DOI 10.1007/s11055-021-01077-3
Neuroscience and Behavioral Physiology, Vol. 51, No. 3, March, 2021

Changes in the Expression of Steroidogenic Factor 1 (SF-1) in Neurons in the Ventromedial Nucleus of the Hypothalamus in Rats on Aging

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 106, No. 6, pp. 720–727, June, 2020. Original article submitted February 15, 2020. Revised version received March 6, 2020. Accepted April 3, 2020.

Expression of steroidogenic factor 1 in neurons in the ventromedial nucleus of the hypothalamus in rats was studied using an immunohistochemical method and western blotting in young (2–3 months) and aged (2–2.5 years) male and female rats. Steroidogenic factor 1 was present in a majority of neurons in the ventromedial nucleus of the hypothalamus in young and aged rats, in the dorsomedial part of the nucleus. In aged rats, the proportion of neurons immunoreactive to steroidogenic factor 1 decreased significantly, from 88% to 68% in males and from 82 to 66% in females. There were no significant sex-related differences in the numbers of immunopositive neurons in either age group. Expression of steroidogenic factor 1 was also significantly lower in aged male and female rats in terms of western blot results. Thus, aging was associated with changes in the neurochemical composition of the ventromedial nucleus of the hypothalamus, which is involved in regulating energy metabolism and sexual behavior.

Keywords: hypothalamus, ventromedial nucleus, steroidogenic factor 1, aging.

The hypothalamus is a phylogenetically ancient segment of the diencephalon and plays an important role in maintaining the constancy of the internal milieu and supporting integration of the functions of the autonomous, endocrine, and somatic systems. The hypothalamus has been suggested to be involved in the mechanisms of aging [1, 2], with an important role being ascribed to the middle group of nuclei, including the ventromedial nucleus (VMN) of the hypothalamus [2, 3].

The VMN is known for its involvement in regulating sexual and feeding behavior, though it also regulates blood pressure and pain pathways [3–6]. Lesions to the VMN alter feeding behavior and are associated with hyperphagia and the development of obesity [7]. The internal clocks of the VMN play a role in controlling peripheral circadian rhythms [8].

The ventrolateral part of the VMN (vlVMN) is responsible mainly for sexual behavior in female rats and contains neurons expressing estrogen receptors [9, 10], while the dorsomedial part of the VMN (dmVMN) is more linked with controlling appetite [11]. The VMN contains heterogeneous neuron populations differing in terms of functional identity and biochemical identity. Different subpopulations of neurons in the VMN express different neuropeptides, peptide receptors, and transcription factors, and have different internal properties. The VMN also contains neurons responding to changes in extracellular glucose content [12].

Steroidogenic factor 1 (SF-1) is a nuclear receptor expressed in the adrenals, gonads, anterior pituitary, and VMN of the hypothalamus [13]. SF-1 is vitally important not only for the development of the VMN, but also for its physiological functions [14]. In mice, knockout of SF-1 induce obesity and anomalous development of the VMN [15], while mutations in SF-1 in humans lead to obesity and type 2 diabetes mellitus [16].

The aim of the present work was to study SF-1 expression in the VMN of the hypothalamus in young and aged rats.

Methods. Studies were performed on 40 white female ($n = 20$) and male ($n = 20$) Wistar rats aged 2–3 months (young,

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$n = 20$) and 2–2.5 years (aged, $n = 20$) in compliance with the Regulations for Studies Using Experimental Animals (USSR Ministry of Health Order No. 775 of August 12, 1977) and the principles of the Basel Declaration. Administration of a lethal dose of urethane (3 g/kg, i.p.) was followed by transcardiac perfusion with a solution of phosphate-buffered saline (PBS, 0.01 M, pH 7.4) (BioloT, Russia).

For immunohistochemical studies, rats ($n = 20$) were then perfused with 4% paraformaldehyde solution (Sigma, USA) in PBS. After perfusion, the brain was extracted and the hypothalamic area, containing the VMN, was harvested in accordance with coordinates from a rat brain atlas [17]. Series of coronal sections of the hypothalamus of thickness 14 μm were cut on a Shandon E cryostat (Thermo Scientific, UK). SF-1 was detected using labeled antibodies: primary monoclonal mouse antibodies to SF-1 diluted 1:150 (Invitrogen, Cat. No. 434200, USA); secondary goat antibodies (Jackson ImmunoResearch Laboratories, USA) against mouse immunoglobulin G conjugated with fluorescein isothiocyanate (FITC), which fluoresces in the green part of the spectrum, were diluted 1:100. Cells of the whole neuron population were stained with a stain fluorescing in the red part of the spectrum, i.e., NeuroTrace Red Fluorescent Nissl Stains (Molecular Probes, USA) diluted 1:200. Sections were then washed with PBS and embedded in VectaShield medium for immunofluorescence (Vector Laboratories, USA). To exclude nonspecific reactions, some sections were incubated without primary and/or secondary antibodies.

Preparations were examined under an Olympus BX43 fluorescence microscope (Tokyo, Japan) with the appropriate set of light filters and a Tucsen 6.1ICE cooled digital CCD camera and ISCapture 3.6 software (China). The proportion of SF-1-immunoreactive (IR) neurons was taken as the ratio of such neurons to the total number of neurons seen with NeuroTrace Fluorescent Nissl Stains, which was taken as 100%. Neurons whose sections passed through the nucleus with visible nucleoli and fluorescence greater than background fluorescence were included in the analysis. The proportion of IR neurons was determined using ImageJ (NIH, USA).

Western blotting was run on 20 rats, five in each group (males, females, young, aged). A vibratome was used to prepare sections of hypothalamus of thickness 300 μm. The VMN was excised under a stereomicroscope and then homogenized with lysis buffer. Each tissue lysate was diluted in sample buffer (BioRad Laboratories Inc., USA) and denatured at 95°C for 5 min. Equivalent quantities of samples were loaded and separated by electrophoresis on polyacrylamide gels and were then transferred to PVDF membranes (AppliChem, Germany). Membranes were blocked with blocking solution containing 3% defatted dried milk (AppliChem, Germany) in TBS-T (0.1% Tween 20, 0.2 mM Tris, 137 mM NaCl) for 30 min at room temperature. After washing with TBS-T, membranes were incubated with primary monoclonal mouse antibodies to SF-1 diluted 1:500 (Invitrogen, Cat. No. 434200, USA) and polyclonal rabbit antibodies to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) diluted 1:2500 (Abcam, UK) at 4°C overnight. After washing with TBS-T, membranes were incubated with secondary antibodies (goat HRP-conjugated anti-rabbit and anti-mouse IgG (Abcam, UK)) diluted 1:3000. Immunoblots were examined by chemiluminescence (ECL Prime western blot detection reagent, BioRad) with a Syngene G: BOX Chemi XR5E gel documentation system (Syngene, UK). Chemiluminescence signals were determined quantitatively using Gene Tools Gel Analysis software (Syngene, UK) and optical densities were expressed relative to GAPDH. Protein molecular weight markers were included in each western blot analysis.

Mathematical processing of data and construction of plots were performed using Sigma Plot (Systat Software, USA). All values are presented as arithmetic means ± errors of the mean ($M \pm m$). Pairs of independent groups were compared using the Mann–Whitney U test and three or more using Kruskal–Wallis rank analysis of variance with subsequent paired comparison of groups using the Mann–Whitney test with the Dunn correction for assessment of p values. Results were regarded as statistically significant at $p < 0.05$.

Results. The results of immunohistochemical studies showed that SF-1 is present in most neurons in the VMN of the hypothalamus in young and aged male and female rats, in the dorsomedial part of the nucleus (Fig. 1). The vlVMN contained only occasional SF-1-IR neurons. Occasional SF-1-IR neurons were also located in the area between the VMN and the arcuate nucleus, as well as in the arcuate nucleus.

In aged rats, the proportion of SF-1-IR neurons in the dmVMN decreased significantly, from 88 ± 2.5% to 68 ± ± 5.5% in males and from 82 ± 2.5% to 66 ± 2.7% in females ($p < 0.05$, Figs. 2 and 3). Neither age group showed any significant sex-related differences in the numbers of SF-1-IR neurons ($p > 0.05$).

Expression of SF-1 in the VMN was determined by western blotting; both nuclei in all animals showed bands corresponding to a protein of molecular weight 55 kDa. Expression of this protein was significantly reduced in aged male rats, from 0.59 ± 0.03 to 0.48 ± 0.01 and in aged female rats, from 0.57 ± 0.02 to 0.48 ± 0.01 ($p < 0.05$, Fig. 4) relative to GAPDH. No sex-related differences were seen in the level of expression of this protein in any of the age groups ($p > 0.05$).

Discussion. Our results on the selective expression of SF-1 in the dmVMN and central part of the VMN in rats are consistent with published data obtained in mice. SF-1 was detected in neurons in the hypothalamus of male mice through the whole extent of the VMN in the embryonic period, though by birth SF-1 was not detected in the vlVMN [18]. Ours is the first demonstration of the presence of SF-1-IR neurons outside the VMN, particularly as occasional neurons in the arcuate nucleus. Nonetheless, the closeness of these neurons to the VMN supports the notion that they

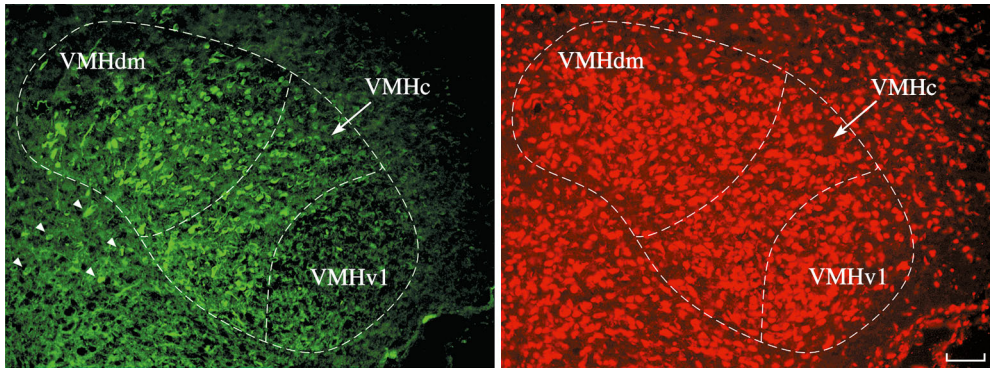

Fig. 1. Fluorescence survey photomicrograph (red, NeuroTrace Red stain, Fluorescent Nissl Stains) in an aged female rat showing SF-1-immunoreactive neurons (green). Arrowheads point to individual neurons located outside the VMN. VMHdm – dorsomedial, VMHc – central, VMHvl – ventrolateral areas of the VMN as per rat brain atlas [17]. Scale bars – 300 μm.

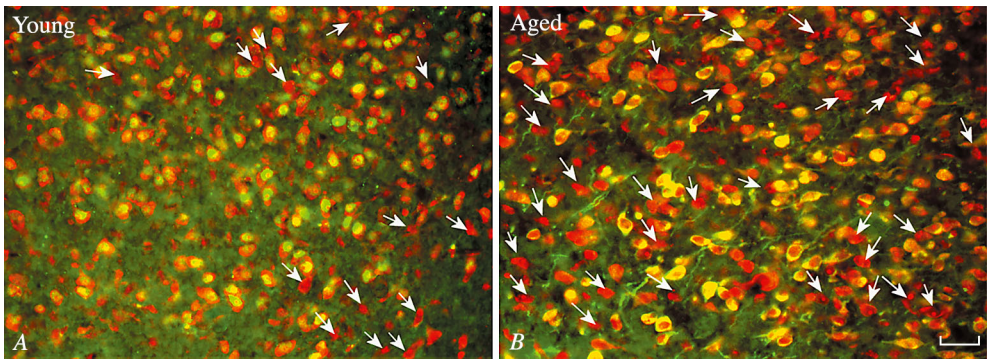

Fig. 2. Photomicrograph of SF-1-containing neurons in the dmVMN in young (*A*) and aged (*B*) female rats. FITC fluorescence (green, SF-1), NeuroTrace Red Fluorescent Nissl Stains (red). Scale bar – 50 μm.

have common origin and functions with the main population in the dmVMN.

We did not find any sex-related differences in the number or distribution of SF-1-IR neurons in the VMN. The vlVMN and dmVMN are structurally and functionally different. Published data provide evidence that sex specificity in rodents is characteristic of the vlVMN. Neurons in the vlVMN and dmVMN express relatively high levels of steroid receptors in females [19], while leptin receptors and corticotropin-releasing hormone type 2 receptors (CRFR2) are found mainly in the dmVMN [20, 21]. Thus, SF-1-containing neurons in the dmVMN play an important role in regulating metabolism.

The age-related decrease in SF-1 expression seen here in the VMN is consistent with previous data on changes in the neurochemical characteristics of rat VMN neurons on aging, particularly in terms of the percentage content of neurons containing nitric oxide synthase and the calcium-binding proteins calbindin and calretinin [22]. In gerontology, the elevation theory of aging holds that age-related increases in the threshold of sensitivity of the hypothalamus to homeostasis signals are of key importance [23]. Aging in mammals, including rodents and humans, is accompanied by increases in body weight and the quantity of fatty tissue. The VMN of the hypothalamus, which is responsible for regulating substance and energy metabolism in the body and is also involved in controlling peripheral circadian rhythms, presumptively also plays an important role in aging processes [24].

Thus, these results support the hypothesis that aging is associated with changes in the neurochemical composition of the ventromedial nucleus of the hypothalamus, which takes part in regulating energy metabolism and sexual behavior. The molecular mechanisms leading to decreased SF-1 expression in the hypothalamus require further study.

This study was carried out with a grant from the Russian Science Foundation (Project No. 19-15-00039).

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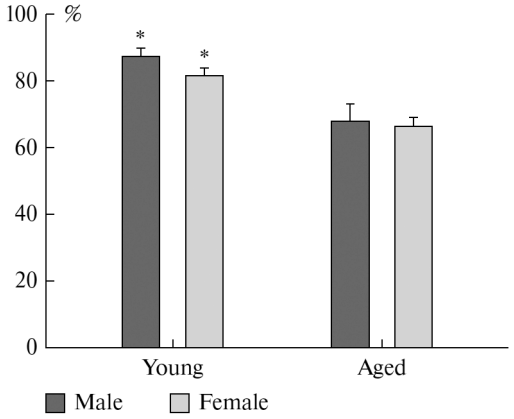

Fig. 3. Proportions of SF-1-IR neurons in the dmVMN of young and aged male and female rats. Significant differences compared with aged animals, $*p < 0.05$.

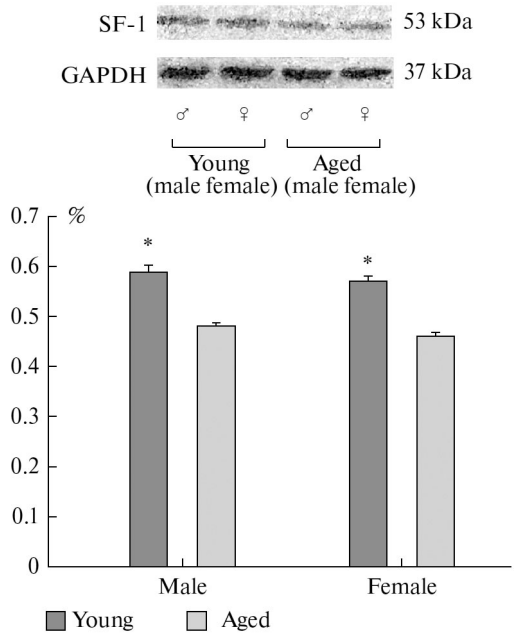

Fig. 4. SF-1 and GAPDH expression in the dmVMN in young and aged male and female rats. Data are presented in relation to the level of GAPDH expression. Immunoblots are shown above plots. Significant differences compared with aged rats, $*p < 0.05$.

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