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Regulation of conditioned and unconditioned fear in rats by 5-HT$_{1A}$ receptors in the dorsal periaqueductal gray

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Abstract

Studies on the involvement of 5-HT$_1$-mediated mechanisms in the dorsal periaqueductal gray (dPAG) of animals with past stressful experiences have not been conducted so far. We investigated the role of 5-HT$_1$ receptors in the dPAG of rats previously submitted to contextual fear conditioning. Defensive behaviors induced by activation of the dPAG were assessed by measuring the lowest electric current applied to this structure (threshold) able to produce freezing and escape responses during testing sessions of contextual fear conditioning, in which animals were placed in a context previously paired to footshocks. The 5-HT$_{1A}$ function of the dPAG was evaluated by local injections of 8-OH-DPAT (4 and 8 nmol/0.2 μL) and WAY-100635 (10 nmol/0.2 μL), selective agonist and antagonist of 5-HT$_{1A}$ receptors, respectively. In accordance with previous studies, 8-OH-DPAT increased aversive thresholds (antiaversive effects) but injections of WAY 100635 into the dPAG did not produce significant effects on the aversive thresholds in naive rats. However, the aversive thresholds of animals exhibiting contextual fear remained unchanged with both treatments. Moreover, 8-OH-DPAT and WAY 100635 did not change the dPAG post-stimulation freezing. The present results suggest that the stressful experience of being fear conditioned has an effect on the role of the 5-HT$_{1A}$ receptors in mediating unconditioned fear. Also, the reduction in the regulation of the defensive behaviors by 5-HT$_{1A}$-mediated mechanisms in the dPAG of these animals may underlie the stress precipitated psychopathology associated with the neural substrates of aversion of the dPAG.

Keywords: 5-HT1A receptors; Dorsal periaqueductal gray; 8-OH-DPAT; WAY-100635; Unconditioned fear; Contextual fear conditioning

1. Introduction

A brain aversion system made up of the dorsal PAG (dPAG), dorsomedial hypothalamus and amygdala has been associated with unconditioned fear (Graeff et al., 1986; Graeff 1990, 2004). Panic attacks have been related to the deregulation of the dPAG (Graeff et al., 1986; Graeff 1990, 2004), dorsomedial hypothalamus (Johnson and Shekhar 2006) and bilateral temporal poles (Reiman et al., 1989). The electrical or chemical stimulation of the dPAG causes a characteristic pattern of active defense reaction, with alertness, freezing and escape responses, along with autonomic changes that resemble this anxiety disorder (Graeff et al., 1986; Brandão et al., 2003; Borelli et al., 2004; Graeff 2004). It has also been suggested that another system comprised of the hippocampus, amygdala and ventrolateral periaqueductal gray (vPAG) is related to conditioned fear (Gray and McNaughton 2000). Malfunctioning of this system appears to be associated with generalized anxiety disorder (Gray and McNaughton 2000). It seems that these two aversive systems are not entirely independent and some interaction between them may exist. For example, it has been proposed that anxiety states generated at the amygdala level may inhibit panic attacks elicited by activation of the neural substrates of aversion in the dPAG (Graeff 2004). In line with this notion, a recent study has shown that rats exposed to conditioned fear stimuli

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present a reduction in the unconditioned fear when concomitantly stimulated in the dPAG at the escape threshold (Magierek et al., 2004).

Defensive behaviors are hierarchically organized and different behaviors within this class are provoked by aversive stimuli of different intensities or distances from the predators (Blanchard and Blanchard, 1990; Schenberg et al., 2005; Santos et al., 2005). In this context, it has been shown that there are two types of freezing behavior induced by direct stimulation of the PAG; one bound to the stimulus and another one that appears when this stimulation terminates (Vianna et al., 2001). The first freezing appears as a preparatory response for escape (immediate defensive responses) and the post-stimulation freezing is related to the processing of aversive information that is relayed to higher structures (Borelli et al., 2005a; Ruiz-Martinez et al., 2006). Considering the premise that different anxiety disorders might be related to distinct defensive systems, which in turn might involve specific neural mechanism, we have proposed that the dPAG-evoked freezing is related to panic attacks whereas the post-stimulation freezing may be related to agoraphobia-like responses associated with panic disorder (Oliveira et al., 2007). The 5-HT (5-hydoxytryptamine) system is highly involved in the modulatory systems underlying generalized anxiety disorder and panic attacks. Several studies have been conducted to disclose how the multiple 5-HT receptors modulate the aversive states induced by stimulation of the dPAG (Jenck et al., 1989; Brandão et al., 1991; Graeff 2004). The dPAG is rich in 5-HT immunoreactive nerve terminals from serotonin-containing cell bodies located mainly in the dorsal raphe nucleus (Clements et al., 1985; Beitz et al., 1986; Lovick et al., 2000). One prominent function of serotonin is to regulate aversive states induced by electrical or chemical stimulation of the dPAG (Graeff et al., 1986; Graeff 2004). Electrophysiological studies have found that the dPAG is rich in 5-HT_{1A} and 5-HT_2 receptors subtypes (Brandão et al., 1991; Lovick 1993). It has been shown that the activation of these receptors has an inhibitory effect on the neural substrates of aversion in the dPAG (Graeff et al., 1986; Coimbra and Brandão 1997; Castilho and Brandão 2001). Recently, it has been found that animals with previous aversive experience and injected locally with 5-HT_2 antagonists into the dPAG show an enhanced sensitivity to the electrical stimulation of this structure in comparison with naïve animals, in which intra-dPAG injections of 5-HT_2 antagonists do not change the freezing and escape thresholds (Oliveira et al., 2007). Taking into account that 5-HT_{1A} and 5-HT_2-mediated mechanisms play a cooperative role in the regulation of fear in the dPAG (Nogueira and Graeff 1995; Zanoveli et al., 2003), it is of relevance to know whether the fear generated at the level of the dPAG in animals with previous experience with stressful events is also regulated by 5-HT_{1A} receptors. In this study, we evaluated the involvement of the 5-HT_{1A}-mediated mechanisms of the dPAG of rats submitted to the electrical stimulation of the dPAG at the freezing and escape thresholds before or after contextual fear conditioning (CFC). Conditioning was evaluated in a neutral context or in the presence of the contextual cues previously paired with footshock. The 5-HT_{1A} function was assessed by local injections into the dPAG of 8-OH-DPAT (DP) and WAY 100635 (WAY), selective agonist and antagonist of 5-HT_{1A} receptors, respectively (Mundey et al., 1996; Fornal et al., 1996; Fletcher et al., 1996; Ahlenius et al., 1999; Avanzi and Brandão 2001; Borelli et al., 2005b).

2. Methods

2.1. Animals

Eighty-six male Wistar rats weighing 250–280 g from the animal house of the Campus of Ribeirão Preto of the University of São Paulo were housed in a temperature-controlled (22± 1 °C) room and maintained on a 12-h light/12-h dark cycle (0700–1900 lights on). These animals were maintained in pairs in Plexiglas-walled cages and given free access to food and water throughout the experiment. The experiments were carried out according to the Brazilian Society of Neuroscience and Behavior Guidelines for Care and Use of Laboratory Animals.

2.2. Surgery

The animals were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame (David Kopf, Tujunga, CA). The rapid induction and recovery, adequate surgical plane of anesthesia, and lack of complications make this anesthetic effective and simple to use in rodents (Papaioannou and Fox, 1993). The upper incisor bar was set at 3.3 mm below the interaural line such that the skull was horizontal between bregma and lambda. A chemitrode made of a stainless steel guide cannula (o.d. 0.6 mm, i.d. 0.4 mm) glued to a brain electrode was aimed at the dPAG. The electrode was made of stainless steel wire, 160 μm in diameter, insulated except at the cross-section, and was introduced with a 16° angle and directed towards midline, with lambda serving as the reference for each plane: antero-posterior (AP)=0.0 mm; medio-lateral (ML)=±1.9 mm; and dorso-ventral (DV)=5.1 mm, according to Paxinos and Watson (1997). For all groups the electrode and cannula were fixed to the skull by means of acrylic resin and two stainless steel screws. The electrode wire was connected to a male pin so that it could be plugged into an amphenol socket at the end of a flexible electrical cable and used for brain stimulation. At the end of the surgery each guide cannula was sealed with a stainless steel wire to protect it from obstruction.

2.3. Microinjection procedure

The injection needle was a thin dental needle (0.3 mm, o.d.) connected to a 5 μL Hamilton syringe by means of a polyethylene tube. The injection needle was introduced through the guide cannula until its lower end was 1 mm below the guide cannula. A total volume of 0.2 μL for a 1 min duration was injected into the dPAG driven by an infusion pump (Harvard Apparatus, South Natick, MA, U.S.A.). The displacement of an air bubble inside the polyethylene (PE-10; Becton-Dickinson, Franklin Lakes, NJ, U.S.A.) catheter connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The needle was held in place for an additional 1 min to maximize diffusion away from the needle tip. We have previously shown that the volume of 0.2 μl has a diameter of diffusion circumscribed to the site of injection (Ferreira-Netto et al., 2007).