GENETICS

Bax and Bcl-X_L Apoptosis Protein mRNA in Rat Brain Stem and Cortex during Ontogeny

A. V. Bannova, P. N. Men'shanov, F. A. Il'inykh, T. S. Kalinina, and N. N. Dygalo

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Bax mRNA level in fetal rat brain stem increases by day 40 of life and then decreases, while level of Bcl-X_L mRNA reaches the adult value over one month. Bax mRNA level in the cerebral cortex decreases from day 8 to day 90 of life, while Bcl-X_L mRNA level does not change. Judging from Bcl-X_L/Bax mRNA ratio, cortical cells exhibit higher readiness to apoptosis than brain stem cells.

Key Words: *Bax; Bcl-X_L; brain; ontogeny; apoptosis*

Apoptosis is an essential component of normal development of the nervous system in mammals [2,7]. More than half of primordial cerebral neurons undergo apoptosis during brain formation [5]. In rats, proliferation and differentiation of brain stem neurons are completed during the embryogenesis period, while in the cortex these processes continue during the first days after birth. Heterochronic maturation suggests different ontogenetic time course of apoptosis in these structures. Obligatory participants in apoptosis are Bcl-2 family proteins: Bcl-X_L antiapoptotic protein and Bax proapoptotic protein [2,8].

The content of Bcl-X_L and Bax mRNA in the frontal compartments of the brain were studied during ontogeny by *in situ* hybridization [3]. Unfortunately, only qualitative changes can be evaluated by this method, which explains disagreement in the data on the ontogenetic changes in Bcl-X_L and Bax proteins [5, 12]. In addition, the proportion between pro- to antiapoptotic representatives of Bcl-2 family determines cell liability to triggering the self-destruction program [9]. Quantitative data are needed for evaluation of this ratio.

We studied the levels of Bax and Bcl-X_L mRNA in rat brain stem and cortex during ontogeny.

MATERIALS AND METHODS

The study was carried out on 20- and 21-day Wistar rat fetuses and on rats aged 5, 8, 24, 40, and 90 days. Fetal brain was dissected into the stem and frontal parts along the plane from the pineal gland to the optic chiasm. Starting from day 5 of life, the brain stem, including the metencephalon and pons area, and 1.5-3.0 mm thick cerebral cortex, including the frontal half of hemisphere surface, were isolated. Blocks of tissue from both compartments increased with the growth of the brain remaining within the specified anatomical limits for all ages.

The level of mRNA was evaluated by semiquantitative reverse transcription PCR (RT-PCR) in total RNA isolated by single-step guanidine isothiocyanate method. cDNA was isolated using Oligo-dT primer and MuLV revertase (Sibenzyme). Amplification of cDNA sites specific for each gene was carried out by the standard method [1] using primers for Bax [11],

Laboratory of Genetic Base of Neuroendocrine Regulation, Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk. ***Address for correspondence:*** anitik@bionet.nsc.ru. A. V. Bannova

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Bcl-X_L [10], or β-actin. The number of PCR cycles for each pair of primers corresponded to the exponential phase of accumulation of optically detectable amplification product, the amounts of cDNA and PCR product were in linear relationship for all genes. The level of Bax and Bcl-X_L mRNA was evaluated relatively the content of β-actin mRNA after UV scanning PCR products (BioDoc II, Biometra GmbH) separated by electrophoresis in 1.5% agarose gel and stained with ethidium bromide followed by computer-assisted densitometry (Scion Image).

The effect of age on the studied parameters was evaluated by unifactorial dispersion analysis (ANOVA), the significance of differences between the age groups was evaluated using LSD test for multiple comparisons.

RESULTS

The level of proapoptotic protein Bax mRNA in the brain of rats changed appreciably during ontogeny (Fig. 1, *a*). The level of this transcript in the cortex of 20-21-day fetuses was somewhat higher than on days 40-90 of life, while the level of Bax mRNA in the brain stem of fetuses was virtually the same as in adult animals. By day 5 of life the content of Bax mRNA somewhat decreased in comparison with the prenatal period in both brain structures, but later the time course of age-associated changes in each compartment was specific. In the brain stem the content of Bax mRNA increased and peaked on day 40 of life, but then significantly decreased ($F_{(6,19)}$=3.8; p<0.01) by day 90 of life. In the cerebral cortex the content of this transcript increased 1.5 times by day 8 of life in comparison with day 5, after which it gradually decreased to day 90 of life ($F_{(6,18)}$=2.4; p<0.07). RT-PCR showed clear-cut age-associated changes (though slight by the amplitude) in Bax mRNA concentrations in the brain stem and cortex of rats, which previously could not be detected by *in situ* hybridization [3]. Our results indicate that ontogenetic changes in the concentration of Bax mRNA contribute to the decrease in the level of this proapoptotic protein observed during the postnatal period in the cortex [12] and in the brain, in general [5].

The level of Bcl-X_L in the cortex of 20-21-day rat fetuses was virtually the same as in 90-day-old animals (Fig. 1, *b*). No appreciable differences in Bcl-X_L mRNA content in the cortex were detected during the studied period ($F_{(6,14)}$=1.24; p<0.35). The level of Bcl-X_L mRNA in fetal brain stem was about 75% of the adult level. After birth the content of Bcl-X_L mRNA in the brain stem slightly decreased by day 5 of life, after which it increased until day 40 of life; then the level of this transcript did not change until the age of 3 months ($F_{(6,18)}$=4.35; p<0.01). RT-PCR, similarly to *in situ* hybridization [3,4], failed to detect ontogenetic changes in Bcl-X_L mRNA in the cortex. These results agree with the constant level of Bcl-X_L protein starting from the period of embryonic development until adult state in the cortex [12] and brain in general [4,5]. Similarly as with Bax mRNA, RT-PCT showed a 1.5-fold increase in Bcl-X_L mRNA level in the brain stem during postnatal ontogeny, while *in situ* hybridization failed to detect this increase [3]. Our data confirm the data on the increase in Bcl-2 protein content in the brain stem during the first month of rat life [6].

The Bcl-X_L/Bax mRNA ratio in the brain stem was 0.5-0.8 in fetuses and 5-day-old rat pups, about 1 on days 8-40 of life, and 1.5 on day 90 of life. The pattern of changes in the ratio of anti- to proapoptotic protein transcripts attests to decreased liability of the

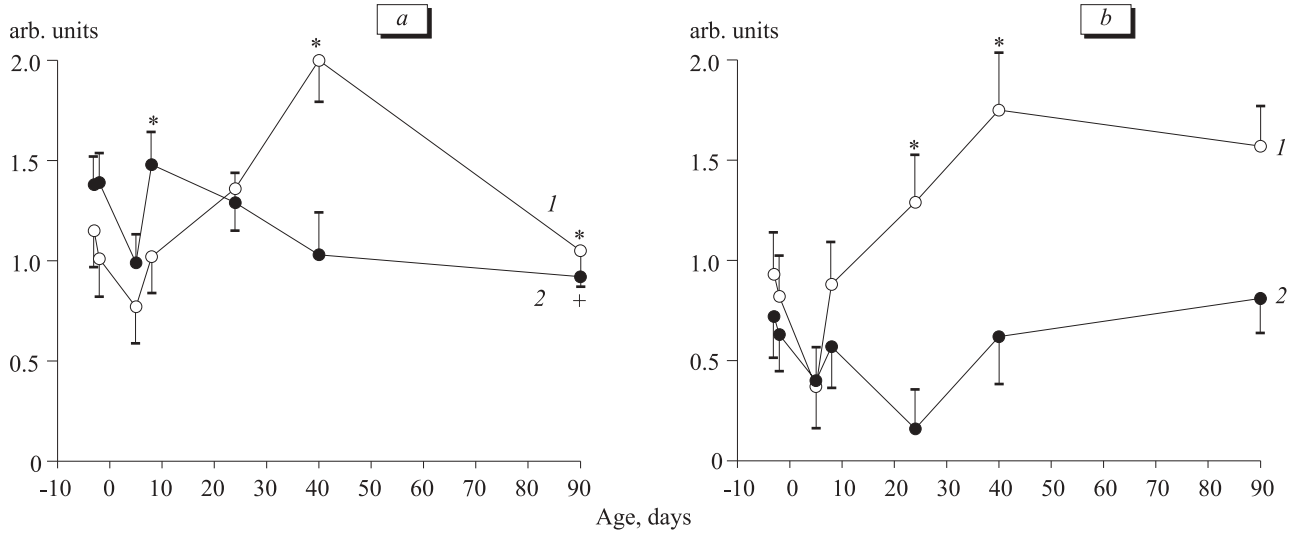

Fig. 1. Levels of proapoptotic Bax (*a*) and antiapoptotic Bcl-X_L mRNA (*b*) in arbitrary units relative to the level of β-actin mRNA in the rat brain stem (*1*) and cortex (*2*) during ontogeny. *p<0.05 compared to previous age; ^+p<0.05 compared to day 8 of life.

brain stem to triggering apoptosis program during ontogeny. This conclusion is confirmed by a previously detected decrease in the level of caspase 3 mRNA (the main apoptosis protease) in the brain stem during ontogeny [1]. In the cortex the Bcl-X_L/Bax mRNA ratio was only 0.1-0.5 during the first 3 weeks of life and gradually increased to about 1 by day 90 of life. Judging from the Bcl-X_L/Bax mRNA ratio, cortical cells are characterized by higher (in comparison with brain stem cells) readiness to self-destruction during the early postnatal period and retain this readiness to the adult state; this was previously demonstrated by caspase 3 mRNA assay [1].

In general, these findings indicate that changes in the levels of Bax and Bcl-X_L apoptotic protein mRNA and their ratio in the brain stem and cortex during rat ontogeny exhibit clear-cut regional differences. These differences during the early ontogeny seem to be due to heterochronic maturation of the studied structures. In older age regional differences can be due the presence of proliferation zones in the cortex in adult age and hence, the necessity to eliminate excessive cells in these structures, while important regulatory centers are located in the brain stem, whose cells should therefore be highly resistant to factors provoking their death.

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