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Molecular cloning of a human gene that is a member of the nerve growth factor family

(brain-derived neurotrophic factor/polymerase chain reaction/neurotrophic factor/mRNA)

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ABSTRACT **Cell death within the developing vertebrate nervous system is regulated in part by interactions between neurons and their innervation targets that are mediated by neurotrophic factors. These factors also appear to have a role in the maintenance of the adult nervous system. Two neurotrophic factors, nerve growth factor and brain-derived neurotrophic factor, share substantial amino acid sequence identity. We have used a screen that combines polymerase chain reaction amplification of genomic DNA and low-stringency hybridization with degenerate oligonucleotides to isolate human BDNF and a human gene, neurotrophin-3, that is closely related to both nerve growth factor and brain-derived neurotrophic factor. mRNA products of the brain-derived neurotrophic factor and neurotrophin-3 genes were detected in the adult human brain, suggesting that these proteins are involved in the maintenance of the adult nervous system. Neurotrophin-3 is also expected to function in embryonic neural development.**

During normal vertebrate development, large percentages (up to 80%) of the neurons born into diverse cell populations within the forming nervous system die (1, 2). This is thought to be a mechanism that ensures that adequate numbers of neurons establish appropriate innervation densities with effector organs or other neuronal populations. In several instances, the innervation target of a population of neurons has been shown to have a crucial role in regulating the number of surviving neurons (for review, see ref. 2). Many observations indicate that targets of neuronal innervation produce a limited supply of neurotrophic factors and that competition between responsive neurons for these factors determines which neurons survive (1). Two distinct neurotrophic factors, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), have been purified and are known to be sufficient *in vivo* to reduce the amount of naturally occurring neuronal cell death in portions of the peripheral nervous system (3, 4). Evidence also suggests that NGF modulates neurotransmitter synthesis and neuronal morphology in the adult nervous system (e.g., refs. 5, 6).

The amino acid and nucleotide sequence similarity of NGF and BDNF suggested that these neurotrophic factors could be part of a larger gene family. Other members of this family could have important roles in the development and maintenance of the nervous system. Therefore, to attempt to isolate genes related to NGF and BDNF, we initiated a screen involving the polymerase chain reaction (PCR) (7). By using this screen, we have isolated and characterized* a third member of the NGF family, neurotrophin-3 (NT-3), and the human BDNF gene.

MATERIALS AND METHODS

PCR and Subcloning of PCR Products. Oligonucleotides 2C1 (AARCARTAYTTYTWYGARAC; where R is a purine, Y is a pyrimidine, and W is A or T) or 2B2 (TAYTTYTWYGARACNAARTG; where N is any nucleotide) and 4A1′ (DATNCKDATRAANCKCCA; where D is G, A, or T and K is G or T) were used at 5 μM to amplify 500 ng of human genomic DNA in a 50-μl PCR mixture (7). After 45 cycles of amplification (temperature profile: 94°C for 1 min; 45°C for 2 min; 55°C for 15 sec; 72°C for 2 min), reaction products were separated on a 3% NuSieve agarose gel (FMC) and a band of the expected molecular size (approximately 165 base pairs) was excised. Approximately 10% of the volume of the gel slice (20 μl) was added to a fresh PCR mixture (final volume, 100 μl) containing either oligonucleotide 2B2 or 2C1 [5′ phosphorylated as described (8)] and oligonucleotide 4A1RI (AGAGAATTCDATNCKDATRAANCKCCA). After 15 cycles of PCR amplification, fresh dNTPs (all four dNTPs, each at a final concentration of 0.1 mM) and 5 units of *Escherichia coli* DNA polymerase I, Klenow fragment, were added. After incubation for 1 hr at 20°C, the reaction mixture was phenol-extracted twice, ethanol-precipitated, and digested with 10 units of *Eco*RI. Restriction fragments were purified on a 3% NuSieve gel and ligated to *Sma* I/*Eco*RI-digested M13mp19. Subsequent sequence analysis of the NT-3 gene showed that oligonucleotides 2C1, 2B2, and 4A1′ had zero, two-, and two-nucleotide mismatches, respectively, with the NT-3 DNA sequence. Human genomic DNA for use in the amplification reactions was purified as described (9), with K.R.J. providing source material.

Plaque Hybridization Screening of M13 Clones. Oligonucleotides 3C1P (RTCDATICCICKGCANCC; where I is inosine) and 3D1P (GCANTRNGARTTCCARTG) were 5′-phosphorylated with [γ-^{32}P]ATP and hybridized (8) with nitrocellulose filter lifts of the M13 plaques obtained by transformation of *E. coli* strain TG1 with the ligation reaction mixture described above. After washing three times at 20°C and once at 30°C in 6× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate)/0.1% SDS, signals were visualized by autoradiography.

Isolation and Sequencing of Genomic Clones. A human genomic library [kindly provided by Mike Blanar (UCSF, San Francisco); constructed in the λ DASH vector by using leukocyte DNA] was screened with single-stranded probes containing BDNF or NT-3 PCR products by using standard hybridization methods (8). Restriction fragments [6-kilobase (kb) *Bam*HI–*Eco*RI (NT-3) or 3-kb *Eco*RI (BDNF)] from the NT-3 and BDNF λ phage clones thus isolated were subcloned into pBluescript KS. DNA sequence was determined from these subclones by using reagents and protocols provided in a kit (United States Biochemical).

RNA Isolation and Analysis. Total RNA was prepared from frozen human brain specimens (patients 89–13 and 90–23, an

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Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; PCR, polymerase chain reaction.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M37762 and M37763).

85-year-old male and a 73-year-old female, respectively) or placenta by extraction with guanidine isothiocyanate and sedimentation through cesium chloride (8). Polyadenylylated RNA was isolated (8), fractionated on a 1.5% agarose/formaldehyde gel (8), and transferred by capillary action to Hybond-N (Amersham). Nucleotides 94–750 of the NT-3 sequence or nucleotides 42–741 of the human BDNF sequence were amplified by using the PCR from plasmids containing the NT-3 or BDNF genes (see above), subcloned into M13mp19, and single-stranded probes were prepared from the resulting templates with [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) by using standard procedures (8). Hybridizations were performed at 65°C in 5× SSC/5× Denhardt's solution/0.5% SDS/salmon-sperm DNA (200 μg/ml). (1× Denhardt's solution is 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin.) After washing for a total of 60 min in four changes of 0.5× SSC/0.1% SDS at 65°C, signals were detected by autoradiography at −70°C with an intensifying screen. To compare RNA amounts in the various samples, we used a restriction fragment from the human β_2-tubulin 3′ untranslated region (10).

RESULTS

Isolation of Neurotrophic Factor Genes. Degenerate oligonucleotides having sequences derived from the amino acid sequence conserved between NGF from several species and porcine BDNF (oligonucleotides 2C1, 2B2, and 4A1′, see Fig. 3) were used to amplify human genomic DNA in PCRs. The resulting PCR products were gel-purified and reamplified with the same 5′ oligonucleotides (2C1 or 2B2) and a 3′ oligonucleotide having the same sequence as 4A1′ as well as an *Eco*RI restriction site at its 5′ end (4A1RI). This procedure of sequential amplification, gel purification, and reamplification with an oligonucleotide having an added restriction site was used to maximize initial amplification specificity and final cloning efficiency.

Sequencing of cloned PCR products identified clones that contained either a portion of the human NGF gene or a gene having very high sequence similarity to porcine BDNF (subsequently referred to as human BDNF). Oligonucleotides specific for the human BDNF sequence thus obtained, the human NGF sequence (11), and two degenerate oligonucleotides (3C1P and 3D1P, see Fig. 3) derived from regions of amino acid sequence conservation lying between the PCR oligonucleotides were used to screen four replicate nitrocellulose filter lifts of the M13 clone pools by plaque hybridization. Clones that hybridized with the degenerate oligonucleotide pools but not with the NGF- or BDNF-specific oligonucleotides were identified and sequenced, and a gene related to both BDNF and NGF (subsequently referred to as NT-3) was thereby identified. Seventeen of 130 clones obtained with the 2C1/4A1′ oligonucleotide pair and one of ≈400 clones obtained with the 2B2/4A1′ oligonucleotide pair contained the NT-3 gene.

Probes derived from the PCR product clones were used to isolate clones from a human genomic library. DNA sequence determined from these clones confirmed that they include open reading frames encoding human BDNF and a putative growth factor (NT-3) highly related to BDNF and NGF. The DNA sequences of human NT-3 and BDNF are presented with their conceptual translations in Figs. 1 and 2, respectively.

Sequence Similarities Between Members of the NGF Family. In Fig. 3, the human BDNF and NT-3 amino acid sequences are compared with the human NGF, porcine BDNF, mouse NGF, and chicken NGF amino acid sequences, revealing several regions of similarity that are conserved among all three members of this gene family and near identity between the human and porcine BDNF sequences. The NGF gene gives rise to two transcripts that predict distinct precursor proteins, differing in the site of translational initiation (16). The shorter of these predicted precursors, shown in Fig. 3, is encoded entirely within a single exon. Translation of the other (longer) precursor is predicted to initiate in a separate upstream exon present in some but not all NGF mRNAs (17). Precursors derived from both NGF transcripts are proteolytically processed to yield the mature biologically active form of NGF that includes essentially the 120 C-terminal amino acids of either precursor. The porcine BDNF cDNA structure described by Leibrock *et al.* (14) is most similar to that of mRNAs encoding the short NGF precursor; an analogous long precursor of BDNF has not as yet been described. Alignment of the human NT-3 and BDNF sequences with other NGF and BDNF sequences predicts that the human NT-3 and BDNF are both synthesized as precursors (257 and 247 amino acids, respectively) having an overall structure very similar to that of the short NGF precursor form (241 amino acids) and are subsequently proteolytically processed

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TAACACAGACTCAGCTGCCAGAGCCTGCTCTTAACACCTGTGTTTCCTTTT   51
* H R L S C Q S L L L T P V F P F
CAGATCTTACAGGTGAACAAGGTGATGTCCATCTTGTTTTATGTGATATTT  102
Q I L Q V N K V M S I L F Y V I F  -130
CTCGCTTATCTCCGTGGCATCCAAGGTAACAACATGGATCAAAGGAGTTTG  153
L A Y L R G I Q G N N M D Q R S L  -113
CCAGAAGACTCGCTCAATTCCCTCATTATTAAGCTGATCCAGGCAGATATT  204
P E D S L N S L I I K L I Q A D I  -96
TTGAAAAACAAGCTCTCCAAGCAGATGGTGGACGTTAAGGAAAATTACCAG  255
L K N K L S K Q M V D V K E N Y Q  -79
AGCACCCTGCCCAAAGCTGAGGCTCCCCGAGAGCCGGAGCGGGGAGGGCCC  306
S T L P K A E A P R E P E R G G P  -62
GCCAAGTCAGCATTCCAGCCGGTGATTGCAATGGACACCGAACTGCTGCGA  357
A K S A F Q P V I A M D T E L L R  -45
CAACAGAGACGCTACAACTCACCGCGGGTCCTGCTGAGCGACAGCACCCCC  408
Q Q R R Y N S P R V L L S D S T P  -28
TTGGAGCCCCCGCCCTTGTATCTCATGGAGGATTACGTGGGCAGCCCCGTG  459
L E P P P L Y L M E D Y V G S P V  -11
GTGGCGAACAGAACATCACGGCGGAAACGGTACGCGGAGCATAAGAGTCAC  510
V A N R T S R R K R Y A E H K S H  +7
     ★              -1⬆+1
CGAGGGGAGTACTCGGTATGTGACAGTGAGAGTCTGTGGGTGACCGACAAG  561
R G E Y S V C D S E S L W V T D K  +24
TCATCGGCCATCGACATTCGGGGACACCAGGTCACGGTGCTGGGGGAGATC  612
S S A I D I R G H Q V T V L G E I  +41
AAAACGGGCAACTCTCCCGTCAAACAATATTTTTATGAAACGCGATGTAAG  663
K T G N S P V K Q Y F Y E T R C K  +58
GAAGCCAGGCCGGTCAAAAACGGTTGCAGGGGTATTGATGATAAACACTGG  714
E A R P V K N G C R G I D D K H W  +75
AACTCTCAGTGCAAAACATCCCAAACCTACGTCCGAGCACTGACTTCAGAG  765
N S Q C K T S Q T Y V R A L T S E  +92
AACAATAAACTCGTGGGCTGGCGGTGGATACGGATAGACACGTCCTGTGTG  816
N N K L V G W R W I R I D T S C V  +109
TGTGCCTTGTCGAGAAAAATCGGAAGAACATGAATTGGCATCTCTCCCCAT  867
C A L S R K I G R T *                +119
ATATAAATTATTACTTTAAATTATATGATATGCATGTAGCATATAAATGTT  918
TATATTGTTTTTATATATTATAAGTTGACCTTTATTTATTAAACTTCAGCA  969
ACCCTACAGTATATAAGCTTTTTTCTCAATAAAATCAGTGTGCTTGCCTTC 1020
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FIG. 1. DNA sequence of human NT-3. The derived amino acid sequence is shown in the one-letter amino acid code, with numbering relative to the expected site of proteolytic processing of the precursor (arrow). The methionine and secretory signal sequence, which are conserved with NGF and BDNF, are underlined. A good match to the −1/−3 signal sequence cleavage consensus predicts cleavage after the glycine at position −121 (12). A consensus N-glycosylation signal, also conserved in other members of the NGF family, is indicated by a star. A consensus polyadenylylation signal is underlined, and two possible splice acceptor sites are indicated by a pair of diamonds. The AG pair at nucleotides 62 and 63 is in the same position relative to the methionine codon (position −13) as an acceptor site used in human NGF. The AG pair at nucleotides 53 and 54 (position −22 relative to the methionine codon) is in the same position as an acceptor signal likely to be used in BDNF (see Fig. 2). Both AG pairs are preceded by pyrimidine-rich sequences, a characteristic of splice acceptor signals (13).

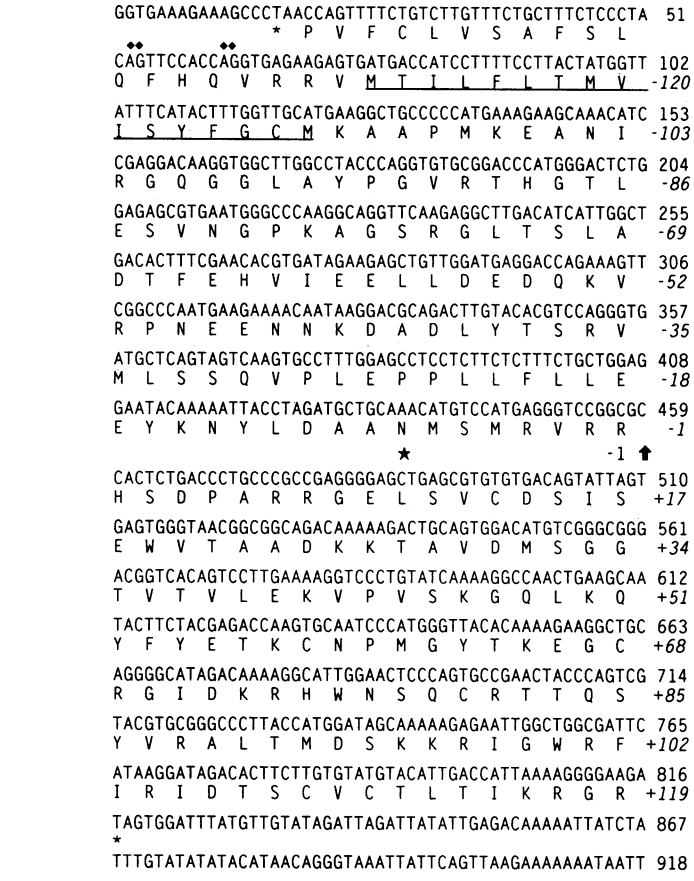

FIG. 2. DNA sequence of human BDNF. The derived amino acid sequence is shown, with numbering relative to the expected site of proteolytic processing of the precursor (arrow). All symbol definitions are as in Fig. 1. The best match with the $-1/-3$ signal sequence cleavage consensus (12) predicts cleavage after the methionine at position -113. The AG pair at nucleotides 62 and 63 is in the same position relative to the methionine codon (position -13) as an acceptor site used in human NGF. Immediately 5′ of the AG pair at nucleotides 53 and 54, the porcine BDNF cDNA sequence (14) diverges completely from this human sequence, whereas the 59 nucleotides 3′ of this AG pair are identical, suggesting that this is a functional splice acceptor site.

to 119-amino acid mature forms. The structures of the human NGF, NT-3, and BDNF precursors are shown in Fig. 3*B*. The putative short precursor forms of human NT-3 and BDNF are encoded entirely within a single exon, as has been reported for NGF and porcine BDNF (Figs. 1 and 2; refs. 11 and 17).

Three regions of homology between human NGF, human BDNF, and human NT-3 are found in this exon. The first region of homology includes the site of translation initiation of the short NGF precursor and a secretory signal sequence (see Fig. 3). The homology in this region also includes several amino acids N-terminal of the initiation methionine (Gln-Val-Xaa-Xaa-Val) and a splice acceptor consensus (see Figs. 1 and 2) at the position where the NGF long precursor upstream exon is joined with the analogous NGF 3′ exon, suggesting that human BDNF and NT-3 may also be synthesized as longer precursors.

The second region of homology between human NGF, BDNF, and NT-3 includes the 45 amino acids N-terminal of the proteolytic cleavage site utilized in the processing of the NGF precursor to the mature form. Where it has been determined, proteolytic cleavage of the NGF and BDNF precursors occurs after the consensus Arg-Xaa-Lys/Arg-Arg (14, 16). This sequence motif is found in a similar location in human NT-3 and BDNF. In addition, in the 40 amino acids on the N-terminal side of the putative proteolytic processing site, the three precursors share 20% amino acid identity and an N-linked glycosylation acceptor site beginning 8 amino acids before the cleavage site. Recent evidence indicates that the pro region is required for proper folding of members of the transforming growth factor β family (18), suggesting an analogous role for the conserved pro regions of the three members of the NGF family.

The third and most extensive region of homology between human NGF, BDNF, and NT-3 includes the mature biologically active portion of NGF. The hypothetical mature portion of NT-3 shares 56% amino acid identity with human NGF and 56% identity with human BDNF. The amino acid sequence of human BDNF is identical with porcine BDNF in the predicted mature portion of the molecule and shares 52% identity with human NGF. There are 53 amino acid identities among all of the mature portions of the NGF, BDNF, and NT-3 sequences shown in Fig. 3, including all six cysteines.

Expression of NT-3 and BDNF in Human Tissues. To obtain information about the tissue distribution of synthesis of human NT-3 and BDNF, we used RNA blot analysis to assess the abundance of each mRNA in several adult human tissues. As shown in Fig. 4, we were able to detect comparatively high levels of the 1.4-kb NT-3 transcript in cerebellum and placenta, and lower amounts in nucleus basalis, basal ganglia, hippocampus, and visual cortex (the latter signal is not clearly visible in Fig. 4). Two BDNF transcripts (1.6 and 4.0 kb) were detected in all brain regions examined. The relative amounts of NT-3 and BDNF transcripts detected in the different samples were calculated and the results of this analysis are presented in Table 1. These data indicate that there are up to 50-fold differences in levels of expression of these transcripts in different brain regions.

DISCUSSION

In this paper, we report the molecular cloning of human BDNF and of NT-3, a gene related to BDNF and NGF. We present evidence that transcripts encoding NT-3 are expressed in the adult human brain and in placenta. Thus, NT-3 appears to be a neurotrophic factor that is likely to regulate the differentiation of populations of neurons in the human central nervous system. To isolate a clone encoding this neurotrophic factor, we used a combination of PCR amplification of genomic DNA, subcloning, and screening by plaque hybridization with degenerate and gene-specific oligonucleotides. This approach enabled us to rapidly identify NT-3 as 1 of ≈400 clones obtained with one of the oligonucleotide pairs used, demonstrating its merit when a gene is a very small percentage of the PCR product. This method should be useful in the isolation of additional members of other gene families, especially when it is undesirable to use restriction endonucleases to eliminate genes that have been previously identified (e.g., when a restriction site may be conserved).

During the preparation of this manuscript, Hohn *et al.* (19) and Maisonpierre *et al.* (20) reported the cloning and characterization of the mouse and rat homologues, respectively, of a member of the NGF family, named NT-3. The amino acid sequences of the predicted mature forms of rat and mouse NT-3 are identical to that of human NT-3, making it clear that these represent homologues of the same neurotrophic factor. When expressed in COS cells, the products of the murine and rat NT-3 genes were shown to be secreted and to support the survival of neural crest-derived spinal sensory and sympathetic neurons and placode-derived nodose ganglion neurons, establishing that this is indeed a neurotrophic factor that supports survival of multiple populations of neurons, some of which can also be supported by other trophic factors (NGF or BDNF). In agreement with our results, these groups reported detection of NT-3 mRNA in the central nervous

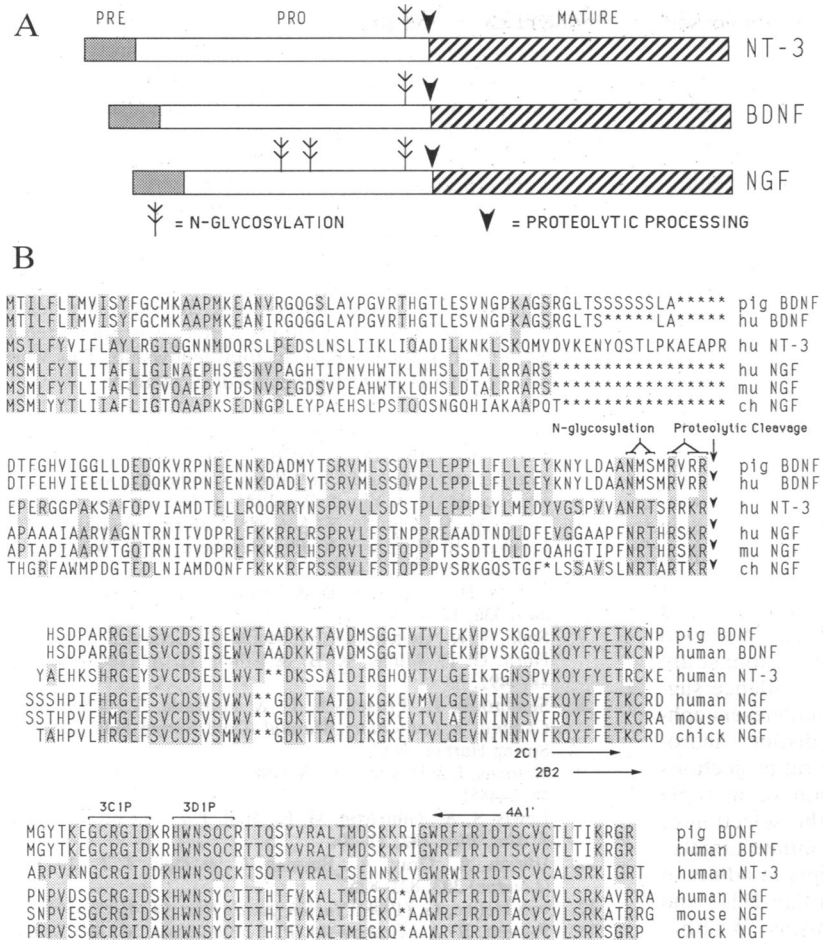

FIG. 3. Similarities between members of the NGF family. (*A*) Schematic representation of the precursor structures predicted for the human members of the NGF family (NGF, BDNF, and NT-3). The secretory signal sequences (pre) are stippled, the mature portions are hatched, and the remainder of the precursor (pro) is open. Predicted sites of N-glycosylation and proteolytic processing are also indicated. (*B*) Amino acid sequence conservation between NGF, porcine BDNF, and human NT-3 and BDNF. Amino acids that are shared by more than one neurotrophic factor type (NGF, BDNF, or NT-3) are shaded. Sequences other than those described in this work were obtained from refs. 11, 14, and 15. Gaps (asterisks) were introduced to optimize the alignment of the amino acid sequences. The positions of the oligonucleotides used in the PCR and oligonucleotide hybridization experiments (2C1, 2B2, 3C1P, 3D1P, and 4A1') are also shown.

system. They also detected this mRNA in many regions outside the central nervous system.

The three members of the NGF gene family have a remarkable degree of similarity at the level of primary structure. Several possible explanations for this conservation are apparent. One possibility is that this family of trophic factors has a recent evolutionary origin. To date, a homolog of NGF in invertebrates has not been reported. Therefore, this gene family may function specifically in vertebrates to enhance the development of their complex nervous systems. The observed conservation may also result from selective constraints that demand tertiary structures capable of interacting with a receptor family that has coevolved with these factors. It is possible that all of the members of the NGF family interact with one protein, the so-called low-affinity NGF receptor (K_d in the nM range), in association with various accessory subunits that would create high-affinity receptors specific for the various factors (K_d in the 10 pM range) (21). Both the observation that many neurons that are not responsive to NGF express the low-affinity NGF receptor (e.g., see refs. 22 and 23) and the finding that NGF and BDNF will compete for binding to this receptor (21) are consistent with this model. If all members of the NGF family interact with the low-affinity NGF receptor, substantial constraints would be

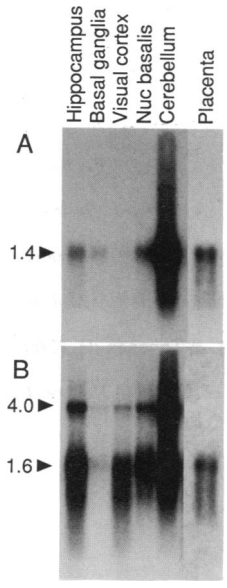

FIG. 4. RNA blot analysis of the expression of NT-3 and BDNF in human tissues. Blots were prepared with ≈5 μg of polyadenylylated RNA (patient 90–23) and hybridized with a single-stranded NT-3 probe (*A*) or BDNF probe (*B*). Hybridization of these filters with a β_2-tubulin probe indicated that the relative RNA levels were (1:0.8:0.5:1:1:0.2, lanes from left to right). The absence of a detectable 4-kb BDNF transcript in placental RNA may have resulted from degradation of this RNA sample. Nuc basalis, nucleus basalis.

Table 1. Relative abundances of NT-3 and BDNF transcripts in the human brain

	Relative abundance		
	NT-3	BDNF (1.6 kb)	BDNF (4 kb)
Cerebellum	100	60	60
Basal ganglia	3	6	1
Visual cortex	2	50	4
Nucleus basalis	10	30	7
Hippocampus	6	50	10
Placenta	≈100	≈100	ND

Signals obtained in RNA blot experiments with probes specific for human NT-3 and human BDNF (see also Fig. 4) were quantitated by scanning densitometry and normalized to the signals obtained from the same set of RNA samples when hybridized with a human β_2-tubulin probe. Numbers are averages of data from two patients and are expressed as percentage of NT-3 signal detected in cerebellum. ND, not detected.

placed on the evolutionary divergence of their amino acid sequences.

The identification of multiple members of the NGF family, all of which appear to promote neuronal survival, has interesting implications for models of the regulation of neuronal population sizes and target innervation density during development. Although it has not yet been conclusively demonstrated, data suggest that NGF and BDNF act on overlapping populations of neurons within the dorsal root ganglion (DRG). For example, either NGF or BDNF is capable of rescuing most of the neurons that die in the DRG during normal chicken development (4). In addition, either BDNF or NGF appears to be sufficient *in vitro* to promote the survival of more than half of the neurons obtained from the embryonic chicken DRG (24), and rat NT-3 has been reported to support the survival of ≈60% of embryonic day 8 chicken DRG neurons (20). These observations imply that some neurons are capable of responding to more than one of the neurotrophic factors—NGF, NT-3, or BDNF. It is possible that responsive cells are presented with multiple neurotrophic factors simultaneously during the course of neural development. However, given the known differences in locations of NGF, BDNF, and NT-3 mRNA accumulation (e.g., ref. 20), it seems likely that a combination of differences in growth factor availability and heterogeneity in neuronal response are critical in neural development. Indeed, some evidence suggests that DRG sensory neurons and trigeminal mesencephalic motor nucleus motor neurons receive distinct neurotrophic signals from their peripheral and central projections (25, 26). Therefore, the combinatorial action of multiple neurotrophic factors could be important in the selection of neurons that have connections with all appropriate targets.

The presence of BDNF and NT-3 transcripts in adults of advanced age (73 and 85 years old) suggests that NT-3 and BDNF may have active roles in aged organisms. Whether they act to maintain the survival or differentiation of responsive neurons (e.g., refs. 5 and 6) remains to be determined. In the adult human brain, NGF mRNA has been detected primarily in areas receiving innervation from the cholinergic neurons of the basal forebrain, which have been shown to respond to this trophic factor (27). Expression of NGF mRNA was comparatively high in hippocampus, neocortex, and striatum, lower in nucleus basalis and thalamus, and not detected in cerebellum (27). In the present study, expression patterns of NT-3 and BDNF were found to be different from that reported for NGF. NT-3 mRNA was most abundant in cerebellum; BDNF mRNA was abundant in cerebellum, hippocampus, nucleus basalis, and visual cortex. BDNF has been shown to function as a trophic factor for retinal ganglion neurons, which innervate the thalamus (28). Its presence in other areas of the brain suggests that it also functions as a trophic factor for additional populations of central nervous system neurons. The fact that NGF, NT-3, and BDNF have different patterns of expression within the adult human brain suggests that they have distinct biological roles.

Intriguingly, all three trophic factors are also detected in human placenta (Fig. 4 and refs. 27 and 29), where transcripts could derive from maternal and/or fetal tissue. Since the placenta is not innervated, it may serve as a source for release of neurotrophic factors into the fetal blood stream, where they could conceivably affect development of many populations of neurons or nonneural cells in the embryo.

The molecular cloning of these growth factors will be quite useful in the analysis of their functions during normal development and in the elucidation of their role in homeostasis in the adult nervous system. The ability to readily obtain pure preparations of multiple neurotrophic factors will also allow an assessment of their therapeutic potential in nerve regeneration and degenerative disease.

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