper panel) shows that inhibitory avoidance was impaired following intra-dorsal periaqueductal gray injection

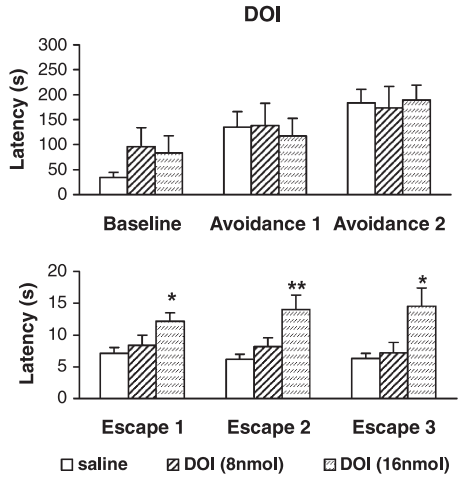

Fig. 3. Effects (mean±S.E.M.) of intra-dorsal periaqueductal gray injection of DOI or saline on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured by the elevated T-maze. For further specification, see legend of Fig. 1. (n=11–12 rats); *$P<0.05$, **$P<0.01$ with respect to control in a same trial.

of 8-OH-DPAT [treatment effect—$F(2,43)=3.66$, $P<0.05$]. There is also a trial effect [$F(2,86)=23.72$, $P<0.001$], but not a trial by treatment interaction [$F(4,86)=0.94$, NS]. The post hoc test showed that 1.6 nmol of 8-OH-DPAT significantly decreased ($P<0.05$) avoidance 1 and 2 latencies. The dose of 3.2 nmol decreased ($P<0.05$) avoidance 1 latency.

In open arm pre-exposed rats, 8-OH-DPAT significantly impaired one-way escape [treatment effect—$F(2,43)=8.67$, $P<0.01$]. There are also a trial effect [$F(2,86)=3.71$, $P<0.05$] and a trial by treatment interaction [$F(4,86)=2.80$, $P=0.05$]. The post hoc test showed that all doses of 8-OH-DPAT increased ($P<0.01$) escape 1 and 2 latencies. Additionally, the highest dose increased ($P<0.05$) escape 1 latency.

The drug did not change locomotion in the arena (see Table 2).

3.2.3. DOI

As it can be seen in Fig. 3 (upper panel) that intra-dorsal periaqueductal gray injection of DOI did not affect inhibitory avoidance [treatment effect—$F(2,32)=0.10$, NS]. Split-plot ANOVA showed a trial effect [$F(2,64)=25.87$, $P<0.001$], but not a trial by treatment interaction [$F(4,64)=1.37$, NS].

Fig. 3 (lower panel) also shows that in open arm pre-exposed rats DOI impaired one-way escape [treatment effect—$F(2,32)=7.61$, $P<0.01$]. There is no trial effect [$F(2,64)=0.03$, NS] nor an interaction between trial and treatment [$F(4,64)=0.76$, NS]. The Duncan test showed that the dose of 16 nmol increased escape 1 ($P<0.05$), 2 ($P<0.01$) and 3 ($P<0.05$) latencies.

Locomotion in the arena was not altered by DOI (see Table 2).

3.2.4. mCPP

As shown at the upper panel of Fig. 4, mCPP facilitated inhibitory avoidance acquisition [treatment effect—$F(1,16)=12.38$, $P<0.01$]. Split-plot ANOVA evidenced a significant trial effect [$F(2,32)=34.50$, $P<0.001$] and a significant trial by treatment interaction [$F(2,32)=9.02$, $P<0.01$]. The Student's t-test showed that mCPP significantly increased ($P<0.01$) avoidance 1 and avoidance 2 latencies.

As in open arm naïve animals, Fig. 4 (lower panel) shows that mCPP impaired one-way escape [treatment effect—$F(1,16)=1.38$, $P<0.01$]. There is no trial effect [$F(2,32)=1.13$, NS] nor interaction between trial and treatment [$F(2,32)=1.43$, NS]. The Student's t-test showed that mCPP increased both escape 2 ($P<0.01$) and escape 3 ($P=0.05$) latencies.

Locomotor activity in the arena was not changed by mCPP (see Table 2).

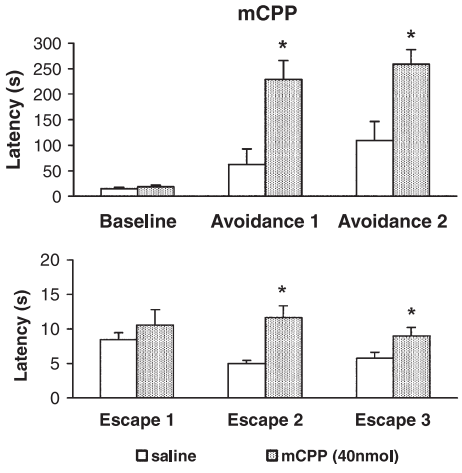

Fig. 4. Effects (mean±S.E.M.) of intra-dorsal periaqueductal gray injection of mCPP or saline on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured by the elevated T-maze. For further specification, see legend of Fig. 1. (n=9, for each group); *$P<0.05$, **$P<0.01$ with respect to control in a same trial.

4. Discussion

In the present study, we investigated the effects of the intra-dorsal periaqueductal gray injection of 5-HT_{1A} and 5-HT_2 receptor agonists on inhibitory avoidance and one-way escape behaviors measured by the elevated T-maze.

Regarding inhibitory avoidance, our results showed that whereas the endogenous agonist 5-HT and the 5-$HT_{2B/2C}$ receptor agonist mCPP facilitated the acquisition of this response, suggesting an anxiogenic effect, the 5-HT_{1A} receptor agonist 8-OH-DPAT had the opposed effect, indicating an anxiolytic effect. The preferential 5-HT_{2A} receptor agonist DOI was ineffective.

The effects of the 5-HT receptor agonists on inhibitory avoidance were similar in animals with or without a previous exposure to one of the elevated T-maze open arms. Therefore, as previously reported (Teixeira et al., 2000), pre-exposure to the open arm did not significantly alter drug effects on this task.

Two of the drugs tested, 5-HT and 8-OH-DPAT, altered baseline latency during inhibitory avoidance measurement, indicating that these drugs may have also affected the locomotor ability of the animals. However, the lack of change in the exploratory behavior measured in the open field seems to disregard nonspecific motor interference as the main cause of the drug effects on inhibitory avoidance. This conclusion is also supported by the fact that although the motor demand of inhibitory avoidance and one-way escape is nearly the same, 8-OH-DPAT shortened avoidance while lengthening escape latencies in animals pre-exposed to the open arm. Similarly, intra-dorsal periaqueductal gray injection of 5-HT, while increasing baseline latency (see Table 1), did not impair one-way escape. Therefore, the effects of 5-HT and 8-OH-DPAT on baseline latency are more likely to reflect, respectively, the enhancement or the reduction of animals' aversive reactions to novelty.

The anxiolytic effect of 8-OH-DPAT on inhibitory avoidance implicates 5-HT_{1A} receptors of the dorsal periaqueductal gray in anxiety conditioning, while the lack of DOI effect disregards a prominent role of 5-HT_{2A} in this process.

Indeed, as observed in inhibitory avoidance performance, other conditioned defensive behavior seems to be refractory to the pharmacological manipulation of 5-HT_{2A} receptors in the dorsal periaqueductal gray. Thus, Castilho and Brandão (2001) showed that intra-dorsal periaqueductal gray injection of ketanserin, a preferential 5-HT_{2A} receptor antagonist, did not change the expression of conditioned freezing using the electrical stimulation of the dorsal periaqueductal gray as unconditioned stimulus. Unfortunately, to our knowledge, no other study has assessed the role of 5-HT_{1A} receptors of the dorsal periaqueductal gray in anxiety conditioning.

In contrast to 8-OHDPAT, intra-dorsal periaqueductal gray administration of 5-HT and mCPP facilitated inhibitory avoidance. In binding assays, 5-HT has higher affinity for 5-HT_{1A} and 5-HT_{2C} when compared with 5-HT_{2A} receptors, whereas mCPP, besides binding to 5-$HT_{2B/2C}$, also interacts with 5-HT_{1B} and 5-HT_{2A} receptors (Engel et al., 1986; Hoyer, 1988; Griebel, 1995). Therefore, since the effect of 5-HT and mCPP on inhibitory avoidance diverge from those observed with 8-OH-DPAT and DOI, we hypothesize that the former two agonists increased conditioned anxiety by acting on 5-HT_{2C} receptors. This idea is supported by reported antagonism studies revealing the importance of 5-HT_{2C} receptors for the pro-aversive effects of mCPP in rats submitted to different animal models of anxiety (Kennett and Curzon, 1988; Kennett et al., 1989).

Regarding one-way escape, our data corroborate previous reported evidence (Teixeira et al., 2000) showing that forced exposure to one of the open arms of the maze resulted in a decrease in the latencies to leave this arm in the test session. This result has been attributed to the habituation of behavioral reactions to novelty (exploration, behavioral inhibition), which are likely to interfere with one-way escape (Teixeira et al., 2000; Sena et al., in press). More importantly, this pre-exposure significantly modified drug effect on one-way escape. Thus, in pre-exposed, but not in open-arm naïve animals, intra-dorsal periaqueductal gray injection of 5-HT, 8-OH-DPAT and DOI impaired one-way escape, suggesting a panicolytic-like effect. Intra-dorsal periaqueductal gray administration of mCPP inhibited one-way escape in both pre-exposed and naïve animals.

As observed in the pre-exposed group, intra-dorsal periaqueductal gray injection of 5-HT, 8-OH-DPAT and DOI, but not mCPP, inhibits escape behavior induced by the electrical and/or chemical stimulation of the dorsal periaqueductal gray, another proposed model of panic disorder (Jenck et al., 1995; Beckett and Marsden, 1995). Both 5HT_{1A} and 5-HT_{2A} receptors in this midbrain area are involved in this effect. Thus, the inhibitory effect of the 8-OH-DPAT on escape induced by intra-dorsal periaqueductal gray microinjection of the excitatory amino acid D,L-homocysteic acid (DLH) was antagonized by the peripheral injection of the selective 5-HT_{1A} receptor antagonist *N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide (WAY-100635; Beckett et al., 1992a; Beckett and Marsden, 1995). Yet, Nogueira and Graeff (1995) showed that intra-dorsal periaqueductal gray administration of the 5-HT_{1A} receptor antagonist 1-[2-methoxyphenyl]-4-[4-(2-phthalimido)butyl]-piperazine (NAN-190) abolished the effect of 8-OH-DPAT in decreasing escape induced by electrical stimulation of this brain area. In the same study, intra-dorsal periaqueductal gray injection of DOI also impaired escape, an effect blocked by the preferential 5-HT_{2A} receptor antagonist spiperone. Interestingly, spiperone also counteracted the effect of 8-OH-DPAT and NAN-190 counteracted the effect of DOI. These results were interpreted in terms of a functional interaction of 5-HT_{1A} and 5-HT_{2A} receptors in the control of escape (Nogueira and Graeff, 1995). Finally, intra-dorsal periaqueductal gray injection of 5-HT also inhibits escape induced by the electrical stimulation of this area and this effect was abolished by local pre-treatment with the preferential 5-HT_{2A} receptor antagonist ketanserin (Schütz et al., 1985).

The inhibitory effect of mCPP on one-way escape is indicative that dorsal periaqueductal gray 5-HT_{2C} receptors also participate in the regulation of this defensive response. However, the generality of this finding may be questioned on the basis of conflicting results of this compound in studies employing the aversive stimulation of the dorsal periaqueductal gray. Thus, intra-dorsal periaqueductal gray injection of mCPP, in the same dose range used in this study, whereas facilitating escape induced by DLH microinjection into the dorsal periaqueductal gray (Beckett et al., 1992b), had no effect on escape induced by electrical stimulation of this area (Nogueira and Graeff, 1995).

In clinic, mCPP has been reported to induce anxiety and panic attacks in panic patients and normal subjects (Bourin et al., 1998). Therefore, the anxiogenic effect of this drug on inhibitory avoidance, but not its effect on one-way escape corroborates these clinical evidences. It may be argued that in these clinical studies anticipatory anxiety rather than panic was enhanced, since mCPP also intensifies anxiety in healthy volunteers (Charney et al., 1987). Moreover, Deakin and Graeff (1991) have hypothesized that the brain mechanisms that underlie anxiety and panic interact, the former exerting inhibitory influence on the latter. Thus, drugs that facilitate avoidance could indirectly decrease escape. Indeed, systemic injection of mCPP also facilitated inhibitory avoidance and attenuated one-way escape in the elevated T-maze (Graeff et al., 1998). Against this argument, however, stand the results obtained with the α_2-adrenergic antagonist yohimbine, which has also been shown to induce panic attacks in panic patients and to enhance anxiety in normal subjects (Bourin et al., 1998). In the elevated T-maze, systemic injection of yohimbine facilitated avoidance without impairing escape (Graeff et al., 1998). In the latter study, however, the effect of yohimbine was assessed in animals without previous exposure to the open arm. Given the low specificity of mCPP for the different 5-HT receptor subtypes and its contradictory effects on different studies, conclusion on the relevance of dorsal periaqueductal gray

5-HT_{2C} receptors in the modulation of escape behavior awaits further experimentation.

Taken together, the results of the present study on one-way escape of animals pre-exposed to the open arm of the elevated T-maze corroborate with Deakin and Graeff's (1991) proposal that in the dorsal periaqueductal gray 5-HT inhibits active defense. As previously reported in other proposed animal model of panic (Jenck et al., 1995; Beckett and Marsden, 1995), both 5-HT_{1A} and 5-HT_{2A} receptors are involved in this response. Additionally, the present findings support the involvement of 5-HT mechanisms in the dorsal periaqueductal gray not only in the modulation of panic-like responses, but also on GAD-related behaviors. In this particular, our results indicate that in this brainstem area 5-HT_{1A} and 5-HT_{2C} receptors exert opposed regulation of inhibitory avoidance. Finally, the results emphasize the importance of pre-exposure to the open arms as a procedure capable of improving escape measurement in the elevated T-maze model.

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