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Research report

Dorsomedial hypothalamus serotonin 1A receptors mediate a panic-related response in the elevated T-maze

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ABSTRACT

The dorsomedial hypothalamus (DMH) has long been associated with the regulation of escape, a panic-related defensive response. Previous evidence has shown that the activation of serotonin (5-HT) 1A and 2A receptors impairs escape behavior induced by the electrical stimulation of the same region. In this study we further explore the relationship of the DMH with defense by investigating the effects of 5-HT1A activation on escape behavior generated in male Wistar rats by an ethologically based aversive stimuli, exposure to one of the open arms of the elevated T-maze (ETM). Aside from escape, the ETM also allows the measurement of inhibitory avoidance, a defensive response associated with generalized anxiety disorder. To evaluate locomotor activity, after ETM measurements animals were submitted to an open field. Results showed that intra-DMH administration of the 5-HT1A receptor agonist 8-OH-DPAT inhibited escape expression. Local administration of the 5-HT1A antagonist WAY-100635 by its own was ineffective, but blocked the panicolytic-like effect of 8-OH-DPAT. Chronic (21 days) systemic treatment with imipramine potentiated the anti-escape effect of 8-OH-DPAT. No significant effects of treatment with 8-OH-DPAT or imipramine on avoidance latencies or the number of lines crossed in the open field were found. These results indicate that 5-HT1A receptors within the DMH may play a phasic inhibitory role on ETM escape expression. As previously proposed, facilitation of 5-HT1A-mediated neurotransmission in the DMH may be involved in the mechanism of action of anti-panic compounds.

1. Introduction

A wealth of evidence supports the involvement of serotonin (5-HT) in the pathophysiology of anxiety disorders, such as generalized anxiety and panic disorder, as well as in the mode of action of drugs used in their treatment (Kahn et al., 1988; Deakin and Graeff, 1991; Hale et al., 2012; Maximino et al., 2013). First option pharmacological treatment for these psychopathologies are antidepressants, which act through facilitation of monoamine neurotransmission, in particular of serotonin, as in the case of drugs such as fluoxetine and sertraline, which selectively inhibit 5-HT re-uptake (Den Boer et al., 2000; Bandelow et al., 2007, 2012; Stein and Lopez, 2011; Andrisano et al., 2013). The nature of the therapeutic effect of these drugs seems to be related to neuroplastic adaptive changes, for they only exert their effect after 3–4 weeks of repeated administration (Bijak et al., 1997, 2001; Nierenberg et al., 2000; Millan, 2005; Veerakumar et al., 2014). The type of neuroplastic changes that occur in response to chronic treatment with antidepressants and the anatomical substrates where they take place have been the subject of an increasing number of studies performed during the past years (Welner et al., 1989; Blier and de Montigny, 1994; Li et al., 1996; Hensler, 2002; Castro et al., 2003; Graeff and Zangrossi, 2010).

Regarding the role played by 5-HT in the modulation of anxiety-related reactions, Deakin and Graeff (1991) proposed a theory suggesting a dual role for this monoamine. According to this theory, 5-HT release in brain regions related to defensive responses induced by potential or distal threats, i.e. the amygdala and the frontal cortex, would increase anxiety and be involved with the pathophysiology of generalized anxiety disorder. GABAergic or serotonergic drugs (that activate 5-HT1A inhibitory receptors in such regions) would thus act as anxiolytics. On the other hand, 5-HT would exert an inhibitory control in regions such as the dorsal

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periaqueductal gray matter (dPAG), which regulate reactions to proximal defense (for instance, escape behavior), by activating 5-HT1A and 2A receptors. In this last case, failure in the inhibitory control induced by 5-HT would increase the susceptibility to panic attacks.

As for the amygdala and the dPAG, the medial hypothalamus has also been related to defense/anxiety (Canteras, 2002). Distinct, well-circumscribed nuclei integrate the region (Canteras, 2002) – i.e., the dorsomedial hypothalamus (DMH), the anterior hypothalamus, the ventromedial hypothalamus and the dorsal premammillary nucleus. The DMH seems to be particularly activated in response to interoceptive challenges that accompany innate fear (Silveira and Graeff, 1992; Shekhar, 1994; Shekhar and Keim, 1997; Canteras, 2002; Graeff and Zangrossi, 2002; Freitas et al., 2009; Johnson and Shekhar, 2012; Canteras and Graeff, in press). Previous evidence indicates that electrical stimulation of this hypothalamic nucleus induces escape, followed by autonomic activation, behavioral and physiological responses that resemble the ones presented by animals when in face of natural threats (Hess and Brugger, 1943; Fernandez de Molina and Hunsperger, 1962; Panksepp, 1971). Administration of agonists of glutamate receptors (Bailey and Dimicco, 2001; Silveira and Graeff, 1992) or of antagonists of GABA receptors into the structure (Schmitt et al., 1986; Nascimento et al., 2010) also evokes a similar defensive behavior. It was additionally shown that anti-panic compounds, such as imipramine and clonazepam, block the behavioral and cardiovascular responses evoked by intra-DMH administration of the GABAA receptor antagonist bicuculline (Shekhar, 1994). Altogether, these results suggest that either electrical or chemical stimulation of the DMH can be used to model some aspects of panic disorder, in particular panic attacks (Graeff and Zangrossi, 2002; Freitas et al., 2009; Johnson and Shekhar, 2012; Moreira et al., 2013; Canteras and Graeff, in press).

If such a proposition is true, the DMH might function as an important neuroanatomical substrate for the mechanism of action of anti-panic compounds. In fact, in a previous study it was shown that injection into the DMH of 5-HT1A and 5-HT2A agonists impaired escape responses observed during electrical current delivery to the structure (de Bortoli et al., 2013). More importantly, chronic treatment with the antidepressants fluoxetine or imipramine, a noradrenaline and a 5-HT-reuptake inhibitor, potentiated the anti-panic effect of these 5-HT agonists (de Bortoli et al., 2013). These results indicate that facilitation of 5-HT1A- and 5-HT2A-mediated neurotransmission in the DMH may be involved in the mechanism of action of anti-panic drugs.

The purpose of the present study was to further explore the relationship of DMH 5-HT neurotransmission to panic-related reactions. More specifically, we evaluated whether activation of 5-HT1A receptors, which are found in the DMH (Li et al., 1997; Clemett et al., 2000), inhibits the escape responses evoked by the elevated T-maze (ETM). In this test, escape behavior is induced by an ethologically relevant threatening stimulus: the exposure of rats to an open and elevated space (Graeff et al., 1993; Viana et al., 1994; Zangrossi and Graeff, 1997; Teixeira et al., 2000; Graeff and Zangrossi, 2002; Poltronieri et al., 2003). Aside from escape, the ETM also allows the measurement of another defensive reaction: inhibitory avoidance (Viana et al., 1994; Graeff and Zangrossi, 2002). The pharmacological validation of this animal model has shown that three different classes of anxiolytics – the benzodiazepine receptor agonist diazepam, the 5-HT1A agonist buspirone, and ritanserin, a nonselective 5-HT_2 antagonist – selectively impair inhibitory avoidance without altering one-way escape (Graeff et al., 1993; Viana et al., 1994; Graeff et al., 1998). These results are compatible with the idea that inhibitory avoidance models generalized anxiety. On the other hand, the escape task is impaired by chronic administration of imipramine (Teixeira et al., 2000), clomipramine and fluoxetine (Poltronieri et al., 2003), drugs used in clinical settings to treat panic. As a result, ETM one-way escape has been used as an animal model of panic disorder (for a review report to Zangrossi and Graeff, 2014).

In the present study, we also investigated whether chronic systemic administration of imipramine altered the anti-escape effect found in the ETM after 5-HT1A receptor activation. For locomotor activity assessment, after tests with the ETM, animals were also tested in an open field.

2. Materials and methods

2.1. Subjects

Male Wistar rats (CEDEME, Universidade Federal de São Paulo, Campus Santos, Brazil), weighing 280–320 g at the beginning of the experiment, were housed in groups of 5–6 per cage. After surgery, animals were housed in pairs in Plexiglas-walled cages until testing. Room temperature was controlled ($22 \pm 1\,^{\circ}C$) and a light–dark cycle was maintained on a 12 h on–off cycle (700–1900 h lights on). Food and water were available all throughout the experiments. The study was approved by the Ethical Committee for Animal Research of the Federal University of São Paulo and was performed in compliance with the recommendations of the Brazilian Society of Neuroscience and Behavior, which are based on the conditions stated in the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996). All efforts were made to minimize animal suffering.

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 was enclosed by 40 cm high walls and was oriented perpendicularly to two opposed open arms. The whole apparatus was elevated 50 cm above the floor. To avoid falls, a 1 cm high Plexiglas rim surrounded the open arms.

The open field was a round arena (60 cm × 60 cm), with the floor divided into 12 parts, and walls 50 cm high.

Luminosity at the level of the T-maze arms or at the open field center was 60 lx. After the experimental session conducted with each animal, the elevated T-maze and the open field were cleaned with a 10% ethanol solution.

2.3. Compounds

The following drugs were used: (±)-8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT; Sigma, USA), N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-2-pyridinyl maleate (WAY-100635; Sigma, USA), imipramine hydrochloride (Sigma, USA). The drugs were dissolved in sterile saline (0.9%).

2.4. Surgery

Three days after their arrival in the laboratory, rats were anaesthetized with an IP injection of ketamine hydrochloride (80 mg/kg; Agribrands, Brazil) and xylazine (10 mg/kg; Agribrands, Brazil) and fixed to a stereotaxic frame (David Kopf, USA). Local anesthesia was also performed (2% lidocaine with a vasoconstrictor; Harvey, Brazil) before the implant of stainless steel guide cannulae into the DMH.

Guide cannulae (0.6 mm outer diameter and 0.4 mm inner diameter) were inserted into the brain through a hole drilled in the skull above the DMH, following the coordinates from the atlas of Paxinos and Watson (2008): AP = −2.8 mm from bregma; ML = −0.5 mm and DV = −7.2 mm from skull. Cannulae were attached to the skull by means of acrylic resin and two stainless steel screws. Stylets with