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NUCLEOTIDE SEQUENCE OF THE RABBIT γ-PREPROTACHYKININ I cDNA

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SUMMARY: We succeded in amplifying tachykinin I specific cDNAs from cerebral tissue of guinea pig, rabbit, rat, hamster, trout and tupaia in the expected size range of 820-980 bp. The amplified 859 bp rabbit γ-preprotachykinin I cDNA was sequenced and consisted of the whole 345 bp γ-PPT I coding sequence including the substance P and neurokinin A coding regions and a 505 bp large 3'-nontranslated region. Both, molecular weight and sequence comparison emphasizes the very high phylogenetic age of the preprotachykinin I gene.

Tachykinins are a family of peptides which share functions as neurotransmitters, neuromodulators and immunomodulators [1].

Extended research about tachykinins are carried out with a wide range of spezies. Therefore the exact primary structure of tachykinins is of great relevance especially for immunocytochemical and immunochemical analysis. In mammals the tachykinins are coded by two genes, called preprotachykinin I gene (PPT I gene) and preprotachykinin II gene (PPT II gene) respectively. The PPT I gene transcript is alternatively spliced into at least four different mRNAs (α–, β–, γ- and δ-PPT I mRNA), coding for specific precursor proteins [2, 3, 4], which are processed to functional peptides (substance P, neurokinin A, neuropeptide K and neuropeptide γ). The precursor mRNAs as well as the tachykinin peptides show a very high degree of homology among the different species. Taking this fact into consideration we constructed and chemically synthesized two PCR-primers corresponding to highly conserved regions of the PPT I mRNAs, which are able to serve as helpful tools for the fast cloning and analysis of many not yet characterized PPT I specific cDNAs. This report concerns rabbit γ–preprotachykinin.

METHODS AND RESULTS

In order to identify highly conserved regions of PPT I specific mRNAs we compared the by that time known mammalian cDNA sequences [2, 5, 6] by means

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of a sequence comparison computer program. Two regions in flanking positions of the cDNA sequences, which seemed to be ideal for our purpose led to the construction and chemical synthesis of the PCR primers SUBP-1 and SUBP-2, which are suitable for the amplification of α-, β– and γ-PPT I cDNAs. The first primer SUBP-1 (5'-CCC GGG AAT TCT AGA TAC AAC ACA TYR TAC AAT RA) corresponds to a region approximately 40 bp upstream of the mRNA poly(A)-tail, whereas the second primer SUBP-2 (5'-CCC GGG AAT TCT AGA AAT CCA ACA TGA AAA TCC TCG TG) includes a short part of the 5'-nontranslated region and 15 nucleotides coding for the first 5 amino acids of PPT I specific precursor proteins. Both primers contain recognition sites for the restriction endonucleases Eco RI and Xba I (underlined). Total RNA was purified from cerebral tissues of several vertebrates (rat, hamster, rabbit, guinea pig, tupaia, trout and hamster) by an automatic nucleic acid extractor (abi 340). After cDNA first strand synthesis (MMLV-RTase, oligo(dT) priming) from 5μg of total RNA 1/15 of each reaction mixture was applied to the following PCR amplification using the primers mentioned above: 94°C, 30 sec (melting of DNA); 45°C, 30 sec (primer annealing); 72°C, 1 min (elongation); 40 cycles; auto segment extension of the elongation step (3 sec/cycle).

Gelelectrophoretical analysis of the PCR products revealed several DNA fragments in the expected size range of 820-980 bp (Fig. 1). Unspecific background bands could not be identified. In the investigated species rat, guinea

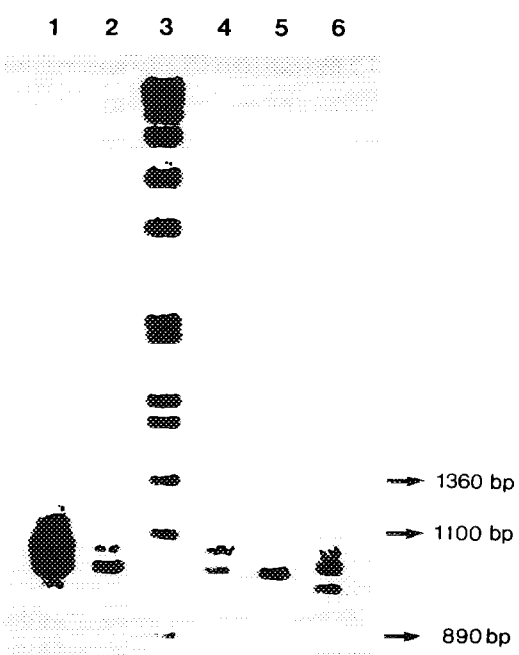

Figure 1. Computer image of an ethidiumbromide-agarose gel analysis of the amplified PCR fragments: rat (lane 1), guinea pig (lane 2), molecular weight marker SPP1/Eco RI (lane 3), rabbit (lane 4), trout (lane 5), tupaia (lane 6).

Figure 2. Nucleotide sequence of rabbit γ-preprotachykinin precursor cDNA.

pig, rabbit and tupaia more than one PCR fragment has become visible, what fits the existence of different PPT I precursor mRNAs. However, we obtained just one PCR fragment from trout and hamster (data not shown).

For further analysis we intend to clone and sequence the PCR fragments using a fluorescence DNA sequencer (abi 373 A), as already occured in the case of rabbit γ-PPT I specific cDNA [7]. The 859 bp large cDNA fragment consists of the whole 345 bp large γ-PPT I coding sequence (for rat sequence see [8]) including the substance P and neurokinin A coding regions and a 505 bp large 3´-nontranslated region down to approximately 40 bp upstream of the poly(A)-tail (Fig. 2). Comparison with the known mammalian PPT I sequences shows about 90% homology in the coding cDNA regions (bovine 89%, rat 91%, human 90%) and a total homology of substance P and neurokinin A on the peptide level.

DISCUSSION

The successful use of the two described primers for the amplification of PPT I cDNA fragments from different vertebrates as well as the high homology of the rabbit cDNA to the bovine, rat and human cDNAs verifies the high age of the tachykinins under phylogenetic aspects. We now attempt to amplify and clone PPT I related cDNAs from other vertebrates (birds, amphibia) and even

nonvertebrates (mollusca). The main task of this research will be the identification of a kind of ancestral tachykinin gene and the investigation of expression and splicing patterns in lower vertebrates and nonvertebrates.

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