Neuroscience 133 (2005) 775–786

RIGHT ATRIAL STRETCH ACTIVATES NEURONS IN AUTONOMIC BRAIN REGIONS THAT PROJECT TO THE ROSTRAL VENTROLATERAL MEDULLA IN THE RAT

A. KANTZIDES, N. C. OWENS, R. DE MATTEO[1] AND E. BADOER*

School of Medical Sciences, Division of Biosciences, RMIT University, PO Box 71, Bundoora 3083, Melbourne, Victoria, Australia

Abstract—Activation of the cardiac mechanoreceptors results in changes in sympathetic nerve activity and plays an important role in the responses elicited by elevated blood volume. Stimulation of the reflex influences several key autonomic regions, namely the paraventricular nucleus (PVN), the nucleus of the tractus solitarius (NTS) and the caudal ventrolateral medulla (CVLM). Neurons in these regions project directly to the rostral ventrolateral medulla (RVLM), a critical region in the generation of sympathetic vasomotor tone. The aim of the present experiments was to determine whether neurons in the PVN, NTS and CVLM that are activated by cardiac mechanoreceptor stimulation also project to the RVLM. Animals were prepared, under general anesthesia, by microinjection of a retrogradely transported tracer into the pressor region of the RVLM, and the placement of a balloon-tipped cannula at the junction of the right atrium and the superior vena cava. On the experimental day, in conscious rats, the balloon was inflated to stimulate cardiac mechanoreceptors (n=9), or left uninflated (control, n=8). Compared with controls, there was a significantly increased number of Fos-immunoreactive neurons (a marker of activation) in both the PVN (2.5-fold) and NTS (two-fold), but this was not seen in the CVLM. Compared with controls, a significant number of the neurons in the PVN (8%) and NTS (4.0%) that projected to the RVLM were activated. The data suggest that subgroups of RVLM-projecting neurons located in the PVN and NTS are involved in the central reflex pathway activated by cardiac mechanoreceptor stimulation. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: Fos-immunohistochemistry, brain, cardiac mechanoreceptors.

Perturbations in body fluid homeostasis elicit a variety of reflex responses that involve hormonal, behavioral and neural components. An elevation of plasma volume causes a rise in atrial pressure which activates the cardiopulmonary mechanoreceptors located on or near the heart (Thames, 1978; Badoer et al., 1997). This stimulus elicits non-uniform compensatory changes in sympathetic nerve activity that include a reduction in renal sympathetic nerve activity and increases in heart rate (HR) (Karim et al., 1972; Morita and Vatner, 1985; Badoer et al., 1998). These contribute to the changes seen in total peripheral resistance, urine flow, sodium excretion, cardiac output and stroke volume (Chen et al., 1979; Fater et al., 1982; Kaufman and Stelfox, 1987; Oren et al., 1993; Badoer et al., 1997). Selective activation of the cardiac mechanoreceptors produces similar reflex responses (Karim et al., 1972; Kappagoda et al., 1979; Kaufman et al., 1981; Linden et al., 1982; Kaufman and Deng, 1993).

The central pathways mediating the reflex effects on the sympathetic nervous system have not been studied in great detail, despite evidence to suggest that dysfunction of the reflex pathways may contribute to the pathophysiology of conditions of chronic fluid overload, such as congestive heart failure (CHF) (Zucker and Wang, 1991; Zucker, 1995; Akama et al., 1998). The afferent information from the cardiac mechanoreceptors is known to travel via the vagus and terminate in the nucleus tractus solitarius (NTS) in the dorsomedial medulla oblongata. Studies using immunohistochemical staining of Fos, the protein product of the early gene *c-fos*, a marker of activated neurons, have suggested particular autonomic brain regions are involved in the cardiac–mechanoreceptor reflex (Badoer et al., 1997; Randolph et al., 1998; Shafton et al., 1999; Potts et al., 2000; Pyner et al., 2002; Dampney and Horiuchi, 2004). Isotonic volume expansion results in increased Fos production in the area postrema, the NTS and the caudal/intermediate ventrolateral medulla oblongata (hereafter denoted CVLM for simplicity) in the brainstem, as well as the paraventricular nucleus (PVN) and supraoptic nucleus (SON) in the hypothalamus (Badoer et al., 1997; Randolph et al., 1998; Shafton et al., 1999; Potts et al., 2000). When volume expansion is preceded by an intrapericardial injection of procaine, Fos-immunoreactive (Fos-IR) neurons are found only in the area postrema and SON, but not in the NTS, CVLM and PVN, implying that these important autonomic brain regions are involved in the brain pathways mediating the reflex responses elicited by activation of the cardiac mechanoreceptors (Cunningham, 2002). Consistent with these conclusions are findings that show volume expansion activates neurons in the PVN, NTS and CVLM in conscious rabbits lacking an intact arterial baroreceptor reflex (Potts et al., 2000). There is now growing evidence suggesting that the PVN is a key brain nucleus involved in the central pathways mediating the reflex responses elicited by stimuli that activate the

[1] Present address: Department of Physiology, Monash University, Melbourne, Victoria, Australia.

*Corresponding author. Tel: +61-3-9925-7081; fax: +61-3-9925-7063.
E-mail address: emilio.badoer@rmit.edu.au (E. Badoer).

Abbreviations: CVLM, caudal ventrolateral medulla; Fos-IR, Fos-immunoreactive; HR, heart rate; MAP, mean arterial pressure; NHS, normal horse serum; NTS, nucleus of the tractus solitarius; PB, phosphate buffer; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; SON, supraoptic nucleus.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.02.038

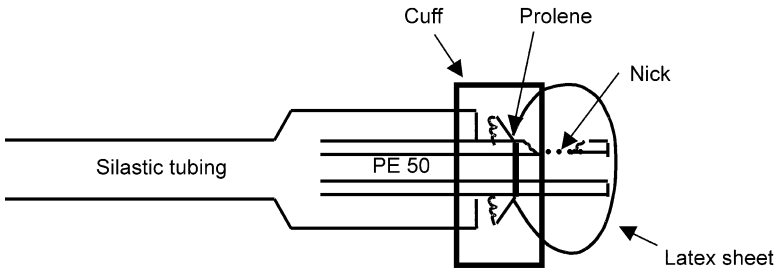

Fig. 1. Schematic diagram of the balloon catheter used in the present study. The manufacture of the balloon involved making a wedge-shaped nick approximately 1 mm from the end of a polyethylene tubing (PE50). This end was smoothed with heat and latex rubber sheet was pulled over this end and over the wedge to form the balloon. The sheet was then pulled taut and tied behind the wedge using a 6.0 Prolene suture. Silastic tubing was pushed over the free end of the PE50 tubing so that it abutted the edge of the latex rubber. A 4 mm length of Silastic tubing was used as a cuff.

cardiac mechanoreceptors (Lovick and Coote, 1988; Haselton et al., 1994; Pyner et al., 2002; Yang and Coote, 2003; Ng et al., 2004).

The PVN, NTS and CVLM contain neurons that project to the critical region in the rostral ventrolateral medulla (RVLM) that is responsible for the tonic generation of the majority of the sympathetic outflow. Neurons in the RVLM project directly to the sympathetic preganglionic neurons in the spinal cord (Ross et al., 1984; Dampney et al., 1987; Strack et al., 1989) and appear to be the major conduit through which cardiovascular reflexes influencing sympathetic nerve activity operate. Thus, these pathways could contribute to the changes in sympathetic nerve activity that can be elicited by influencing the activity of neurons in the PVN, NTS and CVLM. However, whether these neurons are involved in the central pathways that mediate the reflex sympathetic responses initiated by the activation of the cardiac mechanoreceptors is not known.

The aim of the present study was to determine whether neurons in the PVN, NTS and CVLM that project to the

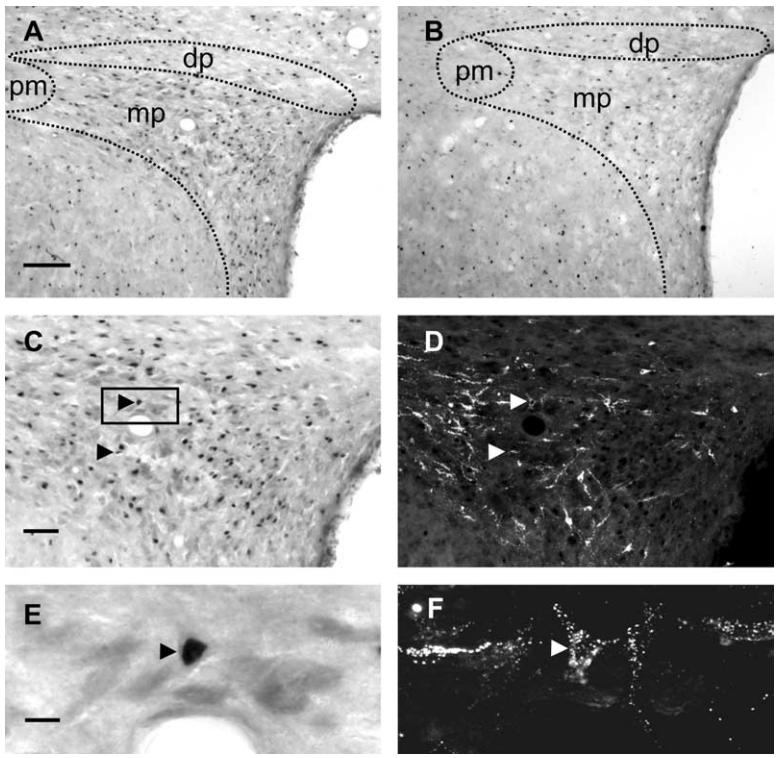

Fig. 2. Photomicrographs of the PVN showing Fos-IR neurons and neurons containing the retrogradely transported fluorescent beads that had been injected into the pressor region of the RVLM. Panel A is a low power magnification showing Fos production in a transverse section of the PVN taken from a rat that had an inflated balloon located at the atrial–vena caval junction. Panel B, shows a similar PVN level in a control animal. In each photomicrograph are shown the outlines of the dorsal parvocellular (dp), medial parvocellular (mp) and the posterior magnocellular (pm) nuclei. Panel C is a higher magnification of the PVN shown in panel A. Panel D is the same section viewed using fluorescent light to identify neurons projecting to the RVLM, the site in which the retrogradely transported fluorescent tracer was microinjected. Arrowheads highlight identical neurons in each panel. Panel E shows the region highlighted in the rectangle in panel C. Panel F is the same region as in E, viewed using fluorescent light. The arrowhead highlights an example of a double-labeled neuron. Scale bars=100 μm in panels A and B, 50 μm in panels C and D, 10 μm in panels E and F.