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Light Microscopical Study of the GABA-expression in the Thalamic Reticular Nucleus of the Rat in the Postnatal Period

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The thalamic reticular nucleus (TRN) is a slender sheet of GABA-ergic neurons that coexist with parvalbumin and somatostatin. In this study we describe the changes in immunopositive neurons and the neuropil in the nucleus during the postnatal period of development, namely on the twentieth and sixtieth day. We found that the immunopositive reaction in twenty-day old animals was considerably stronger than that in sixty-day old ones. This phenomenon is probably associated with the maturation of the neurons, and also their plasticity and synaptogenesis.

Key words: thalamic reticular nucleus, GABA, maturation, plasticity

Introduction

Gamma-aminobutyric acid (GABA) is a biogenic amino acid that is synthesized from glutamic acid with the aid of glutamate decarboxylaze [12, 14]. The thalamic reticular nucleus (TRN) is a thin sheet of cells surrounding the anterolateral surface of the thalamus. It mainly consists of GABA-ergic neurons that coexist with parvalbumin and somatostatin [1]. Due to this specific location it is often regarded as "the gateway" through which the information from the thalamus to the cortex and vice versa has to pass. On the other hand, it is involved in sensory detection, arousal and attention [4, 11, 17, 22]. The disruption of the normal functions of the TRN is associated with the onset of epilepsy [13] and schizophrenia [7]. GABA is considered to be the most important inhibitory neurotransmitter in the CNS, and the excitation of the TRN neurons leads to the induction of a long-lasting period of hyperpolarization in the dorsal thalamic relay nuclei [5, 12, 14]. It has been found that GABA starts its expression very early in the prenatal development of the rat [3], and it is thought that the neurotransmitter role of GABA is highly significant and exerts both a trophic effect and regulation of various developmental processes [21].

The TRN can be divided into a few sectors, and each of them is associated with a different function, i.e. visual, auditory, somatosensory, motor and limbic. The limbic and motors sectors are located in the rostral part of the nucleus, and they are associated

with the anterior thalamic nuclei and the functionally related cortical areas [18, 23]. Cicirata et al. [2] describe the motor sector. All the sensory sectors, associated with thalamic nuclei and cortical structures, are situated in the central and caudal part of the nucleus [20].

Material and Methods

We used 2 groups of 5 rats, 20- and 60-day old respectively. All animals received humane care in compliance with the "Principles of laboratory animal care" formulated by the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the National Institute of Health (NIH publication No. 86-23, revised 1996). The animals were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2-7.4. Immediately after the perfusion, the brains were dissected out and placed in the same fixative for 1 hour at room temperature for postfixation. After a few rinses in 0.1 M phosphate buffer pH 7.4, the material was left to stay overnight in 0.01 M phosphate sodium-chloride buffer (PBS) pH 7.2-7.4 at a temperature of 4ºC. On a freezing microtome coronary brain sections with a thickness of 40 µm were sliced along the anteroposterior axis of the TRN. Free-floating sections were obtained and preincubated for 30 min in 3% H_2O_2 in phosphate-buffered saline, pH 7.4 (PBS) to inactivate endogenous peroxidase. After rinsing in PBS, non-specific sites were blocked in PBS containing 10% normal goat serum (NGS) and 0.2% Triton-X100. The sections were then incubated overnight at room temperature with the following antiserum diluted in 1% NGS in PBS: anti-GABA, 1:6000. After several rinses in PBS, the sections were incubated in biotinylated goat anti-rabbit IgG (Vector) diluted 1:200 in 1% NGS in PBS. The avidin-biotin-peroxidase protocol (ABC; Vector) was followed and finally 3,3´ -diaminobenzidine tetrahydrochloride (DAB; Sigma) was used as a chromogen. Thereafter, the sections were mounted on gelatin-coated glass slides, dried for 24 hours and coverslipped with Entellan. In order to control the specificity of the antibodies, some sections were processed by omitting the primary antibody or replacing it with NGS diluted 1:100. No specific staining was detected in this case. All the sections were examined under the light microscope.

Results

The TRN is a thin layer of cells surrounding the anterolateral surface of the thalamus (**Figs. 1A, 2A**). In twenty-day old rats we found the presence of immunoreactive neurons. The reaction product was confined both to the neuronal perikarya in the different sectors of the TRN, and along the short neuronal processes that formed small varicosities. In the rostral part of the TRN we predominantly observed oval-shaped neurons, and most of them had homogenous immunoreaction. Another part of the neurons expressed GABA in the periphery of their somata, above all in one pole of the cell bodies. The neuropil was moderately stained, albeit intense staining of the neuronal elements was observed (**Fig.1B**). Neurons of different shape and direction were marked in the central sector of the TRN, and they formed smaller or larger cellular groups. Some of the neurons were positioned in the vicinity of blood vessels.

The cellular groups themselves were located at some distance from one another. This sector of the nucleus had the weakest staining of the neuropil when compared to the rostral and caudal parts (**Fig. 1C**). In the caudal sector of the TRN the immunopositive neurons were mainly elongated to oval-shaped and aligned parallel to the long axis of the nucleus. The GABA immunoexpression was positive only in the peripheral parts of the neuronal somata, while the cell processes were longer than in the other neurons of the nucleus, and could only be followed for some distance. The solitary neurons were wholly immunoreactive, while the neuropil displayed weak staining (**Fig. 1D**).

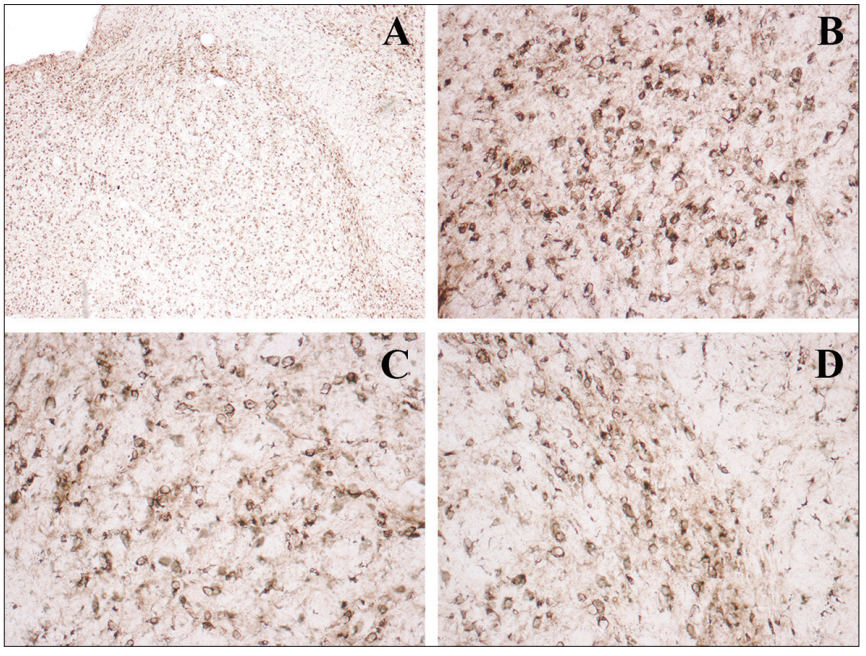

Fig. 1A. Anteroposterior view of the entire nucleus in 20 day animals (x10). **Fig. 1B.** Rostral sector of the TRN (x20). **Fig. 1C.** Central sector of the TRN (x20). **Fig. 1D**. Caudal sector of the TRN (x20)

In sixty-day old animals, the immunoreaction was located only in the peripheral part of the neuronal somata, and the intensity of the observed expression was considerably weaker when compared to the twenty-day old rats. It was solely in the rostral portion of the nucleus that solitary wholly-marked neurons were observed (**Fig. 2B**). In general, the immunostaining both of the neurons and the neuropil at this age was considerably weaker. In the rostral portion of the TRN, the GABA expression was most pronounced in comparison with the other nuclear parts. The spaces in-between the cellular groups in the central portion were significantly larger because of the traversing projection fibers (**Fig. 2C**). The GABA immunoreaction in the caudal portion was similar to that in the central (**Fig. 2D**). On the overall, the nerve fibers showed a weaker intensity of immuno-positivity in all the parts of the nucleus.

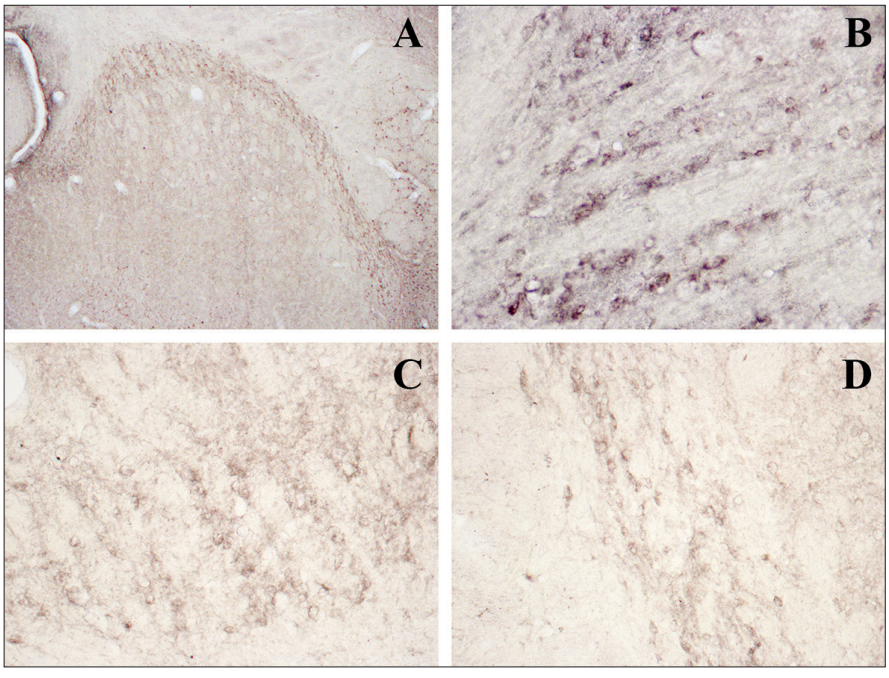

Fig. 2A. Anteroposterior view of the entire nucleus in 60 day animals (x10). **Fig. 2B.** Rostral sector of the TRN (x20). F**ig. 2C**. Central sector of the TRN (x20). **Fig. 2D.** Caudal sector of the TRN (x20)

Discussion

This study aimed to assess the neuronal expression of GABA and the plasticity in the TRN during the postnatal development in the rat. The TRN is extremely rich in GABA-ergic neurons [5, 24]. Since it is located on the border of the afferent and efferent pathways between the cortex and the thalamus [19, 24], the TRN receives collateral projections from both of them. Besides, the TRN also receives direct projections from other thalamic nuclei, though it relays information only to thalamic nuclei and other subcortical areas, and not the cortex [15]. On the other hand, the TRN contains GABA-ergic neurons of two distinct types – ones coexisting with parvalbumin, and others coexisting with somatostatin [1]. These two neuronal types are responsible for various thalamocortical relations. The parvalbumin GABA-ergic TRN neurons generate rhythmic thalamocortical sensory information, while the neurons containing somatostatin exert both anticonvulsive and convulsive effects [9]. Moreover, deep cerebral stimulation of the TRN is applied as a novel therapeutic strategy in laboratory animals to influence the neuronal function of the nucleus; it is also used to modulate the neuronal excitability in animal models [16]. The inhibitory role of GABA in the TRN appears after the second postnatal week, when most of the GABA-ergic terminals reach full development [6]. The vulnerability of the immature brain to epileptic seizures is probably the reason for the disruption of neuronal connectivity. This is associated with

the processes of maturation, synaptogenesis, neuronal migration and differentiation, and to a lesser degree with neuronal death [8]. The decrease in the GABA expression in sixty-day old rats is probably associated with the maturation of the brain structures. When GABA neurotransmission in the TRN is disrupted, a number of various psychiatric disorders, such as schizophrenia and depression, Parkinson's disease, and Alzheimer's disease may develop [10]. The technique of deep cerebral stimulation gives an opportunity to modulate neuronal excitability in experimental animal models for neurological and psychiatric conditions. When the normal development of the TRN neurons is affected in the early stages of the postnatal development of the rat, it results in the disruption of the interneuronal connections within the TRN, and also in communication alterations between the TRN and the thalamus on the one hand, and between the TRN and the thalamocortical and corticothalamic pathways on the other hand. These phenomena lead to the development and onset of the diseases that were described previously.

Conclusion

It is apparent that GABA plays significant roles in the developmental processes of the TRN and the brain in general. These roles are associated with trophic functions, synaptogenesis and maturation, especially in the early postnatal stages. The disruption of the functional modalities in the adult TRN may lead to various neurological and psychiatric conditions, so acquiring further knowledge on the intrinsic processes in the nucleus may offer perspectives for novel therapeutic strategies.

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