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Morphology of NOS-immunoreactive Neurons in the Human Thalamic Reticular Nucleus and its Clinical Implications

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Nitric oxide (NO) is an important signaling molecule that is widely distributed in the central and peripheral nervous system. The aim of this study was to determinate the morphology of the different neurons scattered in the thalamic reticular nucleus (TRN) and close to it, i.e. the internal capsule and external medullary lamina of the thalamus. We discuss the main functions pertaining to the modulation of synaptic transmission in the TRN, the internal capsule and external medullary lamina, the targets of nitrergic signaling, and the involvement of nitric oxide (NO) in the different neurodegenerative diseases, psychiatric disorders, and sensory neurotransmission in the thalamocortical and corticothalamic connections passing through the TRN.

Key words: NOS, thalamic reticular nucleus, NO, neurodegenerative diseases

Introduction

The thalamic reticular nucleus (TRN) belongs to the lateroventral group of thalamic nuclei and it forms a shell enclosing the thalamus anteriorly and along its entire lateral aspect, enveloping the other thalamic nuclei. It consists of cell groups that are traversed by the thalamocortical and corticothalamic pathways [10]. It is located laterally to the external medullary lamina of the thalamus and medially to the internal capsule. The work of Buren and Borke [10] also describes a few main types of neurons, although not in great detail. In the dorsosagittal part of the TRN its largest in size neurons are found, with a diameter of 30-40 μm, while in the anteroinferior part of the nucleus are located its smallest neurons, measuring 20-25 μm. Most of the described cells are long rather than wide, and that difference is 10 to 30 μm. Before these findings, M. E. Scheibel and A. B. Scheibel [29] described in the TRN a population of multipolar neurons of various sizes, similar to the ones in the reticular formation in the brainstem. Berezhnaya [5] describes in detail the neuronal organization of the TRN in the human using silver nitrate impregnation by the Golgi method. Using the Ortolux (Leitz, Germany) microscope, in this study the authors investigated and drew all the sections in sagittal and frontal planes. Due to the fact that there is a similarity between human

neurons and the ones in other species, the classification and terminology of the TRN in the dog was applied [21]. Besides, in this classification the term "reticular cells" is also used to describe the TRN cells as being similar to the cells of the reticular formation in the brainstem [28]. Prior studies on cats report that the dendrites of the TRN neurons form a local inhibitory internal network [13]. Berezhnaya [5] describes in detail the morphology of the TRN neurons, and according to this study when comparing the TRN cells in the human and the other species it can be found that the classical aspiny neurons of types R1 and R2 are indeed identical [21]. Apart from these cells, this study reports that in the human TRN is also found another type of R1 reticular neurons with spine-like processes on their bodies and the proximal parts of their dendrites. The comparison of the large R1 reticular neurons with their identical types in the ventral anterior and ventral lateral nuclei of the human thalamus showed that the nature of branching in the former was the same [3]. Our previous studies on the presence and morphology of NADPH-diaphorase positive neurons in the human TRN [22], and also the study of Berezhnaya [4] on these neurons and the ones in the internal capsule, located lateral to the TRN, demonstrate without any doubt the presence of NADPH-diaphorase positive neurons in these structures of the human brain. Nitric oxide is a small, highly diffusible, and reactive molecule with a short lifespan that is generated by nitric oxide synthase (NOS) through enzymatic conversion of L-arginine to L-citrulline [1, 9]. It is highly reactive, readily diffusible and has limited solubility in water [25]. Neuronal NADPH-diaphorase is identical to the neuronal isoform of nitric oxide synthase (nNOS), and hence NADPH-d histochemistry provides a specific marker for the neurons producing nitric oxide, which has been described in 1991 year by Hope B. et al. [17].

Material and Methods

The brains of 2 males and 1 female (between 30 and 54 years of age) with no evidence of neurogical disorders were obtained at autopsy. The portion of each diencephalic part containing the TRN was dissected out and sectioned into blocks (1-2 cm in the transversal plane), and then fixed for two days in 4% paraformaldehyde in 0.1 M phosphate buffer. Serial coronal sections of 40 μm were cut on a freezing microtome and collected in the same buffer. All planes from the rostral to the caudal pole were examined. Each fifth section was processed for nNOS immunohistochemistry. Free-floating sections were preincubated for 1 hour in 5% normal goat serum in PBS. After that, incubation in the primary antibody was done for 48 hours at room temperature. We used monoclonal anti-NOS1 antibody (Santa Cruz) in a dilution of 1:1000. After rinsing in PBS, the sections were incubated for 2 hours in biotinylated goat anti-mouse IgG antibody (Vector) diluted 1:500. The sections were rinsed in PBS and incubated for 1 hour in avidin-biotin-peroxidase complex (Vector). This was followed by a rinse in PBS and then 0.05M Tris-HCl buffer, pH 7.6. These steps were followed by incubation in 0.05% 3,3′- diaminobenzidine (Sigma) containing 1% H_2O_2 (1:100) for the reaction product visualization. Sections were then collected in Tris-HCl buffer 0.05M, pH 7.6, air-dried for 24 hours, rinsed three times in distilled water (5 minutes per rinse), and air-dried again. Finally, they were mounted on gelatin-coated glass slides, dried for 24 hours and coverslipped with Entellan.

Results

We found NOS-immunopositive neurons everywhere along the longitudinal axis in the transverse sections of the TRN (**Fig. 1A, B**). Some of them were scattered, while others where organized in small groups of cells distant from each other (**Fig. 2C**). This is probably because of the critical position of the TRN, as it is crossed by thalamocortical and corticothalamic pathways. Some neurons that were close to the internal medullary lamina or internal capsule, or even located deep into these structures, were spindle shaped (**Fig. 1D**) or angular (**Fig. 2B**), while others were visualized as multipolar (**Fig. 2A, C, D**). There were even incoming neurons towards the internal capsule or the external medullary lamina of the thalamus. On the other hand, the neuropil showed very weak intensity of staining except for the places where fibers with varicosities along their length traversed akin the longitudinal axis of the nucleus. These fibers comprise part of the afferent and efferent connections of the TRN with the other thalamic nuclei, and also they are part of the thalamocortical and corticothalamic projections and the projectional fibers in the brainstem. Besides, in certain places immunopositive fibers running along the longitudinal axis of the nucleus were observed. They also possessed multiple varicosities, so it can be speculated that they belong to the association fibers connecting the neurons inside the TRN.

The spindle shaped neurons had processes that appeared from the opposite poles of the cells (**Fig. 1D**). After a short distance, the processes divided dichotomously, and while at first they were rather thick, after their division they became thin along their course. When followed visually, the processes further divided into smaller in

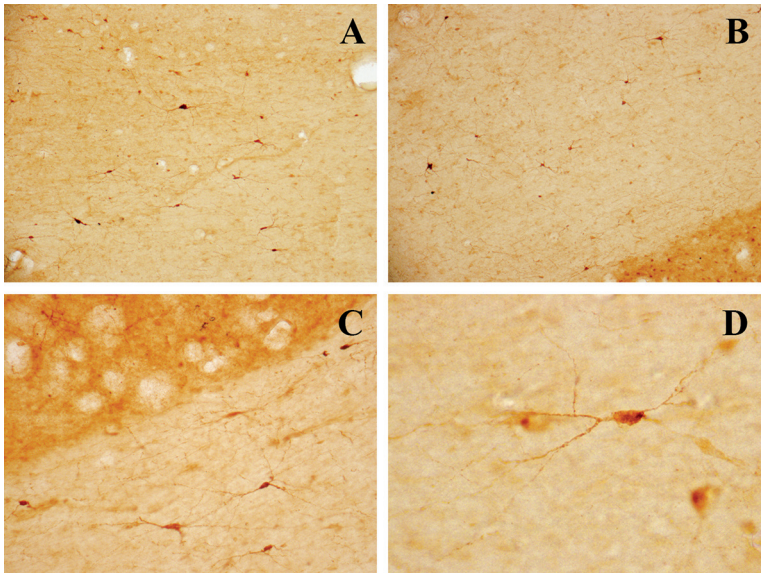

Fig. 1A. Overview of nNOS- imunoreactive neurons in the TRN (x10). **Fig. 1B.** Overview of nNOS- immunoreactive neurons. On the right side is the thalamus. (x10). **Fig. 1C.** Immunopositive neurons of different shape in the TRN (x20). **Fig. 1D.** Elongated reticular neuron with branches extending from the opposite poles of the cell (x40)

size branches. As a whole, the dendritic tree was not with too many arborizations. The axons of these neurons could be followed for some short distance and some of them were seen to end upon blood vessels via a single or a few varicose terminals (**Fig. 1D**). The perikarya of most of these cells demonstrated moderate and more rarely intense nNOS expression (**Fig. 1D**). Along the processes appearing from the neuronal somata, notwithstanding their thickness, varicosities were observed. They were larger in the proximal segments of the processes, while more distally their size diminished.

In the multipolar neurons the arborization of the dendritic tree was much more obvious (**Fig. 2 A, C**). The branches ran in all directions and varicosities of different sizes were seen along their length. The somata of the multipolar neurons demonstrated a considerable immunoreactivity for nNOS (**Fig. 2 A, C, D**).

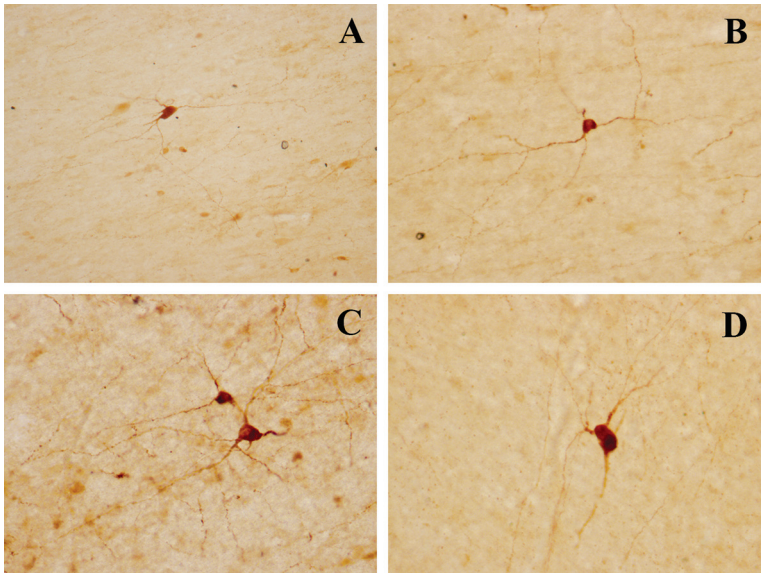

Fig. 2A. Multipolar neuron (x20). **Fig. 2B**. Angular neuron (x20). **Fig. 2C**. Multipolar neurons (x40). **Fig. 2D**. Multipolar neuron (x40)

Discussion

NO is a molecule with pleiotropic effects in the brain. This study describes the morphology and the intensity of the immunoreaction for nNOS in the cells of the TRN and the surrounding brain structures. NO is a signaling molecule in all mammals including the humans, and it is involved in a number of physiological and pathological processes [1]. NO facilitates the synaptic functions by long-term potentiation, maintenance and protein translation at the dendritic spines. It is also critical for the proper supply of blood to neurons and it has been demonstrated to be an antiapoptotic molecule and a regulator of neuronal function by nitrosylation. However, when NO is produced in a pro-oxidant environment, for example during aging, it damages the brain cells [27]. In the TRN there are two main types of reticular neurons, namely long-axon sparsely branched and short–axon smooth dendrite cells, described in our studies [22]

and by Berezhnaya [4] using NADPH-d histochemistry. It should be considered that the positive cellular reaction for NADPH-d indeed is a marker of the presence of nNOS in these cells, described by Hope et al. [17]. Hyperpolarization of the neurons in the thalamic nuclei, in particular the interneurons of the TRN, plays a role as a pacemaker for the transition of visual stimuli to the cortex [26]. This function is regulated by NO [6], whose presence we report in this study. NADPH-d reactivity is co-localized with GABA in a sub-population of local inhibitory interneurons [24]. In that region, the action of NO does not seem to involve cGMP [12]. NO regulates presynaptic plasticity in GABAergic and glutamatergic neurons [16]. Besides its proposed role in protecting NOS neurons from neurotoxicity, NO may itself induce neurotoxicity, especially in the adults. Neurotoxicity associated with cerebral ischemia is thought to involve glutamatergic stimulation via NMDA receptors [11, 19]. The reduction of GABA release from the TRN to other thalamic nuclei, due to inactivation of NMDA receptors on TRN neurons, would increase the firing rate of thalamic relay neurons to the cortex in schizophrenia [14].

NO plays multiple roles in the nervous system. Under physiological conditions, it contributes to regulating the proliferation, survival, and differentiation of neurons. The association of neurons expressing nNOS with blood vessels, which is found in this study, probably proves the intensity of blood supply and the trophic of the long-axon collaterals of the reticular neurons, and also the trophic of the fibers of the projection neurons [1]. NO is involved in synaptic activity, neural plasticity, and memory function; it exerts long-lasting effects through regulation of transcription factors and modulation of gene expression. Abnormal NO signaling could therefore contribute to a variety of neurodegenerative pathologies such as stroke/excitotoxicity, multiple sclerosis [7], Parkinson's and Alzheimer's disease [8], oxidative stress in vascular dementia [2], and intrinsic neuronal excitability [30]. The TRN is referred to as a pacemaker in the thalamus that generates rhythmic activity amongst thalamocortical and corticothalamic chains by mediation of inhibitory postsynaptic potentials [31] and it is regulated by NO [6]. Previous research has shown that the role of NO in sleep regulation is challenged. Actually, the group of Kilduff [18] has reported that long range projecting nNOS-type I GABAergic neurons are specifically activated during sleep [15, 23] by demonstrating that these cells in the cerebral cortex definitely accumulate c-Fos during sleep [15].

Conclusion

The key position of the TRN and its grid structure is involved in all the functional modalities that the nucleus is responsible for (motor, limbic, somatosensory, auditory and visual). All these modalities are associated in one way or another with the expression of NO.

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