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REGIONAL EXPRESSION OF THE NERVE GROWTH FACTOR GENE FAMILY IN RAT BRAIN DURING DEVELOPMENT

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The developmental expression patterns of three members (NGF, NGF-2/NT-3, and BDNF) of the NGF family in rat brain are different. NGF-2/NT-3 mRNA was the first detected during development followed by NGF and BDNF mRNAs. A substantial amount of NGF mRNA was found to be synthesized in the hippocampus and the cortex, and this regional expression pattern did not change during development. In contrast, NGF-2/NT-3 mRNA was detected in almost all the brain regions examined in the early developmental stage. In the late stage, the transcript was found in high concentration only in the hippocampus and the cerebellum. BDNF mRNA was widely distributed, and its level was augmented in the late developmental stage.

Neurotrophic factors are believed to play an important role in the growth, survival, and differentiation of neurons in the nervous system. Nerve growth factor (NGF), a well-characterized neurotrophic factor, is essential for the survival and development of peripheral sensory and sympathetic neurons as well as cholinergic neurons of the basal forebrain (1,2). Recently, the genes coding for brain-derived neurotrophic factor (BDNF)(3,4) and nerve growth factor-2 (NGF-2)(5)/neurotrophin-3 (NT-3)(6,7,8,9) were cloned. Hippocampus-derived neurotrophic factor (HDNF) reported by Ernfors et al. (10) is identical to NGF-2/NT-3. Comparison of the primary structures deduced from their nucleotide sequences indicates that they belong to the same family. Since each of these three members shows a different tissue distribution and a different biological activity with respect to peripheral sensory and sympathetic neurons (7), each

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may have a distinct function in the peripheral nervous system. There are some reports dealing with regional distribution of the mRNAs of these factors in adult rat brain (11,12,13,4,6,8,10), but it is not clear whether the expression of the gene coding for each member is developmentally regulated or not. Therefore, we investigated the regional expression pattern of each of these genes in rat brain during development, using Northern blot analysis.

MATERIALS AND METHODS

Northern blot analysis

Rats (Sprague-Dawley rats from CLEA Japan) were sacrificed and the brains were quickly removed and kept in ice-cold saline. The following regions were then dissected from the brains; the neocortex, the entorhinal cortex and the pyriform cortex, the hippocampus, the striatum, the midbrain, the diencephalon, the cerebellum, and the brain stem. Total RNAs were isolated from these brain regions by the method of Chirgwin et al (14). Poly $(A)^+$ RNAs prepared by oligo-dT cellulose column chromatography (Pharmacia) (15) were electrophoresed on 1.5% agarose gel containing formaldehyde and transferred to a nitrocellulose filter (S&S)(16). DNA probes prepared as described below were labelled to the specific activity of $3x10^8$ cpm/ug. A 0.6 kb fragment containing rat NGF gene was obtained by polymerase chain reaction (PCR) from a rat genomic DNA using synthetic oligonucleotides. A 1.1 kb fragment containing rat NGF-2/NT-3 gene was obtained from a rat genomic library. A 0.7 kb fragment containing rat BDNF gene was obtained by PCR from a rat genomic DNA using synthetic oligonucleotides. These DNA fragments encode both pro-sequences and mature regions of the factors. A DNA fragment containing human actin gene was purchased from Wako Ltd. (Japan). Hybridization was carried out for 16 hr at 42 ^{o}C in 50% formamide, 5xSSPE (1xSSPE contains 180 mM NaCl, 10 mM sodium phosphate, and 1mM EDTA; pH 7.7), 5x Denhardt's solution (1xDenhardt's solution contains 0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), 0.5% SDS, 80 ug/ml sonicated denatured salmon sperm DNA, and 50 ug/ml yeast tRNA. The filters were washed three times each for 5 min at 25 ^{o}C in 2xSSC (1xSSC contains 150 mM NaCl and 15 mM sodium citrate) containing 0.1% SDS and then once for 90 min at 65 ^{o}C in 0.5xSSC containing 0.1% SDS. The filters were autoradiographed onto Kodak X-AR films at -80 ^{o}C with intensifying screens.

RESULTS

Developmental expression of the NGF gene family in rat brain

We have examined the synthesis of NGF, NGF-2/NT-3, and BDNF mRNAs in rat brain from embryonic day 17.5 to 5 weeks postnatally. A 1.3 kb highly expressed NGF mRNA was detected beginning in the postnatal stage, and its synthesis reached a plateau 3 weeks after birth (Fig. 1 a). A 1.5 kb NGF-2/NT-3 mRNA was observed beginning in the embryonic stage, and the level of

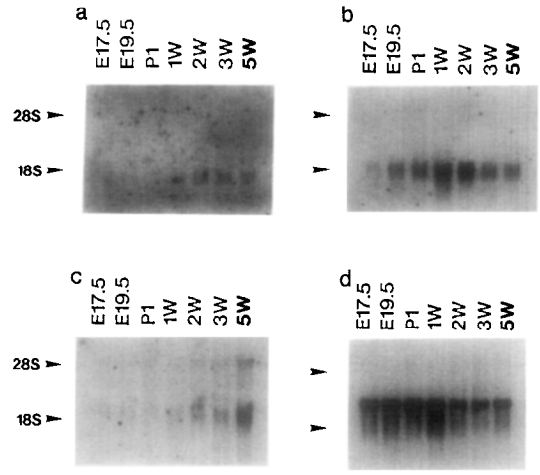

Figure 1. Developmental expression of the NGF gene family in rat brain. Ten micrograms of poly $(A)^+$ RNA from rat brain was loaded in each lane and was analyzed. DNA fragments encoding rat NGF (a), rat NGF-2/NT-3 (b), rat BDNF (c), and human actin (d) were labelled and used as probes. Positions of ribosomal RNA markers are shown on the left. E17.5 and E19.5 indicate embryonic day 17.5 and 19.5, respectively; P1, 1W, 2W, 3W, and 5W indicate 1 day, 1, 2, 3, and 5 weeks postnatally, respectively.

its synthesis reached a maximum 1 to 2 weeks after birth (Fig. 1 b), whereas a 1.6 kb and a 4.0 kb BDNF mRNA were detected also beginning in the embryonic stage, but the level of their synthesis was the highest 5 weeks postnatally (Fig. 1 c). The amounts of mRNA of these three factors gradually decreased toward 71 weeks postnatally (data not shown). Since all the probes used had the same specific activity, the level of NGF-2/NT-3 mRNA synthesis seemed to be the highest among the three members. The level of actin mRNA synthesis was found to be almost equal in all the above experiments (Fig. 1 d).

Regional expression of the NGF gene family during development

The regional expression patterns of the three genes from 2 to 12 weeks postnatally are shown in Fig. 2, 3, and 4. NGF mRNA (1.3 kb) was observed mainly in the hippocampus and the cortex in all the developmental stages examined (Fig. 2), whereas the expression of NGF-2/NT-3 gene appeared to be regulated differently in individual brain regions during development: A 1.5 kb NGF-2/NT-3 mRNA was detected in the hippocampus and the cerebellum as well as in all other brain regions 2 weeks postnatally, but almost all the NGF-2/NT-3 mRNA was detected in the hippocampus and the cerebellum 5 and 12 weeks postnatally

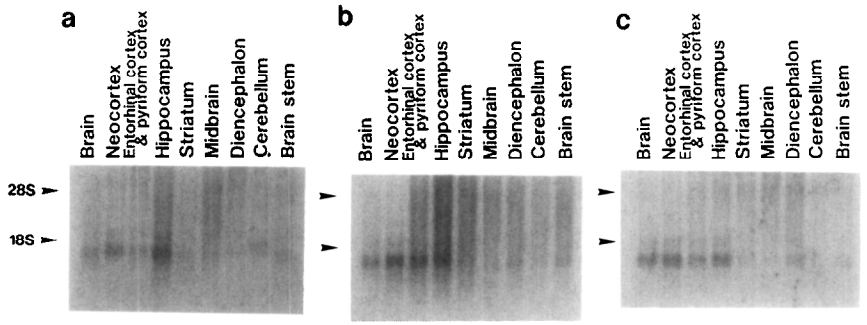

Figure 2. Regional distribution of NGF mRNA during development. Poly $(A)^+$ RNAs (5 ug) from the indicated brain regions 2 (a), 5 (b), and 12 weeks postnatally (c) were analyzed. Rat NGF gene labelled was used as probes. Positions of ribosomal RNA markers are shown on the left.

(Fig. 3). The synthesis of the two BDNF mRNAs (1.6 and 4.0 kb) was also regionally regulated during development (Fig. 4). The levels of BDNF mRNA in many regions were higher 5 or 12 weeks postnatally than 2 weeks postnatally, especially in the cerebellum. Furthermore, at each developmental stage studied, BDNF mRNA was synthesized highly in the hippocampus, and the level was higher in the neocortex than in the entorhinal cortex and the pyriform cortex. Thus the regional distributions of NGF, NGF-2/NT-3, and BDNF mRNAs during development were different. In contrast, the level of actin mRNA synthesis was found to be almost equal in all regions at each developmental stage (Fig. 5).

DISCUSSION

We investigated the developmental and regional expression of three members (NGF, NGF-2/NT-3, and BDNF) of the NGF gene family

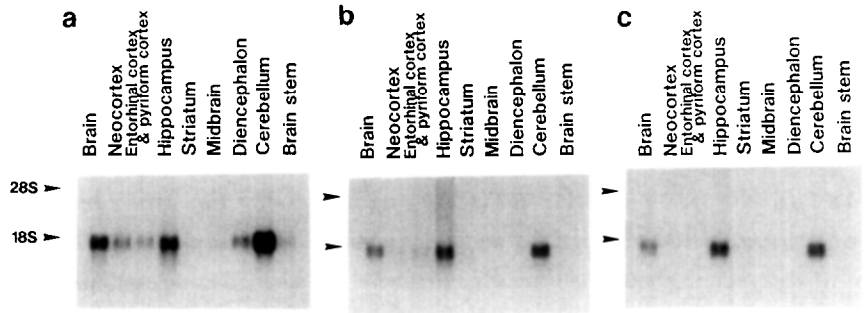

Figure 3. Regional distribution of NGF-2/NT-3 mRNA during development. Rat NGF-2/NT-3 gene labelled was used as probes, and the other conditions were the same as those shown in the legend of Fig. 2.

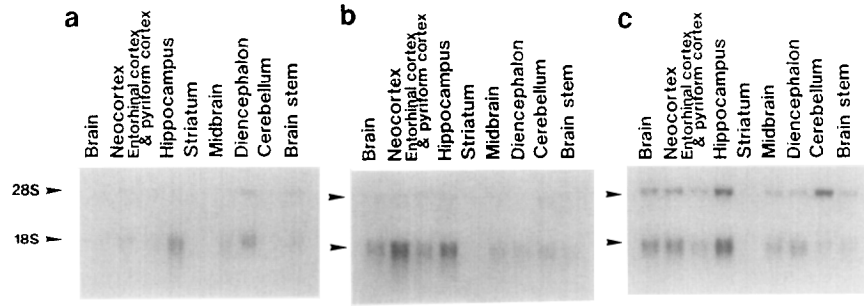

Figure 4. Regional distribution of BDNF mRNA during development. Rat BDNF gene labelled was used as probes, and the other conditions were the same as those shown in the legend of Fig. 2.

in rat brain, using Northern blot analyses. The developmental expression patterns for the members were different, and each mRNA was found to be maximally synthesized at specific stages during the development of the brain: NGF-2/NT-3 mRNA was synthesized maximally in the early stages followed by the syntheses of NGF and BDNF mRNAs. Therefore, each factor may have a distinct function in neural development. It is interesting that the rise in the NGF-2/NT-3 mRNA level coincided with the formation of the neural network. This suggests that NGF-2/NT-3 is the most primordial factor among the members of the NGF family.

The regional distribution of NGF mRNA correlated with cholinergic innervation as has been previously reported (11,12,13). Since the level of NGF mRNA in several brain regions was almost the same in each stage of development, NGF may act on neurons throughout the development. NGF-2/NT-3 mRNA was distributed in all the regions of the brain examined in the early stage of development, but in the late stage almost all the mRNA

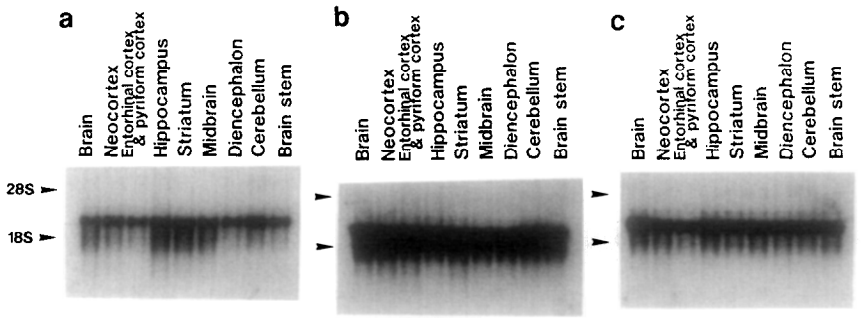

Figure 5. Regional distribution of actin mRNA during development. Human actin gene labelled was used as probes, and the other conditions were the same as those shown in the legend of Fig. 2.

was detected in the hippocampus and the cerebellum. Furthermore, some workers have reported that NGF-2/NT-3 mRNA is distributed in some specific regions of the brain in the adult rat (6,8,10). This suggests that NGF-2/NT-3 has a trophic effect for many species of neurons in the early stage but for limited species in the late stage. BDNF mRNA was widely distributed as has been reported (4,10), and the level of the mRNA was higher 5-12 weeks postnatally than 2 weeks postnatally. The number of neurons which respond to BDNF may increase during the neural network formation. Recently, Maisonpierre et al. have also reported regional expression patterns of the NGF gene family from newborn and adult rat brains, and have noted similarities and differences in the patterns of the NGF gene family (17). These results indicate that each factor has a distinct function in the nervous system during neural development.

It is also interesting that the mRNAs of all the members of the NGF family are expressed highly in the hippocampus which is concerned with memory and learning. This suggests the possibility of clinical application of these factors, especially for the treatment of dementia.

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