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INTERMITTENT ACTIVATION OF PERIPHERAL CHEMORECEPTORS IN AWAKE RATS INDUCES Fos EXPRESSION IN ROSTRAL VENTROLATERAL MEDULLA–PROJECTING NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

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Abstract—Despite the well-established sympathoexcitation evoked by chemoreflex activation, the specific sub-regions of the CNS underlying such sympathetic responses remain to be fully characterized. In the present study we examined the effects of intermittent chemoreflex activation in awake rats on Fos-immunoreactivity (Fos-ir) in various subnuclei of the paraventricular nucleus of the hypothalamus (PVN), as well as in identified neurosecretory preautonomic PVN neurons. In response to intermittent chemoreflex activation, a significant increase in the number of Fos-ir cells was found in autonomic-related PVN subnuclei, including the posterior parvocellular, ventromedial parvocellular and dorsal-cap, but not in the neurosecretory magnocellular-containing lateral magnocellular subnucleus. No changes in Fos-ir following chemoreflex activation were observed in the anterior PVN subnucleus. Experiments combining Fos immunohistochemistry and neuronal tract tracing techniques showed a significant increase in Fos-ir in rostral ventrolateral medulla (RVLM)–projecting (PVN-RVLM), but not in nucleus of solitarii tract (NTS)–projecting PVN neurons. In summary, our results support the involvement of the PVN in the central neuronal circuitry activated in response to chemoreflex activation, and indicate that PVN-RVLM neurons constitute a neuronal substrate contributing to the sympathoexcitatory component of the chemoreflex. Published by Elsevier Ltd on behalf of IBRO.

Key words: chemoreflex, arterial chemoreceptors, Fos protein, paraventricular nucleus, sympathetic activity.

The arterial chemosensitive cells located in the carotid body, at the bifurcation of the carotid arteries, are very sensitive to a reduction in arterial PO_2 (Biscoe and Dunchen, 1990). Potassium cyanide (KCN), used experimentally for activation of chemosensitive cells, causes cytotoxic-hypoxia and produces cardiovascular and respiratory responses similar to those evoked by hypoxic-hypoxia in awake rats (Franchini and Krieger, 1993; Bao et al., 1997; Barros et al., 2002). The activation of the chemoreflex in awake rats produces sympathoexcitation (increase in arterial pressure) and parasympathoexcitation (bradycardia) through activation of independent mechanisms (Haibara et al., 1995).

While it is well established that chemoreceptor primary afferent fibers terminate in the nucleus tractus solitarius (NTS) (Spyer, 1990), the projections from this nuclei to other areas in the brain involved with the sympathoexcitatory component of this reflex are not fully characterized. In this sense, functional evidence indicates that projections from the NTS to the paraventricular nucleus of the hypothalamus (PVN), a key center for autonomic and neuroendocrine integration (Swanson and Sawchenko, 1980), as well as the parabrachial nuclei contribute to the sympathoexcitatory response to chemoreflex activation in awake rats (Berquin et al., 2000a,b; Olivan et al., 2001; Haibara et al., 2002). Moreover, recent data from our laboratory further support an important role for the PVN in the integration of autonomic responses to chemoreflex activation (Olivan et al., 2001; Cruz et al., 2004).

Anatomical and electrophysiological studies demonstrated the presence of direct connections between the NTS and the PVN (Ricardo and Koh, 1978; Kooy et al., 1984; Kannan and Yamashita, 1985; Krukoff et al., 1995; Hardy, 2001), as well as direct connections from the PVN to the rostral ventrolateral medulla [RVLM (Ricardo and Koh, 1978; Spyer, 1990; Koshiya and Guyenet, 1996; Badoer and Merolli, 1998; Badoer, 2001; Coote et al., 1998; Cunningham et al., 1990; Hardy, 2001; Stern and Zhang, 2003)] and the spinal cord (Shafton et al., 1998; Badoer, 2001; Stocker et al., 2004). Recent studies documented that a bilateral electrolytic lesion of the PVN produced a significant reduction in the magnitude and duration of the pressor response evoked by chemoreflex activation with KCN (Olivan et al., 2001). Moreover, an involvement of PVN neurons in the increased renal sympathetic nerve activity produced by chemoreflex activation with KCN was recently demonstrated (Reddy et al., 2005). While these data support the concept that the PVN plays an important role in the central neuronal circuitry underlying the processing of the sympathoexcitatory component of the chemoreflex, the precise PVN neuronal populations activated during the chemoreflex remain to be established.

In the present study, we used Fos immunohistochemistry along with neuronal tract tracing approaches to map

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Abbreviations: DC, dorsal-cap; Fos-ir, Fos-immunoreactivity; HR, heart rate; LM, lateral magnocellular; MAP, mean arterial pressure; NTS, nucleus tractus solitarius; OT, oxytocin; PaA, anterior paraventricular nucleus of the hypothalamus subnucleus; PAP, pulsatile arterial pressure; PaPo, posterior parvocellular; PaV, ventromedial parvocellular; PBS, phosphate-buffered saline; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; VP, vasopressin.

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neuronal populations activated within specific sub-regions of the PVN in response to intermittent chemoreflex activation by i.v. administration of KCN. Our results support a non-homogeneous activation of PVN subnuclei in response to chemoreflex activation, affecting predominantly preautonomic ones, including PVN neurons innervating the RVLM.

EXPERIMENTAL PROCEDURES

Animals

Wistar male rats weighing 270–310 g were used in all experimental protocols. All the experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and Ethical Principles for Animal Experimentation established by the Brazilian Committee for Animal Experimentation and approved by the Animal Care and Ethics Committee of the School of Medicine of Ribeirão Preto, University of São Paulo (Protocol # 057/2003). All experiments also conformed to international guidelines of the ethical use of animals. The number of animals used, as well as their suffering, were minimized as much as possible.

Arterial pressure and heart rate (HR) recordings

One day before the experiments, while rats were under tribromoethanol [2.5%-100 ml/kg (Aldrich Chemical Co., Milwaukee, WI, USA)] anesthesia (i.p.), a catheter [PE-10 connected to PE-50 (Clay Adams, Parsippany, NJ, USA)] was inserted into the femoral artery for measurement of pulsatile arterial pressure (PAP) and a second catheter was inserted into the femoral vein for systemic administration of drugs. PAP was measured with a pressure transducer (model CDX III, Cobe Laboratories, Lakewood, CO, USA) connected to a polygraph (Narcotrace 80, Narco Bio-Systems, Austin, TX, USA). Mean arterial pressure (MAP) was derived from the PAP using a universal coupler (model 7179, Narco Bio-Systems), and HR was quantified from the PAP using a Biotachometer Coupler (model 7302, Narco Bio-Systems) and recorded in the same polygraph.

Activation of the chemoreflex with KCN

Twenty-four hours after catheter implantation, the cardiovascular parameters were recorded simultaneously in pairs of animals: one rat received intermittent injections of KCN (80 μg/0.05 ml/injection, performed every 3 min during a total period of 30 min) for stimulation of the chemoreflex and the second rat received intermittent injection of saline (0.9%) as a volume control. Before the experimental protocols, rats from both groups received an i.v. injection of KCN to verify if the venous catheter was patent. When compared with control rats, no differences in PVN Fos-immunoreactivity (Fos-ir) were observed following the single injection of KCN used to test the venous catheter (results not shown). The values of MAP and HR are representative of the peak values obtained in response to chemoreflex activation. The magnitude of peak changes in MAP (56±5 vs. 46±5 mm Hg, $F=0.35$, $P<0.8$) and HR (−266±21 vs. −270±12 bpm, $F=1.0$, $P<0.4$) in response to the initial and final KCN injections, respectively, were not statistically different.

Baroreflex activation

In a separate group of rats ($n=6$) the baroreflex was intermittently activated in awake rats using phenylephrine [1.0 or 1.5 μl/0.05 ml, i.v., activated every 3 min during 30 min], while control rats received an i.v. injection of the same volume of saline (0.9%). The magnitude of the pressor response to phenylephrine injection was similar to that produced by chemoreflex activation with KCN. Thirty minutes after the last activation, the animals were anesthetized and transcardially perfused with fixative as described elsewhere.

Retrograde labeling of NTS- and RVLM-projecting PVN neurons

Unilateral microinjections of Fluorogold (2%, 100 nl, Fluorochrome Inc., Denver, CO, USA) were performed in the NTS or in the RVLM ($n=4$ for each case) in rats under tribromoethanol anesthesia 4–6 days before intermittent chemoreflex activation. The stereotaxic coordinates used for microinjections into the caudal aspect of the commissural subnucleus of the NTS or in the RVLM were in accordance with the atlas of Paxinos and Watson (2007). Fig. 1 shows representative examples of the injection site following a unilateral microinjection of Fluorogold in the caudal aspect of the commissural NTS or the RVLM. In all cases, RVLM injection sites were contained within the caudal pole of the facial nucleus to

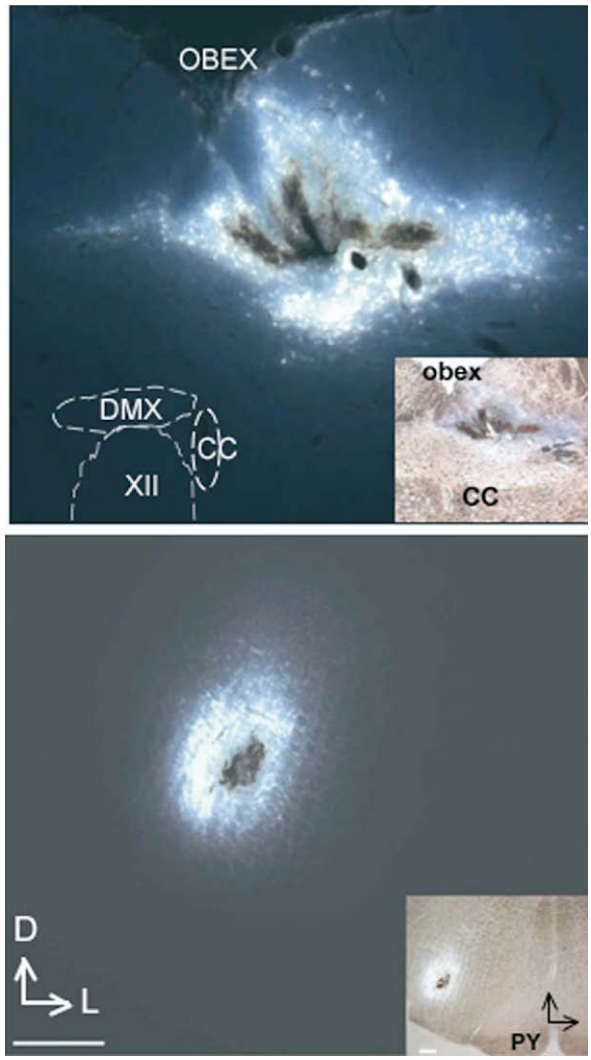

Fig. 1. Photomicrographs showing representative examples of injection sites following unilateral microinjection of Fluorogold into the caudal aspect of the commissural NTS (upper panel) and the RVLM (lower panel). Insets: NTS and RVLM injection sites shown at a lower magnification. Scale bars=200 μm. DMX=dorsal motor nucleus of vagus; CC=central canal; XII=hypoglossal nucleus, PY=pyramidal tract; vertical and horizontal arrows point to dorsal (D) and lateral (L) aspects of the brainstem.