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Comparison of mammalian, chicken and *Xenopus* brain-derived neurotrophic factor coding sequences

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We have used the polymerase chain reaction (PCR) to amplify and clone coding sequences of the mature region of brain-derived neurotrophic factor (BDNF) from monkey, rat, chicken and *Xenopus* genomic DNA. Consistent with previous reports, the predicted amino acid sequences obtained in this manner from monkey and rat were identical to other mammalian BDNF sequences. The chicken and *Xenopus* BDNF sequences are also highly conserved, but contain 7 and 8 amino acid substitutions, respectively, compared to mammalian BDNF. Comparison of these sequences with the homologous NGF and NT3 coding sequences provides further insight into amino acid residues that may be responsible for the different receptor specificities of these factors.

Nerve growth factor; Brain-derived neurotrophic factor; Polymerase chain reaction; Chicken; *Xenopus*

1. INTRODUCTION

Nerve growth factor (NGF), which is critical for the survival of developing sympathetic and neural crest derived sensory neurons, is the most thoroughly studied example of a target-derived neurotrophic factor [1,2]. Another neurotrophic factor, brain-derived neurotrophic factor (BDNF), has been recently identified that is also capable of maintaining survival of developing peripheral neurons [3]. BDNF and NGF bind to distinct high-affinity receptors and have neurotrophic effects on different as well as overlapping neuronal populations [4–7]. BDNF has been purified and a complete sequence has been obtained by cDNA analysis [3,8]. The similarities in sequence between NGF and BDNF have led to the identification of an additional member of this neurotrophic factor family, neurotrophin-3 (NT3), which is also structurally similar and 50% identical within the biologically active carboxy-terminal region to NGF and BDNF [9–13]. NGF, BDNF, and NT3 are all synthesized as precursor proteins containing a signal peptide, an amino-terminal precursor region and a highly conserved carboxy-terminal region. Proteolytic processing of these precursors releases 119-amino-acid mature forms of BDNF [8] and NT3 [13] from the carboxy-terminal region and a 120-amino-acid form of NGF which is further processed at the carboxy terminus in the mouse submandibular gland to produce the well-characterized 118 amino acid β-NGF [14]. Mammalian forms of NGF, BDNF and NT3 are 50% identical within this biologically active carboxy-terminal region [9,12,13]. The three neurotrophic factor family members also have similar genomic organizations in that the entire coding sequence of the predominant transcript (corresponding to transcript B of mouse NGF [15]) is contained on a single exon [12,16].

Identical amino acid sequences encoding mature BDNF have been reported from porcine [8], mouse [9], rat [12] and human [13,17] sources. The amino-acid sequences reported for mature NT3 from mouse [9], rat [12] and human [11,13] sources were also identical. In contrast, NGF is less well conserved between mammalian sources [16,18–21]. Here, we have determined the DNA coding sequences for the mature forms of monkey, rat, chicken and *Xenopus* BDNF.

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2. MATERIALS AND METHODS

BDNF cDNA clones were obtained by polymerase chain reaction (PCR) amplification of rat, monkey, chicken and *Xenopus* genomic DNA with two oligonucleotides prepared based on the published cDNA sequence of porcine BDNF [8]. The 5′ oligonucleotide (5′-GTCGACAACATGTTCATGAGGGTCCG-3′) contained a *Sal*I restriction site and bases 542–561 of the cDNA sense strand of porcine BDNF. The 3′ oligonucleotide (5′-CTATCTTCCCCTCTTA-ATGGTCTGGAC-3′) contained a *Pst*I restriction site and 21 bases complementary to bases 905–925 of the porcine BDNF cDNA. These oligonucleotides are predicted to span 384 bases of BDNF which contains the coding region for 10 amino acids of the amino-terminal precursor region and the 119-amino-acid mature BDNF. Genomic DNA (1 μg) from *Xenopus laevis*, chicken (White Leghorn), rat (Lewis) and monkey (*Macaca mulatta*) was amplified with Taq polymerase as described by Saiki et al. [22] with 35 cycles of 95°C denaturation, 55°C annealing and 72°C extension. PCR products migrating at the expected size after polyacrylamide gel electrophoresis were purified, ligated to *Hinc*II-digested pBS (Stratagene) and

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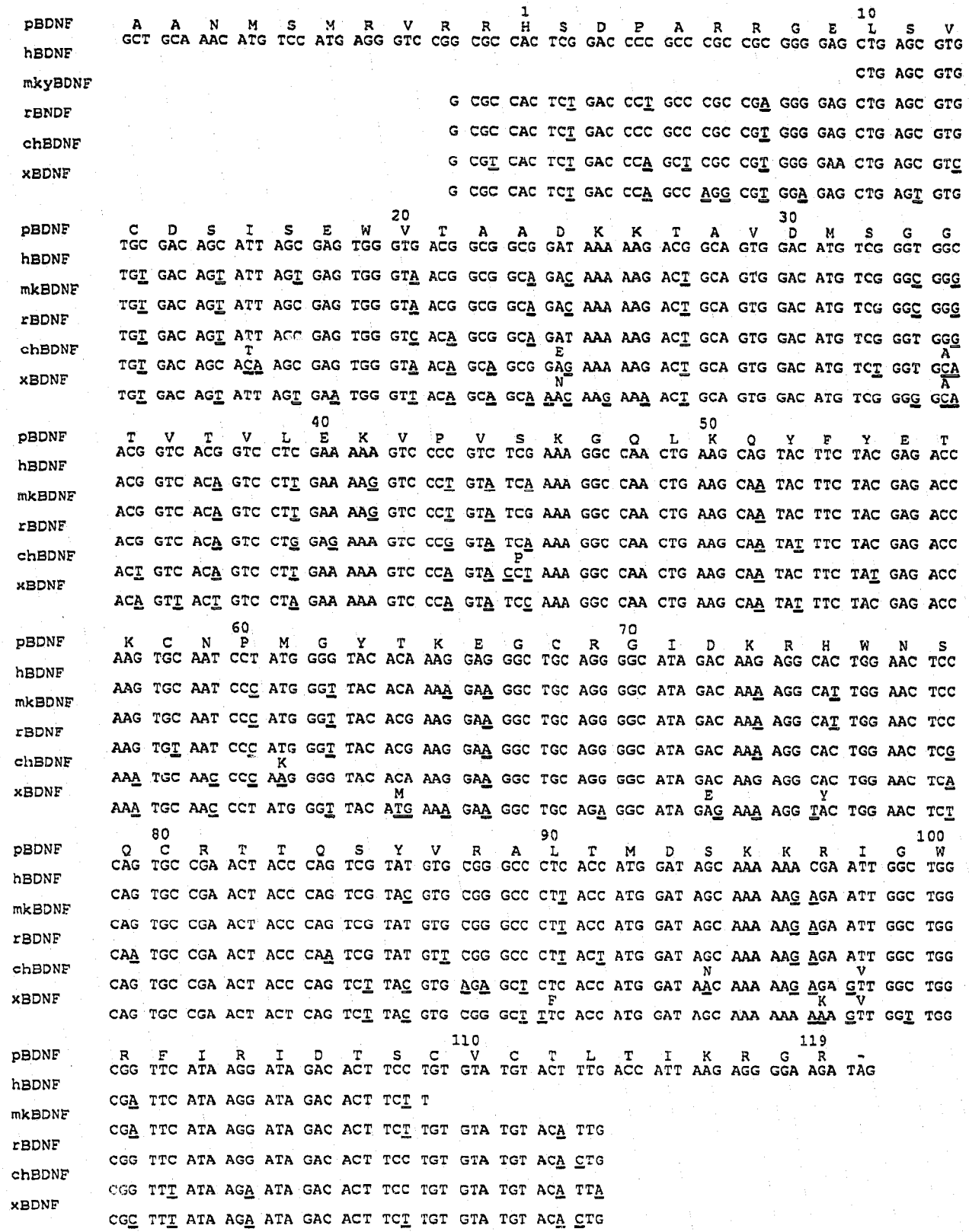

Fig. 1. Comparison of nucleotide sequences encoding mature BDNF from different species. Numbers above the sequences refer to amino-acid residues of the mature 119-amino-acid BDNF protein. Amino-acid substitutions predicted for chicken and *Xenopus* BDNF are indicated above the nucleotide sequences. Nucleotide sequence differences compared to the porcine BDNF sequence are underlined. Nucleotide sequences shown for porcine (pBDNF) [8] and human BDNF (hBDNF) [17] are compared to monkey (mBDNF), rat (rBDNF), chicken (cBDNF) and *Xenopus* BDNF (xBDNF) sequences obtained in this study.

transformed into JM105. Four independent BDNF clones of each species were characterized by dideoxy sequence analysis with T7 DNA polymerase (USB). The inserts contained 343 bp of sequence within the two oligonucleotide primers that was derived from rat, monkey, chicken or *Xenopus* BDNF genes.

3. RESULTS AND DISCUSSION

We have taken advantage of the high degree of BDNF sequence conservation between species to obtain BDNF clones from monkey, rat, chicken and *Xenopus* DNA. Oligonucleotide primers were synthesized based on the porcine cDNA sequence [8] which flank the mature BDNF coding region. Amplified DNA between the primers contains the coding sequence for the entire 119-amino-acid mature BDNF except for the carboxy-terminal 6 amino acids. The fact that the primers efficiently produced amplification products from each of the species examined suggests that the carboxy-terminus is also highly conserved. The predicted amino acid sequence of mature rat and monkey BDNF from these

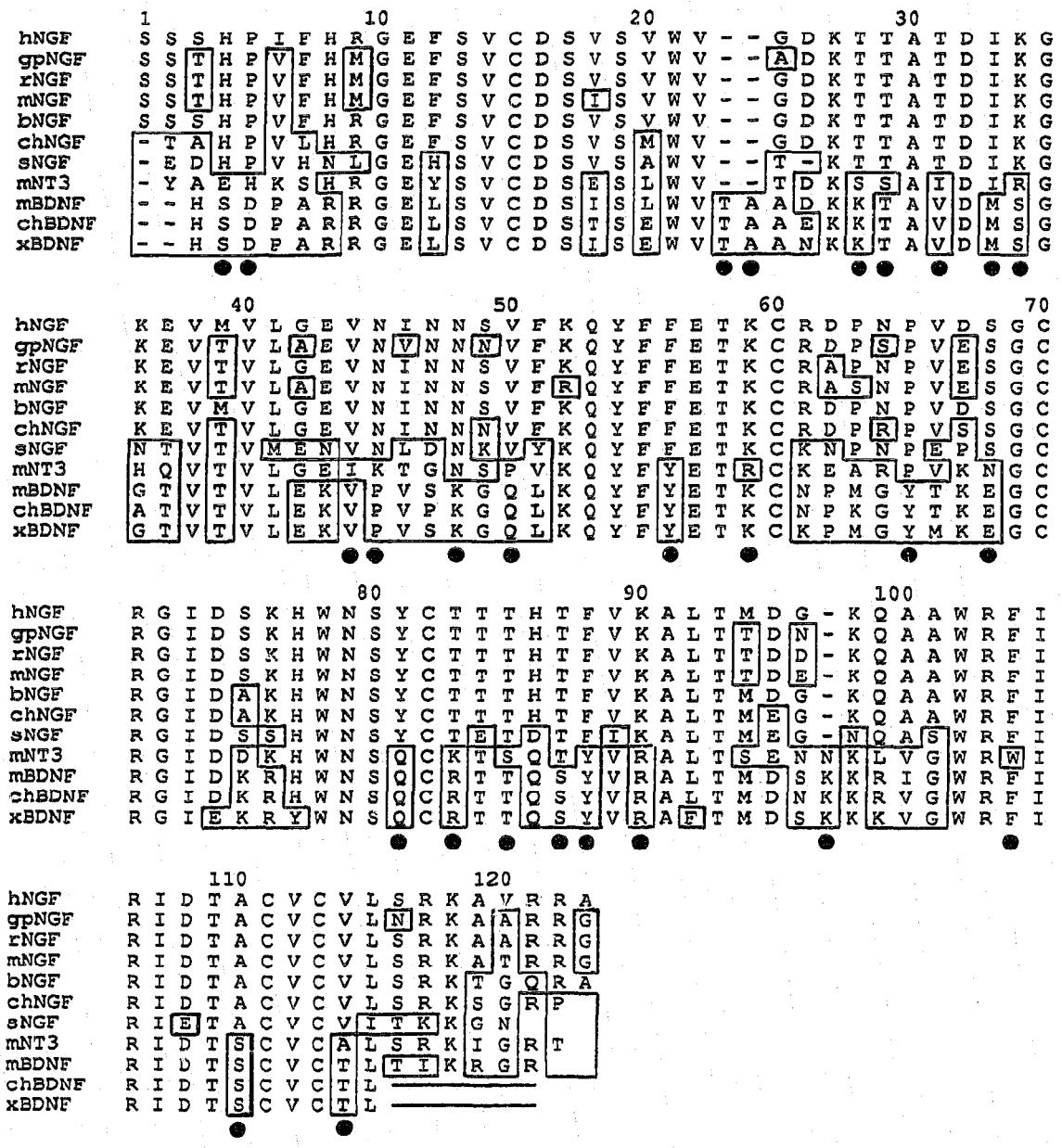

Fig. 2. Comparison of amino acid sequences of the mature regions of NGF, NT3 and BDNF from different species. Amino acid sequences are shown for human NGF (hNGF) [16], guinea pig NGF (gpNGF) [21], rat NGF (rNGF) [20], mouse NGF (mNGF) [16,18], bovine NGF (bNGF) [19], chicken NGF (chNGF) [19,27,28], cobra NGF (sNGF) [29], mammalian NT3 (mNT3) [9–13], mammalian BDNF (mBDNF) [8,9,12,13], and chicken BDNF (chBDNF) and *Xenopus* BDNF (xBDNF) sequences predicted from this study. Amino-acid residues different from the human NGF sequence are boxed. Residues different between NGF, BDNF and NT3 but conserved between species comparisons of NGF and BDNF are indicated below with filled-in circles.

clones is identical to the published porcine sequence [8] as expected from previous reports of identical amino-acid sequences of porcine, mouse [9], rat [12] and human [13,17] BDNF. Within the 343-bp region amplified between the primers, the monkey BDNF sequence contained 28 base changes compared to the published porcine cDNA sequence [8], 18 base substitutions relative to the amplified rat sequence and 4 base changes within a 298 bp region reported for human BDNF [17] (Fig. 1). One of the isolated monkey BDNF clones contained a single A to T substitution that would change Gln^{48} to His^{48}. This single base change is likely to be the result of a polymerase error during the amplification. The rat BDNF sequence contained 32 base changes compared to the porcine sequence.

The chicken and *Xenopus* BDNF sequences were more divergent with 45 and 58 base substitutions, respectively, compared to the porcine BDNF cDNA [8]. The chicken and *Xenopus* mature BDNF proteins are predicted to contain 7 and 8 amino-acid substitutions, respectively, compared to the mammalian form of BDNF. Together, the chicken and *Xenopus* sequences identify 12 amino-acid residues at which substitutions can occur relative to the mammalian BDNF. Three of these substitutions are at residues that are otherwise absolutely conserved in all of the other NGF, BDNF and NT3 sequences (Fig. 2). Although not yet proven, it is expected that chicken and *Xenopus* forms of BDNF will have cross-species biological activity as occurs within the NGF family. This is further suggested by the fact that mammalian BDNF has neurotrophic activities on chicken and quail neurons [4,23].

The receptor binding site of NGF has not been clearly identified. Removal of residues 1–9 or the carboxy-terminal Arg^{118} of mouse β-NGF have no effect on its biological activity [14]. A previous comparison of NGF sequences has implicated the region surrounding residue 33 as a possible receptor binding site since this region is hydrophilic and also conserved between species [19]. Peptides synthesized corresponding to this region have been reported to induce neurite extension from PC12 cells at concentrations 1000-fold higher than native NGF [24]. A previous comparison of rat NGF, BDNF and NT3 sequences has indicated 61 invariant residues that presumably are important for correct folding of the molecule but do not play a role in determining the different receptor specificities of the factors [9]. A comparison of NGF from different species with mammalian NT3 and BDNF, chicken BDNF and *Xenopus* BDNF identifies only 42 residues that are absolutely conserved (Fig. 2). These include the six cysteine residues and Trp^{21}, Trp^{78} and Trp^{102}. Conservation of the tryptophan residues is consistent with previous experiments demonstrating that oxidation of tryptophan residues of mouse β-NGF results in a loss of receptor binding [14,25]. Chemical modification of His^{75} and His^{84} (His^{77} and His^{86}, numbering scheme in Fig. 2) of mouse β-NGF also results in a loss of receptor binding [26]; however, these residues are not conserved. His^{77} is absolutely conserved except in *Xenopus* BDNF where it is changed to Tyr^{77}. There are 24 amino-acid residues that are different between NGF, BDNF and NT3 but do not vary between species. In Fig. 2 these variable residues can be seen to be predominantly clustered in two regions at residues 23–34 and 81–90. These residues may be expected to be important for determining the distinct receptor binding specificities of the different factors.

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