Neurobiology of Learning and Memory 121 (2015) 52–58

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Dorsal periaqueductal gray post-stimulation freezing is counteracted by neurokinin-1 receptor antagonism in the central nucleus of the amygdala in rats

M.C. Carvalho [a,b,*], J.M. Santos [a,c], M.L. Brandão [a,b]

[a] Instituto de Neurociências e Comportamento, Campus USP, Ribeirão Preto, SP, Brazil
[b] Laboratório de Neuropsicofarmacologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
[c] Faculdade de Educação Física e Fisioterapia, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil

ARTICLE INFO

Article history:
Received 13 January 2015
Revised 4 April 2015
Accepted 6 April 2015
Available online 13 April 2015

Keywords:
Amygdala
NK1 receptor
Spantide
Dorsal periaqueductal gray
Fear
Anxiety

ABSTRACT

Electrical stimulation of the dorsal periaqueductal gray (dPAG) in rats generates defensive responses that are characterized by freezing and escape behaviors, followed by post-stimulation freezing that resembles symptoms of panic attacks. dPAG post-stimulation freezing involves the processing of ascending aversive information to prosencephalic centers, including the amygdala, which allows the animal to evaluate the consequences of stressful situations. The basolateral nucleus of the amygdala (BLA) is thought to act as a filter for innate and learned aversive information that is transmitted to higher structures. The central (CeA) and medial (MeA) nuclei of the amygdala constitute an output for the expression of fear reactions through projections to limbic and brainstem regions. Neurokinin (NK) receptors are abundant in the CeA, MeA, and BLA, but their role in the expression of defensive responses and processing of aversive information that is evoked by electrical stimulation of the dPAG is still unclear. In the present study, we examined the role of NK1 receptors in these amygdala nuclei in the expression of defensive responses induced by electrical stimulation of the dPAG in rats and fear memory of this aversive stimulation. Rats were implanted with an electrode into the dPAG for electrical stimulation and one cannula in the CeA, MeA, or BLA for injections of vehicle (phosphate-buffered saline) or the NK1 receptor antagonist spantide (SPA; 100 pmol/0.2 μl). Injections of SPA into the CeA but not BLA or MeA reduced the duration of post-stimulation freezing evoked by electrical stimulation of the dPAG, without changing the aversive thresholds of freezing or escape. Twenty-four hours later, exploratory behavior was evaluated in the elevated plus maze test (EPM) in the CeA group of rats. Electrical stimulation of the dPAG rats that received vehicle exhibited higher aversion to the open arms of the EPM than sham rats that did not receive any dPAG stimulation. SPA injections into the CeA prevented the proaversive effects of electrical stimulation of the dPAG assessed in the EPM 24 h later. The present results suggest that neurokininergic modulation via NK1 receptors in the CeA but not BLA or MeA is involved in the processing of aversive information derived from dPAG stimulation. The long-lasting consequences of electrical stimulation of the dPAG may be prevented by NK1 receptor antagonism in the CeA.

1. Introduction

Chemical or electrical stimulation of the dorsal periaqueductal gray (dPAG) in rats generates defensive responses that are characterized by alertness, freezing, and escape behavior (Bandler, Depaulis, & Vergnes, 1985; Brandao, de Aguiar, & Graeff, 1982; Krieger & Graeff, 1985; Schenberg, Costa, Borges, & Castro, 1990; Vianna, Graeff, Landeira-Fernandez, & Brandao, 2001b), responses that resemble those that are displayed by animals when confronted with natural predators (Bandler & Depaulis, 1991; Brandao, Anseloni, Pandossio, De Araujo, & Castilho, 1999; Fernandez de Molina & Hunsperger, 1959; Olds & Olds, 1962). In humans, electrical stimulation of the dPAG has been reported to be extremely unpleasant, with feelings and autonomic changes that are similar to those that occur during a panic

Abbreviations: ANOVA, analysis of variance; SP, Substance P; SPA, spantide; dPAG, dorsal periaqueductal gray matter; BLA, basolateral nucleus of the amygdala; MeA, medial nucleus of the amygdala; CeA, central nucleus of the amygdala; EPM, elevated plus maze; ES, electrical stimulation; PSF, post-stimulation freezing.

* Corresponding author at: Laboratório de Neuropsicofarmacologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto 14090-901, SP, Brazil. Fax: +55 16 3315 4830.
E-mail address: mccarva@usp.br (M.C. Carvalho).

http://dx.doi.org/10.1016/j.nlm.2015.04.001
1074-7427/

attack (Amano et al., 1978; Nashold, Wilson, & Slaughter, 1969). Given the similarities between behavioral responses in rats and symptoms of panic attacks in human, electrical stimulation of the dPAG has been effectively used as a model of panic attacks (Graeff, 1990; Graeff & ZanPlease check whether the given reference 'Graeff, 2002; Jenck, Moreau, & Martin, 1995; Lovick, 2000; Schenberg, Bittencourt, Sudre, & Vargas, 2001).

Although escape and freezing behaviors that are evoked by electrical stimulation of the dPAG have been the main research focus, dPAG post-stimulation freezing has also received growing interest. This sort of behavior emerges immediately after the cessation of electrical stimulation of the dPAG (Carvalho, Santos, Bassi, & Brandao, 2013; Martinez, de Oliveira, & Brandão, 2006; Vianna, Graeff, Brandao, & Landeira-Fernandez, 2001a; Vianna et al., 2001b). In contrast to context-conditioned freezing, dPAG post-stimulation freezing is not context-dependent. The context that is paired with electrical stimulation of the dPAG does not evoke dPAG post-stimulation freezing (Vianna, Borelli, Ferreira-Netto, Macedo, & Brandao, 2003; Vianna et al., 2001a,b). This process involves ascending aversive information that is transmitted to prosencephalic centers, including the amygdala, via the medial forebrain bundle, which allows the animal to evaluate the consequences of aversive situation and aids in the recognition of threatening stimuli in fear-experienced animals (Brandao, Zanoveli, Ruiz-Martinez, Oliveira, & Landeira-Fernandez, 2008).

The inter-relationship between the PAG and amygdala in the expression of unconditioned defensive reactions related to anxiety and fear is well established (Canteras, 2002; Comoli, Ribeiro-Barbosa, & Canteras, 2003; Graeff, 1990; Olds & Olds, 1963; Strauss, Maisonnette, Coimbra, & Zangrossi, 2003; Sullivan, Apergis, Gorman, & LeDoux, 2003). The basolateral nucleus of the amygdala (BLA) is predominantly involved in filtering aversive stimuli. The central (CeA) and medial (MeA) nuclei of the amygdala constitute the output for autonomic and somatic components of defensive reactions via major projections to the hypothalamus and brainstem regions (Canteras, Simerly, & Swanson, 1995; Sah, Faber, Lopez De Armentia, & Power, 2003). The excitability of these output neurons is regulated by a tonic inhibitory influence from the BLA (Nitecka & Ben-Ari, 1987). The amygdala synthesizes stimulus inputs from the environment; depending on the type of threat, it acts in concert with the neural substrate of fear in the dPAG (Fanselow, 1991; Gross & Canteras, 2012; Ledoux, 1994; Zhao, Yang, Walker, & Davis, 2009).

Several studies have shown that the amygdala influences affective behaviors related to fear and anxiety, at least partially through actions of substance P (SP; (Bassi, de Carvalho, & Brandao, 2014; Carvalho et al., 2013; Ebner, Rupniak, Saria, & Singewald, 2004; Smith et al., 1999; Zhao et al., 2009). Substance P is involved in the regulation of such behavioral processes as reinforcement, learning, memory, fear, and anxiety and also mediates stress responses (Chahl, 2006; Ebner et al., 2004; Hasenohrl et al., 2000; Huston & Hasenohrl, 1995). Three neurokinin (NK) receptors have been identified to date: NK1, NK2, and NK3. Despite the fact that SP binds to all three receptor subtypes, it has higher affinity for NK1 receptors (Hokfelt, Bartfai, & Bloom, 2003; Mantyh, 2002; Mussap, Geraghty, & Burcher, 1993; Quartara & Maggi, 1998). Many studies have investigated the participation of the SP/NK1 receptor system in the CeA, MeA, and BLA in the expression of defensive responses in rats (Bassi et al., 2014; Boyce, Smith, Carlson, Hewson, Rigby, O'Donnell, Harrison, & Rupniak, 2001; Ebner et al., 2004; Kertes, Laszlo, Berta, & Lenard, 2009a; Smith et al., 1999; Zhao et al., 2009), but it is not known whether or not this amygdale system modulates the expression of defensive behaviors evoked by electrical stimulation of the dPAG and the fear memory of this aversive stimulation. Thus, the present study investigated the effects of the NK1 receptor antagonist SPA injected into the CeA, MeA, and BLA on freezing, escape, and dPAG post-stimulation freezing responses elicited by electrical stimulation of the dPAG in rats and on the exploratory behavior in the elevated plus maze (EPM) 24 h later. With the EPM test we assessed whether the long lasting aversive consequences of the electrical stimulation of the dPAG can be prevented by NK1-receptors antagonism in the amygdala. According to several studies, this time window is enough for memory consolidation process (Colley & Routtenberg, 1993; Izquierdo & Medina, 1997; Izquierdo et al., 2006).

2. Materials and methods

2.1. Animals

The experiments were performed in accordance with the Brazilian Society of Neuroscience and Behavior (SNeC) Guidelines for the Care and Use of Laboratory Animals. The procedures were approved by the Committee on Animal Research and Ethics (CEUA) of the University of Sao Paulo (no. 09.1.84.54.7). All efforts were made to minimize the number of animals used and their suffering. A total of 48 male Wistar rats, weighing 250–270 g, were obtained from the animal house of the Campus of Ribeirão Preto, University of São Paulo, and housed in a temperature-controlled room (22 ± 1 °C) under a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals were kept in Plexiglas-walled cages and given free access to food and water throughout the experiment. The rats were randomly assigned to one of three surgery groups: BLA, MeA, and CeA. An additional sham group for the CeA (not exposed to electrical stimulation of the dPAG) served as a control for the EPM test.

2.2. Surgery

The animals were intraperitoneally anesthetized with 100 mg/kg ketamine/4.5 mg/kg xylazine (Agener União, Embu-Guaçu, SP, Brazil) and fixed in a stereotaxic frame (David Kopf, Tujunga, CA, USA). The upper incisor bar was set 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. A unilateral guide cannula was implanted over the right BLA, MeA, or CeA. The right amygdala was chosen because the right hemisphere is specialized in emotional behavior, particularly negative affect, compared with the left hemisphere (Adamec, Burton, Shallow, & Budgell, 1999; Michelgard et al., 2007). According to the atlas of Paxinos and Watson (2005) and with bregma serving as the reference point, the coordinates were the following: BLA (anterior/posterior [AP], 2.3 mm; medial/lateral [ML], 5.3 mm; dorsal/ventral [DV], 8.6 mm), MeA (AP, 1.9 mm; ML, 3.4 mm; DV, 8.7 mm), and CeA (AP, 1.9 mm; ML, 4.1 mm; DV, 8.0 mm). A bipolar brain electrode was then implanted into the midbrain aimed at the dPAG. The electrodes were made of two twisted stainless-steel wires, each 50 μm in diameter, that were insulated except at the cross-section of the tip. The electrode was introduced at a 22° angle inclined medially, with lambda serving as the reference for each plane (AP, 0 mm; ML, 1.9 mm; DV, 5.3 mm). For all of the groups, the cannulae and electrode were fixed to the skull with acrylic resin and two stainless-steel anchor screws. Each guide cannula was sealed with a stainless-steel wire to protect it from blockage. At the end of surgery, the animals received an injection of a polyvalent veterinary antibiotic (Pentabiótico, 0.2 ml, intramuscular; Fort Dodge, Campinas, SP, Brazil) and an injection of the antiinflammatory and analgesic flunixin meglumine (Banamine, 2.5 mg/kg, subcutaneous; Schering-Plough, Cotia, SP, Brazil). Afterward, the rats were allowed 5 days to recover from the surgical procedure.