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SEROTONIN IN THE DORSAL PERIAQUEDUCTAL GRAY INHIBITS PANIC-LIKE DEFENSIVE BEHAVIORS IN RATS EXPOSED TO ACUTE HYPOXIA

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Abstract—It has been proposed that spontaneous panic attacks are the outcome of the misfiring of an evolved suffocation alarm system. Evidence gathered in the last years is suggestive that the dorsal periaqueductal gray (dPAG) in the midbrain harbors a hypoxia-sensitive suffocation alarm system. We here investigated whether facilitation of 5-HT-mediated neurotransmission within the dPAG changes panic-like defensive reactions expressed by male Wistar rats submitted to a hypoxia challenge (7% O_2), as observed in other animal models of panic. Intra-dPAG injection of 5-HT (20 nmol), (±)-8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT) (8 nmol), a 5-HT1A receptor agonist, or (±)-2,5-dimethoxy-4-iodo amphetamine hydrochloride (DOI) (16 nmol), a preferential 5-HT2A agonist, reduced the number of upward jumps directed to the border of the experimental chamber during hypoxia, interpreted as escape attempts, without affecting the rats' locomotion. These effects were similar to those caused by chronic, but not acute, intraperitoneal administration of the antidepressant fluoxetine (5–15 mg/kg), or acute systemic administration of the benzodiazepine receptor agonist alprazolam (1–4 mg/kg), both drugs clinically used in the treatment of panic disorder. Our findings strengthen the view that the dPAG is a key encephalic area involved in the defensive behaviors triggered by activation of the suffocation alarm system. They also support the use of hypoxia-evoked escape as a model of respiratory-type panic attacks.

Key words: respiratory panic, serotonin, periaqueductal gray, hypoxia.

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Abbreviations: 8-OH-DPAT, (±)-8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide; DOI, (±)-2,5-dimethoxy-4-iodo amphetamine hydrochloride; dPAG, dorsal periaqueductal gray; KCN, potassium cyanide; NTS, nucleus of tractus solitarius; SFA, suffocation false alarm theory.

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INTRODUCTION

Panic attacks are an abrupt surge of intense fear or marked discomfort accompanied by physical and cognitive symptoms that reach a peak within approximately 15 min. The recurrence of unexpected panic attacks and/or fear of having a panic attack are the main diagnostic criteria of panic disorder (APA, 2013). In addition, evidence amassed in the last decades suggests nevertheless the existence of two types of panic, i.e. respiratory or non-respiratory, depending on the prominence of respiratory symptoms (Briggs et al., 1993; Nardi et al., 2003; Freire et al., 2010). The proposal of respiratory or non-respiratory types of panic was corroborated by latent class analysis of the temporal stability, psychiatric comorbidity and treatment outcome in a large-scale epidemiological survey (Roberson-Nay et al., 2012).

Among the many hypotheses on the mechanisms of panic disorder (e.g. Clark, 1986; Pyke and Greenberg, 1986; Reiss, 1991; Margraf et al., 1993; Busch et al., 1996; Clark et al., 1997; Gorman et al., 2000; Austin and Richards, 2001; Hasler et al., 2008), Deakin and Graeff (1991) proposed that dysfunction of (5-HT)-mediated neurotransmission in the dorsal periaqueductal gray matter (dPAG) renders the subject vulnerable to panic attacks. Ever since, the dPAG has been widely accepted as a possible substrate of panic attacks (for reviews see Schenberg et al., 2001; Graeff, 2002; Del-Ben and Graeff, 2009; Canteras and Graeff, 2014; Schenberg, 2014). Indeed, it is long known that electrical stimulation of the dPAG in humans elicits marked autonomic changes accompanied by strong feelings of fear that resemble symptoms of panic (Nashold et al., 1969; Amano et al., 1978). In laboratory animals, chemical or electrical stimulation of this midbrain area evokes vigorous escape reactions, similar to those observed under natural conditions (e.g. confrontation with an approaching predator), which have been pharmacologically validated as an animal model of panic attack (Schenberg et al., 2001; Moreira et al., 2013).

In particular, microinjection in the dPAG of either 5-HT or drugs that mimic its effects, such as the 5-HT1A or the preferential 5-HT2A receptor agonist (±)-8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT) and (±)-2,5-dimethoxy-4-iodo amphetamine hydrochloride (DOI), respectively, reduced the escape response evoked by electrical stimulation of the dPAG (Nogueira and Graeff, 1995; Mongeau and Marsden, 1997; Jacob et al., 2002). Importantly as well, studies with experimental

animals also support a role of dPAG 5-HT in the mode of action of antipanic drugs. Thus, facilitation of both 5-HT1A and 5-HT2A-receptor-mediated neurotransmission in this area is observed after treatment with different classes of panic-relieving drugs such as the antidepressants fluoxetine and imipramine or the benzodiazepine receptor agonist alprazolam (for review see Graeff and Zangrossi, 2010).

On the other hand, Klein (1993a) proposed that spontaneous panic attacks are the outcome of the misfiring of an evolved suffocation alarm system. Briefly, in a preliminary presentation of the suffocation false alarm theory (SFA), Klein (1993b) suggested that the march of symptoms of clinical panic "*appears to be a three-layer cake. The first layer is the reaction of the smothering alarm system, as it had received an increment of CO_2, by breathlessness and increased tidal volume. When the control system keeps getting signals interpreted as predictive of asphyxiation, then the panic attack, with its feeling of suffocation and urge to flee is released, followed by the increase in respiratory frequency*". Importantly, panic attacks can be triggered by both infusions of 0.5 M sodium lactate and inhalations of 5–7% carbon dioxide (CO_2) in panic-prone patients but not in healthy subjects or patients suffering from other anxiety disorders (Drury, 1920; Gorman et al., 1984; Liebowitz et al., 1985; Klein, 1993a). Panic attacks are also precipitated by hyperventilation (Nardi et al., 2004), breath-holding (Nardi et al., 2003), and hypoxia (Beck et al., 1999, 2000) in predisposed individuals and by a two-tidal volume inhalation of 35% CO_2 in both patients and healthy subjects (Argyropoulos et al., 2002; Perna et al., 2004; Van Duinen et al., 2007). Additionally, the prevalence of panic disorder is significantly higher in asthmatics (Perna et al., 1997; Goodwin et al., 2010) and patients with chronic obstructive pulmonary disease (Porzelius et al., 1992; Pollack et al., 1996; Vögele and von Leupoldt, 2008; Nardi, 2009).

Recent evidence obtained by Schimitel et al. (2012) showed that although rats are unresponsive to CO_2 challenge, selective cytotoxic hypoxia by intravenous injection of low doses of potassium cyanide (KCN) evoked marked defensive behaviors in this species. Based on the finding that chemistry hypoxia facilitated PAG-evoked escape response, and that these behaviors were attenuated or even suppressed by discrete lesions of the PAG, Schimitel and coworkers (2012) suggested that the PAG harbors a hypoxia-sensitive suffocation alarm system which spontaneous activation could both precipitate panic and render the subject hyperresponsive to respiratory challenges. This suggestion is compatible with evidence obtained by Casanova and colleagues (2013) showing that rats that expressed escape reactions to severe hypoxia (approximately 6% O_2) showed significant increases in c-Fos protein expression in the nucleus of tractus solitarius (NTS) and in the dorsolateral and lateral columns of the dPAG.

Accordingly, the present study examines whether intra-dPAG microinjection of either 5-HT or the 5-HT1A and the preferential 5-HT2A receptor agonists 8-OH-DPAT and DOI, respectively, inhibits the escape response of rats to severe ambient hypoxia (7% O_2) as much as they do in escape responses of mice exposed to an approaching predator (Pobbe et al., 2011) or rats subjected to electrical or chemical stimulation of the dPAG (Nogueira and Graeff, 1995; Mongeau and Marsden, 1997; de Bortoli et al., 2006). The behavioral effects of these 5-HT agonists were compared to those caused by systemic treatment with two standard panic-relieving drugs, fluoxetine and alprazolam.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (University of Sao Paulo, Campus of Ribeirao Preto) weighing 300 ± 20 g on the day of the experiment, were housed in groups of four per cage under a 12-h light–dark cycle (lights on at 7:00 a.m.), maintained at 22 ± 1 °C, and with free access to food and water. Procedures were conducted in conformity with the guidelines of the Brazilian Council for the care and use of laboratory animals (COBEA), which are in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and were approved by our local ethics committee.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulfate (5-HT; Sigma, St. Louis, MO, USA), 8-OH-DPAT (Sigma, St. Louis, MO, USA) and DOI (Sigma, St. Louis, MO, USA) dissolved in sterile saline (0.9%). Fluoxetine hydrochloride (EMS, Hortolândia, SP, Brazil) and alprazolam (EMS, Hortolândia, SP, Brasil) were dissolved in a solution containing sterile saline with 2% Tween-80.

Apparatus

The gas chamber was a roof-sealed cylinder (25-cm diameter 35-cm height) made of transparent Plexiglas. A removable rubber floor was used to prevent the animals from skidding. The cage floor was divided in four equal quadrants by a pen-mark in order to assess animals' locomotion in the cage. The chamber had a flow valve connected to both an air pump and a nitrogen (N_2) cylinder. Hypoxia (7% O_2) was produced by N_2 administration at a flow rate of 4.5 L/min during approximately 270 s. Chamber concentrations of both O_2 and CO_2 were monitored using a gas analyzer (ML206 Gas Analyzer, AdInstruments, Bella Vista, NSW, Australia) and scanned online with the PowerLab Chart 5 software (AdInstruments, Bella Vista, NSW, Australia).

Surgery

Rats were anesthetized with 2,2,2-tribromoethanol (250 mg/kg, i.p; Sigma–Aldrich, USA) followed by local anesthesia (2% lidocaine with a vasoconstrictor; Harvey, Brazil) and fixed in a stereotaxic instrument. A stainless-steel guide-cannula (12 mm length; outer diameter

0.6 mm) was implanted 2 mm above the dPAG, according to the coordinates based on the rat brain atlas (Paxinos and Watson, 2007), as follows: holding the incisor bar 2.5 mm below the horizontal plane, 2.04 mm anterior from the interaural plane, 1.9 mm lateral from the midline, 3.2 mm below the surface of the skull along an angle of 22°. The guide-cannula was fixed to the skull with acrylic resin and two stainless-steel screws. A stainless-steel wire was introduced into the guide cannula to protect it from obstruction. At the end of surgery, all animals were injected (i.m., 1.0 ml/kg) with a pentabiotic preparation (benzylpenicillin and streptomycin; Forte Dodge, Campinas, SP, Brazil) to prevent possible infections. In addition, flunixin meglumine (Banamine®, Schering – Plough, Sao Paulo, SP, Brazil), a drug with analgesic, antipyretic and anti-inflammatory properties, was administered (2.5 mg/kg) subcutaneously for post-surgery analgesia. Surgery for guide cannula implantation was performed 5–7 days before the hypoxia challenge in Experiment 1.

Procedures

Intra-dPAG injections. Injections in the dPAG were performed through a needle (0.3 mm outer diameter, 12 mm length) inserted into the guide-cannula. Drugs were microinjected in a volume of 0.2 μL over 120 s with the aid of a 10 μL microsyringe (Hamilton, Reno, NV, USA) coupled to a microinfusion pump (KD Scientific, Holliston, MA, USA). The microinjection was monitored by the displacement of an air bubble inside a polyethylene tubing connecting the microsyringe to the needle. The needle was removed 60 s after the end of the injection.

Behavioral test. One day before the hypoxia challenge, the rats were placed in the experimental cage for 15 min. During this habituation session, room-air was flushed into the chamber at a flow rate of 4.5 L/min in order to familiarize the rats to gas flow and to the air jet sound, and to reduce neophobic reactions to the cage environment. Twenty-four hours later, the rats were randomly allocated in different experimental groups according to the drug treatment.

In Experiment 1, the animals were microinjected (n = 7–9) in the dPAG with serotonin (20 nmol), 8-OH-DPAT (8 nmol), DOI (16 nmol) or saline. Ten minutes after the injection, the animals were placed in the experimental cage and tested as described below. These doses were selected based on previous studies in dPAG-stimulation and elevated T-maze models of panic (Nogueira and Graeff, 1995; Jacob et al., 2002; de Paula Soares and Zangrossi, 2004).

In Experiment 2A, independent groups of rats were intraperitoneally injected with fluoxetine (5, 10, or 15 mg/kg) or vehicle, either acutely (n = 8, for each group) or daily throughout 21 days (n = 8). In chronically treated rats, the injection was done after the habituation session. In both acute and chronic studies, animals were tested 30 min after the last injection. The doses of fluoxetine were selected based on previous studies with the elevated T-maze (Poltronieri et al., 2003; Vicente and Zangrossi, 2012). At the selected post-injection time, acute systemic administration of fluoxetine raises 5-HT levels in different brain regions (Perry and Fuller, 1992; Bymaster et al., 2002; Koch et al., 2002), whereas chronic injection of the same drug increases 5-HT release in the dPAG (Zanoveli et al., 2010).

In Experiment 2B, independent groups of rats (n = 8, for each group) received a single intraperitoneal injection of alprazolam (1, 2, or 4 mg/kg) or vehicle 30 min before the test session. These doses and the post-injection time were selected based on the alprazolam anti-escape effect in both dPAG electrical stimulation and elevated T-maze tests (Jenck et al., 1995; Gobira et al., 2013).

During the test session, the animals were placed individually into the chamber and acclimated to it under normoxic condition (21% O_2) for 5 min. For this, room air was flushed into the chamber at a flow rate of 4.5 L/min. Subsequently, N_2 was flushed into the chamber (4.5 L/min) for approximately 4 min up to the production of hypoxia (7% O_2). Next, N_2 infusion was suspended and the hypoxia condition (7% O_2) was maintained for 6 min.

The animal behavior was recorded throughout the experiment using a video camera connected to a DVD recorder. The number of upward jumps and the time spent in prostrated immobility during the hypoxic challenge were computed offline by video analysis. Non-specific drug effects on locomotion were assessed over the first 5 min of test-sessions in normoxic conditions. Activity was measured by the number of transitions across the floor quadrants.

Histology

Animals of Experiment 1 were sacrificed under deep anesthesia with urethane and a dental needle was reinserted in the guide-cannula for marking the injected site with 0.2 μL of Evans Blue. The brain was perfused through the heart with saline solution followed by 10% formalin before being removed and stored in formalin. Frozen coronal slices of 40 μm were cut using a cryostat in order to localize the site of drug injection, according to Paxinos and Watson's atlas (2007). Only rats with injection sites located inside the dPAG, which includes the dorsolateral and dorsomedial columns of the PAG, were included in the statistical analysis.

Statistical analysis

A one-way ANOVA was used to analyze both the number of crossings under normoxic condition and the number of jumps during hypoxia. When appropriate, post hoc comparisons were performed by Tukey's test.

RESULTS

Behavioral effects of hypoxia

Fig. 1 shows the effect of hypoxia on jumping behavior. During the first 5 min of normoxia there were no changes in either gas concentrations (1% CO_2 and 21% O_2) or rat behavior. After 4-min administration of N_2, O_2 concentration was reduced from 21% to 7%. At this

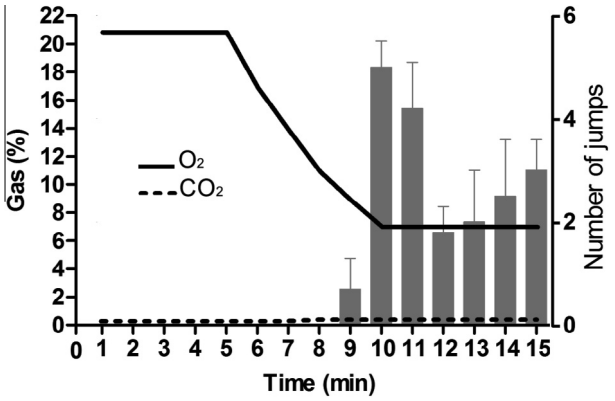

Fig. 1. Relationship among the number of jumps, the levels of oxygen (O_2) and carbon dioxide (CO_2) into the hypoxia chamber along the experimental time. The gray bars represent the number of jumps (mean ± SEM) expressed by the control group of the alprazolam experiment. The lines represent the gases levels: black line (O_2) and dashed line (CO_2).

point, in all animals tested, hypoxia produced manifest increases in depth of ventilation, based on visual observation, and active escape behaviors characterized by bouts of upward leaps directed to the border of the chamber interspersed with periods of prostrated immobility.

Experiment 1: behavioral effects of dPAG microinjection of 5-HT receptor agonists

Fig. 2 shows a diagrammatic representation of injection sites within the dPAG.

A one-way ANOVA showed that hypoxia-evoked jumps were significantly altered by microinjection of 5-HT, 8-OH-DPAT or DOI into the dPAG [$F(3,28) = 14.46$; $p < 0.05$]. All these agonists decreased the expression of this defensive behavior (Fig. 3), without changing locomotion during normoxia or the time spent in prostrated immobility during hypoxia (see Table 1).

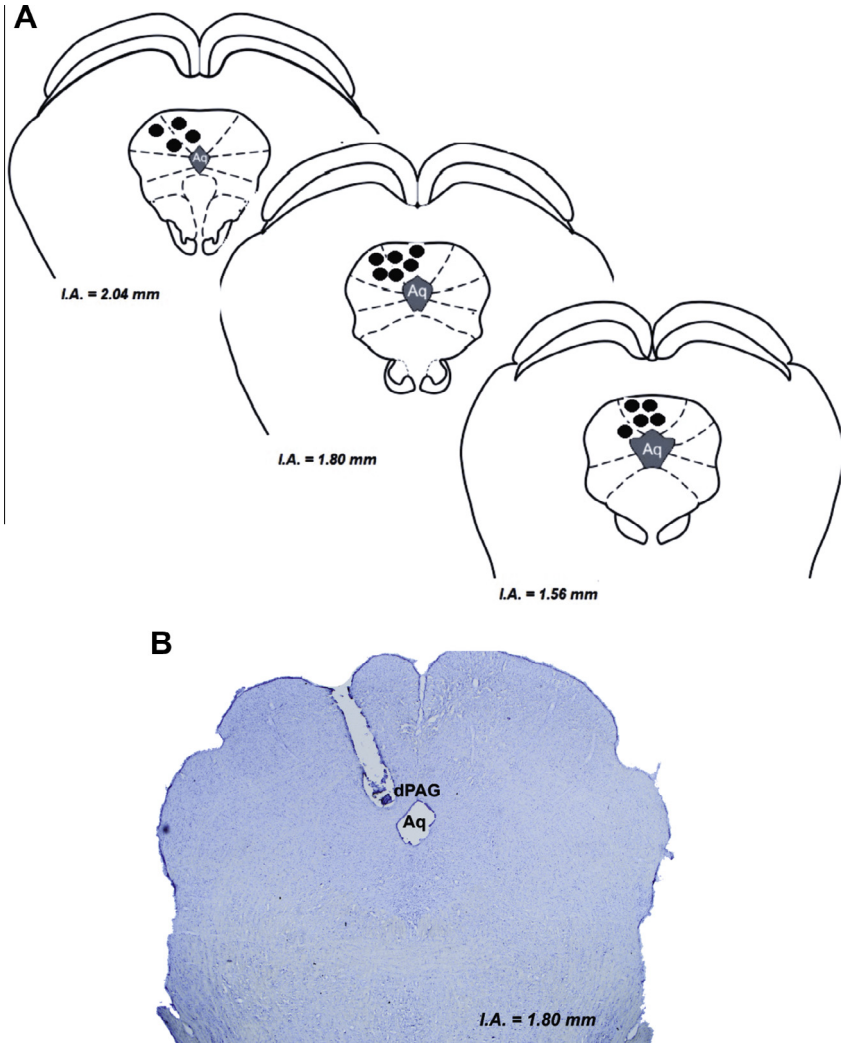

Fig. 2. (A) Diagrammatic representation of coronal sections of the rat brain (Paxinos and Watson, 2007) showing the microinjection sites inside of the dPAG (filled circles). Due to overlaps, the number of points represented is fewer than the number of rats actually injected. (B) Photomicrographs showing typical injection sites (arrow) in the dPAG. I.A. = interaural, Aq = aqueduct.

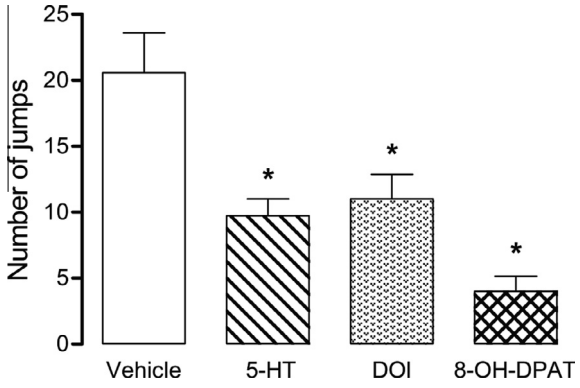

Fig. 3. Effects (mean ± SEM) of intra-dPAG injection of serotonin (20 nmol), 8-OH-DPAT (8 nmol), DOI (16 nmol) or saline on the number of jumps expressed during hypoxia. $^{*}p < 0.05$ compared with the control group.

Table 1. Mean ± SEM of the number of transitions across the floor quadrants over the first 5 min of test-sessions in normoxic conditions, and the total time (*s*) spent in prostrated immobility under 7% O_2 hypoxia

Experiment	Number of crossing	Prostrated immobility
Experiment 1		
Vehicle	32.6 ± 4.1	145.6 ± 19.4
5-HT 20 nmol	37.2 ± 6.0	148.4 ± 17.3
8-OH-DPAT 8 nmol	35.2 ± 3.0	153.5 ± 15.4
DOI 16 nmol	19.7 ± 7.4	114.9 ± 12.3
Acute fluoxetine (i.p)		
Vehicle	36.6 ± 4.3	161.1 ± 26.7
5 mg/kg	37.2 ± 4.2	171.0 ± 24.7
10 mg/kg	30.0 ± 5.7	186.1 ± 21.8
15 mg/kg	20.4 ± 4.5	167.1 ± 18.3
Chronic fluoxetine (i.p)		
Vehicle	32.2 ± 3.1	194.1 ± 14.8
5 mg/kg	31.7 ± 4.2	161.5 ± 23.5
10 mg/kg	39.6 ± 3.9	183.8 ± 28.1
15 mg/kg	23.1 ± 4.9	164.1 ± 18.7
Acute alprazolam (i.p)		
Vehicle	40.0 ± 5.8	170.3 ± 17.5
1 mg/kg	37.6 ± 4.9	182,8 ± 18.3
2 mg/kg	30.4 ± 7.0	174.0 ± 22.0
4 mg/kg	18.4 ± 4.5*	233.6 ± 17.3

* $p < 0.05$, when compared with the control group.

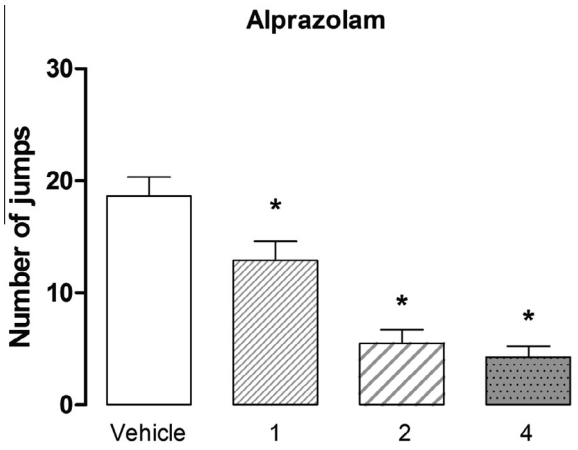

Fig. 5. Effects (mean ± SEM) of an acute injection of alprazolam (0; 1; 2 or 4 mg/kg, i.p) or saline on the number of jumps expressed during hypoxia. $^{*}p < 0.05$ compared with the control group.

Experiment 2: chronic or acute treatment with fluoxetine

A one-way ANOVA revealed that the number of jumps was significantly affected by chronic [$F(3,28) = 14.46$; $p < 0.05$] but not acute [$F(3,28) = 0.92$; NS] injections of fluoxetine. The post hoc analysis showed that chronic fluoxetine at 10 and 15 mg/kg significantly reduced the escape response during hypoxia (Fig. 4). Treatment with fluoxetine did not interfere with locomotion evaluated during normoxia or with the time spent in prostrated immobility during hypoxia (see Table 1).

Experiment 3: acute treatment with alprazolam

Treatment with alprazolam also changed the number of jumps expressed by the rats during hypoxia [$F(3,28) = 21.62$; $p < 0.05$]. Post-hoc comparisons showed that all doses of this benzodiazepine decreased this behavioral index (Fig. 5). As shown in Table 1, treatment with alprazolam also affected locomotion during normoxia [$F(3,28) = 3.17$; $p < 0.05$]. Tukey's test showed that the highest dose tested significantly decreased locomotion when compared with the control group. This benzodiazepine also tended to increase the time spent in prostrated immobility during hypoxia [$F(3,28) = 2.42$; $p = 0.08$].

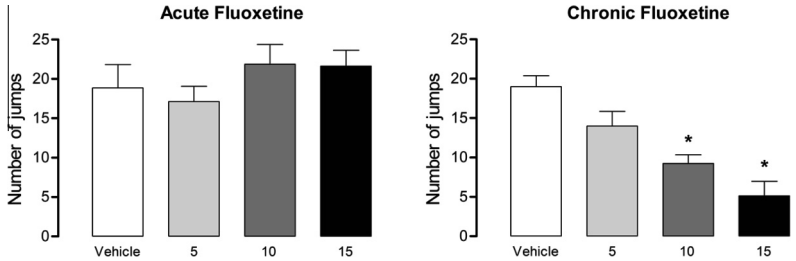

Fig. 4. Effects (mean ± SEM) of acute or chronic (21 days) treatment with fluoxetine (0; 5; 10 or 15 mg/kg, i.p.) or vehicle solution on the number of jumps expressed during hypoxia. $^{*}p < 0.05$ compared with the control group.

DISCUSSION

The purpose of this study was to investigate whether facilitation of 5-HT-mediated neurotransmission within the dPAG changes the defensive reaction expressed by rats under hypoxia conditions. Although the anti-aversive effect of 5-HT has already been reported in different animal models that associate escape expression with panic attacks (see below), none of these experiments addressed the respiratory-type panic attack that is the basis of Klein's (1993a) SFA.

Our results showed that intra-dPAG microinjection of serotonin, 8-OH-DPAT or DOI decreased the number of jumps toward the border of the experimental chamber. Because Wistar rats hardly jump, these results were interpreted as attempts to escape from hypoxia. Microinjection effects were similar to those observed after both chronic and acute treatments with the clinically-effective panicolytics, fluoxetine and alprazolam. Therefore, facilitation of 5-HT1A- and 5-HT2A-mediated neurotransmission within the dPAG causes panicolytic-like effects much as is observed in other models that associate escape expression with panic attacks, such as the elevated T-maze (Zanoveli et al., 2003; de Paula Soares and Zangrossi, 2004), chemical or electrical stimulation of the dPAG (Beckett and Marsden, 1997; de Bortoli et al., 2006; de Oliveira Sergio et al., 2011) and mouse defense test battery (Pobbe et al., 2011). This inhibitory response does not seem to be attributed to a non-specific effect of these 5-HT receptor agonists on locomotor activity of these animals, as none of them altered the number of crossings in the experimental cage under normoxia or the time spent in prostrated immobility during hypoxia.

Altogether, the current findings are consonant with previous studies suggesting that the dPAG harbors a hypoxia-sensitive suffocation alarm system (Schimitel et al., 2012; Casanova et al., 2013). Furthermore, the demonstration that escape responses to low concentrations of O_2 are likewise attenuated by chronic but not acute administration of fluoxetine is in agreement with previous findings reported by Schimitel and coworker (2014) with cytotoxic hypoxia induced in rats by intravenous injection of low doses of KCN, and those reported in other animal models that associate active escape responses with panic disorder. More specifically, only chronic treatment with fluoxetine, in the same dose range used here, lengthened latencies to escape in the elevated T-maze (Poltronieri et al., 2003) or reduced escape expression evoked by an approaching predator (Griebel et al., 1995). Taken together, these studies suggest that at least part of the panicolytic effect promoted by fluoxetine may be due to 5-HT inhibition of a dPAG-inbuilt suffocation alarm system, thereby bridging the theories on panic attacks of Deakin and Graeff (1991) and Klein (1993a). Consistent with this notion is the observation that previous administration of the 5-HT1A receptor antagonist WAY-10635 in the dPAG blocked the anti-escape effect caused by chronic systemic administration of fluoxetine in rats tested in the elevated T-maze (Zanoveli et al., 2010).

Regarding alprazolam, although the highest dose impaired ambulatory activity, suggesting a sedative effect, the anti-escape effect occurred with lower doses. Our results are consistent with recently obtained results with the high-potency benzodiazepine, clonazepam, also a clinically effective panic-relieving drug, in the escape task of the elevated T-maze (Pobbe et al., 2014), and in the flight response induced in rats by intravenous injection of low doses of KCN (Schimitel et al., 2014).

Concerning PAG engagement with the suffocation alarm system, there are few studies on afferents to this midbrain area from the NTS second order neurons that are recipients of glossopharyngeal fibers conveying information from carotid chemoreceptors. Nonetheless, the pioneering study of Bandler and Tork (1987) reported retrogradely labeled neurons in both the ventrolateral and the ventromedial subnuclei of the NTS following an injection of horseradish peroxidase (HRP) centered into the central district of the PAG of the cat. These subnuclei were shown to be the major recipients of glossopharyngeal fibers in both cats and rats (Panneton and Loewy, 1980; Finley and Katz, 1992). In contrast, the NTS commissural subnucleus believed to process information from carotid baroreceptors was not labeled (Claps et al., 1989). The latter observation suggests that NTS projections to PAG are mainly involved with respiratory responses. Indeed, a recent study with c-fos immunohistochemistry showed significant activations of both dorsolateral and lateral PAG, but not the dorsomedial or ventrolateral PAG, of rats that showed escape responses to severe hypoxia (Casanova et al., 2013).

CONCLUSIONS

In conclusion, the present data showed that the facilitation of serotonergic neurotransmission into the dPAG impairs escape reactions produced by severe hypoxia. Our results also strength the view that the dPAG is a key encephalic area involved in the defensive behaviors triggered by activation of the suffocation alarm system. In addition, data from systemic injection with panic-relieving compounds support the use of hypoxia-evoked escape as a model of respiratory-type panic attacks.

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REFERENCES

Amano K, Tanikawa T, Iseki H, Kawabatake H, Notani M, Kawamura H, Kitamura K (1978) Single neuron analysis of the human midbrain tegmentum. Rostral mesencephalic reticulotomy for pain relief. Appl Neurophysiol 41(1):66–78.

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders. 5th ed.

Argyropoulos SV, Bailey JE, Hood SD, Kendrick AH, Rich AS, Laszlo G, Nash JR, Lightman SL, Nutt DJ (2002) Inhalation of 35% CO (2) results in activation of the HPA axis in healthy volunteers. Psychoneuroendocrinology 27(6):715–729.

Austin DW, Richards JC (2001) The catastrophic misinterpretation model of panic disorder. Behav Res Ther 39(11):1277–1291.

Bandler R, Tork I (1987) Midbrain periaqueductal grey region in the cat has afferent and efferent connection with solitary tract nuclei. Neurosci Lett 74:1–6.

Beck JG, Ohtake PJ, Shipherd JC (1999) Exaggerated anxiety is not unique to CO_2 in panic disorder: a comparison of hypercapnic and hypoxic challenges. J Abnorm Psychol 108(3):473–482.
Beck JG, Shipherd JC, Ohtake PJ (2000) Do panic symptom profiles influence response to a hypoxic challenge in patients with panic disorder? A preliminary report. Psychosom Med 62 (5):678–683.
Beckett S, Marsden CA (1997) The effect of central and systemic injection of the 5-HT1A receptor agonist 8-OHDPAT and the 5-HT1A receptor antagonist WAY100635 on periaqueductal grey-induced defence behaviour. J Psychopharmacol 11:35–40.
Briggs AC, Stretch DD, Brandon S (1993) Subtyping of panic disorder by symptom profile. Br J Psychiatry 163:201–209.
Busch F, Milroad B, Cooper A, Shapiro T (1996) Psychodynamic approaches to panic disorder. J Psychother Pract Res 5 (1):72–83.
Bymaster FP, Zhang W, Carter PA, Shaw J, Chernet E, Phebus L, Wong DT, Perry KW (2002) Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. Psychopharmacology 160(4):353–361.
Canteras NS, Graeff FG (2014) Executive and modulatory neural circuits of defensive reactions: implications for panic disorder. Neurosci Biobehav Rev 46(3):352–364.
Casanova JP, Contreras M, Moya EA, Torrealba F, Iturriaga R (2013) Effect of insular cortex inactivation on autonomic and behavioral responses to acute hypoxia in conscious rats. Behav Brain Res 253:60–67.
Claps A, Torrealba F, Calderon F (1989) Segregation of coarse and fine glossopharyngeal axons in the visceral nucleus of the tractus solitarius of the cat. Brain Res 489:80–92.
Clark DM (1986) A cognitive approach to panic. Behav Res Ther 24:461–470.
Clark DM, Salkovskis PM, Ost LG, Breitholz KA, Koehler E, Westling BE, Jeavons A, Gelder MG (1997) Misinterpretation of body sensations in panic disorder. J Consult Clin Psychol 65:203–213.
de Bortoli VC, Nogueira RL, Zangrossi Jr H (2006) Effects of fluoxetine and buspirone on the panicolytic-like response induced by the activation of 5-HT1A and 5-HT2A receptors in the rat dorsal periaqueductal gray. Psychopharmacology 183(4):422–428.
de Oliveira Sergio T, de Bortoli VC, Zangrossi Jr H (2011) Serotonin-2A receptor regulation of panic-like behavior in the rat dorsal periaqueductal gray matter: the role of GABA. Psychopharmacology 218(4):725–732.
de Paula Soares V, Zangrossi Jr H (2004) Involvement of 5-HT1A and 5-HT2 receptors of the dorsal periaqueductal gray in the regulation of the defensive behaviors generated by the elevated T-maze. Brain Res Bull 64(2):181–188.
Deakin JF, Graeff FG (1991) 5-HT and mechanisms of defence. J Psychopharmacol 5(4):305-1.
Del-Ben CM, Graeff FG (2009) Panic disorder: is the PAG involved? Neural Plast 108135.
Drury AN (1920) The percentage of carbon dioxide in the alveolar air, and the tolerance to accumulating carbon dioxide in cases of so-called ‘‘irritable heart” of soldiers. Heart 7(4):165–173.
Finley JC, Katz DM (1992) The central organization of carotid body afferent projections to the brainstem of the rat. Brain Res 572:108–116.
Freire RC, Perna G, Nardi AE (2010) Panic disorder respiratory subtype: psychopathology, laboratory challenge test, and response to treatment. Harv Rev Psychiatry 18(4):220–229.
Gobira PH, Aguiar DC, Moreira FA (2013) Effects of compounds that interfere with the endocannabinoid system on behaviors predictive of anxiolytic and panicolytic activities in the elevated T-maze. Pharmacol Biochem Behav 110:33–39.
Goodwin RD, Pagura J, Cox B, Sareen J (2010) Asthma and mental disorders in Canada: impact on functional impairment and mental health service use. J Psychosom 68(2):165–173.
Gorman JM, Askanazi J, Liebowitz MR, Fyer AJ, Stein J, Kinney JM, Klein DF (1984) Response to hyperventilation in a group of patients with panic disorder. Am J Psychiatry 141(7):857–861.
Gorman JM, Kent JM, Sullivan GM, Coplan JD (2000) Neuroanatomical hypothesis of panic disorder, revised. Am J Psychiatry 157(4):493–505.
Graeff FG (2002) On serotonin and experimental anxiety. Psychopharmacology 163(3–4):467–476.
Graeff FG, Zangrossi Jr H (2010) The dual role of serotonin in defense and the mode action of antidepressants on generalized anxiety and panic disorders. Cent Nerv Syst Agents Med Chem 10(3):207–217.
Griebel G, Blanchard DC, Agnes RS, Blanchard RJ (1995) Differential modulation of antipredator defensive behavior in Swiss-Webster mice following acute or chronic administration of imipramine and fluoxetine. Psychopharmacology 120(1):57–66.
Hasler G, Nugent AC, Carlson PJ, Carson RE, Geraci M, Drevets WC (2008) Altered cerebral gamma-aminobutyric acid type A-benzodiazepine receptor binding in panic disorder determined by [11C] flumazenil positron emission tomography. Arch Gen Psychiatry 65(10):1166–1175.
Jacob CA, Cabral AH, Almeida LP, Magierek V, Ramos PL, Zanoveli JM, Landeira Fernandez J, Zangrossi H, Nogueira RL (2002) Chronic imipramine enhances 5-HT(1A) and 5-HT(2) receptors-mediated inhibition of panic-like behavior in the rat dorsal periaqueductal gray. Pharmacol Biochem Behav 72(4):761–766.
Jenck F, Moreau JL, Martin JR (1995) Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. Psychiatry Res 57(2):181–191.
Klein DF (1993a) False suffocation alarms, spontaneous panics, and related conditions. An integrative hypothesis. Arch Gen Psychiatry 50(4):306–317.
Klein DF (1993b) Panic may be a misfiring suffocation alarm. In: Montgomery SA, editor. Psychopharmacology of Panic. New York: Oxford University Press. p. 67–73.
Koch S, Perry KW, Nelson DL, Conway RG, Threlkeld PG, Bymaster FP (2002) R-fluoxetine increases extracellular DA, NE, as well as 5-HT in rat prefrontal cortex and hypothalamus: an in vivo microdialysis and receptor binding study. Neuropsychopharmacology 27(6):949–959.
Liebowitz MR, Fyer AJ, Gorman JM, Dillon D, Davies S, Stein JM, Cohen BS, Klein DF (1985) Specificity of lactate infusions in social phobia versus panic disorders. Am J Psychiatry 142:947–950.
Margraf J, Barlow DH, Clark DM, Telch MJ (1993) Psychological treatment of panic: work in progress on outcome, active ingredients, and follow-up. Behav Res Ther 31(1):1–8.
Mongeau R, Marsden CA (1997) Effect of imipramine treatments oh the 5-HT1A-receptor mediated inhibition of panic-like behaviours in rats. Psychopharmacology 131(4):321–328.
Moreira FA, Gobira PH, Viana TG, Vicente MA, Zangrossi H, Graeff FG (2013) Modeling panic disorder in rodents. Cell Tissue Res 354(1):119–125.
Nardi AE (2009) Panic disorder is closely associated with respiratory obstructive illnesses. Am J Respir Crit Care Med 179(3):256–257.
Nardi AE, Nascimento I, Valença AM, Lopes FL, Mezzasalma MA, Zin WA, Versiani M (2003) Respiratory panic disorder subtype: acute and long-term response to nortriptyline, a noradrenergic tricyclic antidepressant. Psychiatry Res 120(3):283–393.
Nardi AE, Lopes FL, Valença AM, Nascimento I, Mezzasalma MA, Zin WA (2004) Psychopathological description of hyperventilation-induced panic attacks: a comparison with spontaneous panic attacks. Psychopathology 37(1):29–35.
Nashold Jr BS, Wilson WP, Slaughter DG (1969) Sensations evoked by stimulation in the midbrain of man. J Neurosurg 30:14–24.
Nogueira RL, Graeff FG (1995) Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray generated aversion. Pharmacol Biochem Behav 52(1):1–6.
Panneton WM, Loewy AD (1980) Projections of the carotid sinus nerve to the nucleus of the solitary tract in the cat. Brain Res 191:239–244.
Paxinos G, Watson C (2007) The Rat Brain in Stereotaxic Coordinates. 6th ed. Amsterdam: Elsevier.
Perna G, Bertani A, Politi E, Colombo G, Bellodi L (1997) Asthma and panic attacks. Biol Psychiatry 42(7):625–630.

Perna G, Casolari A, Bussi R, Cucchi M, Arancio C, Bellodi L (2004) Comparison of 35% carbon dioxide reactivity between panic disorder and eating disorder. Psychiatry Res 125(3):277–283.

Perry KW, Fuller RW (1992) Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. J Pharm Pharmacol 44(12):1041–1042.

Pobbe RL, Zangrossi Jr H, Blanchard DC, Blanchard RJ (2011) Involvement of dorsal raphe nucleus and dorsal periaqueductal gray 5-HT receptors in the modulation of mouse defensive behaviors. Eur Neuropsychopharmacol 21(4):306–315.

Pobbe RL, Lopes MA, Vasconcelos AT, Yamashita PS, de Bortoli VC, Zangrossi H (2014) Influence of procedural variables on rat inhibitory avoidance and escape behaviors generated by the elevated T-maze. Behav Brain Res 273:45–51.

Pollack MH, Kradin R, Otto MW, Worthington J, Gould R, Sabatino SA, Rosenbaum JF (1996) Prevalence of panic in patients referred for pulmonary function testing at a major medical center. Am J Psychiatry 153(1):110–113.

Poltronieri SC, Zangrossi Jr H, de Barros Viana M (2003) Antipanic-like effect of serotonin reuptake inhibitors in the elevated T-maze. Behav Brain Res 147(1–2):185–192.

Porzelius J, Vest M, Nochomovitz M (1992) Respiratory function, cognitions, and panic in chronic obstructive pulmonary patients. Behav Res Ther 30(1):75–77.

Pyke RE, Greenberg HS (1986) Norepinephrine challenges in panic patients. J Clin Psychopharmacol 6(5):279–285.

Reiss S (1991) Expectancy model of fear, anxiety, and panic. Clin Psychol Rev 11(2):141–153.

Roberson-Nay R, Latendresse SJ, Kendler KS (2012) A latent class approach to the external validation of respiratory and non-respiratory panic subtypes. Psychol Med 42(3):461–474.

Schenberg LC (2014) Panic disorder. Neurosci Biobehav Rev 46 (3):343–344.

Schenberg LC, Bittencourt AS, Sudré EC, Vargas LC (2001) Modeling panic attacks. Neurosci Biobehav Rev 25(7–8):647–659.

Schimitel FG, de Almeida GM, Pitol DN, Armini RS, Tufik S, Schenberg LC (2012) Evidence of a suffocation alarm system within the periaqueductal gray matter of the rat. Neuroscience 200:59–73.

Schimitel FG, Müller CJ, Tufik S, Schenberg LC (2014) Evidence of a suffocation alarm system sensitive to clinically-effective treatments with the panicolytics clonazepam and fluoxetine. J Psychopharmacol 28(12):1184–1188.

Van Duinen MA, Schruers KR, Maes M, Griez EJ (2007) CO_2 challenge induced HPA axis activation in panic. Int J Neuropsychopharmacol 10:797–804.

Vicente MA, Zangrossi H (2012) Serotonin-2C receptors in the basolateral nucleus of the amygdala mediate the anxiogenic effect of acute imipramine and fluoxetine administration. Int J Neuropsychopharmacol 15(3):389–400.

Vögele C, von Leupoldt A (2008) Mental disorders in chronic obstructive pulmonary disease (COPD). Respir Med 102 (5):764–773.

Zanoveli JM, Nogueira RL, 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(2–3):153–161.

Zanoveli JM, Pobbe RL, de Bortoli VC, Carvalho MC, Brandão ML, Zangrossi Jr H (2010) Facilitation of 5-HT1A-mediated neurotransmission in dorsal periaqueductal grey matter accounts for the panicolytic-like effect of chronic fluoxetine. Int J Neuropsychopharmacol 13(8):1079–1088.

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