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INVOLVEMENT OF DORSAL HIPPOCAMPUS GLUTAMATERGIC AND NITRERGIC NEUROTRANSMISSION IN AUTONOMIC RESPONSES EVOKED BY ACUTE RESTRAINT STRESS IN RATS

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Abstract—The dorsal hippocampus (DH) is a structure of the limbic system that is involved in emotional, learning and memory processes. There is evidence indicating that the DH modulates cardiovascular correlates of behavioral responses to stressful stimuli. Acute restraint stress (RS) is an unavoidable stress situation that evokes marked and sustained autonomic changes, which are characterized by elevated blood pressure (BP), intense heart rate (HR) increase and a decrease in cutaneous temperature. In the present study, we investigated the involvement of an *N*-methyl-D-aspartate (NMDA) glutamate receptor/nitric oxide (NO) pathway of the DH in the modulation of autonomic (arterial BP, HR and tail skin temperature) responses evoked by RS in rats. Bilateral microinjection of the NMDA receptor antagonist AP-7 (10 nmol/500 nL) into the DH attenuated RS-evoked autonomic responses. Moreover, RS evoked an increase in the content of NO_2/NO_3 in the DH, which are products of the spontaneous oxidation of NO under physiological conditions that can provide an indirect measurement of NO production. Bilateral microinjection of *N*-propyl-L-arginine (0.1 nmol/500 nL; *N*-propyl, a neuronal NO synthase (nNOS) inhibitor) or carboxy-PTIO (2 nmol/500 nL; c-PTIO, an NO scavenger) into the DH also attenuated autonomic responses evoked by RS. Therefore, our findings suggest that a glutamatergic system present in the DH is involved in the autonomic modulation during RS, acting via NMDA receptors and nNOS activation. Furthermore, the present results suggest that NMDA receptor/nNO activation has a facilitatory influence on RS-evoked autonomic responses.

Key words: hippocampus, restraint stress, glutamate, nitric oxide.

INTRODUCTION

The hippocampus is part of the limbic system and is directly involved in the modulation of emotional, learning and memory processes (Maclean, 1952; Siegel and Flynn, 1968; Papez, 1995). Anatomically, the hippocampus includes CA1, CA2, CA3, and dentate gyrus sub regions with the pattern of efferent and afferent connectivity changing between dorsal and ventral hippocampus (Brodal, 1998). Petrovich and co-workers have divided the hippocampus in terms of afferent connectivity into five parallel zones with Zone 1 encompassing the dorsal half of CA1 and Zones 2–5 the ventral CA1/subiculum (Petrovich et al., 2001).

In many mammals, electrical stimulation of the hippocampus induces behavioral changes and defensive reactions, such as attack responses (Siegel and Flynn, 1968), attention (Kaada et al., 1953), agitation, and growl escape reactions (Maclean and Delgado, 1953), as well as lightheadedness and confusion (Maclean, 1957). It has been reported that autonomic responses caused by electrical stimulation of the dorsal hippocampus (DH), which are characterized by respiratory inhibition (Kaada and Jasper, 1952; Andy and Akert, 1955; Liberson and Akert, 1955; Anand and Dua, 1956), either increased blood pressure (BP) or decreased heart rate (HR) (Smith, 1944; Anand and Dua, 1956) and increased sympathetic activity (Carlson et al., 1941; Andy and Akert, 1953). In addition, DH chemical stimulation using glutamate caused a reduction in BP and HR that was similar to those evoked by its electrical stimulation in rats (Ruit and Neafsey, 1988), suggesting that these cardiovascular responses are due to activation of hippocampal neurons and not to stimulation of fibers of passage. Thus, these studies indicate an involvement of the hippocampus, especially its dorsal portion, in the modulation of autonomic activity, and in particular in effects on the cardiovascular system.

Restraint stress (RS) is a widely used experimental model of acute stress, being an inescapable stressor

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Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; AP-7, 2-amino-7-phosphonoheptanoic acid; BNST, bed nucleus of stria terminalis; BP, blood pressure; BSA, bovine serum albumin; c-PTIO, carboxy-PTIO(S)-3-carboxy-4-hydroxyphenylglicine; DH, dorsal hippocampus; HR, heart rate; L-glu, glutamate; LSA, lateral septal area; MAP, mean arterial pressure; MPFC, medial prefrontal cortex; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; *N*-propyl, N^{ω}-propyl-L-arginine; RS, restraint stress; SEM, standard error of mean.

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stimulus where animals are placed into a tube of plastic or metal, which restricts their movements (Conti et al., 2001; Yoshino et al., 2005), resulting in several autonomic responses. These autonomic responses include: mean arterial pressure (MAP) and HR increases (Taylor et al., 1989; Kubo et al., 2002; Tavares et al., 2007; Busnardo et al., 2010), skeletal muscle vasodilatation and cutaneous vasoconstriction, which are accompanied by a rapid skin temperature drop and are followed by body temperature increases (Blessing and Seaman, 2003; Vianna and Carrive, 2005).

The DH has connections to several brain structures that are involved in the modulation of responses associated with RS, such as the medial prefrontal cortex (MPFC), the bed nucleus of stria terminalis (BNST), the lateral septal area (LSA) and the medial amygdala (MeA) (Kubo et al., 2002; Tavares and Correa, 2006; Fortaleza et al., 2009; Crestani et al., 2010). Previous work has shown that during RS, neurons are activated in the DH (Chen et al., 2006), suggesting its involvement in the modulation of these RS-evoked responses. Furthermore, results from our laboratory (unpublished) indicate that inhibition of neurotransmission in the DH reduces the magnitude of RS-evoked autonomic responses, such as increased BP and HR, as well as the decreased skin temperature caused by RS, suggesting that the DH plays an important role in the modulation of autonomic responses associated with RS. However, which neurotransmitters in the DH are involved in the modulation of RS-evoked autonomic responses has not yet been identified.

Glutamate (L-glu) is an important central nervous system (CNS) neurotransmitter (Fleck et al., 1993; Khodorov, 2004), which is involved in the modulation of the autonomic system. It has been demonstrated that administration of L-glu into the DH of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) caused decrease in BP, which was blocked by prior administration of AP-5 (*N*-methyl-D-aspartate (NMDA) receptors antagonist). Furthermore, administration of CNQX (non-NMDA receptors antagonist), did not change the depressor response caused by L-glu (Wang and Ingenito, 1994) suggesting that the glutamatergic system into the DH through the NMDA receptors modulates cardiovascular responses. In addition, Moghaddam (1993) demonstrated that during RS there is an increased release of L-glu in the DH of rats. These results support a possible involvement of the DH glutamatergic system in the modulation of responses associated with RS.

Nitric oxide (NO), a free radical gas, is synthesized from L-arginine by different enzymes (Garthwaite et al., 1988, 1989). In the CNS the major isoform of these enzymes is the neuronal form (neuronal nitric oxide synthase (nNOS)), which is present in hippocampal neurons (Blackshaw et al., 2003), and whose activity is regulated by Ca^{2+} influx induced by the activation of glutamate receptors, mainly of NMDA receptors (Garthwaite et al., 1989). Joca and Guimaraes (2006) demonstrated that inhibition of nNOS in the DH of rats caused an anxiolytic effect, which was characterized by a reduction in the immobility observed in the Forced Swim Test model. In the hippocampus, the activation of NMDA receptors caused increased formation of NO (Garthwaite et al., 1989), suggesting an interaction between NMDA receptors and NO formation in this structure.

Considering the above evidence, an involvement of both the glutamatergic and nitrergic systems of the DH in the modulation of RS-evoked cardiovascular responses can be proposed. In the present study, we used the RS model to test the hypothesis that the glutamatergic/nitrergic systems in the DH modulate stress-evoked autonomic responses.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats weighing 230–250 g were used (total $n = 57$). The animals were kept in the animal care unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. The rats were housed individually in plastic cages with free access to food and water under a 12-h light/dark cycle (lights on at 06:30 h). Experimental procedures were carried out following protocols approved by the Ethics Review Committee of the School of Medicine of Ribeirao Preto (protocol n. 063/2010), which complies with the guidelines laid down by the National Institutes of Health (NIH, Guide for the Care and Use of Laboratory Animals).

Surgery procedure

Five days before the experiment, the rats were anesthetized with 2,2,2-tribromoethanol (Sigma, St. Louis, MO, USA) (250 g/kg, i.p.). After scalp anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulae (0.55 mm) were implanted bilaterally into the DH using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Stereotaxic coordinates for cannulae implantation in the DH were chosen based on the rat brain atlas of Paxinos and Watson (1997): AP: −4 mm from bregma, L: +2.6 mm from the medial suture, V: −2.1 mm from the skull. The incisor bar position was set at −2.5 mm. Cannulae were fixed to the skull with dental cement and one metal screw.

Twenty-four hours before the RS session, a polyethylene catheter was implanted into the left femoral artery for BP recording. The arterial catheter consisted of a piece of PE-10 tubing (4.0 cm) heat-bonded to a longer segment of PE-50 tubing (10–12 cm). The catheter was filled with 0.3% heparin (5000 UI/ml) in sterile saline (0.9% NaCl). The PE-10 piece was introduced into the femoral artery until the tip reached the aorta. The catheter was secured in position with thread, and the PE-50 part was passed under the skin to be extruded at the dorsum of the animals. The catheter was extruded at the dorsum and attached to the skin. After each surgery, animals were treated with a poly-antibiotic combination of streptomycins and penicillins i.m. (Pentabiotico, Fort Dodge, Brazil) to