Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres
Brain Research

Research Report

Caudal ventrolateral medulla mediates baroreceptor afferent inputs to subfornical organ angiotensin II responsive neurons

*John Ciriello**

Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada N6A 5C1

ARTICLE INFO

Article history:
Accepted 31 October 2012
Available online 6 November 2012

Keywords:
Circumventricular organs
Extracellular fluid balance
Cardiovascular reflex pathways
Blood pressure regulation

ABSTRACT

Although anatomical data indicates that the caudal ventrolateral medulla (CVLM) projects directly to the subfornical organ (SFO), little is known about the afferent information relayed through the CVLM to SFO. Experiments were done in the anesthetized rat to investigate whether CVLM neurons mediate baroreceptor afferent information to SFO and whether this afferent information alters the response of SFO neurons to systemic injections of angiotensin II (ANG II). Extracellular single unit recordings were made from 78 spontaneously discharging single units in SFO. Of these, 32 (41%) responded to microinjection of L-glutamate (L-Glu; 0.25 M; 10 nl) into CVLM (27/32 were inhibited and 5/32 were excited). All 32 units also were excited by systemic injections of ANG II (250 ng/0.1 ml, ia). However, only those units inhibited by CVLM ($n=27$) were found to be inhibited by the reflex activation of baroreceptors following systemic injections of phenylephrine (2 μg/kg, iv). Activation of CVLM or arterial baroreceptors in conjunction with ANG II resulted in an attenuation of the SFO unit's response to ANG II. Finally, microinjections (100 nl) of the synaptic blocker $CoCl_2$ or the non-specific glutamate receptor antagonist kynurenic acid into CVLM attenuated (10/13 units tested) the SFO neuron's response to activation of baroreceptors, but not the unit's response evoked by systemic ANG II. Taken together, these data suggest that baroreceptor afferent information relayed through CVLM functions to modulate of the activity of neurons within SFO to extracellular signals of body fluid balance.

Abbreviations: 3V, 3rd ventricle; Amb, nucleus ambiguus; ANG II, angiotensin II; AT1, angiotensin type 1 receptors; BARO, baroreceptor activation; $CoCl_2$, cobalt chloride; CVLM, caudal ventrolateral medulla; ia, intra-arterial; ION, inferior olivary nucleus; KYN, kynurenic acid; L-Glu, L-glutamate; LRN, lateral reticular nucleus; PVT, paraventricular nucleus of the thalamus; py, pyramidal tract; SFO, subfornical organ; sm, striamedullaris; SPVC, spinal trigeminal nucleus, caudal division; vhc, ventral hippocampal commissure

*Corresponding author. Fax: +1 519 661 3827.
E-mail address: john.ciriello@schulich.uwo.ca

0006-8993/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.10.064

1. Introduction

The subfornical organ (SFO) is a circumventricular organ that lacks a functional blood-brain barrier (Dellman, 1987; Gross, 1985) and that functions as a major site within the central nervous system at which angiotensin II (ANG II) within the circulation activates neuronal circuits involved in the regulation of fluid and sodium balance, and blood pressure (Antunes-Rodrigues et al., 2004; Ciriello and Gutman, 1991; Fitzsimons, 1998; McKinley et al., 1998, 2001). SFO neurons have been shown to contain angiotensin type 1 (AT1) receptors (Phillips et al., 1993; Rowe et al., 1992; Song et al., 1991). The activation of these receptors mediates the dipsogenic and blood pressure response induced by ANG II (Gutman et al., 1988; Krause et al., 2008; Mangiapane and Simpson, 1980; Saad et al., 2008; Simpson et al., 1978). Blockade of these receptors within SFO inhibits the fluid intake and autonomic responses to blood-borne ANG II (Antunes-Rodrigues et al., 2004; Fitzsimons, 1998). Electrical and chemical stimulation of SFO neurons have also been reported to activate neuronal circuits that are involved in correcting changes in blood volume, plasma osmolality and blood pressure (Antunes-Rodrigues et al., 2004; Ciriello and Gutman, 1991; Fitzsimons, 1998; Gutman et al., 1986; Miselis, 1981). Activation of SFO has been shown to elicit pressor responses, increases in the release of vasopressin and drinking responses (Ciriello and Gutman, 1991; Ferguson and Kasting, 1986; Gutman et al., 1985; Lind and Johnson, 1982; Mangiapane et al., 1984; Robertson et al., 1983).

It has been suggested that neuronal inputs to SFO may be involved in the modulation of the response of SFO neurons to ANG II (Babic et al., 2004; Ciriello et al., 1996; Ciriello, 1997; Gu and Ju, 1995; Kawano and Masuko, 2001; Rosas-Arellano et al., 1995, 1996a,b; Shioya and Tanaka, 1989; Tanaka and Seto, 1988a,b; Tanaka et al., 1993, 2001, 2002; Zardetto-Smith and Gray, 1987). Several anatomical studies have shown that neurons within the brainstem's caudal ventrolateral medulla (CVLM) innervate SFO neurons. Injections of the retrograde tract-tracer wheat germ agglutinin horseradish peroxidase into the SFO region resulted in the labeling neurons within CVLM (Kawano and Masuko, 2001). Additionally, injections of the anterograde tract-tracer Phaseolus vulgaris leucoagglutinin into cardiovascular responsive sites in the VLM resulted in the labeling of axons and presumptive axonal terminals within SFO (Babic et al., 2004). Interestingly, an electrophysiological study has also demonstrated that electrical stimulation of the CVLM region alters the activity of SFO neurons (Tanaka et al., 2002). Although this finding in conjunction with the observation that some SFO neurons also responded to hemorrhage was interpreted to suggest that CVLM neurons directly mediate information about blood volume to SFO, no evidence to support this conclusion exists (Tanaka et al., 2002). CVLM neurons have been shown to relay cardiovascular afferent information to several forebrain sites involved in blood pressure regulation (Kawano and Masuko, 1996; Maeda et al., 1991; Marchenko and Sapru, 2003; Shioda and Nakai, 1996; Smith et al., 1995), and in the maintenance of intra- and extracellular fluid homeostasis, which in turn may relay cardiovascular afferent information to SFO (Gu and Ju, 1995; Rosas-Arellano et al., 1995, 1996a,b; Smith et al., 1995; Tanaka et al., 1986). Additionally, it is well known that electrical stimulation does not discriminate between activation of neuronal cell bodies and fibers of passage through the region of stimulation, thus the response of SFO neurons to selective activation of CVLM neurons remains to be determined.

This study was done in the anesthetized rat to investigate whether SFO neurons that respond to systemic injections of ANG II alter their discharge rate to selective activation of CVLM neurons using L-glutamate (L-Glu). In addition, SFO neurons responsive to ANG II and CVLM were further tested for their responses to reflex activation of arterial baroreceptors following the iv injection of phenylephrine. Furthermore, the effect of either CVLM or baroreceptor activation on the SFO neuron's responses to ANG II was tested. Finally, the effect of injections of the synaptic blockers cobalt chloride ($CoCl_2$) or kynurenic acid (KYN) into the CVLM region on the response of SFO neurons to activation of baroreceptors was also investigated.

2. Results

Extracellular recordings were made from 78 histologically verified (Fig. 1) spontaneously active (3.7 ± 0.5 spikes/s) single units in SFO excited (11.7 ± 0.2 spikes/s) by intra-carotid injection of ANG II with a peak latency of 41.7 ± 11.5 s and with a response duration of 65.3 ± 8.1 s (Figs. 2–3). Of these neurons, 32 (41%) SFO neurons responded to activation of the CVLM by microinjection of L-Glu (Fig. 1a and Fig. 4). Twenty-seven neurons were inhibited (peak latency, 5.7 ± 1.2 s; duration, 21.3 ± 5.5 s) (Figs. 2 and 4) and five were excited (peak latency, 4.2 ± 2.3 s; duration, 15.5 ± 6.1 s by stimulation of CVLM) (Fig. 4). All 27 neurons inhibited by CVLM were also inhibited by the reflex activation of arterial baroreceptors (peak latency, 21.7 ± 3.2 s; duration, 51.3 ± 7.5 s) (Figs. 2–4). None of the SFO single units excited by stimulation of CVLM responded to baroreceptor activation. These data are summarized in Fig. 4. Unexpectedly, when the rate-meter record was further examined for these 27 units, in some cases ($n=9$), the discharge rate of the single unit was observed to decrease below resting discharge levels immediately after the units response to ANG II (Figs. 2–3), although this effect was not statistically significant ($p<0.65$) when the responses of all 27 units were analyzed.

Single units responding to ANG II were found throughout SFO (Fig. 1b). However, those that responded to CVLM and baroreceptor stimulation were preferentially located in the dorsolateral aspects of SFO (Fig. 1b). Both single units that were inhibited or excited by CVLM were found in similar areas throughout the SFO (Fig. 1b).

To investigate whether the response of single units in SFO to baroreceptor activation was mediated by CVLM neurons, the CVLM region was injected with either the synaptic blocker $CoCl_2$ ($n=6$) or the non-specific glutamate receptor antagonist KYN ($n=4$). A representative experiment is shown in Fig. 3. When $CoCl_2$ was microinjected into the CVLM, the response to baroreceptor activation was attenuated (Fig. 5a). The effects evoked by KYN were similar to those observed by the $CoCl_2$ injections into CVLM (Fig. 5b). Injections of $CoCl_2$ (Fig. 3) or KYN appeared to elicit an increase in the discharge