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NMDA AND NON-NMDA GLUTAMATE RECEPTORS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS MODULATE DIFFERENT STAGES OF HEMORRHAGE-EVOKED CARDIOVASCULAR RESPONSES IN RATS

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Abstract—Here we report the involvement of *N*-Methyl-D-Aspartate (NMDA) and non-NMDA glutamate receptors from the paraventricular nucleus of the hypothalamus (PVN) in the mediation of cardiovascular changes observed during hemorrhage and post-bleeding periods. In addition, the present study provides further evidence of the involvement of circulating vasopressin and cardiac sympathetic activity in cardiovascular responses to hemorrhage. Systemic treatment with the V_1-vasopressin receptor antagonist dTyr $(CH_2)_5$(Me)AVP (50 μg/kg, i.v.) increased the latency to the onset of hypotension during hemorrhage and slowed post-bleeding recovery of blood pressure. Systemic treatment with the β_1-adrenergic receptor antagonist atenolol (1 mg/kg, i.v.) also increased the latency to the onset of hypotension during hemorrhage. Moreover, atenolol reversed the hemorrhage-induced tachycardia into bradycardia. Bilateral microinjection of the selective NMDA glutamate receptor antagonist LY235959 (2 nmol/100 nL) into the PVN blocked the hypotensive response to hemorrhage and reduced the tachycardia during the post-hemorrhage period. Systemic treatment with dTyr$(CH_2)_5$(Me)AVP inhibited the effect of LY235959 on hemorrhage-induced hypotension, without affecting the post-bleeding tachycardia. PVN treatment with the selective non-NMDA receptor antagonist NBQX (2 nmol/100 nL) reduced the recovery of blood pressure to normal levels in the post-bleeding phase and reduced hemorrhage-induced tachycardia. Combined blockade of both NMDA and non-NMDA glutamate receptors in the PVN completely abolished the hypotensive response in the hemorrhage period and reduced the tachycardiac response in the post-hemorrhage period. These results indicate that local PVN glutamate neurotransmission is involved in the neural pathway mediating cardiovascular responses to hemorrhage, via an integrated control involving autonomic nervous system activity and vasopressin release into the circulation.

Key words: cardiovascular system, glutamate neurotransmission, hemorrhagic shock, paraventricular nucleus of hypothalamus, sympathetic activity, vasopressin.

INTRODUCTION

Hemorrhagic shock is a serious complication that may occur as a result of trauma, surgery, gastrointestinal disease, and anticoagulant therapy (Levi et al., 2002; Siqueira and Schmidt, 2003; Gutierrez et al., 2004). Tissue perfusion is reduced during hemorrhagic shock, due to a loss of circulating blood volume (Garrioch, 2004), which can lead to death. Indeed, hemorrhage is implicated in millions of deaths worldwide (Levi et al., 2002; Siqueira and Schmidt, 2003; Gutierrez et al., 2004), and is the leading cause of trauma-associated deaths (Bellamy, 1984; Abjean, 1986; Moore et al., 1996; Cuschieri et al., 2012; Malinoski et al., 2012). Early emergency care and treatment for severe trauma are extremely important, because about 40% of trauma-induced deaths occur 5–30 min after trauma (Cherkas, 2011; Liu et al., 2013).

Blood loss triggers a complex set of neural and hormonal responses intended to preserve blood flow to vital organs and to reduce tissue energy consumption (Garrioch, 2004). These responses are triggered by arterial baroreceptors and atrial volume receptors, which transmit information about changes in blood volume and pressure to the nucleus tractus solitarius (NTS) (Loewy, 1990; Jaworski and Blair, 2004). These sensory signals are transmitted to supramedullary structures through ascending projections that originate in the NTS and ventrolateral regions of the medulla (Loewy, 1990). However, the central organization of neural networks responsible for the nervous and hormonal regulation of blood pressure during hemorrhage remains unclear.

The paraventricular nucleus of the hypothalamus (PVN) is comprised of magnocellular neurosecretory

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Abbreviations: ACF, artificial cerebrospinal fluid; ACTH, adrenocorticotropin; AVP, vasopressin; CMM, caudal midline medulla; HR, heart rate; MAP, mean arterial pressure; NMDA, *N*-Methyl-D-Aspartate; NTS, nucleus tractus solitarius; PAP, pulsatile arterial pressure; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; vlPAG, ventrolateral column of the periaqueductal gray.

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neurons as well as preautonomic and neuroendocrine parvocellular neurons (Swanson and Kuypers, 1980). The preautonomic parvocellular region of the PVN contains neurons projecting directly to the intermediolateral cell column of the thoracolumbar spinal cord and the rostral ventrolateral medulla (RVLM), which constitute important regions involved in the regulation and generation of sympathetic activity (Kuypers and Maisky, 1975; Shafton et al., 1998; Pyner et al., 2001). Magnocellular neurons synthesize the neurohypophysial hormones oxytocin and vasopressin, which are axonally transported down to the neurohypophysis and subsequently secreted into the bloodstream (Bisset and Chowdrey, 1988). Labor and milk ejection involve an oxytocin release, whereas vasopressin is released in response to decreased blood pressure or blood volume and increased plasma osmolality (Cunningham and Sawchenko, 1991; Renaud and Bourque, 1991; Cunningham et al., 2002, 2004).

It has been reported that hemorrhage causes a massive activation of both magnocellular neurosecretory and parvocellular neurons in the PVN (Roberts et al., 1993; Li and Dampney, 1994; Petrov et al., 1995; Badoer, 1996; Badoer and Merolli, 1998; Krukoff, 1999). Accordingly, previous studies reported an involvement of the PVN in the elevation of circulating corticosterone and adrenocorticotropin (ACTH) induced by hemorrhagic stimuli (Darlington et al., 1988; Blair et al., 1998). However, the possible role of this hypothalamic nucleus in the control of cardiovascular function during hemorrhage is poorly understood (Darlington et al., 1988; Blair et al., 1998).

We have previously reported that glutamate neurotransmission is an important local signaling mechanism in the PVN, being involved in the regulation of cardiovascular function (Busnardo et al., 2009, 2013). We observed that PVN stimulation with L-glutamate (L-glu) caused pressor and tachycardiac responses that were mediated by activation of *N*-Methyl-D-Aspartate (NMDA) glutamate receptors and subsequent sympathetic stimulation. When NMDA receptors were blocked, the microinjection of L-glu into the PVN caused pressor and bradycardiac responses that were mediated by activation of local non-NMDA glutamate receptors with a vasopressin release into the circulation (Busnardo et al., 2009). Therefore, the control exerted by PVN glutamate neurotransmission on the cardiovascular system is mediated by both neural (sympathetic) and humoral (vasopressin) factors. Nevertheless, to the best of our knowledge, the involvement of PVN glutamate neurotransmission in the control of cardiovascular responses evoked by hemorrhagic stimuli has never been evaluated. Thus, our hypothesis is that glutamate neurotransmission in the PVN modulates the cardiovascular system during hemorrhage by integrating sympathetic and vasopressin mechanisms.

EXPERIMENTAL PROCEDURES

Subjects

Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (Protocol number 075/2015), which comply with requirements established by the National Institutes of Health (NIH). 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. Animals were kept under a 12:12-h light–dark cycle (lights on between 06:00 and 18:00 h). Animals had free access to water and standard laboratory food, except during the experimental period.

Surgical preparation

To implant guide cannulas bilaterally in the PVN, five days before the trial, the animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and their heads fixed to a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The skull was surgically exposed and trepanned with a dental drill at 1.9 mm from the medial line and 7.2 mm anterior to the interaural line (Paxinos and Watson, 1986). Bilateral stainless steel guide cannulas (24G, 13 mm-long) were lowered 8 mm from the skull, at a 12° angle to both sides. Guide cannulas were positioned 1 mm above the intended stimulation sites, and fixed to the skull with a metal screw and dental cement. After surgery, the animals received a poly-antibiotic formulation with streptomycins and penicillins to prevent infection (80.000 UI, i.m.) and the nonsteroidal anti-inflammatory flunixine meglumine for post operation analgesia (2.5 mg/kg, s.c.).

Twenty-four hours before the experiment, animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and polyethylene catheters were implanted into the right femoral artery for cardiovascular recording and into the left femoral artery for blood withdrawal (hemorrhage) and drug injection when it was necessary. The catheters were exposed on the dorsum of the animals and attached to the skin, allowing cardiovascular recording and blood withdrawal of unanesthetized rats in their own cage. Flunixine meglumine (2.5 mg/kg s.c.) was used for post operation analgesia.

Cardiovascular recording

The catheter was connected to a pressure transducer and pulsatile arterial pressure (PAP) was recorded using a HP-7754A pre-amplifier (Hewlett Packard, Palo Alto, CA, USA) and an acquisition board (MP100A, Biopac Systems Inc., Goleta, Santa Barbara, CA, USA) connected to a personal computer. Mean arterial pressure (MAP) and heart rate (HR) values were derived from PAP recordings using the Acknowledge III software (Biopac, USA). MAP was calculated according to the equation: diastolic pressure + (systolic − diastolic)/3. HR (bpm) was calculated from PAP peak intervals integrated every 6 s.

Hemorrhage

All animals underwent a fixed volume hemorrhage of 24 mL/kg (estimated as 30% of total blood volume) over