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Research report

The distribution of neural nitric oxide synthase-positive cerebrospinal fluid-contacting neurons in the third ventricular wall of male rats and coexistence with vasopressin or oxytocin

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Abstract

The detailed distribution of neural nitric oxide synthase (nNOS)-positive cerebrospinal fluid-contacting neurons (CSF-CN) was studied in the wall of the third ventricle of rats by anti-nNOS immunohistochemistry. The coexistence of nNOS and 8-arginine vasopressin (AVP) or oxytocin (OT) was also investigated in the CSF-CN using double labeling immunohistochemistry. The results demonstrated a widespread occurrence of nNOS-CSF-CN throughout the wall of the hypothalamic third ventricle. The vast majority of nNOS-CSF-CN cell bodies were of magnocellular type, commonly classified as oval, fusiform, multipolar, and inverted pear shape. These cell bodies were located in the ependyma, the subependyma, or the parenchyma, and their processes inserted in the ependymal layer or directly contacted with the CSF space. Electron microscopy demonstrated many nNOS-immunoreactive somas, dendrites, and/or axons that were situated at the subependyma, the ependyma, or the supraependyma. Generally, the distribution of OT-CSF-CN in the third ventricular wall was similar to the nNOS-CSF-CN and the ratio of NOS/OT co-expression was approximately 88%. In comparison, the distribution of AVP-CSF-CN was mainly restricted to the rostral part of the third ventricle and the ratio of nNOS/AVP co-expression was only about 6%. The widespread presence of nNOS-CSF-CN-expressing OT in the third ventricular region suggests that NO is an important messenger in the CSF-hypothalamo-hypophyseal neuroendocrine regulation that may in part act in concert with OT.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Other neurotransmitter

Keywords: Cerebrospinal fluid-contacting neurons; Coexistence; Nitric oxide; Oxytocin; Rat; Vasopressin

Abbreviations: ABC, avidin and biotinylated-horseradish peroxidase complex; ac, anterior commissure; aca, anterior limb of anterior commissure; acp, posterior limb of anterior commissure; AM, anterior magnocellular part (PVN); ap, anterior parvocellular part (PVN); AVP, 8-arginine vasopressin; BDA, biotinylated dextran amine; CSF, cerebrospinal fluid; CSF-CN, CSF-contacting neurons; dc, dorsomedial cap (PVN); DIC, differential interference contrast; D3V, dorsal third ventricle; f, fornix; FITC, fluorescein isothiocyanate; icv, intracerebroventricular; LM, lateral magnocellular part (PVN); LV, lateral ventricle; ME, median eminence; mp, medial parvocellular part (PVN); NADPH-d, nicotinamide adenine dinucleotide phosphate-diaphorase; NO, Nitric oxide; NOS, NO synthase; opt, optic tract; ox, optic chiasma; OT, oxytocin; PaV, ventral part of the PVN; Pe, periventricular hypothalamic nucleus; PM, posterior magnocellular part (PVN); Po, posterior part (PVN); PVN, paraventricular nucleus; Rch, retrochiasmatic nucleus; Sch, suprachiasmatic nucleus; SFO, subfornical organ; SO, supraoptic nucleus; SOR, retrochiasmatic part of the SO; sox, supraoptic decussation; 3V, third ventricle

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1. Introduction

Growing evidence demonstrates that the cerebrospinal fluid (CSF)-hypothalamo-hypophyseal recruit forms a special neurohormonal network and plays an important role in hypothalamic neuroendocrine regulations [1,8,17,23,49,50]. As a key component of this recruit, a large number of CSF-contacting neurons (CSF-CN) are present in the wall of the third ventricle in low vertebrates and mammals [19,22,34,35,47]. These neurons contact the ventricular CSF via their dendrites, axons, or perikarya, and constitute a CSF-contacting neuronal system [5,33,47,48,55]. They may act as a "message modulator" in the CSF-hypothalamo-hypophyseal recruit, which can register physical or chemical changes in the CSF, and transmit interrelated information to neighboring structures, such as the paraventricular nucleus (PVN) which projects to the neurohypophysis [1,44]. Furthermore, the CSF-CN may also secrete neurotransmitters or neurohormones into the CSF or act on the lamina terminalis by transport of the CSF [21,49].

Recent evidence suggests that nitric oxide (NO) is a ubiquitous modulator molecule produced from L-arginine by NO synthase (NOS) and takes part in regulating the function of a variety of neuroendocrine systems [26,40]. Many neuroanatomical studies have shown that neural NOS (nNOS) is present in several different cellular locations in the hypothalamus and coexists with oxytocin (OT) or 8-arginine vasopressin (AVP) [24,27,28,37,45]. The involvement of NO in the regulation of the release of OT and AVP has been investigated by a series of physiological and pharmacological experiments [6,12,13,14,18,29,42,46,54]. However, the complicated mechanisms of NO involved in the regulation of synthesis and release of these peptides under physiological and pathological conditions remain unclear [18,46]. For example, there are two conflicting views of NO in modulating the release of AVP. By intracerebroventricular (icv) injection of NO-related agents, some experiments demonstrated that NO showed an excitatory effect on AVP release [7,18,29,46]. In contrast, other studies revealed that NO played an inhibitory role in the regulation of AVP release [6,13,41,54]. These discrepancies may be attributable to different NO agents and may result from different dose, rate, and route of administration, and effects in different time points after injection among these experiments [52]. In addition, NO may act at various cerebral sites to induce diverse effects, because nNOS or its mRNA is distributed throughout the brain [26]. In order to understand the role of NO in the central regulation of OT/AVP and other phenomena, it seemed necessary to further clarify the distribution of nNOS and its association with OT and AVP in the hypothalamo-ventricular system. This is considered to be the most potential place for NO action by icv injection of NO agents [18]. The detailed organization of nNOS-, AVP-, and OT-immunoreactive cell bodies in the hypothalamic magnocellular neurosecretory nuclei of rats has been described by many neuroanatomical studies [24,27,28,37]. To our knowledge, no studies have shown the localization of nNOS-CSF-CN in the third ventricular wall in rats, although a few reports referred to the nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-positive neuron contacting the CSF in the brain [1,3,31,36,39].

In this study, a detailed localization and distribution of nNOS-positive CSF-CN (nNOS-CSF-CN) in the third ventricular region have been mapped by immunofluorescence-combined confocal differential interference contrast (DIC) scanning technique. Cellular and subcellular localization of nNOS-CSF-CN and their relationships with the ependymal cells were also observed on the light and electron microscopic level. The coexistence of NOS and AVP or OT was further investigated in the CSF-CN by using double labeling methods in the same section.

2. Materials and methods

2.1. Animals and tissue preparation

Thirty male Sprague–Dawley rats (200–250 g body weight) were purchased form the Animal Center of Nanjing Medical University. The experimental protocols were approved by the Animal Ethics Committee, Nanjing Medical University. The rats had free access to water and standard pellets, and were housed under controlled conditions with 12 h of light and 12 h of darkness. During the light period, the animals were deeply anesthetized by intraperitoneal administration of pentobarbital sodium (60 mg/kg body weight). The animals were then perfused transcardially through the ascending aorta, with 100 ml of warm (37 °C) 0.9% NaCl containing heparin (10 000 U/l) followed by 400 ml of an ice-cold, freshly prepared solution of 4% formaldehyde without (for immunofluorescent histochemistry) or with 0.5% glutaraldehyde (for immunoelectron microscopy) in phosphate-buffered saline (PBS, 0.1 M, pH 7.4). The brains were dissected out, and coronal slices were cut in the brain blocker (WPI, Sarasota, FL, USA) containing hypothalamus (5 mm thick) and immersion fixed in the same fixative for 24 h. The specimens were then rinsed in an ice-cold solution of 20% sucrose in PBS for 24 h. For immunofluorescence histochemistry, the specimens were totally embedded in OCT compound (Fisher Scientific, Pittsburgh, PA, USA). Serial coronal sections (about 70–80 in number) were cut on a cryostat (−20 °C) (Leica, Germany) at a thickness of 25 μm. In order to retain the ependymal layer without modifications, sections were carefully mounted onto gelatin-coated slides in sequence and divided into two series. The first set of sections was stained with cresyl violet in order to define the cytoarchitecture of the hypothalamus and the third ventricular wall; the second set was used for immunohistochemistry. For immunoelectron microscopic processing, the specimens were cut in the coronal plane