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Differential distributions of neuropeptides in hypothalamic paraventricular nucleus neurons projecting to the rostral ventrolateral medulla in the rat

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HIGHLIGHTS

- Several peptides are expressed in the PVN-RVLM neurons.
- The large majority of PVN-RVLM neurons expressed OT and Dyn.
- The PVN-RVLM neurons predominantly co-expressed multiple peptides.
- Peptides were differentially distributed between subdivisions of PVN-RVLM neurons.

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ABSTRACT

The hypothalamic paraventricular nucleus (PVN) exerts a major regulatory role in the endocrine and the autonomic system. It has anatomically and functionally distinct cell populations, one of which projects to the rostral ventrolateral medulla (PVN-RVLM) and are involved in the sympathetic regulation of the cardiovascular system. To investigate the expression and the distributional patterns of peptides in the PVN-RVLM neurons of rats, we used single cell RT-PCR analysis in combination with retrograde tracing. Approximately 80% of PVN-RVLM neurons expressed oxytocin (OT) and 60% expressed dynorphin (Dyn). There were differential distributions of peptides between the subdivisions of PVN-RVLM neurons. The posterior parvocellular subdivision predominantly expressed OT; the dorsal cap subdivision contained much less vasopressin (VP) compared to other subdivisions. In addition, PVN-RVLM neurons preponderantly co-expressed multiple peptides. These results suggest that peptides may contribute to elaborate responses of PVN to various stimuli.

1. Introduction

Hypothalamic paraventricular nucleus (PVN) is an important integrating site for endocrine and autonomic regulation [26]. It consists of the magnocellular and the parvocellular cell group. The former projects to the posterior pituitary gland, which is responsible for secretions of oxytocin (OT) and vasopressin (VP). Some of the latter project to the median eminence to regulate secretions of several hormones, and others of the latter cell group project to the brain stem or the spinal cord, regulating the autonomic functions [2,26]. Within the autonomic-related cell group, specifically those involved in sympathetic control, two subgroups were characterized by their different projecting sites. One group projects directly to sympathetic centers in the spinal cord, and the other gives rise to indirect connections via relays in the rostral ventrolateral medulla (RVLM) [3,19]. The critical role of the PVN neurons projecting to the RVLM (PVN-RVLM) in the regulation of cardiovascular sympathetic outflows has been well demonstrated [1,5,6].

Peptides functions, as a neurotransmitter, in the nervous system are well known [11]. In the PVN, peptides, including OT and VP, were detected not only in the magnocellular and parvocellular cells associated with endocrine functions, but also in the parvocellular neurons with long descending projections to the autonomic centers in the brain-stem and the spinal cord [26]. Subsequently, accumulating evidence has indicated that more peptides exist in the PVN

Abbreviations: ACSF, artificial cerebrospinal fluid; CRH, corticotropin-releasing hormone; DC, the dorsal cap subdivision; Dyn, dynorphin; Enk, enkephalin; GHRH, growth hormone-releasing hormone; OT, oxytocin; PaPo, the posterior parvocellular subdivision; PaV, the ventral parvocellular subdivision; PVN, the hypothalamic paraventricular nucleus; RVLM, the rostral ventrolateral medulla; Sst, somatostatin; TRH, thyrotropin-releasing hormone; VP, vasopressin.

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and that they are differentially distributed according to functionally or anatomically distinct cell groups within the PVN [8,21,22]. However, such information remains unknown for PVN-RVLM neurons.

The present study aimed to investigate the expression and the differential distributions of peptides in PVN-RVLM neurons. We used single cell RT-PCR for detection of peptides, as it is able to detect multiple genes in a single cell simultaneously but independently. Eight representative peptides known to be prominent in the PVN were selected: dynorphin (Dyn), enkephalin (Enk), somatostatin (Sst), growth hormone-releasing hormone (GHRH), corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), oxytocin (OT), and vasopressin (VP). This study aimed to provide important information for understanding the central mechanisms of the PVN-RVLM pathway.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats ($n = 4$, 7 weeks old) were used (Orient Bio Inc., Kyonggi-do, Korea). The rats were maintained under a 12 h light/dark cycle (lights on at 9:00 a.m.) and given free access to food and water until sacrifice. All surgical procedures were performed after anesthesia induced by an intraperitoneal injection of Zoletil (25 mg/kg) and xylazine (10 mg/kg). Every effort was made to minimize the number of animals and their suffering. The experimental protocols were performed in accordance with the guidelines set by the Institutional Animal Care and Use Committee of Seoul National University and were approved by the Institute of Laboratory Animal Resources of Seoul National University.

2.2. Retrograde tracing

The retrograde-transported fluorescent tracer, FluoSphere-Red (Molecular Probes, Eugene, OR, USA), was injected into the RVLM of rats to identify PVN-RVLM neurons as previously described [9]. The injection point was, on average, 12.3 mm caudal, 1.7 mm lateral, and 8.0 mm ventral from bregma. Verification of the RVLM injection site was assessed by examining serial medulla slices (100 μm thickness) of all animals after sacrifice. Injections were located ventral to the nucleus ambiguus, within ~1 mm caudal of the facial nucleus.

2.3. Hypothalamic slice preparation

Hypothalamic brain slices were prepared according to the methods described previously [9]. After anesthesia, the rat was transcardially perfused with an ice-cold sucrose-rich artificial cerebrospinal fluid (ACSF) containing (in mM) 210 sucrose, 26 $NaHCO_3$, 5 KCl, 1.2 NaH_2PO_4, 1.2 $CaCl_2$, 2.4 $MgCl_2$, and 10 glucose. The brains were briefly removed and dissected into two or three coronal slices (300 μm thickness) including PVN regions, using a vibrating tissue slicer (Vibratome 1000 plus, Vibratome Company, St. Louis, MO, USA). The slices were incubated in an oxygenated normal ACSF containing (in mM) 126 NaCl, 26 $NaHCO_3$, 5 KCl, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 1.2 $MgCl_2$, and 10 glucose for at least 1 h at 30 °C until used.

2.4. Single cell RT-PCR

To gain RNAs from single PVN-RVLM neurons for RT-PCR, the hypothalamic brain slice was transferred to the recording chamber used for electrophysiological recording, perfused continuously with an oxygenated ACSF (4–5 ml/min), and maintained at 30–32 °C. Fluorescent-labeled PVN-RVLM neurons were visualized by combining differential interference contrast video microscopy

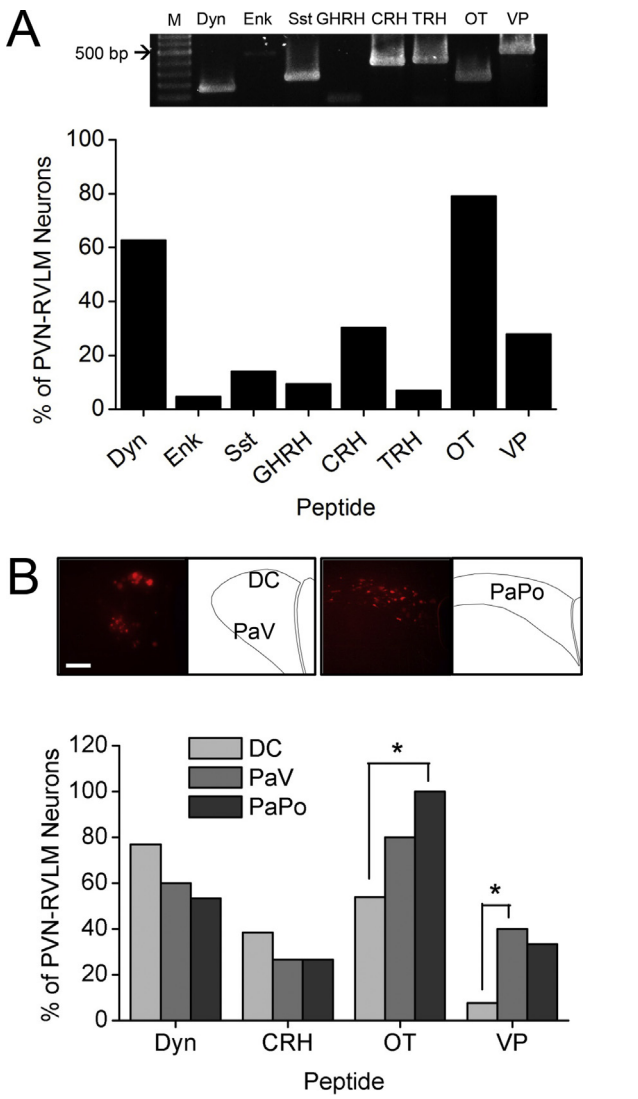

Fig. 1. Expressions of various peptides in PVN-RVLM neurons. (A) Upper panel, a representative gel image for Dyn, Enk, Sst, GHRH, CRH, TRH, OT, and VP. Lower panel, percentages of cells expressing each peptide in PVN-RVLM neurons. OT and Dyn were predominantly detected in the largest number of PVN-RVLM neurons, and CRH and VP were detected in the intermediate number. (B) Upper panel, representative fluorescent images showing retrograde-labeled PVN-RVLM neurons. The PVN-RVLM neurons are preponderantly distributed in three subdivisions: the dorsal cap (DC), the ventral parvocellular (PaV), and the posterior parvocellular (PaPo). Scale bar = 0.2 mm. Lower panel, bar graphs represent percentages of cells expressing Dyn, CRH, OT, and VP in each subdivision. The OT-expressing cells and the VP-expressing cells are distributed differently among subdivisions (*$p < 0.05$ by a chi-squared test).

with fluorescence microscope equipped with a green filter cube (WG, Olympus, Tokyo, Japan). The healthy cells that had apparent boundaries shaped by fluorescent dots and were located within three subdivisions of PVN, including the dorsal cap (DC), the ventral parvocellular (PaV), and the posterior parvocellular (PaPo) [23] (Fig. 1B), were chosen. The cells were approached with micropipettes pulled from borosilicate glass capillaries (1.7 mm diameter and 0.5 mm wall thickness) and filled with nuclease-free water (Qiagen, Valencia, CA, USA) using a micromanipulator (MP-225, Sutter Instruments, Novato, CA, USA). After the tip of the micropipettes attached to a cell, a slight negative pressure was applied to pull the cytoplasmic contents out of the cells into the pipette, taking care to avoid the nucleus. A mixture of cytoplasm and water was immediately removed into the tube containing (in