Primary Structure of Bovine Endothelin ET\(_B\) Receptor and Identification of Signal Peptidase and Metal Proteinase Cleavage Sites*

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A cDNA clone corresponding to the entire coding region of the bovine ET\(_B\) endothelin receptor mRNA was isolated from a lung cDNA library and sequenced. The cDNA encodes 441 amino acids: 26 constituting an NH\(_2\)-terminal signal peptide and 415 constituting the mature receptor. The signal peptidase cleavage site was determined by direct amino acid sequencing of purified receptor. A comparison of the predicted amino acid sequence with the available bovine ET\(_A\) and rat ET\(_B\) endothelin receptor sequences revealed 63 and 85% homology, respectively. Endothelin receptors of various species are known to be very sensitive to a certain metal proteinase(s) and have been shown to be converted to a lower M\(_r\) form in the absence of EDTA. The metal proteinase cleavage site was also determined by direct protein sequencing of the proteolysis product. The amino acid sequence (Ala-Gly-X-Pro-Pro-Arg) surrounding the cleavage site (between Ala-79 and Gly-80) is conserved among the ET\(_B\) endothelin receptors, explaining the above mentioned proteolytic conversion from the higher to lower M\(_r\) forms observed in various species.

Endothelins are novel 21-amino acid contractile peptides which are thought to function as local hormones. Endothelin-1 was first identified as a potent vasoactive agent produced by vascular endothelial cells (1) and subsequently shown to act on nonvascular tissues as well (2-5). Gene analysis using an endothelin-1 cDNA probe demonstrated the presence of two other members in the endothelin family (6): endothelin-2 and endothelin-3. It is therefore supposed that by cooperative action of the three members, endothelin plays a major role in finely tuning vascular and nonvascular smooth muscle tone. To understand this complicated regulatory system, it is essential to identify and characterize the receptors mediating the diverse effects of endothelins.

In a previous paper (7), we described the purification and partial amino acid sequencing of the bovine lung endothelin receptor. During the course of cDNA cloning based on the partial amino acid sequence, two reports appeared on cloning and expression of cDNAs encoding endothelin receptors. Arai et al. (8) have cloned, by expression cloning using Xenopus oocytes, an endothelin receptor cDNA from a bovine lung cDNA library and showed that the receptor is highly specific for endothelin-1 and widely distributed in the heart, lung, brain, kidney, and intestine. Sakurai et al. (9) have also cloned, by expression cloning using COS-7 cells, a cDNA encoding a nonselective endothelin receptor from a rat lung cDNA library constructed in the mammalian expression vector pCDM8. The latter type of receptor, termed ET\(_A\), has also been demonstrated to be widely distributed throughout the body including the brain, lung, kidney, heart, adrenal, liver, stomach, and uterus but not in vascular smooth muscles. The term ET\(_A\) is recommended for the former specific receptor by a receptor nomenclature subcommittee (10). Sequence comparison revealed that our purified receptor is very similar to the rat ET\(_B\) receptor (9) and considerably different from the bovine ET\(_A\) receptor (8); we therefore considered it worth the effort to determine the complete amino acid sequence of the bovine ET\(_B\) receptor we purified. We further determined the cleavage sites by the signal peptidase and an EDTA-sensitive metal proteinase. Sequence comparison indicated that the bovine ET\(_A\) and ET\(_B\) receptors have diverged in their NH\(_2\)- and COOH-terminal regions.

EXPERIMENTAL PROCEDURES AND RESULTS AND DISCUSSION

Deduced Amino Acid Sequence and Its Comparison with Other Endothelin Receptors—The complete nucleotide sequence of the bovine ET\(_B\) endothelin receptor cDNA is presented in Fig. 3. The cDNA has an open reading frame of 441 codons capable of encoding a protein of 49,371 Da. The tryptic fragments whose amino acid sequences are presented in a previous paper (7) were all present in the translated sequence (Fig. 3). As expected from the biochemical analysis of the endothelin signal transduction system, which indicated that endothelin receptors belong to the G protein-coupled receptor family, the bovine ET\(_B\) receptor has seven putative transmembrane hydrophobic domains.

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** The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D890456.

1 The abbreviations used are: ET\(_A\), isopeptide-nonselective type A endothelin receptor; ET\(_B\), type B endothelin receptor; bp, base pairs; kb, kilobase pairs; SSC, saline sodium citrate (15 mM, pH 7); SSPE, saline sodium phosphate (10 mM, pH 7.4) EDTA (1 mM).

2 Portions of this paper (including "Experimental Procedures," part of "Results," and Figs. 1, 2, 5, and 6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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The translation initiation codon starts at nucleotide 1015.

Fig. 4. Amino acid homologies among bovine ET<sub>a</sub> receptor (bb), rat ET<sub>a</sub> receptor (rb), and bovine ET<sub>h</sub> receptor (ba). The sequences of bovine ET<sub>a</sub>, rat ET<sub>a</sub>, and bovine ET<sub>h</sub> were aligned by visual inspection and the assistance of SDC-GENETYX computer software (Tokyo, Japan). Identical amino acids are shaded. Putative transmembrane domains are overlined. The signal peptidase cleavage site (*) and metal proteinase-sensitive site (†) are indicated.

Fig. 3. Nucleotide and deduced amino acid sequence of the bovine ET<sub>a</sub> receptor. Nucleotide and amino acid numbers are shown on the right. The nucleotides are numbered in the 5'-3' direction, beginning with the first nucleotide of the cDNA clone bETR5; the translation initiation codon starts at nucleotide 1015.

Cell culture and assay of proteinase cleavage sites.

Identification of the Cleavage Site Highly Susceptible to a Metal Proteinase—In a previous paper (7), we demonstrated that the bovine ET<sub>a</sub> endothelin receptor is very sensitive to a metal proteinase and rapidly converted to a low molecular weight form in the absence of EDTA. NH<sub>p</sub>-terminal sequence analysis of the low molecular weight form revealed the following.

Peptide sequences obtained from the purified receptor (7) are indicated.
sequence (7): Gly-Ile-Pro-Pro-X-Thr-Pro-Pro-Pro-Cys-. This sequence is located near the NH$_2$-terminal end of the first transmembrane domain (Fig. 3). The proteolytic cleavage site was, therefore, determined to be between Ala-79 and Gly-80. Similar sequences (i.e. Ala-Gly followed by proline-rich sequences) are also found in the rat ET$_B$ receptors (9), suggesting that a similar limited proteolysis is a common feature characteristic of the ET$_B$ receptors. In fact there are many reports describing the presence of lower molecular mass forms (35-45 kDa) of endothelin receptor (11-13), and in particular Schwartz et al. (14) have demonstrated, using rat brain samples, a rapid conversion of a 52-kDa native form of endothelin receptor to a 30-kDa form, which could be inhibited with EDTA.

NH$_2$-terminal Domain and Ligand Specificity—Generally the extracellular NH$_2$-terminal domain is expected to be involved in determining the ligand specificity; however, this was not the case in the bovine ET$_A$ receptor as shown in a previous paper (7) in which we have demonstrated the presence of lower molecular mass forms (35-45 kDa) of endothelin receptor (11-13), and in particular Schwartz et al. (14) have demonstrated, using rat brain samples, a rapid conversion of a 52-kDa native form of endothelin receptor to a 30-kDa form, which could be inhibited with EDTA.

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EXPERIMENTAL PROCEDURES

Northern Analysis—The estimated size of the mRNA for bovine ET, receptor by Northern blot analysis was about 4.8 kb (Fig. 6). Therefore, the longest cDNA clone (5524 bp, Fig. 3) covers the entire complementary sequence of the corresponding mRNA. In the case of rat ET, and bovine ET, 5524 nt, the values of 4.8 kb and 3.3 and 4.2 kb, respectively, have been reported.

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Bovine Type B Endothelin Receptor

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Fig. 5. Hydrophathy profile of bovine ETβ endothelin receptor. The profile was obtained by the algorithm of Kyte and Doolittle (1982) using a window of 11 residues. The numbers at the bottom indicate the amino acid residues in the sequence presented in Fig. 3.

Fig. 6. Northern blot analysis of total RNA and mRNA from bovine lung. To estimate the size of the bovine ETβ mRNA, total (lane 1) and poly(A)+ mRNA (lane 2) were prepared from bovine lung, denatured, electrophoresed, transferred to nylon membrane, and probed with 32P-labeled cDNA as described under "Experimental Procedures".