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The endothelin-converting enzyme from human umbilical vein is a membrane-bound metalloprotease similar to that from bovine aortic endothelial cells

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ABSTRACT **A phosphoramidon-sensitive, membrane-bound metalloprotease that cleaves big endothelin 1 (big-ET-1) to ET-1 was obtained from human umbilical vein endothelial cells and also from bovine aortic endothelial cells by isolation of plasma-membrane vesicles free of lysosomes. The enzyme was characterized by RIA with an antibody specific for ET-1 and also by reverse-phase HPLC. For both sources, the pH rate profile of the membrane fraction had a very sharp maximum at pH 7.0; little or no activity was seen at more acidic pH values. In contrast, the cytosolic fraction had a major peak at acidic pH values, as well as a broad peak in the neutral region. The activity at pH 7.0 in the membrane fraction was shown by reverse-phase HPLC to produce ET-1 and C-terminal fragment as products. This activity was abolished by phosphoramidon, EDTA, and 1,10-phenanthroline but was not inhibited by pepstatin A, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, leupeptin, or E-64—consistent with the characteristics of a metalloprotease. These results suggest that this activity is from the physiologically relevant, phosphoramidon-inhibitable, endothelin-converting enzyme. The activity found at neutral pH values in the cytosolic fraction was only partially inhibited by EDTA and 1,10-phenanthroline but was not inhibited by phosphoramidon. The membrane-bound endothelin-converting enzyme from human umbilical vein endothelial cells and bovine aortic endothelial cells showed marked similarities, including IC_{50} values for phosphoramidon of 2.7 and 1.8 μM and K_m values for big-ET-1 of 45.4 and 20.9 μM, respectively. The apparent molecular mass by gel filtration was ≈300–350 kDa for the enzyme from either source. This report characterizes human endothelin-converting enzyme, which may be an important therapeutic target for cardiovascular disease.**

Endothelin 1 (ET-1) is a peptide with potent vasoconstrictor activity first isolated from the medium of cultured porcine aortic endothelial cells (1). Based upon sequence analysis of cDNA for ET-1, Yanagisawa *et al.* (1) proposed that ET-1 is generated by an unusual processing involving a cleavage between Trp-21 and Val-22 of big ET-1 (big-ET-1) by a putative endothelin-converting enzyme (ECE). Because the vasoconstrictor activity of big-ET-1 is much lower than that of ET-1 (2, 3), the conversion from big-ET-1 to ET-1 appears essential for physiological activity.

Two different types of proteases have most often been reported as possible candidates for ECE. One of these is an acid protease with a pH optimum at 3.5 that is inhibited by pepstatin A (4, 5). The other is a metalloendopeptidase that is active at neutral pH and inhibited by phosphoramidon, a metalloprotease inhibitor (6, 7). There is growing evidence indicating that the phosphoramidon-inhibitable enzyme is the physiologically relevant one. Phosphoramidon can inhibit the pressor and airway contractile effects of big-ET-1 *in vivo* (8, 9) and suppress the secretion of ET-1 from cultured endothelial cells (10, 11). In contrast, Bird *et al.* (12) showed that inhibitors of cathepsin E did not inhibit the big-ET-1-induced pressor response. Our study, therefore, was initiated to search for a phosphoramidon-sensitive enzyme capable of specifically converting big-ET-1 to ET-1. Because human umbilical vein endothelial cells (HUVEC) have recently been shown to secrete ET-1 in a manner inhibitable by phosphoramidon (13), we wished to determine whether these cells possess an ECE activity comparable to that initially described in cells from other species. This question was of particular interest because the enzyme from human endothelial cells could be an important therapeutic target for cardiovascular diseases.

We report here evidence that HUVEC and bovine aortic endothelial cells (BAEC) contain a phosphoramidon-inhibitable ECE that is a membrane-bound metalloprotease. Also presented are data that show marked similarities between the ECE activities from these two different sources.

MATERIALS AND METHODS

Materials and Buffers. Human big-ET-(1–38), ET-1-(1–21), the C-terminal fragment-(22–38) (CTF) of big-ET-1, and antibody against CTF were purchased from Peptides International (Louisville, KY). ^{125}I-labeled ET-1 and human ^{125}I-labeled big-ET-1-(1–38) were from Amersham. HUVEC and medium for their growth were from Clonetics (San Diego). Polyclonal antibody against ET-1 was from Biodesign International (Kennebunkport, ME). Phosphoramidon, pepstatin A, and leupeptin were from Boehringer Mannheim. E-64, 1,10-phenanthroline, and soybean trypsin inhibitor were obtained from Sigma. Triton X-100, CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), octyl glucoside, and sodium deoxycholate were from Calbiochem. Molecular mass standards and the Superose-12 fast protein liquid chromatography column were from Pharmacia LKB. The protein-concentration-determination agents were from Bio-Rad. Buffer A was 10 mM Tris·HCl, pH 7.5/0.25 M sucrose/20 mM KCl; buffer B was 60 mM KP_i, pH 7.4/10 mM EDTA/8 mM NaN_3/0.3% bovine serum albumin; buffer C was 25 mM KP_i, pH 7.0/50 mM NaCl/0.5% Triton X-100 (hydrogenated)/0.1% NaN_3.

Cell Culture. BAEC were grown at 37°C in a CO_2 incubator (95% air/5% CO_2) in Dulbecco's modified Eagle's medium/10% fetal calf serum/2 mM L-glutamine/penicillin at 100 units per ml/streptomycin at 0.1 mg/ml. The typical enzyme prep-

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Abbreviations: ET-1, endothelin 1; big-ET-1, big ET-1; ECE, endothelin-converting enzyme; CTF, C-terminal fragment-(22–38) of big-ET-1; HUVEC, human umbilical vein endothelial cells; BAEC, bovine aortic endothelial cells; ir, immunoreactive; RP-HPLC, reverse-phase HPLC.

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aration was performed after obtaining confluent cells at five to eight passages from 30–50 roller bottles.

HUVEC were grown at 37°C in a CO_2 incubator (95% air/5% CO_2) in endothelial cell basal medium/10% fetal calf serum/0.4% bovine brain extract/heparin at 40 ng/ml/human epidermal growth factor at 0.1 ng/ml/gentamicin at 50 ng/ml/amphotericin B at 0.05 ng/ml. A typical enzyme preparation was done from confluent cells at less than five passages from ≈60 T-175 flasks.

Preparation of Cytosolic and Membrane Fractions. Endothelial cells were washed with phosphate-buffered saline and gently scraped. All operations were done at 0–4°C unless otherwise noted. Typically ≈10^9 BAEC or 10^8 HUVEC were washed further with phosphate-buffered saline followed by buffer A. Cells suspended in 100 ml of buffer A were homogenized via nitrogen cavitation [600 psi (1 psi = 6.9 kPa) for 10 min] and centrifuged at 5000 × *g* for 20 min. The supernatant was then centrifuged at 20,000 × *g* for 35 min. The resulting supernatant was further centrifuged at 100,000 × *g* for 1 hr. The supernatant of the final centrifugation was used as the cytosolic fraction. The pellet was washed with buffer A, resuspended in the same buffer, and then used as the membrane fraction.

Measurement of ET-1-Converting Activity. The typical reaction mixture (50 μl) contained 10 μM big-ET-1, 100 mM Tris·HCl (pH 7.0), 0.25% Triton X-100, 0.01% NaN_3, and the indicated amount of protein. The membrane fraction was resuspended in 0.5% Triton X-100 before use. After incubation for the indicated period at 37°C, the reaction was stopped by adding 4 μl of protease inhibitor mixture to give final concentrations of 9 mM, 90 μM, 0.4 mM, and 0.2 mM for EDTA, pepstatin A, phenylmethylsulfonyl fluoride, and leupeptin, respectively. This final mixture was diluted with buffer B (usually 10- to 1000-fold) and used for RIA. One unit of enzyme is defined as the amount generating 1 pmol of immunoreactive (ir) ET-1 per min.

RIA. To measure ir-ET-1, the assay mixture (250 μl) contained the antibody against ET-1, an ET-1 sample, and ^{125}I-labeled ET-1 (7000 cpm) in buffer B. The order of addition was ET-1 sample, then antibody, and finally ^{125}I-labeled ET-1. After incubation at 4°C for 2–3 days, unbound ET-1 was coprecipitated by adding 2.4% (wt/vol) charcoal/0.24% (wt/vol) dextran suspension (125 μl) in buffer B with bovine serum albumin replaced by 0.25% (wt/vol) gelatin. The amount of ir-ET-1 was measured by counting the radioactivity in the supernatant and then using the standard curve. The cross reactivity to big-ET-1 was <0.01%, and the detection limit was 1 fmol. For the measurement of ir-CTF of big-ET-1, the assay conditions were the same as above, but the antibody against ET-1 and the ^{125}I-labeled ET-1 were replaced by the antibody against CTF and ^{125}I-labeled big-ET-1, respectively. Cross reactivity for big-ET-1 was 100%, and the detection limit was 100 fmol.

Reverse-Phase HPLC (RP-HPLC). RP-HPLC analysis was done with a Rainin HPLC model Dynamax and a Vydac C_{18} column (0.46 × 25 cm). Chromatograms were obtained by using a 10-min isocratic elution with water/0.1% trifluoroacetic acid followed by a 60-min linear gradient from 0 to 60% acetonitrile in 0.1% trifluoroacetic acid. The flow rate was 1 ml/min. Retention times were 36.7, 48.7, and 50.1 min for the CTF of big-ET-1, big-ET-1, and ET-1, respectively. For the identification of ET-1 and CTF, four fractions (0.2 min per fraction) around their retention times were collected and analyzed.

Protein Assay. Protein concentrations were determined with the Bio-Rad protein assay dye reagent and bovine serum albumin as standard.

RESULTS

ECE Is a Membrane-Bound Metalloprotease. When ET-1-converting activities were measured in a pH range of 2–11, marked differences between the cytosolic and membrane fractions of both HUVEC and BAEC were seen (Fig. 1). In the cytosolic fraction, there was a major activity peak in the acidic as well as the neutral region. The activity at the lower pH range had a broad pH optimum and was completely inhibited by a mixture of protease inhibitors including pepstatin A, phenylmethylsulfonyl fluoride, and leupeptin. The cytosolic fraction from BAEC yielded a much higher activity at low pH than did that from HUVEC. The neutral activity in the cytosolic fraction also had a broad pH optimum. In

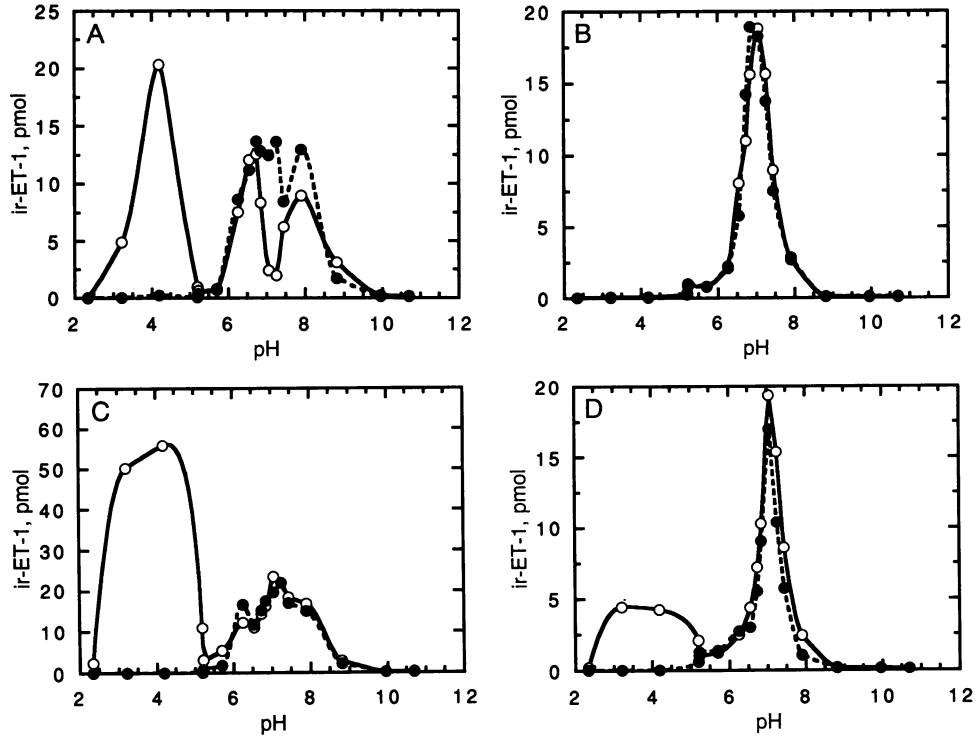

FIG. 1. pH dependence of ET-1-converting activity. The cytosolic (*A* or *C*) or membrane (*B* or *D*) fraction from HUVEC or BAEC was incubated at 37°C for 2 hr with (●) or without (○) protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, 20 μM pepstatin A, and 0.1 mM leupeptin) in a reaction mixture (50 μl) containing 10 μM big-ET-1, 0.25% Triton X-100, 0.01% NaN_3, and a buffer at 100 mM as follows: pH 2.35–5.21 (citric acid·KOH), pH 5.71–6.26 (Mes·KOH), pH 6.55–7.91 (Hepes·KOH), pH 8.82–10.71 {2-[*N*-cyclohexylamino]ethanesulfonic acid (Ches)·KOH}. Quantities of cytosolic fraction used were 29.5 μg (HUVEC) and 11.4 μg (BAEC). Quantities of membrane fraction used were 6.3 μg (HUVEC) and 7.3 μg (BAEC). The amount of ET-1 generated was analyzed by RIA (1 pmol of ir-ET-1 corresponds to 8.3 × 10^{-3} activity units).

Table 1. Inhibition profile of the ET-1-converting activity in the cytosolic or membrane fractions from HUVEC or BAEC

Inhibitor	ET-1-converting activity, % of control			
	HUVEC		BAEC	
	Cytosolic	Membrane	Cytosolic	Membrane
Phosphoramidon (0.1 mM)	94.9	6.2	89.6	3.8
EDTA (0.5 mM)	65.2	8.8	81.1	3.1
1,10-Phenanthroline (1 mM)	36.1	3.7	18.9	1.5
EGTA (2 mM)	61.4	11.6	56.3	4.6
Captopril (0.1 mM)	127.2	98.0	98.6	94.7
Enalaprilat (0.1 mM)	98.7	99.7	95.9	100.0
Phenylmethylsulfonyl fluoride (0.2 mM)	162.7	104.8	110.4	88.5
Soybean trypsin inhibitor (6.5 μg)	99.4	108.8	88.7	106.1
Pepstatin A (0.02 mM)	119.6	100.3	110.4	86.3
Leupeptin (0.1 mM)	86.1	100.0	100.0	101.5
E-64 (0.5 mM)	112.7	111.6	111.7	102.3

The cytosolic or membrane fraction from HUVEC or BAEC was incubated at 37°C for 2 hr with or without the indicated protease inhibitors. Quantities of cytosolic fraction used were 35.4 μg (HUVEC) or 11.4 μg (BAEC). Quantities of membrane fraction used were 10.6 μg (HUVEC) or 13.0 μg (BAEC). The amount of ET-1 generated was analyzed by RIA. The amounts of ir-ET-1 in controls for the cytosolic fraction were 15.8 pmol (HUVEC) or 22.2 pmol (BAEC). The amounts of ir-ET-1 in controls for the membrane fraction were 35.4 pmol (HUVEC) or 13.1 pmol (BAEC). Final concentration of the organic solvent (dimethyl sulfoxide, ethanol, or methanol) was 1%.

contrast, the membrane fraction showed one major ET-1-converting activity with a pH optimum of 7.0 that was extremely sharp, probably indicating the presence of one major ET-1-converting activity. The width of the peak at half-maximum activity was <1 pH unit. This activity was not affected by the same mixture of protease inhibitors described above. To eliminate the possible effects of different buffers on the activity at neutral pH, the same buffer (Hepes·KOH) was used for the entire range covered by the sharp peak (pH 6.6–7.9). When KP_i was used in this range (pH 6.4–7.7), the activities were generally slightly higher than those in Hepes·KOH, but the pH optimum was also very sharp with a maximum at 7.0 (data not shown).

To further evaluate the nature of the ET-1-converting activity found at neutral pH, individual protease inhibitors were tested on both cytosolic and membrane fractions. Table 1 shows that activity in the membrane fraction was inhibited >90% by metalloprotease inhibitors (EDTA, phosphoramidon, and 1,10-phenanthroline) and was not affected by serine, cysteine, and acid protease inhibitors (phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, E-64, leupeptin, and pepstatin A). Captopril and enalaprilat, inhibitors of angiotensin-converting enzyme, did not inhibit this ECE activity. EGTA also inhibited the activity completely. These data strongly indicate that the phosphoramidon-sensitive ECE is located primarily in the membrane fraction and is a metalloprotease. The K_m data and the inhibition titration curves were consistent with equations representing one enzyme rather than several with various K_i or K_m values (see below).

In contrast, the cytosolic fraction was not inhibited by most protease inhibitors tested (Table 1). Only partial inhibition was observed with EDTA, EGTA, and 1,10-phenanthroline, but no inhibition was obtained with phosphoramidon. These data, in addition to the broad pH optimum, indicate that the ET-1-converting activity at neutral pH in the cytosolic fraction is probably due to multiple proteases.

Table 2 shows that the ratio of the phosphoramidon-sensitive ET-1-converting activity at pH 7.0 (putative ECE) in the membrane fraction to that in the cytosolic fraction was ≈30 and ≈17 for both HUVEC and BAEC, respectively. The specific phosphoramidon-sensitive activity of the membrane fraction was significantly higher than that of the cytosolic fraction: 98 and 36 times higher for HUVEC or BAEC, respectively. Interestingly, the specific activities for the cytosolic and membrane fractions from HUVEC were comparable to those from BAEC (not only from two different species but also from two different tissues). These results on activity recovery and specific activities were reproducible in multiple preparations of the enzyme.

Authenticity of ir-ET-1. To determine whether ir-ET-1 is authentic ET-1, the reaction mixture was fractionated by RP-HPLC after incubation with samples of either the cytosolic or membrane fraction. An RIA was then done for the fractions with retention times corresponding to an ET-1 standard. For the membrane fraction, these amounts of ir-ET-1 were 82% (BAEC) and 101% (HUVEC) of those detected without HPLC separation after correcting for recovery from the reverse-phase column (Table 3). The molecular mass of the peptide in the product peak was then measured with electrospray mass spectrometry and found identical to that measured for authentic ET-1.

Table 2. Total ET-1-converting activities and specific activities in the cytosolic and membrane fractions from HUVEC and BAEC

Source	Fraction	Total protein, mg	Total activity, units	Phosphoramidon-inhibitable total activity, units	Specific phosphoramidon-inhibitable activity, units per mg
HUVEC*	Cytosolic	24.7	135.9	6.9	0.3
	Membrane	7.0	219.1	205.5	29.4
BAEC†	Cytosolic	77.0	463.3	48.2	0.6
	Membrane	37.4	846.7	814.5	21.8

*Approximately 1×10^8 cells were used.
†Approximately 5×10^8 cells were used.

Table 3. Authenticity of immunoreactive ET-1

Source	Fraction	ir-ET, pmol	
		Before HPLC	After HPLC*
BAEC	Cytosolic	8.4	5.0
	Membrane	109.4	89.4
HUVEC	Cytosolic	ND	ND
	Membrane	126.9	127.6

The cytosolic or membrane fraction from HUVEC or BAEC was incubated at 37°C for 2 hr in a reaction mixture (50 μl) containing 10 μM big-ET-1, 100 mM Tris·HCl (pH 7.0), and 0.01% NaN_3. At the end of incubation, 200 μl of 0.1% trifluoroacetic acid was added to the reaction mixture, which was then injected onto RP-HPLC. The quantity of cytosolic fraction used was 8.6 μg (BAEC); the quantity of membrane fraction used was 40.0 μg (HUVEC) or 19.5 μg (BAEC). The amount of ET-1 generated was analyzed by RIA. ND, not determined.

*Data were corrected for the average 50% recovery from the RP-HPLC column; three samples of ET-1 standard (between 10–100 pmol) were injected onto RP-HPLC, and the recovery was determined as described.

To additionally confirm that the membrane-bound ECE cleaves big-ET-1 specifically at Trp-21–Val-22, an RIA was developed that measured the CTF of big-ET-1 (see *Materials and Methods*). Because cross reactivity of the antibody against CTF with big-ET-1 is 100%, this RIA could be used to quantify CTF after separation by HPLC. The results showed a stoichiometric conversion of big-ET-1 to ET-1 and CTF for the membrane-bound ECE from BAEC. (Under the conditions where ≈20% of big-ET-1 was converted, as shown in Table 3, 89.4 pmol and 107.2 pmol of ET-1 and CTF, respectively, were detected.) In addition, the identity of the CTF peak was confirmed by electrospray mass spectrometry.

From all data presented above, the ir-ET-1 generated by the membrane fraction from either HUVEC or BAEC was concluded to be authentic ET-1.

Characterization of Membrane-Bound ECE. When big-ET-1 was incubated with the membrane fraction from either HUVEC or BAEC, ir-ET-1 was generated in both a dose- and time-dependent manner (shown for BAEC in Fig. 2, with comparable data obtained for HUVEC). All experiments described in this report were done in the linear range of these curves. At a 0.1% concentration, octyl glucoside, CHAPS, and sodium deoxycholate inhibited the ECE activity by 50, 46, and 73%, respectively. Triton X-100 was not inhibitory at up to 0.5%. KCl inhibited by 50% at 0.5 M and inhibited still more at higher levels. The velocity versus big-ET-1 concentration curves were fit to Michaelis–Menten kinetics and, thus, were shown to be consistent with a single-enzyme species. The K_m values for big-ET-1 were 45.4 ± 6.6 and 20.9 ± 4.5 μM with V_{max} values of 160.0 ± 11.1 and 46.0 ± 4.3 pmol/mg of membrane fraction per min for HUVEC and BAEC, respectively. Phosphoramidon inhibited the ECE activity with IC_{50} values of 2.7 ± 0.36 and 1.8 ± 0.31 μM for HUVEC and BAEC, respectively, as shown in Fig. 3. The curves were consistent with the inhibition of a single-enzyme species, as was shown by fitting them to a four-parameter titration equation by nonlinear least squares and finding exponential values close to 1.0.

The apparent molecular mass for ECE was estimated at ≈300–350 kDa for ECE from both sources by comparing the Superose-12 elution volume of the ECE activity with those of reference proteins. This size is larger than the apparent molecular mass measured for the enzyme from bovine carotid artery endothelial cells (7). The ECE activity from BAEC eluted in a broader peak than that from HUVEC, and ≈65% and 20% of the activities were recovered for HUVEC and BAEC, respectively (Fig. 4).

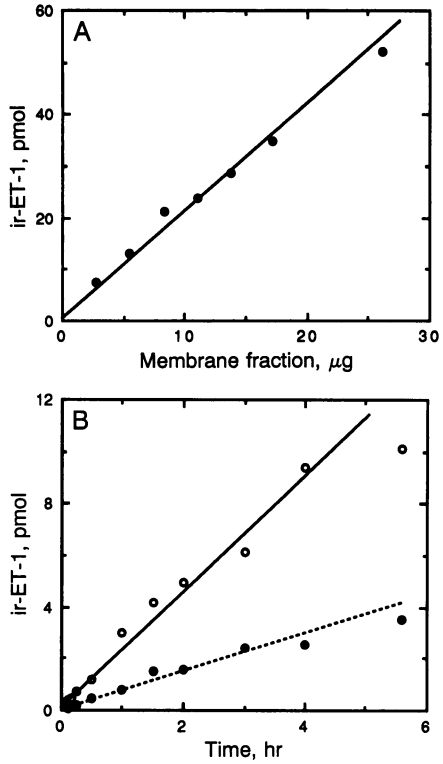

FIG. 2. Dose–response curve (*A*) and time course (*B*) for ECE from BAEC. The indicated amount of membrane fraction was incubated with 10 μM big-ET-1 for 2 hr (*A*). The membrane fraction (2.5 μg) was incubated with either 10 μM (○) or 3 μM (●) big-ET-1 for the indicated period (*B*). The amount of ET-1 generated was analyzed by RIA.

DISCUSSION

ET-1-converting activities in HUVEC and BAEC have been characterized with regard to subcellular fraction, pH dependence, protease inhibitor effect, elution volume on gel-filtration chromatography, K_m values for substrate, and product determination by HPLC in combination with RIA. The subcellular fractionation method described gives a lysosome-free membrane fraction with ECE activity at pH 7.0 virtually free of other contaminating protease activities.

Although the pH rate profiles suggest the presence of a variety of ET-1-converting activities, the sharp peak centered

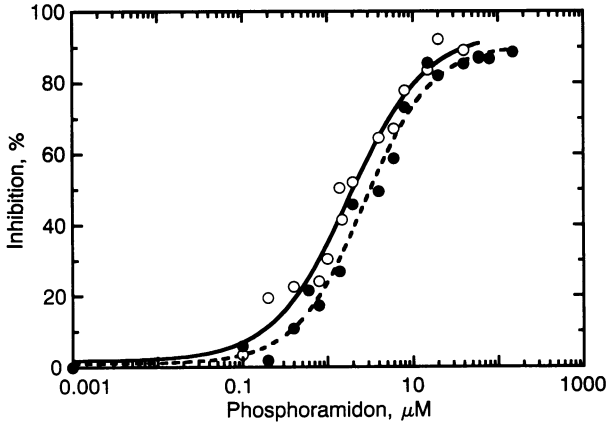

FIG. 3. Inhibition of ECE activity by phosphoramidon. The membrane fraction [6.5 or 7.1 μg of HUVEC (●) or BAEC (○), respectively] was incubated with 10 μM big-ET-1 for 2 hr with the indicated phosphoramidon concentrations. The amount of ET-1 generated was analyzed by RIA.

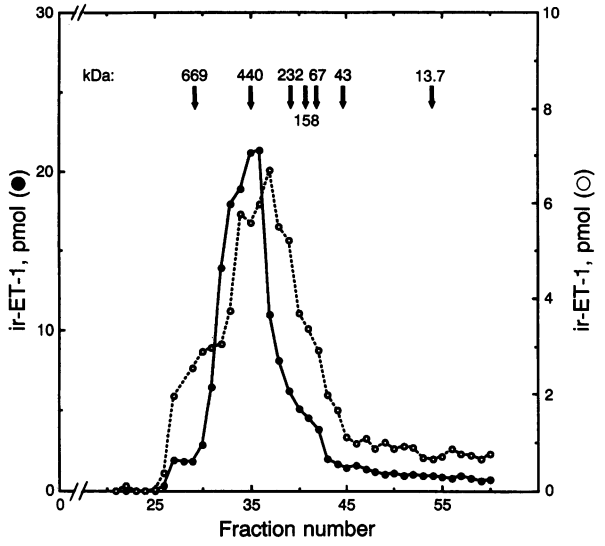

FIG. 4. Gel filtration of ECE. The membrane fraction [1.0 or 1.5 mg for HUVEC (●) or BAEC (○), respectively] was applied to a Superose-12 fast liquid chromatography column equilibrated with buffer C. Elution was done with the equilibration buffer at a flow rate of 0.3 ml/min; 0.3-ml fractions were collected. ECE activity was determined by using 30 μl of each fraction. The amount of ET-1 generated was analyzed by RIA. Marker proteins were detected by monitoring UV absorbances (A_{280}); the marker proteins were ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), and ribonuclease A (13.7 kDa).

at pH 7.0 in the membrane fraction is probably the authentic ECE activity because it is inhibitable by phosphoramidon and because a membrane-bound enzyme is a more likely candidate than a lysosomal enzyme for the physiologically relevant cleavage reaction. Because phosphoramidon inhibits the pressor and airway contractile effects of big-ET-1 *in vivo* (8, 9) and suppresses the secretion of ET-1 from cultured endothelial cells (10, 11), it has been inferred that there is a putative ECE that is inhibited by phosphoramidon. Indeed, several research groups have reported (7, 14–16) ET-1-converting activities from various sources that were inhibitable by phosphoramidon. Other *in vivo* studies also indicate that inhibitors of cathepsin E and angiotensin-converting enzyme (enalaprilat) do not inhibit the big-ET-1-induced pressor response (12, 17). Our results on the ECE activity in the membrane fraction at pH 7.0 are completely consistent with these observations, in that the activity is abolished by phosphoramidon but is not affected by enalaprilat.

In this study, the ET-1-converting activities in the cytosolic fraction (at low and neutral pH) are not inhibited by phosphoramidon. The activity at low pH is probably due, at least in part, to cathepsin D, as reported by Sawamura *et al.* (5) for BAEC. These workers also showed that cathepsin D cleaves the Asn-18–Ile-19 bond in addition to the Trp-21–Val-22 bond of big-ET-1 and, therefore, that cathepsin D is probably not a physiologically relevant ECE (18). The evidence we find for membrane-bound enzyme agrees with that of Matsumura *et al.* (16), who found that ECE from porcine aortic endothelial cells is also a membrane-bound metalloprotease. However, Takada *et al.* (15) detected phosphoramidon-inhibitable neutral metalloproteases with ET-1-converting activity in both the membrane and cytosolic fractions from bovine carotid artery endothelial cells in a ratio of 5:1. This discrepancy might be from the difference in tissue source or from a difference in subcellular-fractionation conditions.

Our work suggests that ECE from HUVEC resembles that from BAEC because both have an unusually sharp pH rate profile and an identical inhibitor sensitivity. We have observed complete inhibition of the ECE activity by metalloprotease inhibitors but not by serine, cysteine, and acid protease inhibitors and, therefore, conclude that ECE is a metalloprotease. ECE appears to be a membrane-bound enzyme because significantly higher specific activity is found in the membrane fraction than in the cytosolic fraction from both sources. The methods described in this study provide the basis for a purification scheme for human ECE, allowing better characterization of the enzyme, cloning of the gene, and the production of antibodies useful for localization studies.

This paper is dedicated to Prof. Arthur Kornberg on his 75th birthday. We thank Drs. R. Panek and E. Reynolds for providing BAEC for initiating tissue culture. We also thank Mr. Tracy Stevenson for electrospray mass spectrometry analysis.

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