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Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain

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Brain-derived neurotrophic factor (BDNF) is a protein that allows the survival of specific neuronal populations. This study reports on the distribution of the BDNF mRNA in the adult mouse brain, where the BDNF gene is strongly expressed, using quantitative Northern blot analysis and *in situ* hybridization. All brain regions examined were found to contain substantial amounts of BDNF mRNA, the highest levels being found in the hippocampus followed by the cerebral cortex. In the hippocampus, which is also the site of highest nerve growth factor (NGF) gene expression in the central nervous system (CNS), there is ~50-fold more BDNF mRNA than NGF mRNA. In other brain regions, such as the granule cell layer of the cerebellum, the differences between the levels of BDNF and NGF mRNAs are even more pronounced. The BDNF mRNA was localized by *in situ* hybridization in hippocampal neurons (pyramidal and granule cells). These data suggest that BDNF may play an important role in the CNS for a wide variety of adult neurons.

Key words: brain-derived neurotrophic factor/hippocampus/*in situ* hybridization/nerve growth factor/trophic interactions

Introduction

During the development of the vertebrate nervous system, large numbers of apparently redundant neurons are eliminated as part of the normal developmental program (Oppenheim, 1981). An interesting characteristic of this phenomenon is that it takes place at about the time when the axons of the developing neurons first contact their target cells, and a variety of experiments suggest that these target cells play a major role in determining their own degree of innervation (Oppenheim, 1981). The secretion by the target cells of specific neurotrophic factors that are absolutely required by the neurons for further survival is the current molecular explanation of how this control is achieved (for recent reviews see Davies, 1988; Purves, 1988; Barde, 1989).

Up until recently, only one such neurotrophic factor had been characterized, nerve growth factor (NGF) (for reviews, see Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980). NGF has all the characteristics of a target-derived factor that is required for the survival of some classes of embryonic neurons, like the peripheral sympathetic neurons (Cohen, 1960; Korsching and Thoenen, 1983; Heumann *et al.*, 1984; Shelton and Reichardt, 1984; Davies *et al.*, 1987). The availability of this protein has allowed the demonstration that in fact, the role of NGF is not limited to a survival function during development. For example, the injection of NGF into adult rats increases the number of sympathetic axons in the target tissue (Olson, 1967; Olson and Malmfors, 1970). Conversely, the neutralization by antibiotics of endogenous NGF in adult animals dramatically reduces the levels of enzyme that are rate-limiting in the synthesis of neurotransmitters in NGF responsive neurons (Goedert *et al.*, 1978).

While NGF is mostly present outside the central nervous system, evidence has accumulated in recent years for its presence in the adult central nervous system (CNS) (Korsching *et al.*, 1985). Though the physiological role of NGF during the development of the CNS is still not entirely clear (the effects of neutralizing antibodies being far less dramatic than in the PNS), its role after lesion of adult CNS neurons is now well-established (Hefti, 1986; see Thoenen *et al.*, 1987 for a review). For example, after section of the axons of basal forebrain cholinergic neurons, the shrinkage, loss of function or even death of these neurons can be prevented by administration of NGF (Hefti, 1986). These results underscore the functional significance of neurotrophic factors in the adult CNS.

The recent elucidation of the full primary structure of BDNF has established that this protein is closely related to NGF (Leibrock *et al.*, 1989). BDNF was isolated from brain tissue and has been shown to play a role during the development of the nervous system that appears to be quite similar to that of NGF, albeit on different neurons (Hofer and Barde, 1988). We report here on the distribution of BDNF mRNA in the adult CNS, using a mouse cDNA clone. Surprisingly high levels of mRNA were found in some brain areas, and in the hippocampus, neurons (pyramidal and granule cells) were identified as the cells expressing the BDNF gene. These results are discussed and compared with those obtained previously on the distribution of the NGF mRNA.

Results

Characterization of the mouse BDNF cDNA sequence

The mouse BDNF cDNA sequence was determined on the basis of clones derived from a mouse brain cDNA library (see Materials and methods). On Southern blot analysis with mouse genomic DNA, only one size-fragment was revealed, indicating that the BDNF gene is unique. No cross-hybridization was found with the related genes NGF and NT-3 (data not shown). The deduced amino acid sequence (Figure 1) shows that the sequence of mature mouse BDNF (the biologically active protein) is 100% identical with that of the pig (Leibrock *et al.*, 1989). A high degree of conservation can also be observed in the prepro-sequence of BDNF, as well as in the 5′- and 3′-untranslated regions (see Discussion).

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                                                                  GTCTTCC
   CCAGAGCAGC TGCCTTGATG TTTACTTTGA CAAGTAGTGA CTGAAAAAGT TCCACCAGGT GAGAAGAGTG     77
1                                    10                                   20
Met Thr Ile Leu Phe Leu Thr Met Val Ile Ser Tyr Phe Gly Cys Met Lys Ala Ala Pro
ATG ACC ATC CTT TTC CTT ACT ATG GTT ATT TCA TAC TTC GGT TGC ATG AAA GCG GCG CCC    137
                                    30                                   40
Met Lys Glu Val Asn Val His Gly Gln Gly Asn Leu Ala Tyr Pro Gly Val Arg Thr His
ATG AAA GAA GTA AAC GTC CAC GGA CAA GGC AAC TTG GCC TAC CCA GGT GTG CGG ACC CAT    197
                                    50                                   60
Gly Thr Leu Glu Ser Val Asn Gly Pro Arg Ala Gly Ser Arg Gly Leu Thr Thr Thr Ser
GGG ACT CTG GAG AGC GTG AAT GGG CCC AGG GCA GGT TCG AGA GGT CTG ACG ACG ACA TCA    257
                                    70                                   80
Leu Ala Asp Thr Phe Glu His Val Ile Glu Glu Leu Leu Asp Glu Asp Gln Lys Val Arg
CTG GCT GAC ACT TTT GAG CAC GTC ATC GAA GAG CTG CTG GAT GAG GAC CAG AAG GTT CGG    317
                                    90                                  100
Pro Asn Glu Glu Asn His Lys Asp Ala Asp Leu Tyr Thr Ser Arg Val Met Leu Ser Ser
CCC AAC GAA GAA AAC CAT AAG GAC GCG GAC TTG TAC ACT TCC CGG GTG ATG CTC AGC AGT    377
                                   110                                  120
Gln Val Pro Leu Glu Pro Pro Leu Leu Phe Leu Leu Glu Glu Tyr Lys Asn Tyr Leu Asp
CAA GTG CCT TTG GAG CCT CCT CTA CTC TTT CTG CTG GAG GAA TAC AAA AAT TAC CTG GAT    437
                                   130                                  140
Ala Ala Asn Met Ser Met Arg Val Arg Arg His Ser Asp Pro Ala Arg Arg Gly Glu Leu
GCC GCA AAC ATG TCT ATG AGG GTT CGG CGC CAC TCC GAC CCT GCC CGC CGT GGG GAG CTG    497
                                   150                                  160
Ser Val Cys Asp Ser Ile Ser Glu Trp Val Thr Ala Ala Asp Lys Lys Thr Ala Val Asp
AGC GTG TGT GAC AGT ATT AGC GAG TGG GTC ACA GCG GCA GAT AAA AAG ACT GCA GTG GAC    557
                                   170                                  180
Met Ser Gly Gly Thr Val Thr Val Leu Glu Lys Val Pro Val Ser Lys Gly Gln Leu Lys
ATG TCT GGC GGG ACG GTC ACA GTC CTA GAG AAA GTC CCG GTA TCC AAA GGC CAA CTG AAG    617
                                   190                                  200
Gln Tyr Phe Tyr Glu Thr Lys Cys Asn Pro Met Gly Tyr Thr Lys Glu Gly Cys Arg Gly
CAG TAT TTC TAC GAG ACC AAG TGT AAT CCC ATG GGT TAC ACC AAG GAA GGC TGC AGG GGC    677
                                   210                                  220
Ile Asp Lys Arg His Trp Asn Ser Gln Cys Arg Thr Thr Gln Ser Tyr Val Arg Ala Leu
ATA GAC AAA AGG CAC TGG AAC TCG CAA TGC CGA ACT ACC CAA TCG TAT GTT CGG GCC CTT    737
                                   230                                  240
Thr Met Asp Ser Lys Lys Arg Ile Gly Trp Arg Phe Ile Arg Ile Asp Thr Ser Cys Val
ACT ATG GAT AGC AAA AAG AGA ATT GGC TGG CGA TTC ATA AGG ATA GAC ACT TCC TGT GTA    797
                                   250
Cys Thr Leu Thr Ile Lys Arg Gly Arg End
TGT ACA CTG ACC ATT AAA AGG GGA AGA TAG     TGGATTTATG TTGTATAGAT TATATTGAGA        857

CAAAATTATC TATTTGTATA TATACATAAC AGGATAAATT ATTCAGTTAA GAAAAAATAA TTTTATGAAC    917
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of mouse BDNF. The sequence corresponding to the probe used in this study is underlined, and the only consensus sequence for *N*-glycosylation is doubly underlined. The arrow indicates the start of mature BDNF.

Northern blot analysis

The sequence 224−734 (Figure 1) was cloned into a Bluescript plasmid vector, and a single strand cRNA probe prepared using T7 polymerase. In Northern blot analysis, this probe revealed two transcripts (1.5 and 4.2 kb in size) in all brain regions studied that were found to be RNase resistant (Figure 2). With the exception of the cerebellum, the ratio of both transcripts was found to be relatively constant in the various regions analysed. Quantitative measurements were performed by including a known amount of sense transcript to all samples at the time of tissue homogenization. The highest levels were found in the hippocampus, followed by the cerebral cortex (Figure 3). In addition to the brain, BDNF mRNA was also clearly detectable in the spinal cord.

In situ hybridization

Mouse brain sagittal sections were hybridized with a [^{32}P]-cRNA probe and examined by autoradiography. This study revealed that the BDNF gene is expressed throughout the brain gray matter (Figure 4). The highest levels of expression were seen in the hippocampus, and within the hippocampal formation, in the dentate gyrus and CA2−CA4 areas. Amongst the brain areas prominently labelled with the cRNA antisense probe were the cerebral and cerebellar cortex, the thalamic and hypothalamic nuclei and the pontine nuclei (Table I). In these experiments, no signal above background was observed using the sense cRNA probe as a control (Figure 5B). The strong signal observed over the large hippocampal pyramidal cells and the granule cells of the dentate gyrus allowed the localization of the BDNF mRNA in neurons (Figure 5D). It was striking to see that although some neurons were strongly labelled, such neurons were found adjacent to neurons that were not labelled over background (Figure 5D). In the cerebral cortex, the

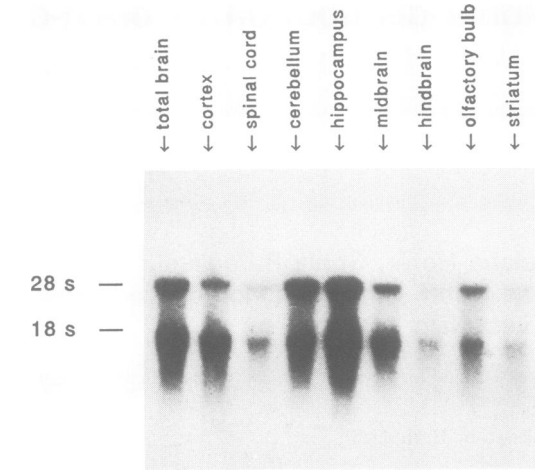

Fig. 2. Distribution of the BDNF mRNA in various brain regions of the adult mouse. 20 μg total RNA from the indicated regions were electrophoresed on a formaldehyde−agarose gel, transferred to Nylon membranes and hybridized with a cRNA probe, followed by RNase treatment.

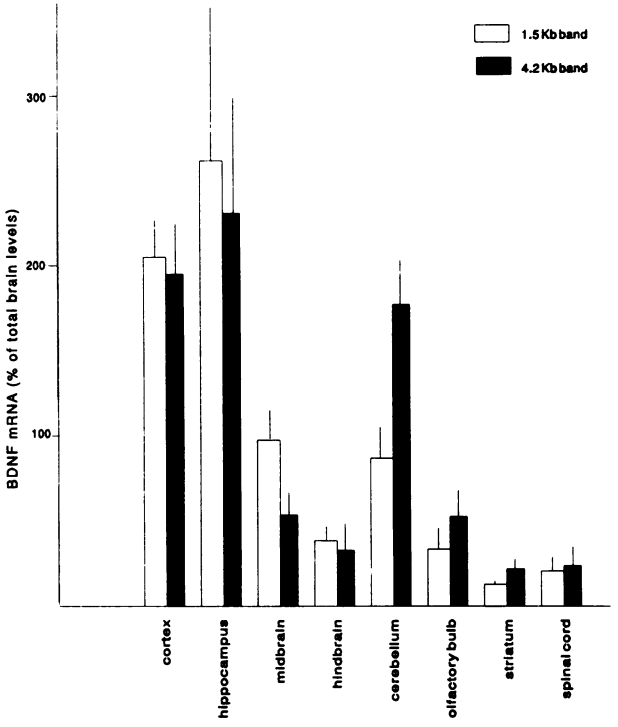

Fig. 3. Quantification of the relative amounts of BDNF mRNA in various brain regions of adult mouse. The values are the means ± SD of 4−5 experiments. For quantification purposes, sense-probe standards were added to the samples (see Materials and methods), and the blots exposed for autoradiography for various lengths of time so that the intensities of the signals to be measured (by densitometric scanning) were within the linear range of those given by serial dilutions of the sense standards. The mean values obtained for total brain samples were 0.68 ± 0.15 pg/mg for the 1.5 kb and 1.0 ± 0.49 pg/mg wet weight of tissue for the 4.2 kb band and are arbitrarily set at 100%.

pyriform, frontal, parietal, occipital and entorhinal cortical areas were all labelled. In the parietal cortex (Figure 6A), layers II and V were strongly labelled, III, IV and VI slightly less. In the olfactory bulb, the following cells could be iden-

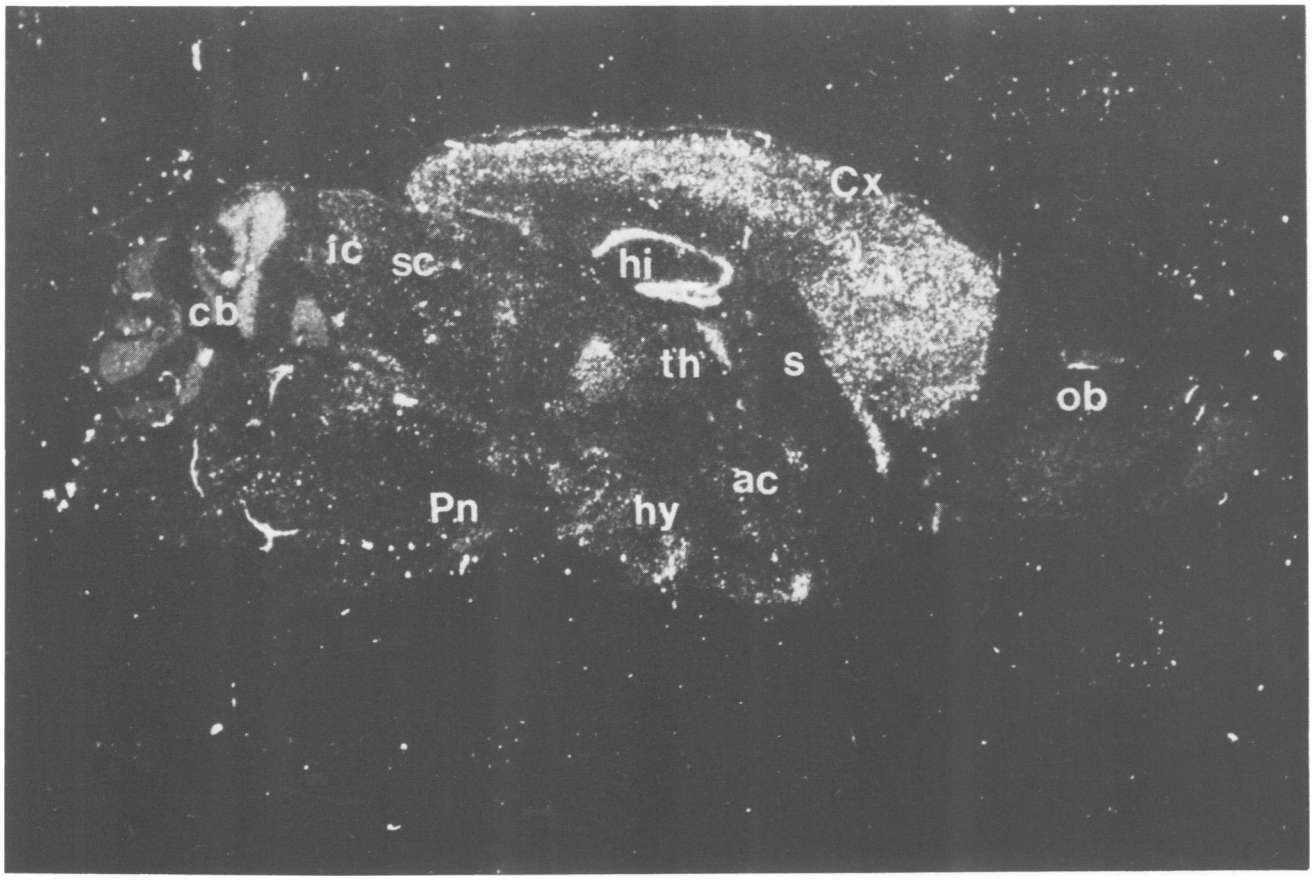

Fig. 4. Sagittal section of the adult mouse brain showing the regional expression pattern of BDNF mRNA by *in situ* hybridization. Dark field view of an autoradiogram of a section hybridized with ^{32}P-labelled BDNF cRNA antisense probe and exposed for 5 weeks. Frontal is to the right and ventral to the bottom. Abbreviations: cb, cerebellum; ic and sc, inferior and superior colliculus; hi, hippocampus; Cx, cerebral cortex; th, thalamus; s, septum; ob, olfactory bulb; ac, anterior comissure; hy, hypothalamus; and Pn, pontine nuclei.

tified as being labelled: periglomerular, tufted, mitral and granule cells (Figure 6B) and in the cerebellar cortex (Figure 6C and D), the internal granule cell layer.

Discussion

Sequence conservation between mouse and pig BDNF

The comparison of the deduced amino acid sequences of mouse and pig BDNF indicates that there is not a single amino acid substitution in the mature (i.e. biologically active) portion of BDNF (Leibrock *et al.*, 1989). This shows that the sequence conservation of BDNF in these two species is even higher than that of the various mammalian NGFs characterized so far, already known to be high (90% amino acid conservation in the mature part of human and mouse NGF, Ullrich *et al.*, 1983). In this context, it is interesting to note that recently, a third protein belonging to the NGF-BDNF gene family has been isolated, neurotrophin-3 (NT-3) (Hohn *et al.*, 1990; Maisonpierre *et al.*, 1990). This neurotrophic factor, that has a neuronal specificity different from that of NGF and BDNF, also shows 100% sequence conservation in the mature portion in two species (rat and mouse). These complete primary structure conservations of the mammalian NT-3 and BDNF indicate that presumably, a strong pressure has been exerted to preserve intact the entire primary structure of the biologically active proteins during evolution. This conservation, together with the close structural relatedness of NGF, BDNF and NT-3 invites the speculation that even small changes in the primary structures of these three neurotrophic proteins might alter their neuronal specificities.

It is interesting to note that a very high degree of sequence conservation has been reported in another family of

Table I. Overview of the BDNF mRNA expression levels in the adult mouse brain by *in situ* hybridization

Brain area	Level of expression
olfactory bulb	+
periglomerular/tufted/mitral/granule cells	+
cerebral cortex	+++
hippocampus: CA1	+++
hippocampus: CA2/CA3/CA4/dentate gyrus	++++
n.anterodorsal th.	++
n.parafascicular th.	++
n.medial th.	+
medial preoptic area	+++
ant. hypoth. area	++
n.ventromedial hypoth.	++
mamillary nuclei	++
inferior and superior colliculi	+
pontine nuclei	+++
cerebellar granule cell layer	++

++++very high, +++high, ++moderate, +low (arbitrary units)

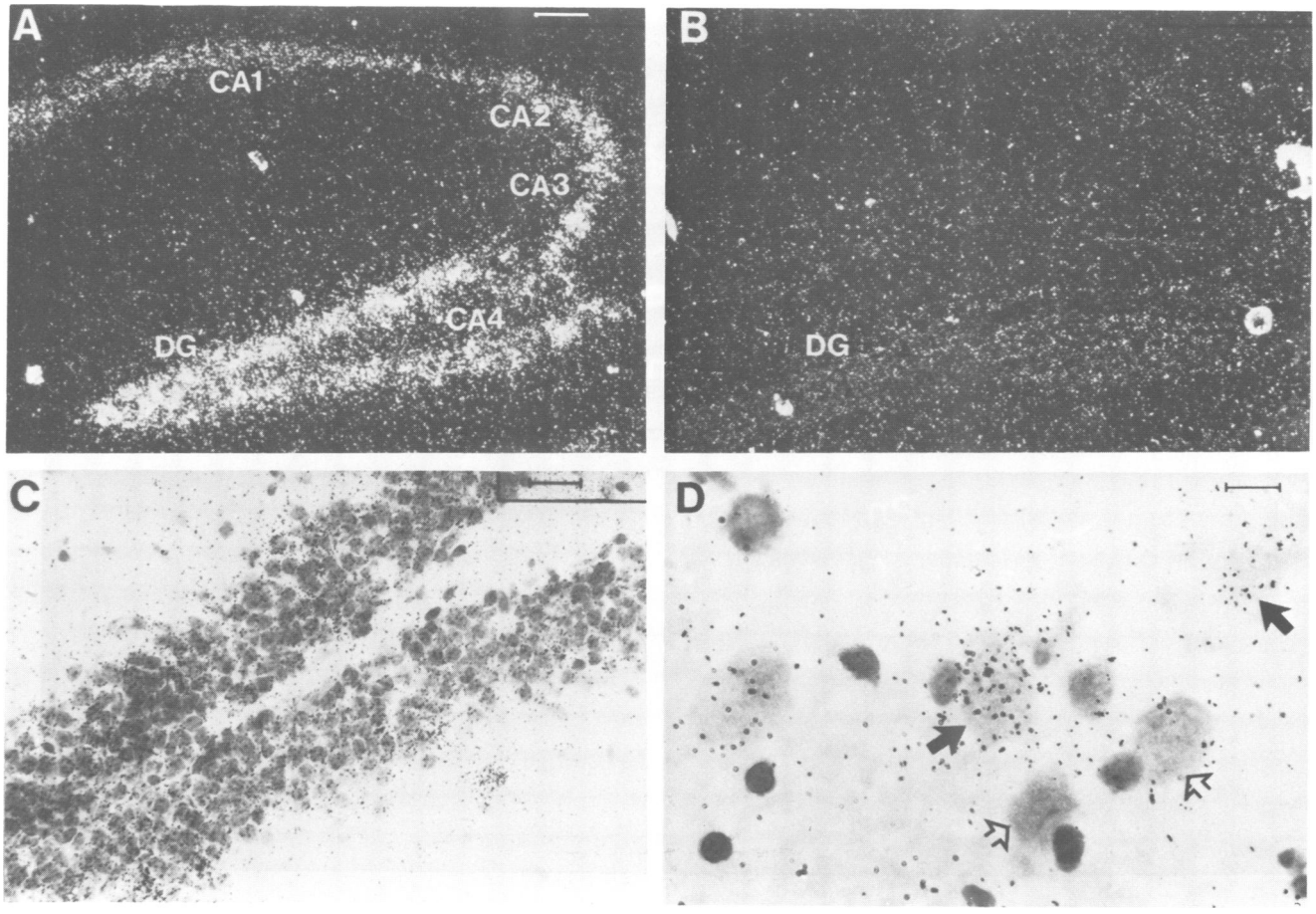

Fig. 5. Expression pattern of BDNF mRNA in the hippocampal formation. Dark field photomicrographs of hybridizations with (**A**) antisense and (**B**) sense BDNF cRNA probes on dentate gyrus (DG) and cornus ammonis (CA) areas (bar = 100 μ). (**C**) higher magnification of dentate gyrus (bar = 25 μ) and (**D**) of CA4 area, showing strong hybridization signals confined to a subset of neurons (arrows) while adjacent neurons (open arrows) show no specific signal over background (bar = 10 μ).

sequence-related factors, the transforming growth factors beta (TGFβ) (see Roberts and Sporn, 1990 for a recent review). The TGFβ 1 to 5 are even more closely related to one another than NGF, BDNF and NT-3 are, and those which have been sequenced in different species show an extremely high degree of sequence conservation (Roberts and Sporn, 1990).

The structure conservation of BDNF is also high outside the mature portion of the protein. In the precursor region, out of a total of 130 amino acids, only nine amino acids are substituted and the primary structure of the putative signal sequence is identical in both species. In fact, we note that in the 5′-region, the nucleotide sequence homology is also very high, no base substitution being present in the first 130 nucleotides (Figure 1). Also, the 3′ untranslated region is extraordinarily well conserved, only four bases being substituted out of a total of 100. We note, that as with the pig sequence, no polyadenylation consensus sequence has been found so far.

Northern blot analysis

In all brain samples examined, we found two BDNF mRNA transcripts, ~1.5 and 4.2 kb in length. This is in contrast to our earlier report (Leibrock *et al.*, 1989), where only the shorter transcript was seen. This discrepancy is most likely due to the use of a different probe which contained, in addition to the probe used here, a 150 bp sequence not related to the BDNF sequence. For reasons that are unclear, this additional sequence (the origin of which is not known) amplified the signal resulting from the shorter transcript. The two transcripts described are seen only with the antisense probe (as opposed to the sense probe), are RNase resistant, and are also found in poly-$(A)^+$ preparations. On a molar basis, the shorter transcript is about three times more abundant, and this ratio is similar in the different brain regions, with the exception of the cerebellum. Since a Southern blot analysis using mouse genomic DNA reveals that the BDNF gene is unique (no cross-hybridization being seen with the NGF and NT-3 genes), it is likely that these two RNase resistant transcripts both derive from the BDNF gene, and represent two differently spliced messages and/or messages using alternative polyadenylation signals.

One of the goals of this study was to determine not only qualitatively, but also quantitatively, the levels of BDNF mRNA in the brain. Indeed, our initial study has established that the CNS was a predominant site of BDNF gene expression in the adult mouse (Leibrock *et al.*, 1989). We could confirm this fact using the probe described here, though it is also clear that the 1.5 and 4.2 kb transcripts can also be found in some non-CNS tissues, heart and lung in particular,

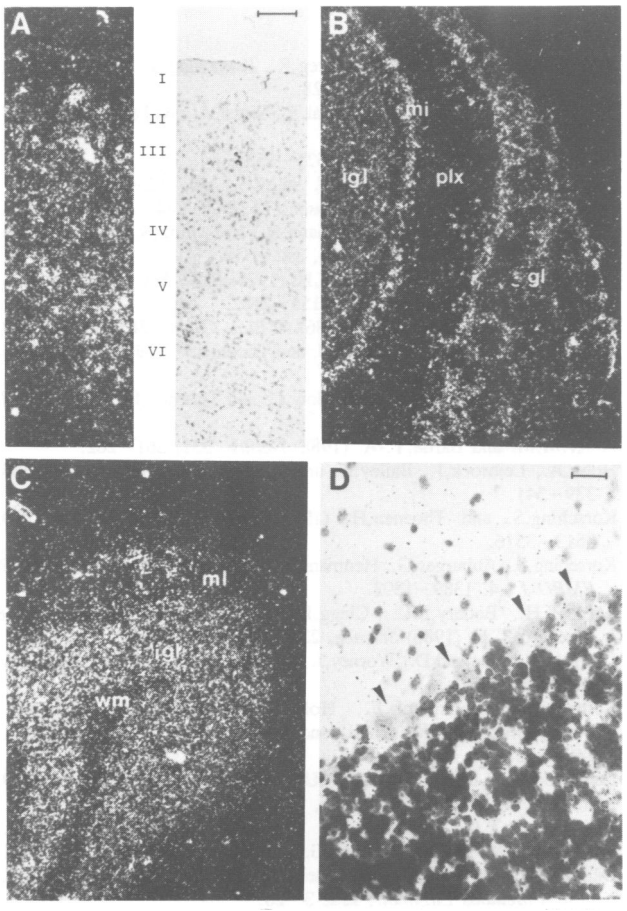

Fig. 6. Laminar expression of BDNF mRNA in various brain regions of adult mouse. Dark (A, B and C) and bright field (A and D) photomicrographs showing (**A**), cortical layers and (**B**), olfactory bulb glomeruli (gl), external plexiform layer (plx), mitral cell layer (mi) and internal granular layer (igl). (**C**), cerebellar molecular layer (ml), internal granular layer (igl), and white matter (wm) (bar = 100 μ; A,B and C: same magnification); and (**D**), grain density over granule neurons and Purkinje neurons, the latter indicated by arrow heads and showing no labelling over background (bar = 25 μ).

albeit at lower levels, (Maisonpierre *et al.*, 1990; Hofer *et al.*, unpublished). In addition, quantitative data on the NGF mRNA distribution in the adult rodent brain are available. We find that the steady state BDNF mRNA levels are far higher than those of NGF in all brain areas studied. For example, although the values reported for NGF in the literature vary somewhat, we find at least 50-fold more BDNF mRNA in the hippocampus than NGF mRNA (Korsching *et al.*, 1985; Shelton and Reichardt, 1986; Large *et al.*, 1986; Auburger *et al.*, 1987). In the cerebellum, the mRNA ratio BDNF/NGF is even higher. The level of BDNF mRNA in the total brain (0.68 pg/mg wet weight) is in the same range as that of NGF mRNA in peripheral organs densely innervated by the sympathetic system like the vas deferens (0.36 pg/mg) or the iris (0.35 pg/mg) (Heumann *et al.*, 1987). It remains to be seen to which extent the levels and distribution of BDNF protein corresponds to that of its mRNA. Although no data are yet available on the regional distribution of BDNF in the brain, the protein purification results using whole pig brain as starting material do indeed suggest that the levels of BDNF are higher than those of NGF: ~1 ng BDNF can be isolated from 1 g of brain (Hofer and Barde, 1988), which corresponds to ~5 ng BDNF/g assuming an overall yield of 20%. In the adult rat brain, ~0.3 ng/g NGF has been reported using immunoassays (Korsching *et al.*, 1985; Whittemore *et al.*, 1986).

In situ hybridization

In general, there is a good correlation between the *in situ* hybridization data and the Northern blot analysis (compare Table I and Figure 3). However, some regions expressing BDNF mRNA at relatively low but detectable levels in the Northern blot analysis like the striatum did not give a signal that could be clearly distinguished from the background in the *in situ* hybridization study. BDNF mRNA was found over gray matter areas, and the large size of the hippocampal pyramidal and granule cells allowed the localization of the message to these neurons. Neither all pyramidal cells, nor all granule cells are labelled, and it is interesting to note that in a previous study with a labelled NGF cDNA probe, exactly the same conclusion has been reached (Ayer-Lelièvre *et al.*, 1988). These findings raise the question of whether NGF and BDNF producing neurons are intermingled during hippocampal formation, or if one neuron co-expresses both genes. It is intriguing that the hippocampal formation seems to be a privileged site of synthesis for neurotrophic factors. In addition to NGF and BDNF discussed here, the third member of this family, NT-3, has also been localized to the hippocampus (by Northern blot analysis using adult mouse brain tissue, Hohn *et al.*, 1990). In future studies, it will be particularly interesting to study the role and interplay of these three neurotrophic factors in the hippocampal formation, and their involvement (if any) in synaptic plasticity.

The expression pattern of BDNF mRNA in the olfactory bulb, compared with that of NGF mRNA, revealed some interesting differences. While NGF mRNA was only detected in periglomerular neurons (Bandtlow *et al.*, in preparation), BDNF mRNA was also detected in several other cell types in this structure (Table I). Again, as in the hippocampus, not all cells were equally labelled.

Though nothing is known about the role of BDNF in the intact adult nervous system, it is already clear that BDNF does act on adult neurons *in vitro*. For example, BDNF is able to prolong the survival of retinal ganglion cells in explanted rat retinae, and to stimulate the rate of axonal elongation from these cells (Thanos *et al.*, 1989). The rate of fibre outgrowth from isolated dorsal root ganglion neurons is also accelerated by BDNF in culture (Lindsay, 1988).

In conclusion, this study shows that in the adult mouse CNS, substantial amounts of BDNF mRNA can be found in a variety of areas. Among others, these include the spinal cord and the superior colliculus, areas that receive projections from BDNF-responsive neurons, such as primary sensory neurons and retinal ganglion cells. In all regions studied, the levels of BDNF mRNA are considerably higher than those of NGF mRNA, even though the overall regional and cellular localization of BDNF mRNA shows some striking similarities to that of the NGF mRNA. To the extent that the presence of BDNF mRNA can be taken as an indication of the distribution of the BDNF protein, these results indicate that a wide variety of neurons contacting those producing BDNF might be responsive to this neurotrophic factor.

Materials and methods

Cloning of the mouse BDNF cDNA

A mouse specific sequence was obtained by sequencing a segment of mouse genomic DNA (1 μg template) amplified using the polymerase chain reaction method (Saiki *et al.*, 1985) and two 17mer primers corresponding to all possible codons of the sequence Ala-Ala-Asp-Lys-Lys-Thr (sense) and Lys-Gln-Tyr-Phe-Tyr-Glu (antisense) (Leibrock *et al.*, 1989). Based on the nucleotide sequence thus obtained, two specific oligonucleotides (GCGGGACGGTCACAGTCCTAGAGAA and GGATACCGGGACTTTCTCTAGGACT) were synthesized, [^{32}P]-end-labelled and used to screen 600 000 phage plaques of a mouse brain λ gt11 cDNA library (Clontech). Seven double-positive clones were obtained and the sequence of one of them is depicted in Figure 1.

Northern blot analysis

Total RNA was extracted from various brain regions of adult mice according to Okayama *et al.* (1987). Electrophoresis was performed according to Lehrach *et al.* (1977) on a 1.3% agarose–formaldehyde gel. Twenty μg total RNA were loaded per lane. The RNA was transferred to nylon membranes (Hybond-N, Amersham) and hybridized overnight at 65°C in 2 ml hybridization buffer (6.5 × SSC, 50% formamide, 5 × Denhardt's, 0.25% SDS, 250 μg/ml salmon sperm DNA, 5 mM EDTA in 50 mM sodium phosphate, pH 7.2) with a [^{32}P]-cRNA mouse BDNF probe (10^7 c.p.m./ml). After washing for 60 min in 0.1 × SSC containing 0.5% SDS at 75°C, the blots were incubated for 60 min at room temperature with 0.1 μg/ml RNase A (Pharmacia) and exposed to an X-ray film (Fuji) at −70°C with intensifying screens.

For quantification, a sense transcript of the probe was included in the RNA preparation as a recovery standard. A serial dilution of this transcript was electrophoresed in separate lanes. Various exposure times were chosen so that the intensities of the signals to be quantified were within the linear range of those given by the serial dilutions of the standards.

After autoradiography the films were densitometrically scanned and the data obtained for the BDNF mRNA were corrected for variable recoveries (Heumann and Thoenen, 1986). When expressed in pg/mg wet weight, the results were corrected for the size of the transcripts.

The mouse probe was prepared from a Bluescript SK$^+$ vector containing the sequence of mouse BDNF cDNA from nucleotides 224–734 in the *ApaI* site. The vector was linearized with *XhoI* and a single-stranded RNA probe was transcribed using the Promega *in vitro* transcription system with T7 polymerase. The specific activity of this probe was $2–5 \times 10^8$ c.p.m./μg.

In situ hybridization

In situ hybridization was performed essentially as described by Shivers *et al.* (1986), with some modifications. Briefly, frozen brain tissues were sectioned on a cryostate (10 μm), thaw mounted onto RNase free silanized glass microscope slides (2% 3-aminopropyltrietoxysilane, Sigma, in acetone), fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline pH 7.5, dehydrated in a graded ethanol series and stored at −70°C. The ^{32}P-radiolabelled BDNF cRNA sense or antisense probes (specific activity $2–5 \times 10^8$ c.p.m./μg) were subjected to 30 min alkaline digestion, neutralized and subsequently diluted in hybridization buffer (4 × SSC, 1 × Denhardt's, salmon sperm DNA, 1 mM EDTA, 0.05% sodium pyrophosphate, 10 mM Tris–HCl pH 7.5, 50% formamide) at a final concentration of 1×10^4 c.p.m./μl. One drop of hybridization buffer (30–50 μl, depending on the area of the section) was then applied, covered with a glass cover slip, and sealed with rubber cement. After overnight hybridization at 62°C in a humidified box, the tissue sections were washed twice for 50 min at room temperature in 2 × SSC containing 0.05% sodium pyrophosphate, followed by two washes for 20 min at 72°C in 0.1 × SSC containing 0.05% sodium pyrophosphate. A post-hybridization RNase treatment was omitted, as it was found to decrease the hybridization signal. The sections were finally dehydrated in ethanol and air-dried. The sections were coated with emulsion (Kodak NTB3 in 50% aqueous solution) and exposed for 1–6 weeks. After development of the autoradiograms, the tissue sections were stained with fast green and cresyl violet, cleared in xylene and coversliped with Entellan mounting medium (Merck).

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References

Auburger,G., Heumann,R., Hellweg,R., Korsching,S. and Thoenen,H. (1987) *Dev. Biol.*, **120**, 322–328.
Ayer-LeLièvre,C., Olson,L., Ebendal,T., Seiger,Å. and Persson, H. (1988) *Science*, **240**, 1339–1341.
Barde,Y.-A. (1989) *Neuron*, **2**, 1525–1534.
Cohen,S. (1960) *Proc. Natl. Acad. Sci. USA*, **46**, 302–311.
Davies,A.M. (1988) *Trends Neurosci.*, **11**, 243–244.
Davies,A.M., Bandtlow,C., Heumann,R., Korsching,S., Rohrer,H. and Thoenen,H. (1987) *Nature*, **326**, 353–358.
Goedert,M., Otten,U. and Thoenen,H. (1978) *Brain Res.*, **148**, 264–268.
Hefti,F. (1986) *J. Neurosci.*, **6**, 2155–2162.
Heumann,R. and Thoenen,H. (1986) *J. Biol. Chem.*, **261**, 9246–9249.
Heumann,R., Korsching,S., Scott,J. and Thoenen,H. (1984) *EMBO J.*, **3**, 3183–3189.
Heumann,R., Korsching,S., Bandtlow,C. and Thoenen,H. (1987) *J. Cell Biol.*, **104**, 1623–1631.
Hofer,M.M. and Barde,Y.-A. (1988) *Nature*, **331**, 261–262.
Hohn,A., Leibrock,J., Bailey,K. and Barde,Y.-A. (1990) *Nature*, **344**, 339–341.
Korsching,S. and Thoenen,H. (1983) *Proc. Natl. Acad. Sci.*, **80**, 3513–3516.
Korsching,S., Auburger,G., Heumann,R., Scott,F. and Thoenen,H. (1985) *EMBO J.*, **4**, 1389–1393.
Large,T.H., Bodary,S.C., Clegg,D.O., Weskamp,G., Otten,U. and Reichardt,L.F. (1986) *Science*, **234**, 352–355.
Lehrach,H., Diamond,D., Worney,J. and Boedtker,H. (1977) *Biochemistry*, **16**, 4743–4751.
Leibrock,J., Lottspeich,F., Hohn,A., Hofer,M., Hengerer,B., Masiakowski,P., Thoenen,H. and Barde,Y.-A. (1989) *Nature*, **341**, 149–152.
Levi-Montalcini,R. and Angeletti,P.U. (1968) *Physiol. Rev.*, **48**, 534–569.
Lindsay,R.M. (1988) *J. Neurosci.*, **8**, 2394–2405.
Maisonpierre,P.C., Belluscio,L., Squinto,S., Ip,N.Y., Furth,M.E., Lindsay,R.M. and Yancopoulos,G.D. (1990) *Science*, **247**, 1446–1451.
Okayama,H., Kawaichi,M., Brownstein,M., Lee,F., Yokota,T. and Akai,K. (1987) *Methods Enzymol.*, **154**, 3–28.
Olson,L. (1967) *Zellforsch. Mikrosk. Anat.*, **81**, 155–173.
Olson,L. and Malmfors,T. (1970) *Acta Physiol. Scand. Suppl.*, **348**, 1–112.
Oppenheim,R.W. (1981) In Cowan,W.M. (ed.), *Studies in Development Neurobiology*, Oxford University Press, Oxford, pp. 74–133.
Purves,D. (1988) *Body and Brain. A Trophic Theory of Neural Connections*. Harvard University Press, Cambridge, MA.
Roberts,A.B. and Sporn,M.B. (1990) In Sporn,M.B. and Roberts,A.B. (eds), *Peptide Growth Factors and their Receptors. Handbook of Experimental Pharmacology*, Springer Verlag, Heidelberg, Volume 95, pp. 419–472.
Saiki,R.K. *et al.* (1985) *Science*, **230** 1350–1354.
Shelton,D.L. and Reichardt,L.F. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7951–7955.
Shelton,D.L. and Reichardt,L.F. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2714–2718.
Shivers,B.D., Schachter,B.S. and Pfaff,D.W. (1986) *Methods Enzymol.*, **124**, 497–510.
Thanos,S., Bähr,M., Barde,Y.-A. and Vanselow,J. (1989) *Euro. J. Neurosci.*, **1**, 19–26.
Thoenen,H. and Barde,Y.-A. (1980) *Phsyiol. Rev.*, **60**, 1284–1335.
Thoenen,H., Bandtlow,C. and Heumann,R. (1987) *Rev. Physiol. Biochem. Pharmacol.*, **109**, 145–178.
Ullrich,A., Gray,A., Berman,C. and Dull,T.J. (1983) *Nature*, **303**, 821–823.
Whittemore,S.R., Ebendal,T., Lärkfors,L., Olson,L., Seiger,Å, Strömberg,I. and Persson,H. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 817–821.

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