Original Article

POSTNATAL DEVELOPMENT OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY AND NADPH-D-REACTIVITY IN THE THALAMIC RETICULAR NUCLEUS OF MALE AND FEMALE RATS: QUANTITATIVE DOUBLE-STAINING STUDY

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Summary

Dopamine (DA) is synthesized from tyrosine in a two step reaction, implicating first tyrosine hydroxylase (TH) generating L-DOPA and second DOPA decarboxylase, generating DA. Expression of the enzyme TH and NADPH-d-reactivity in the thalamic reticular nucleus (TRN) of male and female rats during postnatal developing was studied by means of TH-immunohistochemistry and NADPH-d- histochemistry. The sameness of NADPH-d- reactive neurons and fibers with those which were has proved TH in double labeling experiments. Morphometric analysis discovered sexual dimorphism in the thickness of NADPH-d- reactivity and TH- immunoreactivity of the neurons and neuronal elements in the TRN. The measuring of sex differences was done by using Student's t-test. The present results of this study suggests that has sex differences of TH-immunoreactivity and NADPH-d-reactivity in the TRN, probably related to epigenetic effects of gonadal hormones in the postnatal development of TRN.

Key words: thalamic reticular nucleus, tyrosine hydroxylase, NADPH-diaphorase, sex differences

Introduction

The thalamic reticular nucleus (TRN) is a thin layer of GABA- ergic neurons surrounding the anterolateral surface of the dorsal thalamus. The TRN is located between the internal capsule (capsula interna), which forms its outer margin, and the external medullary plate (lamina medullaris externa thalami). Neurons of TRN receive synaptic imput from collaterals of thalamocortical and corticothalamic neurons [1, 2]. These fibers pass through specific regions of the TRN [3, 5]. The TRN sends reciprocal projections to its associated nuclei in the dorsal thalamus [3]. Located at the intersection of thalamo-cortical and cortico-thalamic axons, the TRN occupies a strategic position between the neocortex and the thalamus [4]. Nitric oxide (NO) is a gaseous neurotransmitter, which become involved in the sexual behavior via the maturation of the nervous system [6,7,8,9,10,11] and activity of NADPH-d can be altered by steroid hormones [12].

Materials and Methods

We used 6 male and 6 female Sprague-Dawley rats to study the histochemistry for NADPH-d and the immunocytochemistry for TH in the developing thalamic reticular nucleus of intact animals at postnatal day-20. This period that considered as „critical period" of brain sexual differentiation [6]. The animals were anesthetized with thiopental (40 mg/kg b.w.). Transcardial perfusion

was done with 4% paraformaldehyde in 0,1 phosphate buffer, pH 7,2. Coronal sections were cut on a freezing microtome (Reichert-Jung) at 40 m. First, every fifth section was processed for double staining NADPH-d and TH [see 6]. Second, coronal sections were used for estimate of the neuronal thickness in the thalamic reticular nucleus of male and female rats. Morphometric analysis was performed using a microanalysis system (primary magnification 40 x objective). Data of complete drawings were entered in the computer program (Olympus CUE-2) recorded automatically and calculated. Findings from males and females rats were compared by the Student's t- test. All values are presented as means ± standard error of the mean (S.E.M).

Results and Discussion

Dopamine and NO played a key role in the regulation and control of the sexual differentiation in the rat. Double- stained neurons for TH and NADPH-d identified (Fig. 1). The first type neurons seen in the caudal part of TRN. This neurons was determinated by fusiform cells body with straight and long rarely branched dendrites. The second type of double-stained neurons varied in form, oval or slightly elongated, multipolar cells with short relatively unramified dendrites that were stretched out along the axis of the TRN, which seen in the rostal and the middle part of the TRN, like their density was greater in the rostral then the middle part (Fig. 2, 3).

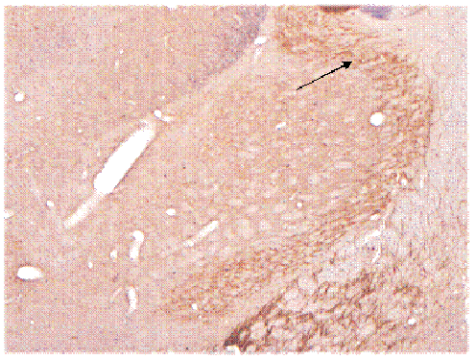

Fig. 1. TRN (arrow) double staining for TH immunoreactive neurons (brown) and NADPH-d-reactive fibers. x 40.

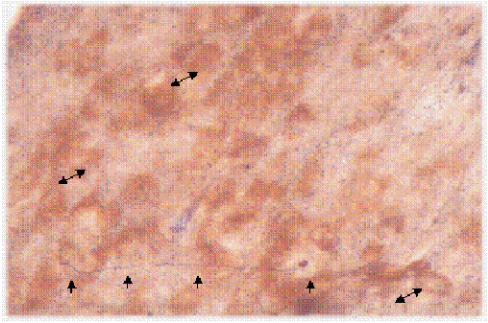

Fig. 2. TH-immunoreactive neurons (double head arrows) and NADPH-d-reactive fibers in the rostral part of the TRN (arrows). x120

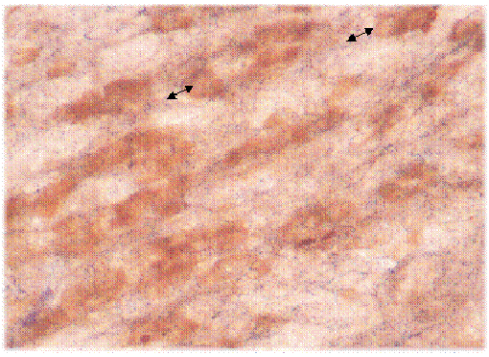

Fig. 3. TH-immunoreactive neurons (double head arrows) and NADPH-d-reactive fibers in the middle part of the TRN (arrows). x120

The lineaments of measures for the thickness of NADPH-d-reactive fibers in the TRN were performing using the computer-assisted program. The bundle of these fibers is presented for the total density of the TRN in **the postnatal developing of male and female rats.** The average density of the NADPH-d-reactive fibers (as mean area) per μm^2 and percentage of measured area in the TRN for male rats is greater than for female. There is a statistically significant decrease in neuronal density from male to female ($p< 0.01$).

The packing density of TH-immunoreactive neurons is presented for the total thickness in the TRN in 20 day old male and female rats. The average number of NADPH-d-reactive fiber and axonal elements in prepubertal male rats was measured and compared with the same measures in the female rats. The average density of the TH-immunoreactive neurons per μm^2 in the TRN for 20 days old male rats is greater than for female, (Fig. 4). There is a statistically significant decrease in neuronal density from male to female ($p< 0.01$).

The principal findings in the present study were as follows. First, TH immunohistochemistry in combination with the NADPH-d reaction allowed us to determine the specific distribution pattern for neurons [6, 8, 7, 9, 13, 14, 21]. TH-immunoreactive neurons in the TRN are located outside of classically-described catecholaminergic system and probably this distribution can be species-specific [15].

Second, our data provide the evidence that there are sex differences in the density of NADPH-d reactive fibers and TH-immunoreactive neurons of the rat TRN at 20 days of age. Males have greater density of NADPH-d reactive fibers than females. These results suggest that sex differences in the density of NADPH-d-reactive fibers, in the TRN can be related to the epigenetic action of gonadal hormones during the early stages of the development [10, 11]. This conclusion corresponds to results that reported such correlation between androgens and expression of different neuroactive substances in various brain regions [6, 7, 8, 9, 17, 18, 19, 20, 21].

Thurth, however, the exact mechanism, by which sex differences in NADPH-d-reactivity are settled

during the development, remains an intriguing question. Our new data emphasize the need to examine the NADPH-d-reactivity in sectors of the postnatal TRN at different days of ages after experimental manipulations of the hormonal environment.

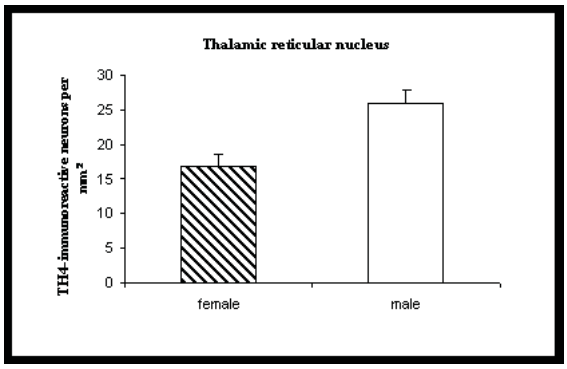

Fig. 4. The number of TH-immunoreactive neurons in чm2 in the TRN of male and female 20 days old rats. Values are presented as means + S.E.M.

References

1. Ohara PT, Lieberman AR. The thalamic reticular nucleus of the adult rat: experimental anatomical studies. J Neurocytol. 1985;14(3):365-411.
2. Clemance AM, Mitrofanis J. Cytoarchitectonic heterogeneities in the thalamic reticular nucleus of cats and ferrets. J Comp Neurol. 1992;322(2):167-80.
3. Jones E. G. Some aspects of the organisation of the thalamic reticular complex. L Comp Neurol. 1975;162(3):285-308.
4. Pinault D. The thalamic reticular nucleus: structure, function and concept. Brain Res Rev. 2004;46(1):1-31.
5. Crabtree JW, Killackey H P. The topographic organization and axis of projection within the visual sector of the rabbit's thalamic reticular nucleus. Eur J Neurosci. 1989;1(1):94-109.
6. Ovtscharoff W, Bozhilova- Pastirova A, Christova T, 2002. Postnatal development of neurons expressing NADPH-diaphorase and parvalbumin in the parietal cortex of male and female rats. Acta Histochemica. 2002;104(1):23-8.
7. Landzhov B, Bozhilova-Pastirova A, Ovtscharoff W. Compt Rend Acad Bulg Sci. 2004;57(2):99-102.
8. Bozhilova-Pastirova A, Landzhov B, Izacov B, Malinova L, Ovtscharoff W. NADPH-d-reactivity and parvalbumin-immunoreactivity in the postnatal piriform cortex. Compt Rend Acad Bulg Sci. 2004;57(3):67-70.
9. Landzhov B, Bozhilova-Pastirova A, Ovtscharoff W. NADPH-d-reactivity and parvalbumin-immunoreactivity in thalamic reticular nucleus of male and female rats. In: Anatomical collection, 85th anniversary of department of anatomy and histology, Sofia; eds. W. Ovtscharoff, 2003;49-51.
10. Malinova L, Bozhilova-Pastirova A. Postnatal development of the NADPH-diaphorase-reactivity and parvalbumin immunoreactivity in the visual sector of the thalamic reticular nucleus of male and female rats. Compt Rend Acad Bul. Sci. 2009 (in press)
11. Malinova L, Bozhilova-Pastirova A. Postnatal development of the dopamine d4 receptor immunoreactivity in the thalamic reticular nucleus of male and female rats. Compt Rend Acad Bulg Sci. 2009 (in press)
12. Okamura H., Yokosuka M, Hayashi S.Estrogenic induction of NADPH-diaphorase activity in the preoptic neurons containing estrogen receptor immunoreactivity in the female rat. J Neuroendocrinol. 1994;6(6):597-601.
13. Nagaeva DV, Akhmadeev AV. Structural organization, neurochemical characteristics, and connections of the reticular nucleus of the thalamus. Neurosci Behav Physiol. 2006;36(9):987-95.
14. Ovtscharoff W, Eusterschulte B, Zienecker R, Reisert I, Pilgrim C.Sex differences in densities of dopaminergic fibers and GABAergic neurons in the prenatal rat striatum. J Comp Neurol. 1992;323(2):299-304.
15. Asmus SE, Newman SW. Tyrosine hydroxylase mRNA-containing neurons in the medial amygdaloid nucleus and the reticular nucleus of the thalamus in the Syrian hamster. Brain Res Mol Brain Res. 1993;20(3):267-73.
16. Sanghera MK, Simpson ER, McPhaul MJ, Kozlowski G, Conley AJ, Lephart ED. Immunocytochemical distribution of aromatase cytochrome P450 in the rat brain using peptide-generated polyclonal antibodies. Endocrinology. 1991;129(6):2834-44.
17. Bozhilova-Pastirova A, Ovtscharoff W. The inferior oilvary complex. Adv Anat Embryol Cell Biol. 2000;155(III-VI):1-84.
18. Stefanova N, Ovtscharoff W. Sexual dimorphism of the bed nucleus of the stria terminalis and the amygdala. Adv Anat Embryol Cell Biol. 2000;158(III-X):1-78.
19. Stefanova N, Bozhilova-Pastirova A, Ovtscharoff W. Sex and age differences of neurons expressing GABA-immunoreactivity in the rat bed nucleus of the stria terminalis. Int J Dev Neurosci. 1998;16(6):443-8.
20. Stefanova N, Bozhilova-Pastirova A, Ovtscharoff W. Distribution of GABA-immunoreactive nerve cells in the bed nucleus of the stria terminalis in male and female rats. Eur J Histochem. 1997;41(1):23-8.
21. Koleva K, Landzhov B, Malinova L, Ilieva E, Ovtscharoff W, Bozhilova-Pastirova A. Postnatal development of tyrosine-hydroxylase-immunoreactive neurons in brain of male and female rats. Praemedicus since 1924. 2004;25(1-2):71-5.