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Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: identity of human and porcine endothelin

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A cDNA encoding a human endothelium-derived vasoconstrictor peptide, endothelin, was isolated from a human placenta cDNA library. The nucleotide sequence of this cDNA clone showed that the primary structure of the human preproendothelin has 212 amino acid residues and is highly homologous to porcine preproendothelin, and that human endothelin is identical with porcine endothelin.

Endothelin; cDNA cloning; Vasoconstrictor peptide; Peptide processing; Cardiovascular physiology; (Endothelium)

1. INTRODUCTION

We have recently purified and sequenced a 21-residue vasoconstrictor peptide derived from porcine endothelium which was named endothelin. This peptide does not belong to any reported peptide family. As it is one of the most potent vasoconstrictors and the expression of the endothelin gene is regulated by several vasoactive agents, the peptide may function as a messenger in a novel endothelium-mediated cardiovascular control system. Disturbances in the control of endothelin production and/or in the sensitivity of vascular smooth muscle cells to endothelin might be a causative factor of hypertension and spastic disorders of blood vessels. As an initial step in investigating the physiological, pathophysiological and possible clinical implications of endothelin in humans, we cloned preproendothelin cDNA from a human placenta cDNA library. Here we describe the amino acid sequence of human preproendothelin deduced from the cDNA sequence.

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00749

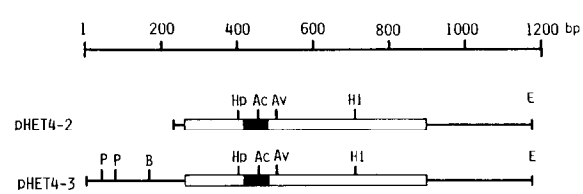

Fig.1. Human endothelin precursor cDNA clones. Lines and boxes indicate the untranslated and coding regions, respectively. The sequences encoding mature endothelin are denoted by closed boxes. Restriction sites: Ac, *Acc*I; Av, *Ava*I; B, *Bgl*II; E, *Eco*RI; Hi, *Hin*dIII; Hp, *Hpa*II; P, *Pst*I.

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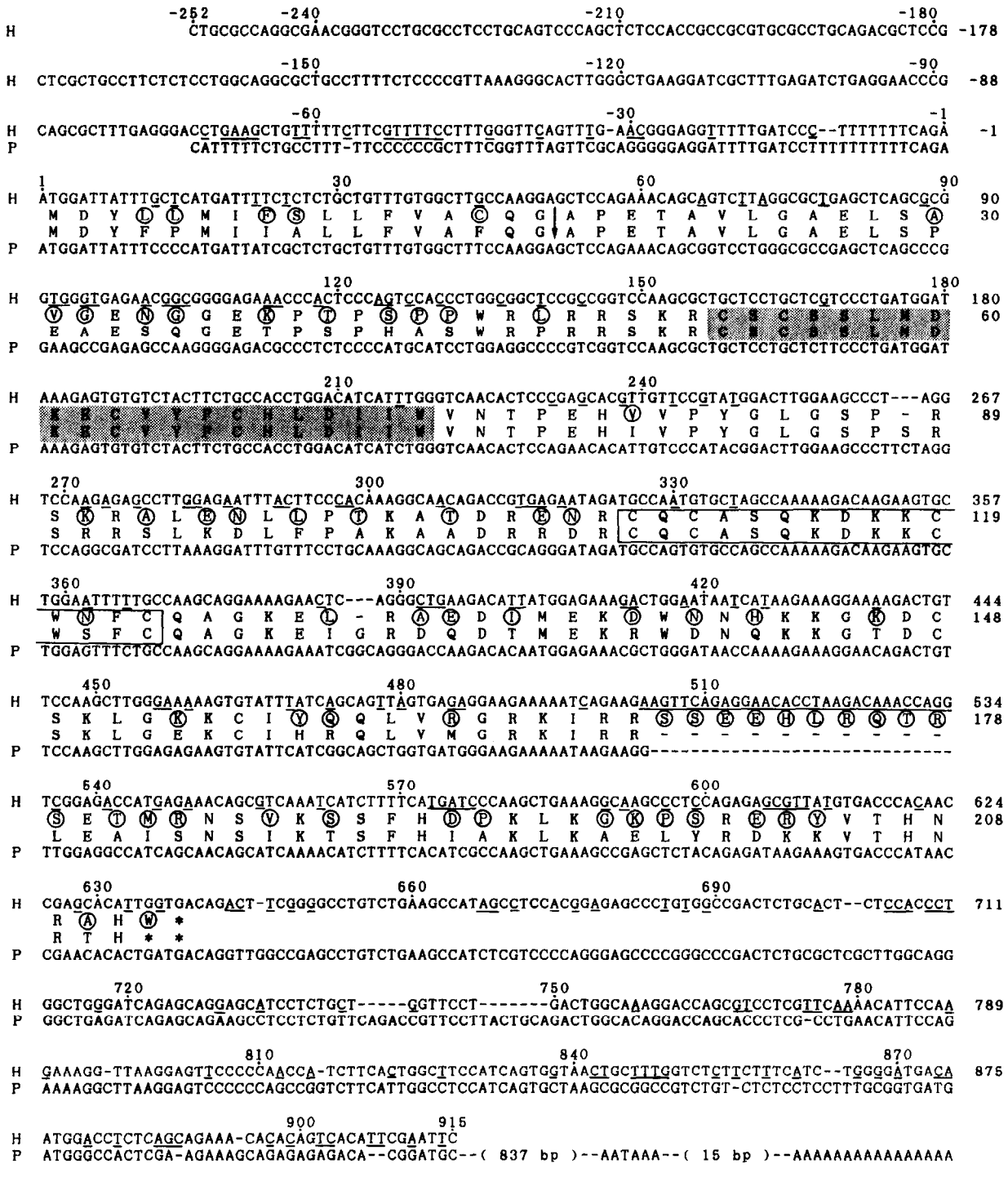

Fig.2. Comparison of nucleotide and deduced amino acid sequences of human and porcine preproendothelin precursor [1]. Base and amino acid substitutions are underlined and circled, respectively. Gaps, denoted by dashes, are introduced for maximal alignment. Amino acid residues corresponding to the mature endothelin and 'endothelin-like' segments are indicated by stippled and open boxes, respectively. The arrow shows the possible cleavage site of the signal peptide.

2. MATERIALS AND METHODS

2.1. *Oligonucleotide probe*

An oligonucleotide 5′-TGGCAGAAGTAGACGCACTC-CTTGTCCAT-3′, containing the coding sequence for Met^{7}–His^{16} of the porcine endothelin [1], was chemically synthesized using a DNA synthesizer, model 380B (Applied Biosystems). This oligonucleotide was labeled to a specific activity of 5×10^8 cpm/μg with T_4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (3000 Ci/mmol; Amersham).

2.2. *cDNA cloning*

A λgt11 human placenta cDNA library (Clontech Laboratories, Inc.) was screened by plaque hybridization [2] with the synthetic probe. Hybridization was carried out in hybridization buffer-S [3] at 45°C for 16 h. The filters were washed once with 2 × SSC (1 × SSC = 150 mM NaCl/15 mM Na citrate) at 45°C for 30 min and then twice with the same solution at 40°C for 30 min. The filters were then dried and autoradiographed onto Kodak X-AR film at −40°C with intensifying screens.

2.3. *DNA sequence analysis*

Nucleotide sequence analysis was carried out by the dideoxy chain-termination method [4] using restriction fragments subcloned in M13 mp18 and mp19.

2.4. *Northern blot analysis*

Venous endothelial cells were obtained from a fresh human umbilical cord and cultured in monolayers as described [5]. Extraction of poly(A)$^+$ RNA and Northern blotting were performed as described [1]. The random-prime-labeled pHET4-3 insert (5×10^8 cpm/μg) was used as a probe.

3. RESULTS

3.1. *cDNA cloning of human preproendothelin*

Two hybridization-positive plaques were isolated by the screening of approx. 8×10^5 plaques from a human placenta cDNA library, and the inserts were subcloned into the *Eco*RI site of plasmid pUC18. The plasmids, designated pHET4-2 and pHET4-3, have cDNA inserts of about 0.9 and 1.17 kb, respectively (fig.1). The *Eco*RI site in the 3′-untranslated region was considered to exist endogenously, since the *Eco*RI-flanking sequences of pHET4-2 and pHET4-3 were identical. Furthermore, this *Eco*RI site was shown to exist also in human preproendothelin gene (unpublished). Fig.2 shows the complete nucleotide sequence of the pHET4-3 insert and the deduced amino acid sequence aligned with the porcine sequences [1]. The nucleotide sequence contained only one long open-reading frame from the 5′-proximal ATG codon. This initiation codon is within the consensus sequence established for eukaryotic translation initiation sites [6]. Furthermore, the encoded amino acid sequence Cys^{53}–Trp^{73} was identical to the porcine endothelin sequence. We concluded from these results that pHET4-3 contains the complete coding region of human endothelin precursor.

Northern analysis showed that a single 2.2–2.3 kb preproendothelin mRNA was expressed in cultured human umbilical vein endothelial cells (fig.3). Assuming that the poly(A) tail is ~200 bases long and the length of the 3′-noncoding region is comparable to that of the porcine mRNA, it follows that the 5′-end of the pHET4-3 insert is very close (within ~50 bases) to the cap site of the mRNA.

3.2. *Structural characteristics*

The predicted human preproendothelin had 212 amino acid residues and was 69% homologous with porcine preproendothelin (fig.2). The sequence differences included both substitutions and insertions/deletions. Out of 55 substitutions, 25

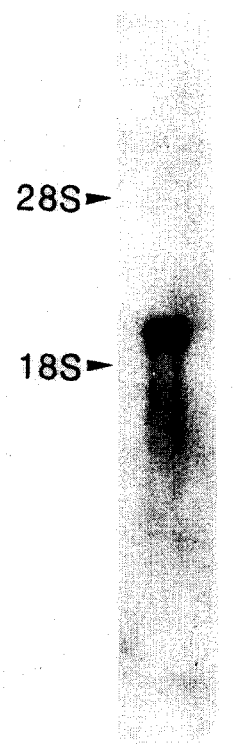

Fig.3. RNA blot hybridization analysis. Positions of human ribosomal RNAs (28 S and 18 S) are indicated.

residues were conservative substitutions [7]. The hydrophobicity profile is, as expected, very similar to that of the porcine precursor (fig.4). Although we have not sequenced human endothelin at the peptide level, human mature endothelin is very likely the 21-residue peptide from Cys^{53} to Trp^{73}, since the sequence between Arg^{48} and His^{79}, which includes the porcine endothelin sequence, is perfectly conserved in human and porcine precursors. In addition to endothelin, the precursor has a cysteine-rich endothelin-like segment (Cys^{109}–Cys^{123}). This region is also particularly conserved when compared with the porcine precursor, although the role of this segment is presently unknown.

The nucleotide sequence is also highly conserved, and the homology in the coding regions is 79%. It is particularly noteworthy that the 5′- and 3′-noncoding regions are also significantly conserved (71% and 75%, respectively), suggesting the functional importance of these regions, e.g., for the regulation of mRNA stability.

4. DISCUSSION

Endothelin and its cDNA were originally isolated from porcine aortic endothelial cells, and we have succeeded in obtaining a human endothelin cDNA from a placenta cDNA library. The presence of preproendothelin mRNA in the placenta was probably due to the high vascularity of this tissue. The predicted amino acid sequence of human mature endothelin did not differ from that of porcine endothelin. This seems to suggest that the preproendothelin gene has evolved under strong pressure to conserve the structure, and hence the function, of the mature peptide.

A possible cleavage site of the signal peptide was located after Gly^{17}, based on a weight-matrix approach [8]. After removal of the signal peptide, the 22478 Da putative proendothelin might be processed to a 2492 Da mature endothelin in a manner similar to that for porcine endothelin, namely, through NH_2-terminal processing at the dibasic-pair Lys^{51}–Arg^{52} and an unusual COOH-terminal

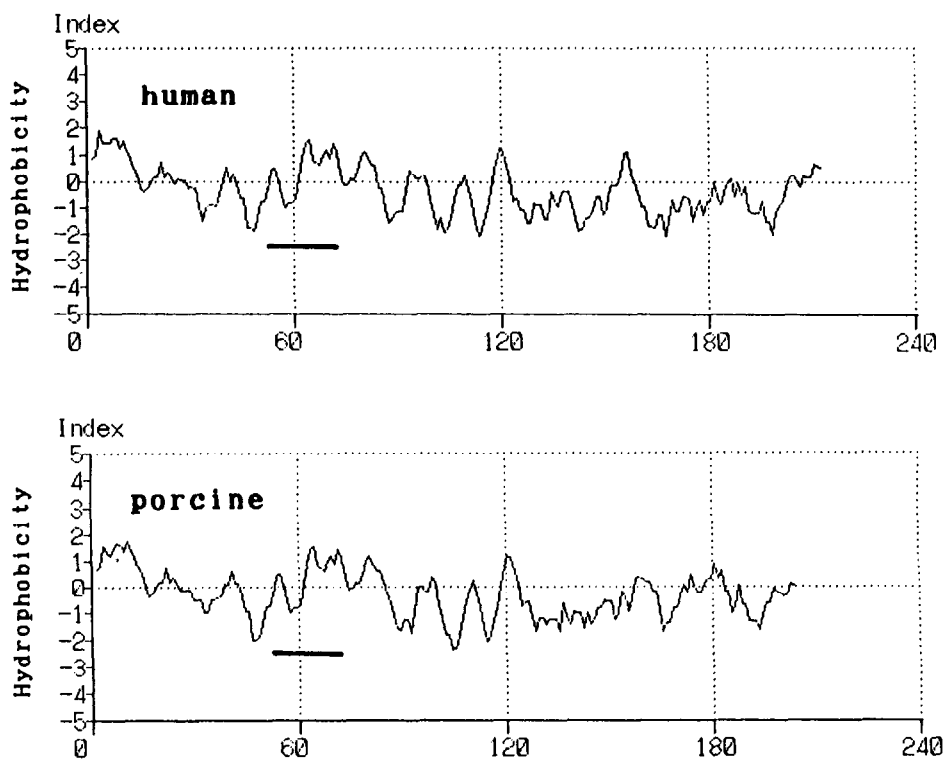

Fig.4. Hydrophobicity index along the amino acid sequence of human and porcine endothelin precursors. The hydrophobicity values were calculated with the hexapeptide averaging method [9]. The positions of endothelin sequences are underlined.

processing between Trp^{73} and Val^{74}. The expression of human preproendothelin cDNA in recombinant mammalian cells would facilitate the characterization of this previously unknown type of peptide processing.

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