BRAIN RESEARCH 1375 (2011) 19–27

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

FoxP2 expression defines dorsolateral pontine neurons activated by sodium deprivation☆

Joel C. Geerling, Matthew K. Stein, Rebecca L. Miller, Jung-Won Shin[1], *Paul A. Gray, Arthur D. Loewy**

Department of Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA

ARTICLE INFO

Article history:
Accepted 5 November 2010
Available online 23 November 2010

Keywords:
Nucleus tractus solitarius
Parabrachial nucleus
Pre-locus coeruleus
Salt appetite
Sodium intake
Transcription factor

ABSTRACT

Two specific groups of neurons in the dorsolateral pons are activated by dietary sodium deprivation. These two groups are the pre-locus coeruleus (pre-LC) and the inner subdivision of the external lateral parabrachial nucleus (PBel-inner). In each site, after rats are fed an extremely low-sodium diet for over a week, neurons increase their expression of an activity-induced transcription factor, c-Fos. Here, we confirm this observation and extend it by demonstrating that these two groups of neurons express a common marker gene, the constitutively-expressed transcription factor Forkhead box protein 2 (FoxP2). That is, virtually all of the c-Fos activated neurons in both regions also express FoxP2. The expression of FoxP2 by both these groups of neurons suggests that they are developmentally-related subsets derived from the same basic population. Given that FoxP2, unlike c-Fos, is expressed independent of sodium deprivation, this marker may be useful in future studies of the pre-LC and PBel-inner. The molecular definition of these neurons, which project to circuits in the forebrain that influence visceral, appetitive, and hedonic functions, may allow direct experimental exploration of the functional role of these circuits using genetic tools.

1. Introduction

In the rostral part of the dorsolateral pons, two groups of neurons exhibit pronounced activation in rats after they have been deprived of dietary sodium (Geerling and Loewy, 2007). This complex region of the brainstem contains several well-defined populations of neurons, including the locus coeruleus (LC), a cluster of noradrenergic neurons adjacent to the fourth ventricle. It also contains the parabrachial nucleus (PB), a collection of subnuclei surrounding the superior cerebellar peduncle, which integrates information from the medulla and spinal cord related to visceral sensation, pain, and temperature and relays it to sites located primarily in the forebrain. In this region of the brainstem, the two groups of neurons with sodium deprivation-associated activity are found, first, in a small cluster immediately rostral to the LC, which we refer to as the pre-locus coeruleus (pre-LC), and second, in a thin band of neurons running along and within the ventrolateral aspect of the superior cerebellar peduncle as part of the inner subdivision of the external lateral PB (PBel-inner). These sodium deprivation-activated groups of neurons are described in anatomical detail in a previous study (Geerling and Loewy, 2007) and are shown in several images below.

☆ National Heart, Lung, and Blood Institute of the National Institutes of Health — Grant number: HL-025449, HL-089742, Bakewell Imaging Center Fund, and National Institutes of Health — Grant number: NS057105 Neuroscience Blueprint Core Grant.

* *Corresponding author*. Fax: +1 314 362 3446.
E-mail address: loewya@pcg2.wustl.edu (A.D. Loewy).
[1] Present address: Department of Neuroscience, Graduate School of East-West Medical Science, Kyung Hee University Yongin, Korea.

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.11.028

Our laboratory identified this novel and highly restricted change in neuronal activity as a natural extension of work involving the expression of an activity-induced transcription factor, c-Fos, in the nucleus of the solitary tract (NTS) after dietary sodium deprivation. Sodium deprivation is a useful, non-invasive experimental manipulation for producing large physiological increases in aldosterone production by the adrenal glands, along with a behavioral change in salt intake (see Geerling and Loewy, 2008). Initially, this experimental paradigm was used to demonstrate activation of the aldosterone-sensitive HSD2 neurons in the NTS (Geerling et al., 2006). Then, several axonal tracing experiments established the dorsolateral pons as a major target of the efferent projections of HSD2 neurons; within this region, their axons appear to synapse primarily within the pre-LC and the PBel-inner (Geerling and Loewy, 2006). Finally, as mentioned above, dietary sodium deprivation — the experimental manipulation we found to induce c-Fos expression in HSD2 neurons in the NTS — was also found to induce prominent c-Fos labeling in two specific regions of the dorsolateral pons, namely the pre-LC and PBel-inner (Geerling and Loewy, 2007). These combined pieces of evidence from tract-tracing and functional–anatomical experiments suggested, in combination, that HSD2 neurons in the NTS, which are activated by sodium deficiency, directly excite their post-synaptic target neurons in the pre-LC and PBel-inner, which integrate this information with other inputs and relay it to the forebrain (Geerling and Loewy, 2008).

The dorsolateral pons is a highly heterogeneous region of the brain, and while these small subsets of neurons do robustly express c-Fos after dietary sodium deprivation, the absence of a more general method to identify them is a major limitation to any further research on their functional and neuroanatomical properties. As these neurons show a distinct cytological response, we hypothesized that they exhibit genetic similarities that could be used as markers to distinguish them from adjacent neurons. Thus, we analyzed a published database of transcription factor expression in the mouse brainstem (see Gray et al., 2004) to search for candidate genes with patterns of expression in the dorsolateral pons that might identify neurons in one or both of these groups. We observed that the transcription factor Forkhead box protein 2 (FoxP2), which is found in several regions of the brain, is expressed by relatively specific subpopulations of neurons in the dorsolateral pons. Here, we show that expression of FoxP2 demarcates virtually all of the c-Fos-activated neurons in the pre-LC and PBel-inner after dietary sodium deprivation.

2. Results

2.1. *FoxP2 in the dorsolateral pons*

FoxP2 protein expression is robust in neuronal nuclei in several parts of the adult rat brainstem, in a pattern generally similar to previous anatomic work in p0 mouse pups (Gray, 2008). The overall pattern of FoxP2 expression in the brain is not the subject of this study, and will not be discussed further here (see Ferland et al., 2003; see Gray, 2008).

The region of interest for the present study, the rostral part of the dorsolateral pons, is shown in Figs. 1A and B. This figure shows immunohistochemical staining for FoxP2, seen as dark, punctate nuclear labeling in two transverse sections through the rostral dorsolateral pons. Within this region, FoxP2-immunoreactive (hereafter referred to as "FoxP2+") nuclei were found only in specific neuronal populations, including several subnuclei of the PB. The dorsal lateral (PBdl) and central lateral (PBcl) subnuclei contained the largest and densest populations of FoxP2+ nuclei. Smaller numbers of FoxP2+ cells were found in several other PB subnuclei, including the medial (PBm), ventral lateral (PBvl), and Kölliker-Fuse (KF). Interestingly, FoxP2 was conspicuously absent from a large region of the external lateral PB (PBel, Saper and Loewy, 1980; Fulwiler and Saper, 1984), throughout its much

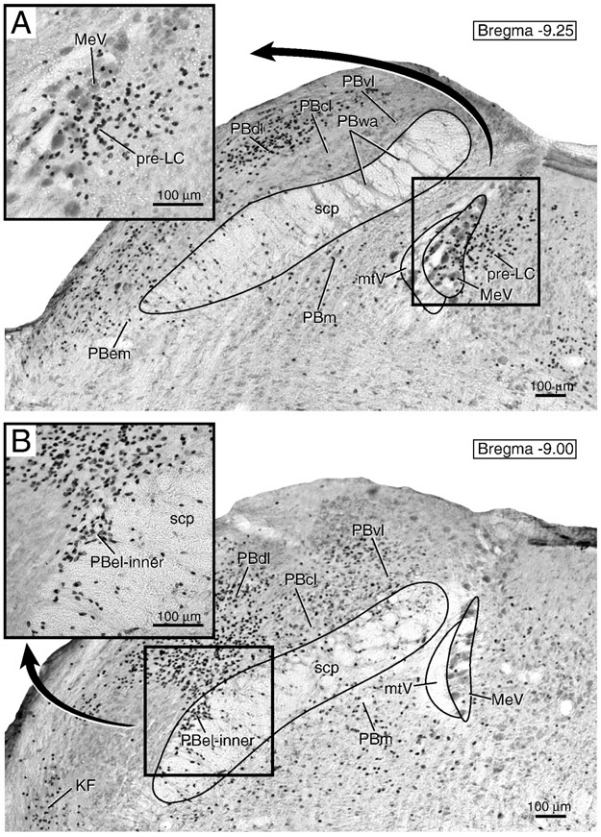

Fig. 1 – FoxP2 immunoreactivity in the rostral part of the dorsolateral pons is shown as dark, punctate nuclear staining. (A) FoxP2+ nuclei in the pre-locus coeruleus (pre-LC), which is located just medial to and intermingled with the large neurons and tract of the mesencephalic trigeminal nucleus (MeV, mtV). Note insert showing pre-LC neurons (black). (B) A more rostral section of the parabrachial nucleus showing the inner subdivision of the external lateral parabrachial nucleus (PBel-inner). Note that the insert contains a high magnification image of the PBel-inner showing labeled nuclei which are distributed along and in the ventrolateral aspect of the superior cerebellar peduncle (scp). There was a prominent void of FoxP2-immunoreactivity throughout most of the PBel, which contrasts with the densely-packed FoxP2+ nuclei in the neighboring dorsal lateral (PBdl) and central lateral (PBcl) subnuclei. Other abbreviations: medial PB (PBm), external medial PB (PBem), ventral lateral PB (PBvl), Kölliker-Fuse nucleus (KF).