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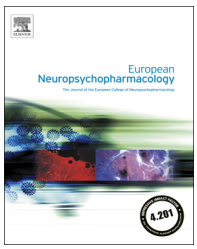

Paraventricular nucleus of the hypothalamus glutamate neurotransmission modulates autonomic, neuroendocrine and behavioral responses to acute restraint stress in rats

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Abstract
In the present study, the involvement of paraventricular nucleus of the hypothalamus (PVN) glutamate receptors in the modulation of autonomic (arterial blood pressure, heart rate and tail skin temperature) and neuroendocrine (plasma corticosterone) responses and behavioral consequences evoked by the acute restraint stress in rats was investigated. The bilateral microinjection of the selective non-NMDA glutamate receptor antagonist NBQX (2 nmol/ 100 nL) into the PVN reduced the arterial pressure increase as well as the fall in the tail cutaneous temperature induced by the restraint stress, without affecting the stress-induced tachycardiac response. On the other hand, the pretreatment of the PVN with the selective NMDA glutamate receptor antagonist LY235959 (2 nmol/ 100 nL) was able to increase the stress-evoked pressor and tachycardiac response, without affecting the fall in the cutaneous tail temperature. The treatment of the PVN with LY235959 also reduced the increase in plasma corticosterone levels during stress and inhibited the anxiogenic-like effect observed in the elevated plus-maze 24 h after the restraint session. The present results show that NMDA and non-NMDA receptors in the PVN differently modulate responses associated to stress. The PVN glutamate neurotransmission, via non-NMDA receptors, has a facilitatory influence on stress-evoked autonomic responses. On the other hand, the present data point to an inhibitory role of PVN NMDA receptors on the cardiovascular responses to stress. Moreover, our findings also indicate an involvement of PVN NMDA glutamate receptors in the mediation of the plasma corticosterone response as well as in the delayed emotional consequences induced by the restraint stress.

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1. Introduction

The paraventricular nucleus of the hypothalamus (PVN) is a complex nucleus, which includes magnocellular neurons that regulate the neurohypophiseal function and the secretion of vasopressin as well as oxytocin into the systemic circulation, and parvocellular neurons that are mainly concerned with the autonomic and endocrine control (Kiss, 1988; Swanson and Sawchenko, 1980, 1983). Specific parvocellular neurons were reported to project either to the median eminence (hypophysiotrophic neurons) or to autonomic targets in the brainstem and spinal cord (preautonomic neurons) (Kuypers and Maisky, 1975; Swanson and Kuypers, 1980). Furthermore, the PVN is also connected with limbic structures (Canteras et al., 1995; Ongur et al., 1998; Risold and Swanson, 1997), and its role in controlling several behavioral responses has been proposed (Blume et al., 2008; Leibowitz, 1978; Norrholm et al., 2005). Furthermore, clinical evidence indicates that morphological changes in the PVN are an important neurobiological substrate for psychiatric disorders (Manaye et al., 2005).

Stress has been shown to be involved in numerous pathologies such as cardiovascular diseases and psychiatric disorders (Post, 1992). The maintenance of the homeostasis during stress requires an appropriate and coordinated set of physiological responses (Dampney et al., 2008; Ulrich-Lai and Herman, 2009). Hormonal and autonomic responses are inserted in the physiological component of behavioral responses to stressful stimuli. Autonomic responses are characterized by an increase in both the blood pressure and the heart rate (HR) (Barron and Van Loon, 1989; Bhatnagar et al., 1998; Irvine et al., 1997; McDougall et al., 2000; Tavares and Correa, 2006). Furthermore, aversive stimuli evoke hemodynamic changes that include vasodilatation in skeletal muscles and a vasoconstriction in the splanchnic, renal and cutaneal territories (Blessing and Seaman, 2003; Vianna and Carrive, 2005; Zhang et al., 1996). The cutaneous vasoconstriction observed during stress leads to a rapid drop in the temperature of the rat-tail skin (Busnardo et al., 2010b; Vianna and Carrive, 2005). Stressful stimuli also evoke an activation of the hypothalamic–pituitary–adrenocortical (HPA) axis, which results in increased levels of circulating glucocorticoids (Hsu et al., 2007; Ulrich-Lai and Herman, 2009). In addition to physiological adjustments, animals submitted to an aversive stimulus also develop emotional changes such as the anxiogenic effect observed in the elevated plus-maze (EPM) 24 h after the stress (Guimaraes et al., 1993; Padovan and Guimaraes, 2000; Reis et al., 2011).

It has been reported that PVN neurons are activated during the exposure to stressful stimuli (Day et al., 2005; Girotti et al., 2006; Imaki et al., 1998), and the role played by the PVN in the modulation of stress-evoked hormonal responses is well established (Evanson et al., 2009; Morton et al., 1989; Ziegler and Herman, 2000). Moreover, previous results from our group indicated that the inhibition of the PVN neurotransmission by a local injection of the unspecific neurotransmission blocker $CoCl_2$ reduced the pressor response, the increase in the plasma corticosterone level and the fall in the tail skin temperature evoked by acute restraint stress (Busnardo et al., 2010b). Although the above findings indicate that a local neurotransmission in the PVN is involved in the modulation of responses to stress, its role has not yet been fully elucidated. Moreover, the involvement of the PVN in the expression of the delayed anxiogenic effect induced by the restraint stress has not yet been investigated.

Glutamate is thought to be an important excitatory signal to PVN neurons (Herman et al., 2004; van den Pol et al., 1990). Several studies have documented high levels of glutamate-immunoreactive synapses and glutamate receptors in the PVN (Crestani et al., 2010; Curras-Collazo et al., 2000; Herman et al., 2000; Khan et al., 2000; Meeker et al., 1994; Singewald and Philippu, 1996; van den Pol et al., 1990). Glutamate acts via two major classes of receptors, namely ionotropic and metabotropic receptors (Hollmann and Heinemann, 1994). The ionotropic receptors are classified as N-methyl-D-aspartate (NMDA) and non-NMDA receptors, according to agonist selectivity and amino acid homology. The non-NMDA receptors are further divided into a-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate (KA) receptor subtypes (Hollmann and Heinemann, 1994). The activation of NMDA glutamate receptor in the PVN increases the renal sympathetic nerve activity, arterial pressure and HR, as well as modulates the baroreflex activity (Busnardo et al., 2009; Crestani et al., 2010; Li et al., 2006). Local non-NMDA glutamate receptors in the PVN may also control the cardiovascular function through the modulation of the vasopressin release into the circulation (Busnardo et al., 2009). Moreover, PVN glutamate receptors also control the neuroendocrine activity (Herman et al., 2004; van den Pol et al., 1990; Ziegler and Herman, 2000). Nonetheless, the roles played by PVN glutamate receptors, and the subtype of receptors involved in the stress-evoked responses are poorly understood.

Therefore, in the present study, we evaluated the involvement of local glutamate ionotropic receptors within the PVN on the autonomic (arterial pressure, heart rate and tail skin temperature), neuroendocrine (plasma corticosterone) and behavioral (delayed anxiogenic effect) response evoked in rats submitted to acute restraint stress. For this purpose, the PVN was bilaterally treated with either the selective NMDA glutamate receptor antagonist LY235959 or the selective non-NMDA glutamate receptor antagonist NBQX, and we verified their effects on the stress-evoked physiological and behavioral responses.

2. Experimental procedures

2.1. Ethical approval

Housing conditions and experimental procedures were approved by the University of São Paulo Animal Ethical Committee, which complies with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society.

2.2. Subjects

Sixty-two male Wistar rats weighing approximately 250 g were used in the present experiment. Animals were housed in plastic cages in a temperature-controlled room (25 °C) in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Animals were kept under a 12:12 h light–dark cycle (lights on between 06:00 am and 6:00 pm). Animals had free access to water and standard laboratory food, except during the experimental period.