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Research Report

Central cholinergic blockade reduces the pressor response to L-glutamate into the rostral ventrolateral medullary pressor area

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ABSTRACT

Injections of the excitatory amino acid L-glutamate (L-glu) into the rostral ventrolateral medulla (RVLM) directly activate the sympathetic nervous system and increase mean arterial pressure (MAP). A previous study showed that lesions of the anteroventral third ventricle region in the forebrain reduced the pressor response to L-glu into the RVLM. In the present study we investigated the effects produced by injections of atropine (cholinergic antagonist) into the lateral ventricle (LV) on the pressor responses produced by L-glu into the RVLM. Male Holtzman rats (280–320 g, $n=5$ to 12/group) with stainless steel cannulas implanted into the RVLM, LV or 4th ventricle (4th V) were used. MAP and heart rate (HR) were recorded in unanesthetized rats. After saline into the LV, injections of L-glu (5 nmol/100 nl) into the RVLM increased MAP (51±4 mm Hg) without changes in HR. Atropine (4 nmol/1 μl) injected into the LV reduced the pressor responses to L-glu into the RVLM (36±5 mm Hg). However, atropine at the same dose into the 4th V or directly into the RVLM did not modify the pressor responses to L-glu into the RVLM (45±2 and 49±4 mm Hg, respectively, vs. control: 50±4 mm Hg). Central cholinergic blockade did not affect baro and chemoreflex nor the basal MAP and HR. The results suggest that cholinergic mechanisms probably from forebrain facilitate or modulate the pressor responses to L-glu into the RVLM. The mechanism is activated by acetylcholine in the forebrain, however, the neurotransmitter released in the RVLM to facilitate the effects of glutamate is not acetylcholine.

1. Introduction

The rostral ventrolateral medulla (RVLM) localized ventrally to the nucleus ambiguus is the main central site that sends projections to activate sympathetic pre-ganglionic neurons in the intermediolateral column (IML) in the spinal cord (Araújo et al., 1999; Arneric et al., 1990; Brown and Guyenet, 1984; de Paula and Machado, 2001; Guertzenstein and Silver, 1974; Guyenet, 1990; Willette et al., 1983). The activity of the RVLM neurons is influenced by excitatory and inhibitory mechanisms (Dampney et al., 2000, 2003). One of the main excitatory neurotransmitters in the RVLM is the excitatory amino acid L-glutamate (Sved and

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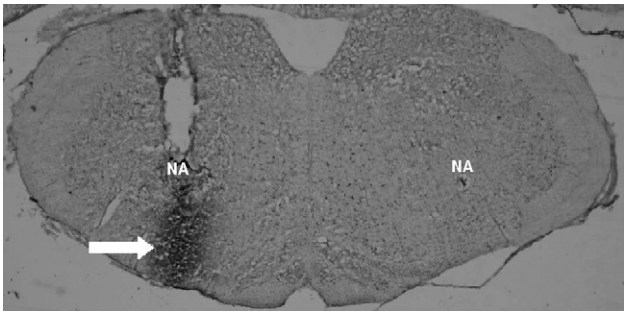

Fig. 1 – Photomicrograph of a brain slice from one rat representative of the groups studied showing (arrow) the typical site of injection into the RVLM. NA = nucleus ambiguus.

Table 1 – Changes in MAP and HR induced by injections of L-glutamate into the RVLM after saline or atropine into the 4th V

Treatment	Δ MAP	Δ HR
Saline 4th V + saline RVLM	1 ± 1	1 ± 1
Saline 4th V + L-glutamate RVLM	46 ± 2*	10 ± 21
Atropine 4th V + L-glutamate RVLM	45 ± 2*	3 ± 18
Saline 4th V + carbachol 4th V	46 ± 3	−48 ± 4
Atropine 4th V + carbachol 4th V	14 ± 1+	−16 ± 18

L-glutamate (5 nmol/100 nl) was injected into the RVLM. Atropine (4 nmol/1 μl) and carbachol (4 nmol/1 μl) were injected into the 4th V. Δ MAP = increase in mean arterial pressure (mm Hg); Δ HR = increase in heart rate (bpm). The results are presented as means ± SEM. $n = 5$ rats. * different from saline 4th V + saline RVLM. + different from saline 4th V + carbachol 4th V.

Gordon, 1994; Morrison, 2002). Injections of L-glutamate into the RVLM in unanesthetized rats increase sympathetic activity and arterial pressure (Araújo et al., 1999; Averill et al., 1994; Sun, 1995; de Paula and Machado, 2001).

The cholinergic mechanism is one of the most important pressor mechanisms in the brain (Buccafusco, 1996; Arneric et al., 1990; Punnen et al., 1986; Giuliano et al., 1989; Kubo et al., 2000). Central cholinergic activation increases sympathetic activity and vasopressin secretion (Hoffman et al., 1977; Imai et al., 1989). Studies with intrathecal injection of the cholinergic agonist carbachol showed a possible interaction between cholinergic and glutamatergic mechanisms in the cardiovascular regulation (Feldman and Buccafusco, 1997). Moreover,

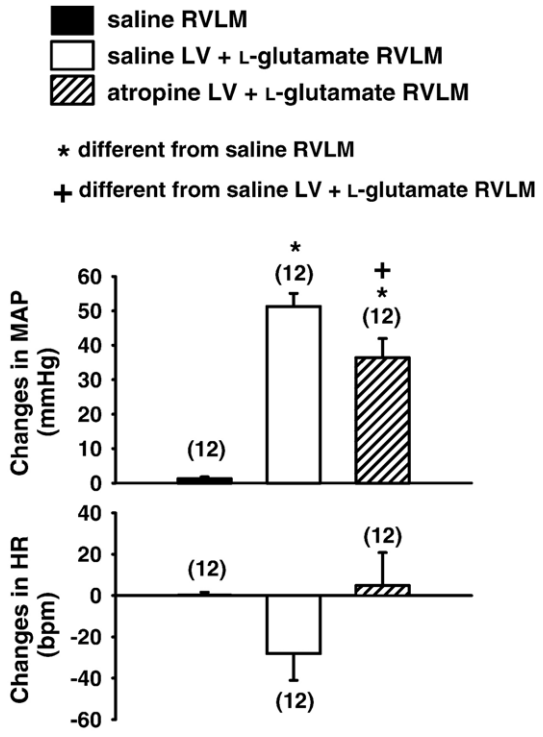

Fig. 2 – Changes in MAP and HR induced by L-glutamate (5 nmol/100 nl) injected into the RVLM after saline or atropine (4 nmol/1 μl) into the LV. The results are represented by means ± standard error of means (SEM). The number of rats is indicated in parenthesis above each bar.

other studies also suggested a possible interactions between cholinergic and glutamatergic mechanisms in the RVLM for the cardiovascular control (Agarwal and Calaresu, 1992; Morrison et al., 1989; Bazil and Gordon, 1991).

A recent study has shown that electrolytic lesions of the preoptic-periventricular tissue surrounding the anteroventral third ventricle (AV3V region) reduced the pressor responses produced by injections of L-glutamate into the RVLM in unanesthetized rats (Vieira et al., 2006). Previous studies have also already shown that forebrain areas and among them the AV3V region have important role on sympathetic control and cardiovascular regulation (Brody et al., 1978, 1984; Brody and Johnson, 1980; Olivan et al., 2001; Vieira et al., 2004). In rats, AV3V lesions reduce sympathetic activation, vasopressin secretion and arterial pressure in different models of experimental hypertension (Brody et al., 1978, 1984; Brody and Johnson, 1980; Menani et al., 1988). AV3V lesions also strongly reduce the pressor responses produced by injections of the cholinergic agonist carbachol intracerebroventricularly or in different forebrain areas, like subfornical organ, medial septal area, lateral preoptic area and ventromedial hypothalamic nucleus (Menani et al., 1990; Colombari et al., 1992a,b; Gonçalves et al., 1992).

Considering that forebrain cholinergic pressor mechanisms are blocked by AV3V lesions which also reduce the pressor responses to L-glutamate into the RVLM, one question is if the activity of central cholinergic mechanisms is important for the pressor response to L-glutamate into the RVLM. Therefore, in the present study we investigated the effects of central muscarinic cholinergic blockade on the pressor responses produced by injections of L-glutamate into the RVLM of unanesthetized rats. In addition, peripheral chemo and baroreflex were also tested in rats with central cholinergic blockade.

2. Results

2.1. *Histological analysis*

Fig. 1 shows the typical site of injections into the RVLM.

Injections into the RVLM were ventral to the nucleus ambiguus and approximately 200 to 400 μm caudal to facial nucleus (Araújo et al., 1999; de Paula and Machado, 2001).