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Research report

Stress-induced alterations in catecholamine enzymes gene expression in the hypothalamic dorsomedial nucleus are modulated by caudal brain and not hypothalamic paraventricular nucleus neurons

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Abstract

The hypothalamic dorsomedial nucleus (DMN) represents an important coordinate center for regulation of autonomic and neuroendocrine systems, especially during stress response. The present study was focused on the gene expression of catecholamine-synthesizing enzymes and the protein levels of tyrosine hydroxylase in DMN, both in control and stressed rats. Moreover, pathways modulating the gene expression of tyrosine hydroxylase in DMN during immobilization (IMO) stress were also investigated. Gene expressions of all catecholamine-synthesizing enzymes were detected in DMN samples. While the levels of tyrosine hydroxylase and phenylethanolamine *N*-methyltransferase mRNA were increased in IMO rats, aromatic L-amino acid decarboxylase and dopamine-β-hydroxylase mRNA remained unchanged. Tyrosine hydroxylase protein levels were significantly elevated in the DMN only after repeated IMO stress. Postero-lateral deafferentations of the DMN, or transections of the ascending catecholaminergic pathways originating in the lower brainstem abolished the IMO-induced increase of tyrosine hydroxylase gene expression in the DMN. Nevertheless, postero-lateral deafferentations of the hypothalamic paraventricular nucleus (PVN), which separate the DMN from the PVN, had no effect on IMO-induced elevation of tyrosine hydroxylase mRNA in the DMN. The present data indicate that certain DMN neurons synthesize mRNA of catecholamine enzymes. The stress-induced increase of tyrosine hydroxylase and phenylethanolamine *N*-methyltransferase mRNA in DMN neurons indicates the involvement of these catecholaminergic neurons in stress response. The gene expression of tyrosine hydroxylase in DMN is modulated by lower brainstem and/or spinal cord, but not by PVN afferents.

Keywords: Aromatic L-amino acid decarboxylase; Dopamine-β-hydroxylase; Phenylethanolamine *N*-methyltransferase; Tyrosine hydroxylase; Norepinephrine; Immobilization stress; Rats

1. Introduction

Neuroanatomical and neurophysiological data accumulated during the last decade indicate that the hypothalamic dorsomedial nucleus (DMN) is a prominent component of hypothalamic visceromotor generator network [2,5,15,24,51]. Reportedly, the DMN is involved in the regulation of processes, such as ingestion, fluid balance, reproduction, circadian rhythms, thermoregulation, and autonomic and neuroendocrine responses to stress [3,4,7,9,19,21,37].

Autonomic and neuroendocrine systems are controlled also by central catecholaminergic (CA) neurons. The catecholamine-synthesizing cells are distributed mainly in the midbrain and brainstem structures (see refs. [35,36]). Although the existence of tyrosine hydroxylase (TH) neurons in the DMN has previously been described by immunohistochemistry [16,20], detailed studies dealing with the gene expressions of CA enzymes in DMN have not been published yet.

In the present study, gene expression levels of tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase

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(AADC), dopamine-β-hydroxylase (DBH), phenylethanolamine *N*-methyltransferase (PNMT), and TH protein were studied in control and immobilized rats. To better understand the regulation of DMN CA cells, postero-lateral deafferentations of the nucleus, surgical isolation of the paraventricular (PVN), and the dorsomedial nuclei, as well as transections of ascending CA pathways were performed to interrupt the possible afferent pathways to the DMN.

2. Materials and methods

2.1. Animals

All experiments were carried out on male Sprague–Dawley rats weighing 330 ± 30 g (Charles Rivers, Gödöllö, Hungary). One week before starting the experiments, the animals were kept under controlled environmental conditions (12 h light/12 h dark cycle with light on at 06:00 h; temperature, 22 ± 1 °C) with free access to tap water and standard pellet rat chow. Experiments were performed between 08:00 and 12:00 h. The animals were avoided of all external noises or other stressful stimuli. Principles of laboratory animal care and all procedures were approved by the Animal Care Committee of the Institute of Experimental Endocrinology (Bratislava, Slovak Republic). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

2.2. Immobilization stress

Immobilization procedure was performed as described previously [25] by taping the limbs of the rats to metal mounts attached to a board.

Single immobilization: animals ($n = 9$–10 per group) were exposed to a single immobilization for 2 h.
Repeated immobilization: animals ($n = 10$ per group) were exposed to immobilization for 7 consecutive days (2 h daily).

Control rats were decapitated immediately after their removal from the home cages.

2.3. Hypothalamic deafferentations and brainstem surgical transections

Deafferentation and transection procedures were performed 2 weeks prior to the initiation of the experiments, i.e., leaving a time for regeneration of animals after brain surgery. Rats were anaesthetized with a mixture containing 60 mg/kg ketamine and 7.8 mg/kg xylazine. Head of the animals was fixed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Holes with 2.0 mm in diameter were drilled into the skull. The hypothalamic deafferentations and transections in the rostral brainstem were performed according to coordinates of a stereotaxic map [41]. In all experiments sham-operated animals served as controls. The heads of these animals were fixed, drilled, but no knife cuts were performed.

2.3.1. Hypothalamic deafferentations

These deafferentations were performed by Halász knife [17]. Heads of the rats were fixed in a 3° nose-down position. Ventral surface of the knife was placed perpendicularly to the midline plane of brain during penetration into the brain tissue. After reaching the appropriate point in the brain, the knife was twisted to the left and right sides by 90°, and then removed.

Stereotaxic coordinates for the postero-lateral deafferentation of the DMN: antero-posterior (from the level of the bregma) = −3.8 mm; vertical (from the top of the skull) = −9.8 mm; medio-lateral (from the midline) = 0.0 mm. The cut transected all of the fibers ascending from caudal and lateral directions (Fig. 1A), but did not disconnect the DMN from anterior direction. The length of the horizontal part of knife was 1.2 mm and the vertical part 1.6 mm.

Stereotaxic coordinates for the postero-lateral deafferentation of the paraventricular nucleus: anterior–posterior = −2.8 mm; vertical = −10.0 mm; medio-lateral = 0.0 mm. This knife cut disrupted all the connections between PVN and DMN (Fig. 1B), but left the caudal projections to the DMN intact. The length of horizontal part of knife was 1.4 mm and the vertical part 2.0 mm.

2.3.2. Bilateral transections through the upper brainstem

The heads of the rats were fixed in a 5° nose-down position and a narrow line-shaped hollow was drilled into the skull 1.0–3.0 mm lateral to the midline on the both side, 8.0–8.5 mm caudal to the level of the bregma. Coordinates: antero-posterior = −8.2 mm; vertical = −11.0 mm, medio-lateral = ±1.8 mm. The transections were performed bilaterally by perpendicular penetrations of a "glass knife" made from histological coverslip [39]. The width of knife was 1.5 mm. The shape of the knife is shown in Fig. 2.

2.4. Tyrosine hydroxylase immunohistochemistry

Animals were rapidly perfused transcardially with 50 ml of 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 10% saturated picric acid. Then the brains were removed, postfixed in the same fixative overnight at 4 °C, and infiltrated with 30% sucrose in 0.1 M PB for 48 h at 4 °C. Serial coronal sections of 20 μm thickness were cut with in a cryocut (Reichert) at −16 °C. Sections were rinsed in 0.1 M phosphate

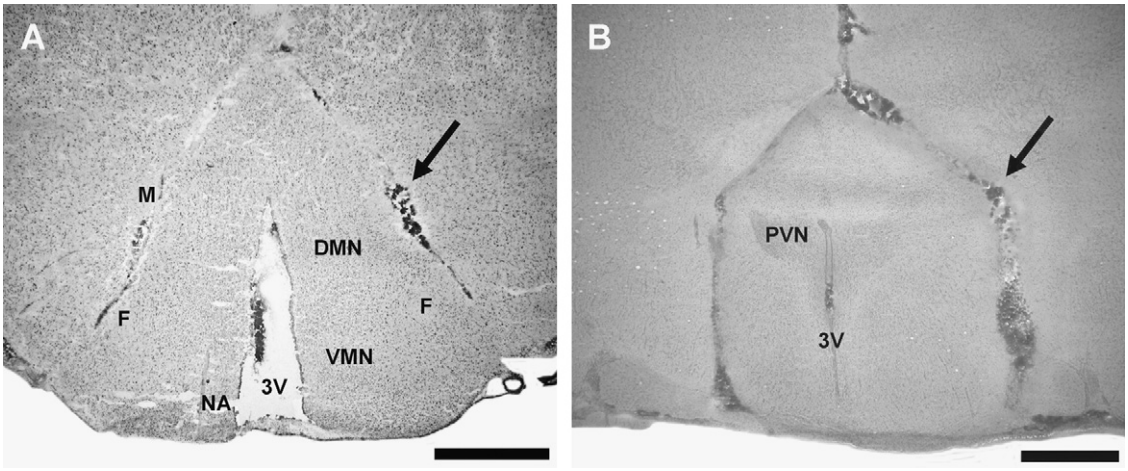

Fig. 1. Coronal sections through the hypothalamus indicating the anatomical location of the surgical deafferentations (arrow) of the dorsomedial (A) and paraventricular nuclei (B). Cresyl violet staining (A) and eosine staining (B). *Abbriviations:* DMN, dorsomedial nucleus; F, fornix; M, mamillothalamic tract; NA, arcuate nucleus; PVN, paraventricular nucleus; VMN, ventromedial nucleus; 3V, third ventricle. Scale bar: 1 mm.