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Molecular studies on the BDNF (Brian-derived neurotrophic factor) from flounder (Paralichthys olivaceus)

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Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that also includes NGF, NT-3, NT-4/5, NT-6 and NT-7 (Hashimoto and Heinrich. 1997). The nerotrophins have been extensively studied in birds and mammals. These studies revealed that neurotrophins play critical roles in the regulation of neuron survival during periods of natural cell death.

Materials and Methods

Construction of flounder pituitary cDNA library

A pituitary cDNA library was constructed from pituitaries that were obtained from male and female flounder at all reproductive stages. cDNA library was constructed as described in the Manufacture's instruction (Stratagene, cDNA library construction kit).

Purification of plasmid DNA and Sequence analysis

The clone obtained from screening of library was purified using Wizard Plus SV minipreps DNA purification system (Promega) and checked for the size of the inserted cDNA. The nucleotide sequences of cloned cDNAs were determined by a chain-termination method using BigDye terminator premix kit (Perkin Elmer). Sequencing was carried out using T3 and T7 Universal primers. The fluorescence-labeled nucleotides were analyzed on an ABI PRISMTM 310 automatic sequencer (Perkin Elmer).

Results and Conclusion

CCAGGTTAGA AGAGTGATGA CCATCCTGTT CCTTACTATG GTTATTTCAT ACTTCAGTTG CATGAGAGCT GCGCCCCTGA GAGACGCCCC GGGCATGCGG GGCCATCGGA CGGAAGGCTA CTTGGGCGCT GCTGCGACGG CCGCAAGAGG CCATGGGACT CCACAGAGTG GTGGTGGACC AGGCCACGGT GGGGAACTGC CCATGGGACT CCACAGAGTG GTGGTGGACC AGGCCACGGT GGGGAACTGC CCTCGCTCGC AGACACGTTT GAGCAAGTGA TAGAGGAGGT GCTGGAGGTG GAGGGAGAGG CGGCACAGCT GGGACAGGGG GCTGATAAGA GCCAGGGAGG TGGGGGCCCA TCCTCTGTGG TCAACGCAGA GAGCAAGGAT GTCGACCTGT ACGACTCACG GGTGATGATC AGCAACCAAG TGCCTTTGGA GCCCCCTTG CCTCTTTCTCC TGGAGGAATA CAAAAACTAT CTGGACGCAG CTAACATGTC CATGAGGGTG CGGCGACACT CCGATCCCTC ACGGCGTGGA GAGCTCAGTG CATGAGGGTG CGGCGACACT CCGATCCCTC ACGGCGTGGA GAGCTCAGTG TGTGTGACAG TATTAGCCAG TGGGTGACAG CTGTGGATAA AAAGACGGCA ATAGACATGT CTGGGCAGAC AGTTACCGTC ATGGAAAAGG TCCCTGTCCC GGAAGCCAAA GGAGGGCTGC AGAGGAATAG ACAACAAGCGGCA TTATAATTCC CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATTA GACACCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATTA AAAAAGAGGA TTATAAGGAT AGACACTTCA TGTGTATGCA 1051 CAATGCACGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATTA AAAAAGAGGA ATAGTGTA TAAAATGTAT AGATTTATATT 1151 GAAGAGTTA AAAAAGAGGA TAAAAAATTAAA AAAATTTATT GAATATTATTC CAATGACCAT TAAAAAGAGGA ATAGAAGAAAAAAATTTATTG GACTACACGT CTATTTATTT 1201 ATAACAGGGT AAATTATTCC GTCAAATGAA AAATTTTATTG ACTGCATGTA AAAAAACATG AAATTATAC AGTAAAAAGTG ATACTACAGT CTATTTATTG AAAAAAAATTCA TGACCTTGTA AACAATTAAA AAAAATTTAAA AAAATTTAAA AAAATTTAAA AAAATTTAAAA AAAATTTAAA AAAATTTAAAA AAAATTTAAA AAAATTTAAA AAAATTTAAAA AAAATTTAAA	1 51 101 151 201 251	GTTGATAGTT AGCAGAGTTT GTCAGGCGAA GATGACTTCA	ACGTATATGT GTCTCCTTAC TAAGCACGGC GTGGTGGGCG AACAAGACAC CACTTGTATC	TGTAGAGCCG CGATATTGCA ATCTACTGAG ATTACCTACC	CCGAGGTGTT AAGGGTTATT CACAGCAGAA AGCATCTGTT	GCCTGCACTG AGATTCATAA GTTCTCGTAT GGAGTGAGTG
401 GGCCATCGGA CGGAAGGCTA CTTGGGCGCT GCTGCGACGG CCGCAAGAGG 451 CCATGGGACT CCACAGAGTG GTGGTGGACC AGGCCACGCGT GGGGAACTGC 501 CCTCGCTCGC AGACACGTTT GAGCAAGTGA TAGAGGAGCT GCTGGAGGTG 551 GAGGGAGAGG CGGCACAGCT GGGACAGGG GCTGATAAGA GCCAGGGAGG 601 TGGGGGCCCA TCCTCTGTGG TCAACGCAGA GAGCAAGGAT GTCGACCTGT 651 ACGACTCACG GGTGATGATC AGCAACCAAG TGCCTTTGGA GCCGCCCTTG 701 CTCTTTCTCC TGGAGGAATA CAAAAACTAT CTGGACGCAG GAGCACAGTG 6801 TGTGTGACAG TATTAGCCAG TGGGTGACAG CTAACATGTC 751 CATGAGGGTG CGGCGACACT CCGATCCCTC ACGGCGTGGA GAGCTCAGTG 801 TGTGTGACAG TATTAGCCAG TGGGTGACAG CTGTGGATAA AAAGACGGCA 851 ATAGACATGT CTGGGCAGAC AGTTACCGTC ATGGAAAAGG TCCCTGTCCC 901 CAATGGCCAA CTGAAGCAAT ACTTTTATGA GACCAAATGC AACCCCATGG 951 GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAAGCGGCA TTATAATTCC 1001 CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA 1101 CATTGACCAT TAAAAGAGGA AGATCGATAT AAAATTTTATT 1151 GAAGACTTA AAAAAGAGAA TGAACAATAA AGATCTATTT GTATATTATC 1201 ATAACAGGGT AAAATTATTCC GTCAAATGAA AATTTTTATTG 1201 AAAAAAAGATT AAAATTATTCC GTCAAATGAA AAATTTTTATTG 1201 AAAAAAAGATT AAAATTATTCC GTCAAATGAA AAATTTTTATTG 1301 AACATATTCA TGACCCTGTT AACAATTAAAAATCAAT CTATTTATTG 1301 AACATATTCA TGACCCTGTT AAAAAAAATTCAT TCAGTCATTTATTTG 1301 AACATATTCA TGACCCTGTTA AACAATTTAAAAAAAATTCAT TCAGTCATTTTTT 1351	301	CCAGGTTAGA	AGAGTG ATG A	CCATCCTGTT	CCTTACTATG	GTTATTTCAT
451 CCATGGGACT CCACAGAGTG GTGGTGGACC AGGCCAGCGT GGGGAACTGC 501 CCTCGCTCGC AGACACGTTT GAGCAAGTGA TAGAGGAGGT GCTGGAGGTG 551 GAGGGAGAGG CGGCACAGCT GGGACAGGGG GCTGATAAGA GCCAGGGAGG 601 TGGGGGCCCA TCCTCTGTGG TCAACGCAGA GAGCAAGGAT GTCGACCTGT 651 ACGACTCACG GGTGATGATC AGCAACCAAG TGCCTTTGGA GCCCCCTTG 701 CTCTTTCTCC TGGAGGAATA CAAAAACTAT CTGGACGCAG CTAACATGTC 751 CATGAGGGT CGGCGACACT CCGATCCCTC ACGGCGTGGA GAGCTCAGTG 801 TGTGTGACAG TATTAGCCAG TGGGTGACAG CTGTGGATAA AAAGACGGCA 851 ATAGACATGT CTGGGCAGAC AGTTACCGTC ATGGAAAAGG TCCCTGTCCC 901 CAATGGCCAA CTGAAGCAAT ACTTTTATGA GACCAAATGC AACCCCATGG 951 GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAACAATGC AACCCCATGG 1001 CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATT GGCTGGCGT TTATAAGGAT AGACACTTCA TGTGTATGCA 1101 CATTGACCTT TAAAAGAGGA AGATCGTATA TAAAATGTAT AGATTTTATT 1151 GAAGACTTA AAAAAGAGAA TGAAGAGAAA AGATCTATTT GTATATTATC 1201 ATAACAGGGT AAAATTATTCC GTCAAATGAA AATTTTTATTG 1201 AAAAAAGAGTA AAAATTATTAC GTCAAATGAA AATTTTTATTG 1301 AACATATTCA TGACCTTGTA AACAATTAAAAAATCAATC ATCAGTCATTTA 1301 AACATATTCA TGACCTTGTA AACAATTAAAAAAAAAA	351	ACTTCAGTTC	CATGAGAGCT	GCGCCCCTGA	GAGACGCCCC	GGGCATGCGG
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TGTGTGACAG TATTAGCCAG TGGGTGACAG CTGTGGATAA AAAGACGGCA ASSI ATAGACATGT CTGGGCAGAC AGTTACCGTC ATGGAAAAGG TCCCTGTCCC GCATGGCCAA CTGAAGCAAT ACTTTATGA GACCACAATGC AACCCCATGG GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAACCCA TGGATAGCAA CCAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA CATTGACCAT TAAAAAGAGGA AGATAGTGTA TAAAATGTAT AGATTTTATT GAAGAGTTA AAAAAAGAGAA TGAAGAGAA AGATCTATTT GTATATATATC ATAACAGGGT AAATTATTCC GTCAAATGAA AATTTTATGG ACTGCATGTA ATAACAGGT AAATTATTAC AGTAAAAAGG ATACTACAGT CTATTTATTG AACATATTCA TGACCTTGTA AACAATTAAA AAAAATCTGA TCAGTCATTTATTG AACATATTCA TGACCTTGTA AACAATTAAA AAAAATCTGA TCAGTCATTTTTTG GGCCCCAGTT TAAAATACTAT ATTCACAGACAT TGTGATTTTTT	701	CTCTTTCTCC	TGGAGGAATA	CAAAAACTAT	CTGGACGCAG	CTAACATGTC
ATAGACATGT CTGGGCAGAC AGTTACCGTC ATGGAAAAGG TCCCTGTCCC CAATGGCCAA CTGAAGCAAT ACTTTATGA GACCAAATGC AACCCCATGG GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAAGCGGCA TTATAATTCC CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA CATTGACCAT TAAAAAGAGGA AGATAGTGTA TAAAATGTAT GATATTATTT CAAAGAGTTTA AAAAAGAAGAA TAAATTTATTG GATAGTATATT AAAAAAGATT AAAAAAGAGAA AAATTTATAG ACTGCATGTA AAAAAAGAT AAAAAAGAAT AGTAAAAGGA AATTTTATGG ACTGCATGTA ACATATTCA TGACCTTGTA AACAATTAAA AAAATCTAGT CTATTTATTG AACATATTCA TGACCTTGTA AACAATTAAA AAAAATCTAG TCAGTCATTTATTG GGCCCCAGTT TAAAATACTAT ATTCACAATTCA TCAGCACATT TGTGTATTTGTT GGCCCCAGTT TAAAATACTAT ATTCACAACACAT TGTGATTTTGTT GGCCCCAGTT TAAAATACTAT ATTCACAACACAT TGTGATTTTGTT	751	CATGAGGGTG	CGGCGACACT	CCGATCCCTC	ACGGCGTGGA	GAGCTCAGTG
901 CAATGGCCAA CTGAAGCAAT ACTTTTATGA GACCAAATGC AACCCCATGG 951 GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAAGCGGCA TTATAATTCC 1001 CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA 1101 CATTGACCAT TAAAAGAGGA GATAGTGTA TAAAATGTAT AGATTTTATT 1151 GAAGAGTTA AAAAAGAGAA TGAAGAGAAA AGATCTATTT GTATATATATA 1201 ATAACAGGGT AAATTATCC GTCAAATGAA AAATTTTATG ACTGCATCTA 1251 AAAAAGATG AAGTTTATAC AGTAAAAGTG ATACTACAGCT CTATTTATTG 1301 AACATATTCA TGACCCTGTA AACAATTAAA AAAAATCTGA TCAGTCATTTGT 1351 GGCCCCAGTT TAAAATACTAT ATTCACAGACAT TGTGATTTGT	801	TGTGTGACAG	TATTAGCCAG	TGGGTGACAG	CTGTGGATAA	AAAGACGGCA
951 GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAAGCGGCA TTATAATTCC 1001 CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA 1051 AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA 1101 CATTGACCAT TAAAAGAGGA AGATGGTA TAAAATGTAT AGATTTTATT 1151 GAAGAGTTTA AAAAAGAGAA TGAAGAGAAA AGATCTATT GTATATATATA 1201 ATAACAGGGT AAATTATTCC GTCAAATGAA AATTTTATG ACTGCATGTA 1251 AAAAAGATG AAGTTTATAC AGTAAAAGT ATACTACAGT CTATTTATTG 1301 AACATATTCA TGACCCTGTA AACAATTAAA AAAAATCTGA TCAGTCATTTGT 1351 GGCCCAGTT TAAAATACTAT ATTCACAGACAT TGTGATTTGT	851	ATAGACATGT	CTGGGCAGAC	AGTTACCGTC	ATGGAAAAGG	TCCCTGTCCC
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1051 AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA 1101 CATTGACCAT TAAAAAGAGGG AGATAGTGTA TAAAATGTAT AGATTTTATT 1151 GAAGAGTTTA AAAAAGAGAA TGAAGAGAA AGATCTATTT GTATATATATC 1201 ATAACAGGGT AAATTATTCC GTCAAATGAA AATTTTATGG ACTGCATGTA 1251 AAAAAAGATG AAGTTTATAC AGTAAAAGGT ATACTACAGT CTATTTATTG 1301 AACATATTCA TGACCCTGTA AACAATTAAA AAAAATCTGA TCAGTCATTTGT 1351 GCGCCCAGTT TAAAATACTAT ATTCACAGACAT TGTGATTTGT	951	GGTACACAAA	GGAGGGCTGC	AGAGGAATAG	ACAAGCGGCA	TTATAATTCC
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1401 TTACGTTGCC AAGAATTAGA AAAAAAAAA AAAAAAA	1201 1251 1301	ATAACAGGGT AAAAAAGATG AACATATTCA GCGCCCAGTT	AAATTATTCC AAGTTTATAC TGACCTTGTA TAAATTACTA	GTCAAATGAA AGTAAAAGTG AACAATTAAA TATCACATTC	AATTTTATGG ATACTACAGT AAAAATCTGA CTCAAGACAT	ACTGCATGTA CTATTTATTG TCAGTCATTT

Fig 1. Sequence of flounder BDNF gene

The gene encoding flounder BDNF was cloned from a flounder brain cDNA library. The probes used for screening the library were cloned cDNA fragments, generated by PCR amplification of cDNA from brain cDNA phage library. The nucleotide sequence of BDNF has shown an open reading frame (ORF) consisting 810 bp that corresponds to a protein, which has 269 amino acids and one stop codon. The nt and peptide sequences were compared to those of other BDNF genes from other species. It showed as high as 93% in southern platy fish. We also constructed a phylogenetic tree base on BDNF amino acid sequences from various species.

References

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