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# Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: Developmental and topographical expression in the brain

(nerve growth factor family/cDNA/neurotrophic factor/hippocampal neurons/nerve growth factor receptor binding)

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ABSTRACT **We have used a pool of degenerate oligonucleotides representing all possible codons in regions of homology between brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) to prime rat hippocampal cDNAs in the polymerase chain reaction. The amplified DNA included a product with significant similarity to NGF and BDNF, which was used to isolate a 1020-nucleotide-long cDNA from a rat hippocampal library. From the nucleotide sequence, a 282-amino-acid-long protein with ≈45% amino acid similarity to both pig BDNF and rat NGF was deduced. In the adult brain, the mRNA for this protein was predominantly expressed in hippocampus, where it was confined to a subset of pyramidal and granular neurons. The developmental expression in brain showed a clear peak shortly after birth, 1 and 2 weeks earlier than maximal expression of BDNF and NGF, respectively. It was also expressed in several peripheral tissues with the highest level in kidney. The protein, transiently expressed in COS cells, was tested on chicken embryonic neurons and readily stimulated fiber outgrowth from explanted Remak's ganglion and, to a lesser extent, the nodose ganglion. A weak, but consistent, fiber outgrowth response was also seen in the ciliary ganglion and in paravertebral sympathetic ganglia. Moreover, the protein displaced binding of NGF to its receptor, suggesting that it can interact with the NGF receptor. Thus, this factor, although structurally and functionally related to NGF and BDNF, has unique biological activities and represents a member of a family of neurotrophic factors that may cooperate to support the development and maintenance of the vertebrate nervous system.**

During development of the vertebrate nervous system, a vast overproduction of neurons is compensated for by naturally occurring neuronal death, which is regulated by their targets (1). Within the targets, specific proteins, referred to as neurotrophic factors, are produced in limiting amounts and the release of these proteins is believed to regulate both the timing and the extent of innervation (2).

In the peripheral nervous system, the most well-characterized neurotrophic factor, nerve growth factor (NGF), supports the development of sympathetic and neural crest-derived sensory neurons, and in the adult the maintenance of the sympathetic nervous system is critically dependent on NGF (3, 4). In agreement with a trophic role of NGF for adult sympathetic neurons, the levels of both NGF mRNA and protein correlate with the density of sympathetic innervation (5, 6). NGF mRNA and protein have also been found in the brain, with the highest levels in hippocampus and cerebral cortex, to which the major cholinergic pathways in the brain project (7–10). Basal forebrain cholinergic neurons can be prevented from dying after axonal transection by addition of NGF (11–15) and they respond to NGF *in vivo* by a marked increase in fiber outgrowth (16).

In addition to NGF, one other protein, termed brain-derived neurotrophic factor (BDNF), has been shown to be present in low amounts (17), secreted from cells (18), and to support survival of embryonic sensory neurons *in vivo* (19). In common with NGF, BDNF supports the survival of neural crest-derived embryonic sensory neurons *in vitro*, but nonoverlapping trophic activities are suggested by the finding that BDNF also supports placode-derived neurons from the nodose ganglia and retinal ganglion cells (20, 21), which are less sensitive to NGF (22, 23). Regulation of neuronal survival *in vivo* in the brain by BDNF has not yet been demonstrated, although its sites of synthesis have recently been mapped by *in situ* hybridization where a high level of labeling was found in hippocampal neurons (24).

NGF is synthesized as a preproprotein and the structure of both the precursor and the mature protein has been deduced from cDNA and genomic clones (25, 26). More recently, a genomic clone has been isolated for porcine BDNF (18). Of considerable interest is the finding that the mature BDNF and NGF proteins show striking amino acid similarities, suggesting that they are structurally related and may be members of a family of neurotrophic factors (18).

In this study, we report on the cloning and expression of an additional member of the NGF family.¶ Due to its restricted expression in the brain, being mostly confined to a subset of pyramidal and granular neurons in the hippocampus, we have named this protein hippocampus-derived neurotrophic factor (HDNF).

## MATERIALS AND METHODS

**RNA Preparation, Molecular Cloning, and DNA Sequencing.** Polyadenylylated RNA [poly(A)$^+$] was prepared as described (27). For cloning, rat hippocampus poly(A)$^+$ RNA (5 μg) was used as a template for synthesis of single-stranded cDNA using Moloney murine leukemia virus reverse transcriptase (Pharmacia). Six separate mixtures of 28-mer oligonucleotides representing all possible codons corresponding to the amino acid sequence KQYFYET (5′-oligonucleotide) and WRFIRID (3′-oligonucleotide) were synthesized on an Applied Biosystems A381 DNA synthesizer. The 5′-oligonucleotide contained a synthetic *Eco*RI site and the 3′-oligonucleotide contained a synthetic *Hin*dIII site. Each mixture of oligonucleotides was then used to prime the amplification of hippocampal cDNA (25 ng) by the polymer-

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Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; HDNF, hippocampus-derived neurotrophic factor; PCR, polymerase chain reaction.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34643).

ase chain reaction (PCR) (Gene Amp, Perkin–Elmer/Cetus). PCR products of the expected size [182 base pairs (bp) including primer and restriction site] were isolated on an agarose gel and cloned in plasmid Bluescript KS$^+$ (Stratagene) followed by nucleotide sequence analysis by the dideoxynucleotide chain-termination method (28). The insert from one clone showing ≈60% nucleotide sequence similarity to both rat NGF and pig BDNF was then used to screen a cDNA library in λgt10 from rat hippocampus constructed with a cDNA synthesis kit (Pharmacia). Screening of 1.2 × $10^6$ independent cDNA clones from the primary library (29) yielded seven positive clones. One clone with a 1020-bp-long insert was sequenced in its entirety on both strands by the chain-termination method.

**RNA Blot Analysis.** Poly(A)$^+$ RNA (20 μg) was electrophoresed in a 1% agarose gel containing 0.7% formaldehyde and was transferred to a nitrocellulose filter. The filter was hybridized to a 355-bp fragment from the 3′ end of HDNF mRNA (nucleotides 665–1020 in Fig. 1), isolated by PCR using one specific primer together with an oligo(dT) primer as described by Frohman *et al.* (30). The fragment was labeled with [α-$^{32}$P]dCTP by nick-translation to a specific activity of ≈5 × $10^8$ cpm/μg. Hybridization was carried out as described (27) followed by washing at high stringency and exposure to Kodak XAR-5 films. The same filters were boiled for 5 min in 1% glycerol, followed by hybridization first to a 185-bp PCR fragment from rat BDNF corresponding to amino acids 183–239 of pig BDNF (18), then to a 770-bp *Bst*E2/*Pst* I fragment from the 3′ exon of rat NGF (31), and finally to a 1.5-kilobase (kb) *Pst* I fragment from a mouse α-actin cDNA (32). Appropriate exposures of all autoradiograms were quantified with a Shimadzu CS-9000 densitometer.

***In Situ* Hybridization.** Cryostat sections (14 μm) from fresh-frozen adult Sprague–Dawley rat brain were processed and used for *in situ* hybridization with deoxyadenosine [α-[$^{35}$S]thio]triphosphate 3′-end-labeled probes as described (33). To detect HDNF-specific mRNA, a 50-mer oligonucleotide complementary to nucleotides 667–717 in Fig. 1 was used. For BDNF-specific mRNA, a 50-mer oligonucleotide complementary to rat BDNF mRNA, corresponding to nucleotides 748–798 in pig BDNF (18), was used.

**Expression of HDNF Protein, Assays of Biological Activities, and Binding to the NGF Receptor.** The 1020-bp HDNF cDNA insert in λgt10 was amplified by PCR using λgt10 sequencing primer and reverse primer (Clontech). The amplified DNA was treated with T4 polynucleotide kinase and 10-mer *Xho* I linkers were ligated, followed by cloning of the fragment into the *Xho* I site of pXM (34). COS cells grown to ≈70% confluence were transfected with 20 μg of the indicated plasmid construct per 10-cm dish by the DEAE dextran-chloroquine method (35). A plasmid expressing the β-galactosidase gene (pCH110, Pharmacia) was transfected in parallel and β-galactosidase activity was measured in cytoplasmic extracts as a control of transfection efficiency. The conditioned medium from transfected cells (36) was then collected and assayed for stimulation of neurite outgrowth from embryonic chicken ganglia as described (37). Conditioned medium from transfected cells was tested for binding to the NGF receptor on PC12 cells as described (36).

## RESULTS

**Molecular Cloning and Structure of HDNF.** A 1020-bp cDNA clone was isolated. Nucleotide sequence analysis of this clone showed an open reading frame encoding a 282-amino-acid-long protein (Fig. 1). The C-terminal part of this protein contained a potential cleavage site for a 119-amino acid protein with 57% amino acid similarity to both rat mature NGF and pig mature BDNF. Included in this similarity were all six cysteine residues, involved in formation of disulfide bridges, and an overall identity to NGF and BDNF of 68 and

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  1  V D V P G N S H T D A M V T S A T I L Q    20
  1  GTCGACGTCCCTGGAAATAGTCATACGGATGCCATGGTTACTTCTGCCACGATCTTACAG    60

 21  V N K V M S I L F Y V I F L A Y L R G I    40
 61  GTGAACAAGGTGATGTCCATCTTGTTTTATGTGATATTTCTTGCTTATCTCCGTGGCATC   120

 41  Q G N N M D Q R S L P E D S L N S L I I    60
121  CAAGGCAACAACATGGATCAAAGGAGTTTGCCAGAAGACTCTCTCAATTCCCTCATTATC   180

 61  K L I Q A D I L K N K L S K Q M V D V K    80
181  AAGTTGATCCAGGCGGATATCTTGAAAAACAAGCTCTCCAAGCAGATGGTAGATGTTAAG   240

 81  E N Y Q S T L P K A E A P R E P E Q G E   100
241  GAAAATTACCAGAGCACCCTGCCCAAAGCAGAGGCACCCAGAGAACCAGAGCAGGGAGAG   300

101  A T R S E F Q P M I A T D T E L L R Q Q   120
301  GCCACCAGGTCAGAATTCCAGCCGATGATTGCAACAGACACAGAACTACTACGGCAACAG   360

121  R R Y N S P R V L L S D S T P L E P P P   140
361  AGACGCTACAATTCACCCCGGGTCCTGCTGAGTGACAGCACCCCTTTGGAGCCCCCTCCC   420

141  L Y L M E D Y V G N P V V T N R T S P R   160
421  TTATATCTAATGGAAGATTATGTGGGCAACCCGGTGGTAACCAATAGAACATCACCACGG   480

161  R K R Y A E H K S H R G E Y S V C D S E   180
481  AGGAAACGCTATGCAGAGCATAAGAGTCACCGAGGAGAGTACTCAGTGTGTGACAGTGAG   540

181  S L W V T D K S S A I D I R G H Q V T V   200
541  AGCCTGTGGGTGACCGACAAGTCCTCAGCCATTGACATTCGGGGACACCAGGTTACAGTG   600

201  L G E I K T G N S P V K Q Y F Y E T R C   220
601  TTGGGAGAGATCAAAACCGGCAACTCTCCTGTGAAACAATATTTTTATGAAACGAGGTGT   660

221  K E A R P V K N G C R G I D D K H W N S   240
661  AAAGAAGCCAGGCCAGTCAAAAACGGTTGCAGGGGGATTGATGACAAACACTGGAACTCT   720

241  Q C K T S Q T Y V R A L T S E N N K L V   260
721  CAGTGCAAAACGTCGCAAACCTACGTCCGAGCACTGACTTCAGAAAACAACAAACTCGTA   780

261  G W R W I R I D T S C V C A L S R K I G   280
781  GGCTGGCGCTGGATACGAATAGACACTTCCTGTGTGTGTGCCTTGTCAAGAAAAATCGGA   840

281  R T End                                 282
841  AGAACATGAATTGGCATCTGTCCCCACATATAAATTATTACTTTAAATTATATGATATGC   900

901  ATGTAGCATATAAATGTTTATATTGTTTTTATATATTATAAGTTGACCTTTATTTATTAA   960
961  ACTTCAGCAACCCTTACAGTATATAAGCTTTTTTCATAATCGGGCTGCTCAAAAAAAAAA  1020
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FIG. 1. Nucleotide sequence and deduced amino acid sequence of rat HDNF. Arrow indicates the presumptive start of mature HDNF. A consensus sequence for N-glycosylation is underlined. A consensus sequence for polyadenylylation is shown in the box, and the stretches of adenosine at the end of the sequence show the poly(A) tail. Vertical bar shows an exon/intron boundary present in the rat NGF gene (31). Stars indicate potential translation start sites.

67 residues, respectively, was seen. The prepro part of the protein showed weak, but significant, homology to NGF. A potential N-glycosylation site located nine amino acids from the start of the mature protein was also conserved between the three proteins.

**Expression of HDNF mRNA in Peripheral Tissues.** A 1.3-kb HDNF-specific mRNA was detected in several adult rat peripheral tissues, with the highest level in kidney (data not shown). Densitometer scanning of autoradiograms from three independent experiments showed that the spleen and heart contained approximately 6- and 8-fold lower levels, respectively, compared with kidney. Lower levels were also found in the adrenal gland, ovary, muscle, and liver. Hybridization of the same filter to a rat NGF probe revealed that the amounts of HDNF mRNA in heart and spleen were comparable to the level of NGF mRNA in these tissues.

**Developmental Expression of HDNF and BDNF in Rat Brain.** In the developing brain, HDNF mRNA was detected already at embryonic day 15, the earliest time point tested (Fig. 2*a*). A sharp increase was seen at birth and maximal levels were found at postnatal day 4. At 3 weeks of age, the amount had decreased to adult levels. Densitometer scanning of autoradiograms from two independent experiments showed that adult levels were 15-fold lower than at postnatal day 4. The level in the adult brain was lower than in kidney but was comparable to the level in heart. A 1.4-kb BDNF mRNA was first seen at embryonic day 19, with a peak level at 2 weeks of age, which was 10-fold higher than the amount in adult brain (Fig. 2*a*). A 4.0-kb BDNF mRNA was also seen and the developmental and regional expression of this mRNA was the same as for the 1.4-kb mRNA.

**Regional Distribution of HDNF and BDNF mRNA in Adult Rat Brain.** The distribution of HDNF mRNA in the adult

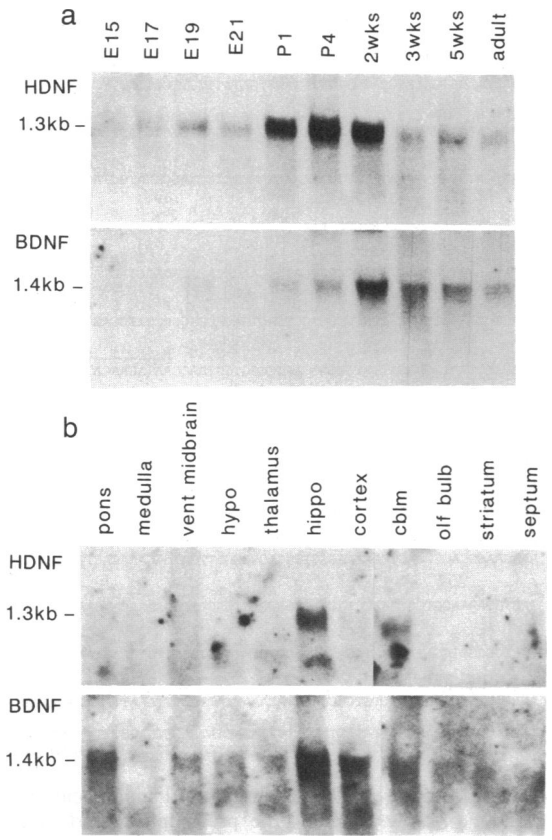


FIG. 2. Developmental and regional expression of HDNF and BDNF mRNA in rat brain. (*a*) Poly(A)$^+$ RNA (20 $\mu$g per slot) isolated from Sprague–Dawley rat brain at the indicated developmental stages was hybridized to the indicated probes (HDNF and BDNF). Adult rats were 12 weeks old. E, embryonic day; P, postnatal day; wks, weeks. (*b*) Same analysis as in *a* using poly(A)$^+$ RNA (20 $\mu$g per slot) isolated from the indicated regions of adult male Sprague–Dawley rat brain. Medulla, medulla oblongata; hypo, hypothalamus; hippo, hippocampus; cortex, cerebral cortex; cblm, cerebellum; olf, olfactory bulb.

brain showed remarkable regional specificity with high levels in hippocampus compared with other brain regions analyzed (Fig. 2*b*). In fact, cerebellum was the only other region where HDNF mRNA was clearly detected, with the exception of cerebral cortex, which showed a weak signal. BDNF mRNA was more widely distributed in rat brain, although hippocampus also contained the highest amount, followed by cerebral cortex, pons, and cerebellum (Fig. 2*b*).

**Neurons Expressing HDNF and BDNF mRNA Are Located in a Distinct Topographical Arrangement in Hippocampus.** Anterior sections of the dorsal hippocampus showed neurons expressing high levels of HDNF mRNA primarily confined to the medial part of CA1 and CA2 (Fig. 3 *a* and *c*). Few HDNF mRNA-expressing neurons were also found in lateral parts of CA1. Granular cells of the dentate gyrus were also highly labeled (Fig. 3*a*). CA3 and hilar cells of the dentate gyrus showed no labeling for HDNF mRNA at any level (Fig. 3*d*). No labeling was seen over any sections after hybridization to a control probe, complementary to the specific HDNF probe. Adjacent sections hybridized to a BDNF-specific probe revealed labeling over granular neurons in the dentate gyrus (Fig. 3*b*), although possibly with lower intensity than that seen after hybridization for HDNF mRNA. Strong labeling with the BDNF-specific probe was found over neurons in the hilar region (Fig. 3*e*), CA3, and part of CA2 (Fig. 3*b*). Few BDNF mRNA-expressing neurons, which appeared to be less intensively labeled, were also detected in CA1 and CA2 (Fig. 3*b*). Intensely labeled neurons were seen in claustrum, located lateral to the external capsule. This region showed no labeling for HDNF mRNA.

**Neurotrophic Activities of HDNF in Explanted Chicken Embryonic Ganglia.** The 1020-bp HDNF cDNA insert was cloned in the expression vector pXM (34), designed for transient expression in COS cells. Two plasmid constructs were isolated, containing the HDNF insert either in the correct or opposite orientation for translation of the HDNF protein. The latter construct was used as a negative control. Included was also a construct containing the rat NGF gene (36). The different constructs were transfected into COS cells and 3 days later conditioned medium was tested for biological activity in bioassays that measured fiber outgrowth from various chicken embryo ganglia. A marked stimulation of neurite outgrowth, consistently resulting in circular or oval fiber halos, was seen in the ganglion of Remak, a ganglionated nerve trunk in the mesorectum of the chicken embryo (38, 39) (Fig. 4*a*). Although NGF is known to stimulate the explanted ganglion of Remak (39), it was far less efficient than HDNF (Fig. 4*b*). A modest stimulation of fiber outgrowth was also seen with HDNF in the nodose ganglion, consisting of neurons exclusively derived from an epidermal placode (22)

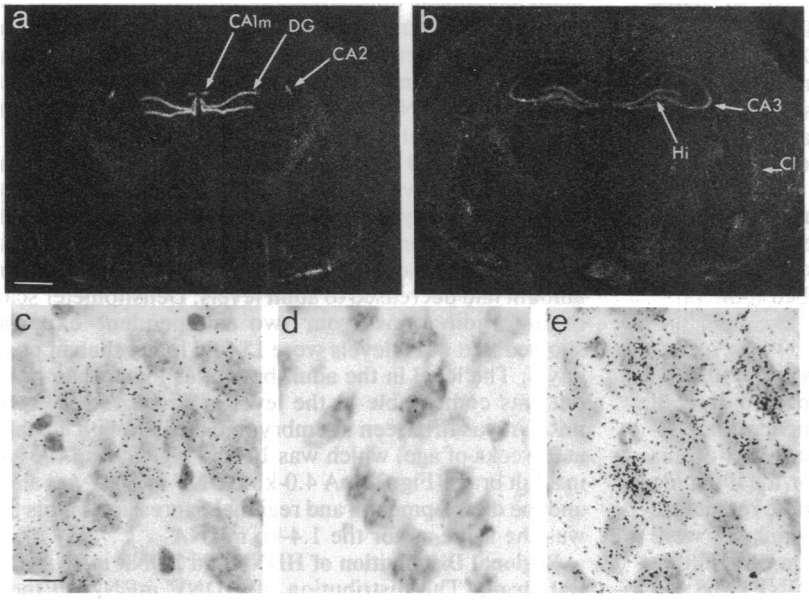


FIG. 3. Expression of HDNF and BDNF mRNA in hippocampal neurons. Rat (Sprague–Dawley) brain sections hybridized to either HDNF- or BDNF-specific oligonucleotide probes. (*a*) Autoradiogram from a section at the level of hippocampus hybridized to the HDNF-specific probe. Note labeling over medial CA1, CA2, and the dentate gyrus. (*b*) Adjacent section hybridized to a BDNF-specific probe. Note labeling over CA2 and CA3 as well as hilar cells and dentate granule layer. (*c*) Pyramidal neurons in medial CA1 labeled with the HDNF-specific probe. (*d*) Nonlabeled hilar neurons after hybridization to the HDNF-specific probe. (*e*) Hilar neurons labeled with the BDNF-specific probe. DG, dentate gyrus; CA1m, CA1 medial; Hi, hilus of dentate gyrus; Cl, claustrum. (*a* and *b*, bar = 1.3 mm; *c–e*, bar = 10 $\mu$m.)

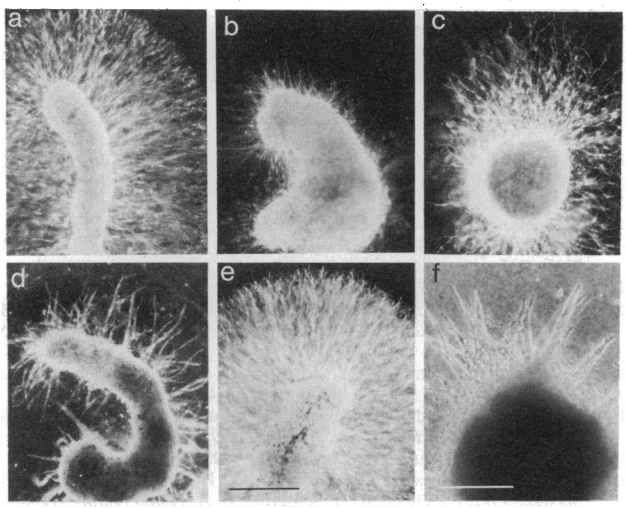


FIG. 4. Stimulation of fiber outgrowth from chicken embryonic ganglia. Biological activity of recombinant HDNF shown as effects on different nerve tissues from the chicken embryo. Remak ganglion stimulated by HDNF (*a*) or NGF (*b*). (*c*) Nodose ganglion with HDNF. Paravertebral sympathetic ganglion in response to HDNF (*d*) and recombinant rat NGF (*e*). (*f*) Ciliary ganglion with HDNF. All figures show ganglia after 1.5 days in culture. Dark-field microscopy. (Bars = 0.3 mm.)

(Fig. 4*c*). Again, HDNF was superior to NGF in evoking this response. A weak, but consistent, fiber outgrowth response with HDNF was seen in paravertebral sympathetic trunk ganglia (Fig. 4*d*), which, however, was much less pronounced compared with the massive response to rat NGF (Fig. 4*e*). In the ciliary ganglion, a weak but consistent fiber outgrowth response, manifested by the projection of short neurite fascicles, was seen with HDNF but never with NGF (Fig. 4*c*). In the dorsal root ganglia, HDNF stimulated neurite outgrowth to the same extent as NGF.

**Displacement of NGF Binding to PC12 Cells by HDNF.** Concentrated conditioned medium from transfected COS cells was tested for its ability to compete for binding $^{125}$I-labeled NGF ($^{125}$I-NGF) to its receptor on PC12 cells. The concentration of $^{125}$I-NGF used allowed ≈80% of the labeled NGF to be bound to the low-affinity receptor site in the absence of competition (40). Twenty-five times concentrated medium containing the HDNF protein displaced ≈70% of the labeled NGF and a 20% displacement was seen after a 25-fold dilution (Fig. 5). In contrast, 25 times concentrated medium from COS cells transfected with the HDNF cDNA in the opposite orientation did not show any displacement. Concentrated medium from cells transfected in parallel with a rat NGF gene displaced 50% of the labeled NGF when diluted 250 times.

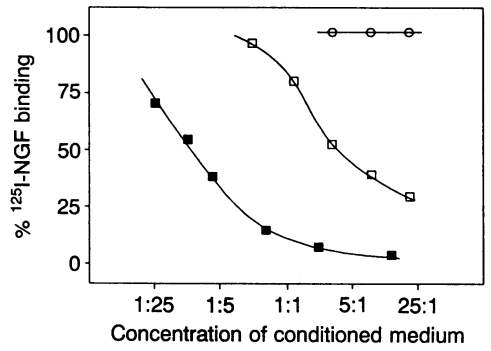


FIG. 5. Displacement of $^{125}$I-NGF from its receptor on PC12 cells by HDNF and NGF. Serial dilutions of transfected COS cell medium with (□) or without (○) HDNF or containing rat NGF (■) were assayed for their ability to displace $^{125}$I-NGF from its receptor on PC12 cells. Data are from two independent experiments that showed a variation of ±20%.

## DISCUSSION

The cDNA clone isolated in this study encodes a protein, HDNF, with a remarkable sequence similarity to both NGF and BDNF and therefore represents an additional member of a family of neurotrophic proteins. Recently (at the time of submission of this manuscript), two groups (41, 42) independent of us isolated genomic clones for a protein (neurotrophin 3) from mouse and rat, respectively, which is identical to the neurotrophic protein characterized in this study. Our cDNA clone predicts a 282-amino-acid-long protein, which is 24 amino acids longer than the protein deduced from the genomic clones (41, 42). Two alternative start sites for translation of the NGF protein have been proposed; the first is located in a separate 5′ exon (43). The second start site, located in the 3′ exon, is also efficiently used for translation of the NGF protein (36, 44) and generates a 68-amino acid shorter protein. Thus, the structure of our cDNA clone indicates that the HDNF protein utilizes two alternative start sites for translation, located in separate exons, and suggests that the genomic organization of HDNF and NGF is very similar.

In peripheral ganglia bioassays, HDNF showed neurotrophic activities that were to some extent reminiscent of both NGF and BDNF. Thus, in similarity to BDNF (20), HDNF stimulated fiber outgrowth from the nodose ganglia and, as for NGF, evoked a fiber outgrowth response in sympathetic ganglia. In the latter case, however, the response was clearly weaker than with NGF. The partially overlapping activities seen *in vitro* may reflect a cooperation of these factors *in vivo*, where two or more proteins from the same family may support the development and/or maintenance of specific neurons. The most striking stimulation of fiber outgrowth evoked by HDNF was seen in the peripheral, autonomic, ganglion of Remak containing mostly cholinergic but also some adrenergic neurons (38, 39). This effect was clearly more pronounced than effects seen with NGF (39), suggesting that HDNF also evokes trophic responses different from both NGF and BDNF. In agreement with this, HDNF showed a weak, but consistent, neurite outgrowth response in the ciliary ganglion, which does not respond to NGF or BDNF. The ciliary ganglion is known to respond to ciliary neurotrophic factor (45), which lacks a signal sequence, but could be released by an as yet unknown mechanism (46). Thus, HDNF is the only secreted neurotrophic factor today that is known to affect fiber outgrowth, at least *in vitro*, from the ciliary ganglion.

The HDNF protein displaced $^{125}$I-NGF from PC12 cells, indicating that it can interact with the NGF receptor. With the assumption that NGF and HDNF were produced in equal amounts in parallel transfections and that the conditioned medium lacks interfering substances, the interaction of NGF to its receptor was 30-fold more efficient. PC12 cells have both low- and high-affinity receptors but only the high-affinity receptor mediates a biological response (47). The fact that recombinant rat NGF readily stimulated neurite outgrowth from PC12 cells, whereas HDNF, even at 30-fold higher concentrations than NGF, did not suggests that HDNF can only interact with the NGF receptor in its low-affinity form. It therefore appears likely that the biological responses elicited by HDNF are mediated by either a separate second messenger system compared with NGF or that the HDNF receptor is different from the NGF receptor.

In similarity with NGF, HDNF mRNA was found in several peripheral rat tissues, with the highest level in kidney. Hybridization of the same filters to a rat NGF probe revealed that the level of HDNF mRNA in kidney was only slightly

higher than the levels of NGF mRNA in peripheral sympathetic target tissues, indicating that HDNF is produced in relatively small amounts in peripheral rat tissues. This is also true for the brain, and the fact that seven positive cDNA clones were isolated from $1.2 \times 10^6$ independent clones suggests that in hippocampus, containing the highest level of HDNF mRNA, this transcript constitutes $\approx 1$ in every 170,000, which clearly represents a rare transcript. Thus, as in the case of NGF, HDNF may be present in limiting amounts and functions *in vivo* as a target-derived factor for a specific subset of both peripheral and central neurons. The regional distribution of HDNF mRNA in the periphery is, however, different from NGF, and, in agreement with the *in vitro* biological assays, HDNF may support a different set of peripheral neurons. Of interest is also that HDNF mRNA was found in the ovary, whereas no mRNA was detected in the testis, where both NGF and its receptor is expressed (48) and where NGF has been suggested to mediate an interaction between Sertoli cells and germ cells (49). This shows that different members of the NGF family are expressed in different reproductive tissues and suggests that they may have nonoverlapping functions outside the nervous system.

Interestingly, the three neurotrophic proteins were maximally expressed at different times of brain development with a peak of HDNF mRNA shortly after birth, BDNF mRNA around 2 weeks, and NGF mRNA around 3 weeks after birth (see ref. 8 for NGF). Moreover, the mRNA's for all three proteins were expressed in hippocampus at levels higher than in other regions, particularly in the case of HDNF. Within hippocampus, all three mRNAs were also confined to neurons (see ref. 10 for NGF) and a clear topographical division was seen, where HDNF mRNA was concentrated to pyramidal neurons in medial CA1, CA2, and granular neurons in dentate gyrus. Strongly labeled BDNF neurons were primarily seen in CA3 and the hilar region of dentate gyrus. Neurons with apparent lower levels of BDNF mRNA were seen in the dentate gyrus. The hilar region, containing neurons with high levels of BDNF mRNA, showed no labeling for HDNF mRNA.

This remarkable concentration of trophic factors in the adult hippocampus suggests that maintenance of plasticity is crucial to its function and may relate to the presumed morphological sequelae of long-term potentiation and memory consolidation processes. The intriguing temporal and spatial expression of the three neurotrophic proteins in the brain suggests that they predominantly support neuronal innervation at different times of development and that they may also exert specific trophic support for different central nervous system neurons, a possibility that will be an interesting topic for future studies.

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