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BRAIN RESEARCH

Research Report

Phox2b expression in the aldosterone-sensitive HSD2 neurons of the NTS

Joel C. Geerling, Peter C. Chimenti, Arthur D. Loewy*

Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110, USA

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ABSTRACT

The transcription factor Phox2b is necessary for the development of the nucleus of the solitary tract (NTS). In this brainstem nucleus, Phox2b is expressed exclusively within a subpopulation of glutamatergic neurons. The present experiments in the adult rat were designed to test whether this subpopulation includes the aldosterone-sensitive NTS neurons, which express the enzyme 11-β-hydroxysteroid dehydrogenase type 2 (HSD2). Nuclear Phox2b was found in virtually all the HSD2 neurons (95–99%, $n = 6$ cases). Unlike the activity-related transcription factor c-Fos, Phox2b expression in the HSD2 neurons was not influenced by dietary sodium deprivation. The ubiquitous expression of Phox2b by the HSD2 neurons suggests that they are developmentally related to other Phox2b-dependent neurons of the NTS and that they release the excitatory neurotransmitter glutamate. This finding also suggests that human Phox2b mutations, which cause the central congenital hypoventilation syndrome (CCHS, also known as Ondine's curse), may also produce deficits in central aldosterone signaling and appetitive or autonomic responses to sodium deficiency.

* *Corresponding author*. Fax: +1 314 362 3446.
E-mail address: geerlinj@msnotes.wustl.edu (J.C. Geerling).

Abbreviations: 4V, fourth ventricle; AP, area postrema; CC, central canal; CCHS, central congenital hypoventilation syndrome; DMX, dorsal motor nucleus of the vagus (Xth) nerve; GAD67, 67kDa isoform of glutamate decarboxylase; HSD2, 11-β-hydroxysteroid dehydrogenase type 2; NTS, nucleus of the solitary tract; PBel, external lateral parabrachial nucleus; pre-LC, pre-locus coeruleus; Phox2b, paired-like homeobox 2b; VGLUT2, vesicular glutamate transporter 2

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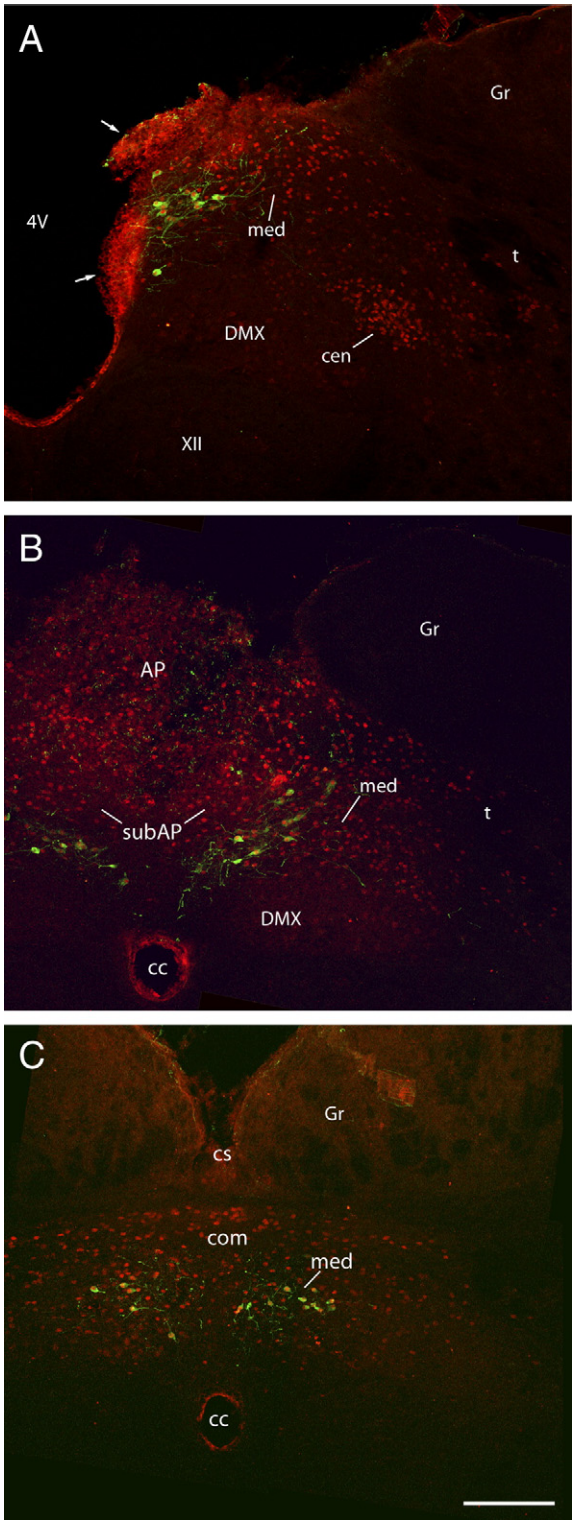
1. Introduction

A unique group of neurons in the brainstem is selectively sensitive to the adrenal mineralocorticosteroid aldosterone (Geerling et al., 2006a, 2006b). These cells, located in the nucleus of the solitary tract (NTS), are identifiable by their prominent expression of the glucocorticoid-inactivating enzyme 11-β-hydroxysteroid dehydrogenase type 2, and are referred to as HSD2 neurons.



In addition to aldosterone-sensitivity, a number of features distinguish this group of cells from other subpopulations of neurons in the surrounding NTS. In association with sodium appetite, the HSD2 neurons are progressively activated by dietary sodium deprivation, and then inactivated when salt is ingested (Geerling et al., 2006a). This activity pattern is very different from surrounding neurons in the medial NTS, most of which are inactive during sodium deprivation, then strongly activated following salt ingestion (Geerling and Loewy, 2006c, 2007b). Likewise, the output connections of the HSD2 neurons are dissimilar from those of most other neurons in the surrounding NTS. Their axons target the bed nucleus of the stria terminalis in the basal forebrain as well as forebrain-projecting relay nuclei in the pons, while providing little or no input to autonomic regions of the brainstem and hypothalamus innervated by many other neurons in the NTS (Geerling and Loewy, 2006a, 2006b).

Other than these attributes, little is known about the molecular phenotype(s) and developmental origins of the HSD2 neurons. Initially, we were unable to find evidence for co-expression of neuropeptides, calcium-binding proteins, and enzymes that define various other subpopulations of neurons in the rat NTS (Geerling et al., 2006b). One exception was the neuropeptide galanin, which was found in a few HSD2 neurons in some cases, but only after intracerebral pre-treatment with colchicine to increase the somatic concentration of axonally-transported peptides.

In the present study, to learn more about the molecular and developmental phenotype of the HSD2 neurons, we tested whether they express Phox2b, a transcription factor that is

Fig. 1 – Low-magnification photomontages showing the relative distributions of Phox2b-like nuclear immunoreactivity (red) and HSD2 neuronal labeling (green) throughout the caudal nucleus of the solitary tract (NTS). Phox2b-immunoreactive nuclei of widely varying intensities were observed throughout the NTS, dorsal motor nucleus of the vagus nerve (DMX), and area postrema; it is difficult to discriminate the more faintly-labeled nuclei from background tissue fluorescence at these low magnifications, particularly in the DMX (for a more detailed account of Phox2b labeling in this region, see Kang et al., 2007). Panel (A) shows the rostralmost cluster of HSD2 neurons, which border the open fourth ventricle (4V) at the obex. At this level, HSD2 neurons comprise the ventromedial extent of intensely Phox2b-immunoreactive neurons found in the medial subnucleus of the NTS (med). Note also the apparent cytoplasmic cross-immunoreactivity in ependymal cells near the obex (arrows), as well as the prominent Phox2b labeling throughout the central subnucleus of the NTS (cen). (B) At levels containing the area postrema (AP), HSD2 neurons comprise the ventromedial extent of Phox2b-labeled cells found in NTSmed. Also, intensely Phox2b-labeled nuclei were found throughout the dorsally-adjacent subpostremal subnucleus of the NTS (subAP). (C) Caudal to the AP, HSD2 neurons comprise part of the ventromedial Phox2b-labeled cells within the caudal NTSmed and commissural (com) subnuclei. Scale bar in (C) is 200 μm, and applies to all three panels. Other abbreviations: 4V, fourth ventricle; cc, central canal; cs, calamus scriptorius; Gr, gracile nucleus/fasciculus; XII, hypoglossal nucleus.