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cDNA CLONING, SEQUENCE ANALYSIS AND TISSUE DISTRIBUTION OF RAT PREPROENDOTHELIN-1 mRNA

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We report the cloning of a full-length cDNA encoding rat preproendothelin-1 (preproET-1). The predicted rat preproET-1 consists of 202 amino acid residues and highly similar to human, porcine and bovine preproET-1, respectively. The deduced 21-residue sequence of mature rat ET-1 is identical to human, porcine, canine and bovine ET-1. As in other mammalian species, the mature ET-1 is predicted to be produced from a 39-residue big ET-1 in the rat. Northern blot analysis showed that a single 2.3-kb preproET-1 mRNA is expressed not only in vascular endothelial cells but also in other rat tissues, including the lung, brain, uterus, stomach, heart, adrenal gland and kidney. These findings suggest that ET-1 may play roles as a local mediator in multiple organs both within and outside the cardiovascular system in the rat.

Endothelin-1 (ET-1) was initially identified as a 21-residue potent vasoconstrictor peptide produced by vascular endothelial cells, but was subsequently found to have a wide variety of effects on both vascular and non-vascular tissues (1, 2). We previously report the partial sequence of a ET-related gene cloned from a rat genomic library (3). However, this gene was subsequently demonstrated to be the rat homologue of endothelin-3 gene (4). Therefore, the structure of rat ET-1 and its precursor has remained to be determined. Here we cloned and sequenced a full-length cDNA encoding rat preproET-1 and analyzed tissue distribution of preproET-1 mRNA by Northern blot analysis with the cloned cDNA as probe.

MATERIALS AND METHODS

Preparation of RNA: Total RNA from rat tissues was prepared with selective precipitation in 3 M LiCl / 6 M urea (5), and poly $(A)^+$RNA was purified by chromatography on oligo(dT) cellulose as described (6).

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cDNA cloning and sequencing: A λgt10 cDNA library was constructed with 2 μg of poly (A)$^+$RNA prepared from cultured rat pulmonary artery endothelial cells (7). Approximately $5x10^5$ individual plaques from the unamplified library were screened by plaque hybridization with a synthetic DNA probe which encodes for Met7-Ile20 of ET-1 (1). Four positive plaques were detected and three of these clones, λrET1-2, λrET1-3, and λrET1-4, were subjected to further characterization. Restriction fragments from the cDNA inserts were subcloned into pUC118/119 phagemids, rescued as single-strand DNA (8), and sequenced by the dideoxy chain termination method (9). Both strands of the cDNAs were completely sequenced from overlapping subclones with no discrepancy.

Northern blot analysis: Poly(A)$^+$RNA (10 μg/lane) from rat tissues were separated by formaldehyde / agarose gel electrophoresis, transferred to a nylon membrane, and hybridized with ^{32}P-labelled cDNA insert of λrET1-2 as described (6). The membrane was washed finally in 0.1 x SSC / 0.1% SDS at 50°C, and autoradiographed at -80°C for 10 h.

RESULTS AND DISCUSSION

Nucleotide sequence of cDNA encoding rat preproET-1: The nucleotide sequence of the cDNA insert of λrET1-2 is shown in Fig. 1. The 5'-most ATG triplet, which is followed by a 606-bp open reading frame, is preceded by an in-frame stop codon (TGA; nucleotide 170-172). The nucleotide sequence around this ATG conform reasonably well to the consensus sequence for translation initiation sites of eukaryotic mRNA (10). The nucleotide sequence of preproET-1 mRNA was highly conserved among mammalian species both in the coding and non-coding regions. The similarities between rat and human cDNA sequences were 75% in the coding regions and 74% in the 3' non-coding regions, respectively. The unusually high similarity within the 3' non-translated nucleotide sequences suggests the possible functional importance of these regions, e.g., involvement in the regulation of mRNA stability. Actually, as in the case of human (11) and porcine (1) preproET-1 mRNA, the 3' non-translated region of rat preproET-1 mRNA has several "AUUUA" motifs (12) known as highly selective mRNA destabilizing signals (data not shown).

Deduced amino acid sequence of rat preproET-1: Fig. 1 also shows the predicted amino acid sequence of rat preproET-1. The deduced rat preproET-1 consists of 202 amino acid residues and is highly similar to human (11), porcine (1) and bovine (13) preproET-1, having 68%, 71% and 78% amino acid identities, respectively. Especially, the deduced 21-residue mature rat ET-1 was identical with human, porcine, canine (14) and bovine ET-1. This suggests that ET-1 had evolved under a strong pressure to conserve the primary structure of the mature peptide. The first 17 residues of rat preproET-1 is predicted by von Heijine's algorithm (15) to be a secretory signal sequence. Paired basic amino acid residues, Lys51-Arg52, which are recognized by processing endopeptidases, directly precede the ET-1 sequence. However, no dibasic pair is found until Lys90-Arg91, indicating that mature rat ET-1 is produced from a 39-residue rat big ET-1 via the putative endothelin converting enzyme (1), as in the case of other mammalian species. The sequence spanning amino acid residues 110-124 contains four Cys residues at relative positions 1, 3, 11 and 15, which are identical to the positions of the Cys residues in mature ET-1, representing an "endothelin-like" motif (1).

Tissue distribution of rat preproET-1 mRNA: Northern blot of poly (A)$^+$RNA from various rat tissues was hybridized with the cloned rat preproET-1 cDNA (Fig. 2). A 2.3-kb preproET-1 mRNA was detected in many of these tissues. The mRNA was also abundantly

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                                                                CGCG -181
ACGCTTCGCTCCGGTGAAGGGGCCACTTTTTGAAGACCGCGCTGAGATCTCCAAAAGCCA -121
GAGGCGATCAGAGCAACCAGACACCATCCTCTTCGTTTTGCATTGAGTTCCATTTGCAAC  -61
CGAGTTTTCTTTCTTTTTTTTTTCTTTTTTTCCCTCCTCTTCTTCTGATCCCTTTGCAGA   -1

ATGGATTATTTTCCCGTGATCTTCTCTCTGCTGTTTGTGGCTTTCCAAGGAGCTCCAGAA   60
MetAspTyrPheProValIlePheSerLeuLeuPheValAlaPheGlnGlyAlaProGlu   20

ACAGCTGTCTTGGGAGCAGAGCTCAGCCCCCGAGCTGAGAAGGAAGTGCAGAGCCCCCCT  120
ThrAlaValLeuGlyAlaGluLeuSerProArgAlaGluLysGluValGlnSerProPro   40

CCCAGCACATCCTGGAGACCCCGCAGGTCCAAGCGTTGCTCCTGCTCCTCCTTGATGGAC  180
ProSerThrSerTrpArgProArgArgSerLysArgCysSerCysSerSerLeuMetAsp   60

AAGGAGTGTGTCTACTTCTGCCACCTGGACATCATCTGGGTCAACACTCCCGAGCGCGTC  240
LysGluCysValTyrPheCysHisLeuAspIleIleTrpValAsnThrProGluArgVal   80

GTCCCGTATGGACTAGGAAGCCCTTCTAGGTCTAAGCGATCCTTGAAAGACTTACTTCCC  300
ValProTyrGlyLeuGlySerProSerArgSerLysArgSerLeuLysAspLeuLeuPro  100

ACAAAGACCACAGACCAAGGGAACAGATGCCAGTGTGCTCACCAAAAAGACAAGAAGTGC  360
ThrLysThrThrAspGlnGlyAsnArgCysGlnCysAlaHisGlnLysAspLysLysCys  120

TGGAATTTCTGCCAAGCAGACAAAGAACTCCGAGCCCAAAGTACCATGCAGAAAGGCGTA  420
TrpAsnPheCysGlnAlaAspLysGluLeuArgAlaGlnSerThrMetGlnLysGlyVal  140

AAAGACTTCAAGAAGGGAAAACCCTGTCCCAAGCTGGGAAAGAAGTGTATCTATCAGCAG  480
LysAspPheLysLysGlyLysProCysProLysLeuGlyLysLysCysIleTyrGlnGln  160

CTGGTGGAGGGAAGAAAACTAAGAAGGTTGGAGGCCATCAGCAACAGCATCAAGACCTCC  540
LeuValGluGlyArgLysLeuArgArgLeuGluAlaIleSerAsnSerIleLysThrSer  180

TTTCGAGTTGCAAAGTTGAAAGCGGAACTCTACAGAGACCAGAAGTTGATACACAACCGA  600
PheArgValAlaLysLeuLysAlaGluLeuTyrArgAspGlnLysLeuIleHisAsnArg  200

GCACATTGACTACAGAGCCCCGTGGTGTTTTGGAAGCCATGACTTACATAGAGCGAGCCC  660
AlaHis***                                                      202

TATGGCCAACTCTGCGCTCTCCATGCTGGCTGGGATCTTAGCAAGAACATCTGTCCGGCT  720
TCTACAGTTTCTTGTTCAGACTGGCAGAGGACCAGCGTCCTTGTTCCAAACATTCCAAGA  780
GAGGTTGAGGTGTTCCCTAACCTGTCTTCGTTTGCATCCGCTGGTAGCAAGTGATTCTCT  840
TGCCTCTTCTTGCTGTCTGGGGATGGCCTCGGACCTCTCGGAGAGCAGAGACACAGTGCC  900
ATTCCTGAGTGGCATCATCCAGAGAGCCAGGAGATTCCATAGCAGGGCGGAGTTTCTGTA  960
GAAAGTCCTTAGGGAGTGTTCGTGTCTGACTCAGGCGCCTGGCACATTTCAGGGAGAAAC 1020
TCCAAAGTCCATGCAAAAAATTTTCTGAGGAATGCACAAATTGAAAACATACTCGAAGGA 1080
CAAACACTTGAGTTTTTTTTTTTTTAAGGACTTTTTTTTAAGTTGTAAAATGCAAAACTC 1140
AAAGCAACTGTTACTACTGTACATTAGGATATGTTTCATGAATATGAGTCTACCCACCTT 1200
T———(0.62 kb)———poly(A)
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Fig. 1. Nucleotide and deduced amino acid sequence of rat preproET-1 cDNA. The putative signal sequence cleavage site predicted by the von Heijne's algorithm (15) is indicated by an arrowhead. Mature ET-1 and big ET-1 sequences are indicated by a box and an underline, respectively. Four Cys residues within the "endothelin-like" region are doubly underscored.

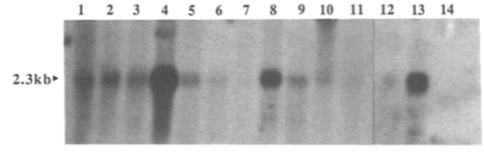

Fig. 2. Northern blot hybridization of preproET-1 mRNA in rat tissues. The 2.3-kb preproET-1 mRNA is indicated by an arrowhead. Lanes: 1, brain; 2, eye ball; 3, submandibular gland; 4, lung; 5, heart; 6, liver; 7, spleen; 8, stomach; 9, small intestine; 10, adrenal gland; 11, kidney; 12, testis; 13, uterus; 14, skeletal muscle (quadriceps femoris). The presence of intact 28S ribosomal RNA was confirmed in all lanes.

expressed in rat pulmonary macro- and microvascular endothelial cells in culture (7) (data not shown). Among native tissues, preproET-1 mRNA was most abundant in the lung, The brain, eye ball, uterus, stomach, submandibular gland and small intestine also contained relatively large amounts of preproET-1 mRNA. Small amounts of preproET-1 mRNA were also detected in the heart, kidney, adrenal gland, liver, spleen and testis, although it is not clear whether these signals represent the expression of the mRNA in the vascular endothelium within these tissues. These findings are in concordance with the previously reported tissue distribution of immunoreactive ET-1 in the rat (16). Taken together with the observations that ET-1 has its specific receptors and pharmacological activities in these ET-1-producing tissues (2, 17, 18, 19), the present observations suggest that ET-1 may play important parts as a locally acting mediator.

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