Human Endothelial Cell Storage Granules
A Novel Intracellular Site for Isoforms of the Endothelin-Converting Enzyme

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Abstract—We have previously shown endothelin (ET)-like immunoreactive staining in Weibel-Palade bodies, storage granules that are an integral component of the regulated secretory pathway in endothelial cells. These structures degranulate after chemical or mechanical stimuli that result in cytosolic calcium influx. We therefore investigated whether the regulated pathway might be an intracellular site involved in the cleavage of big ET-1 to the biologically active peptide ET-1 by determining the ultrastructural localization of endothelin-converting enzyme (ECE)-1. A low level of ECE-like immunoreactivity was detected on the cell surface of human umbilical vein and coronary artery endothelial cells by scanning electron microscopy. Exogenous big ET-1 was added to permeabilized and nonpermeabilized cultured human umbilical vein endothelial cells, and ECE activity was measured by the detection of ET-like immunoreactivity in the culture supernatant. A marked increase in ECE activity was observed in permeabilized cells, indicating that ECE may also be expressed in intracellular compartments. Confocal microscopy revealed intense immunofluorescence staining for big ET-1 and the 2 isoforms of ECE-1 (ECE-1α and ECE-1β) in the perinuclear region and in Weibel-Palade bodies of the human umbilical vein endothelial cells. Stimulated degranulation of storage granules by the calcium ionophore A23187 caused release of ET into the culture supernatants. The findings of this study indicate that big ET-1 is processed to the mature vasoactive peptide by ECEs located within endothelial storage granules. We hypothesize that this activity may be important in the regulated mobilization of ET in human endothelial cells. (Circ Res. 1998;83:314-321.)

Key Words: endothelin ■ Weibel-Palade bodies ■ endothelium ■ endothelin-converting enzyme ■ electron microscopy

Endothelin-1 is a 21–amino acid peptide that is released from vascular ECs and mediates potent vasoconstriction.1 The peptide is generated after proteolytic cleavage of its immediate precursor, big ET-1,2,3 by the putative ECEs (ECE-1 and ECE-2).4 Molecular studies have predicted that 2 isoforms of ECE-1 exist that differ only in their N-terminal sequences: ECE-1α and ECE-1β,5 also called ECE-1b and ECE-1a, respectively.6

ECE expression is high in ECs, and processing of big ET-1 to ET-1 has been attributed to the activity of a converting enzyme that is located on the plasma membrane and within intracellular compartments.7-9 Some studies indicate that ECE is predominantly expressed or has its main activity as an ectoenzyme7,9-12 and therefore acts mainly in a postsecretory processing role. In contrast, other studies have suggested that ECE is either primarily expressed or has predominant activity within intracellular compartments.8,13,14 Intracellular ECE is located within the Golgi complex13 and discrete secretory vesicles,7,8 thus implicating conversion of big ET-1 to ET-1 while in transit through the cell cytosol.

Materials and Methods

Materials
Site-directed antisera were raised against a synthetic peptide corresponding to the deduced amino acids in the C-terminus of bovine
EC-1 (bEC-E-1α-4–593)23 in the C-terminus of human big ET-1 (big ET-1α,3–21)22 and in the N-termini of human ECE-1α (ECE-1α3–10) and human ECE-1β (ECE-1β3–16).23 A mouse monoclonal antibody against human P-selectin (CD62P) was obtained from Novocastra Laboratories Ltd. Goat anti-rabbit IgG(−5 nm gold, goat anti-rabbit IgG/10 nm gold, and goat anti-mouse IgG (H+L)/10 nm gold were from British BioCell International. Polyclonal rabbit anti-human vWF and monoclonal mouse anti-human vWF (clone F8/86) were from Dako. Affinity-purified fluoresceinated anti-rabbit IgG (H+L) and Texas red anti-mouse IgG (H+L) were from Vector Laboratories. Citifluor glycerol solution was from Agar Scientific Ltd. Intense M silver enhancement kit was from Amersham International plc. Synthetic culture medium 199 was obtained from Life Technologies. Chloroquinze was from Promega. The calcium ionophore A23187, compound 48/80, ATP, nonenzymatic cell-dissociation solution, phosphoramidon, and thiorphan were from Sigma-Aldrich Company Ltd. All other reagents were of analytical or electron microscopy grade.

**Tissue Collection**

Human distal epicardial coronary arteries were removed from explanted hearts of 5 patients (40 to 55 years old, 4 men and 1 woman) with chronic heart failure who were undergoing cardiac transplantation at the Papworth Hospital, Cambridgeshire, UK. A segment of artery (1 to 1.5 cm long) was dissected from the heart, and the surrounding fat was removed. The vessel was cleared of blood by perfusing PIPES buffer (0.1 mol/L, pH 7.4) through the lumen, and the vessel was then further dissected into small rings 2 to 3 mm long. Unfixed vessel segments were opened by a longitudinal incision to obtain an en face coronary artery preparation for scanning electron microscopy. The adventitial surface of the arterial bed was adhered to glass coverslips by using a small amount of cyanoacrylate glue, and the exposed endothelial surface was kept moist with culture medium 199. Other vessel segments to be used for immunoelectron microscopy were fixed within 20 minutes of excision in 1% formaldehyde in 0.1 mol/L PIPES buffer (1 hour, 23°C) and then washed in PIPES buffer (1 hour, 23°C) and then washed in PIPES buffer (9 hours, 4°C).

**Cell Culture**

Human umbilical cords were collected from the Rosie Maternity Hospital, Cambridgeshire, UK. ECs were obtained from the umbilical vein by collagenase digestion as previously described24 and were grown and maintained at 37°C in a CO2 incubator (95% air/5% CO2) in medium 199 containing Glutamax (2 mmol/L), penicillin (50 IU/mL), streptomycin (50 μg/mL), fungizone (2.5 μg/mL), EC growth supplement (100 μg/mL), and 30% FCS. Cells were removed by using nonenzymatic cell-dissociation solution and passaged at a split ratio of 1:2 onto gelatin-coated coverslips for confocal microscopy or onto 24-well plates for biochemical experiments. Cells were grown to confluence (4 days, 37°C).

**Scanning Electron Microscopy**

The en face human coronary artery preparations (n = 3) and cultured HUVECs (n = 3) were fixed with 3% formaldehyde in culture medium 199 (30 minutes, 23°C). Tissues were incubated with the general ECE antiserum, preimmune serum (1:100; 2.5 hours, 23°C), or antisera raised against human vWF (1:100; 2.5 hours, 23°C) and then washed in culture medium (3 × 10 minutes, 4°C). Labeling was revealed with goat anti-rabbit/10 nm colloidal gold conjugate (1 hour, 4°C), and tissues were then washed in culture medium (4 × 5 minutes at 23°C) followed by PIPES buffer (4 × 5 minutes, 23°C). Tissues were fixed with 4% glutaraldehyde (30 minutes, 23°C), washed in PIPES buffer (2 × 5 minutes, 23°C), incubated in 2% OsO4 (20 minutes, 23°C), and then washed overnight at 4°C. Immunogold particles were silver enhanced using the Intense M silver enhancement kit. Samples were sputter-coated with 2 nm platinum in an SEM coating unit (model E5000, Polaron Equipment Ltd.). Cell surface staining was visualized with a Philips XL30 FEG scanning electron microscope using secondary electron and backscatter detectors. Confirmation of silver deposition was achieved by using x-ray analysis.

**Converting-Enzyme Activity in Permeabilized and Nonpermeabilized HUVECs**

Cultured HUVECs were grown to confluence in 24-well plates (n = 5, duplicate measurements for each treatment). Cells were incubated with big ET-1 (10 nmol/L; 2 hours, 37°C) in either medium 199 or medium 199 containing 0.1% Triton X-100 to determine cell surface and intracellular conversion of the peptide precursor. Basal release of ET was measured in supernatants of cells to which exogenous big ET-1 had not been added. Selective inhibitors were added to the medium to characterize the enzymes involved in big ET-1 processing. Enzymatic activity was determined by the measurement of ET in the culture supernatants by radioimmunoassay.25 Supernatants were collected and centrifuged at 15 000g for 5 minutes to remove cells that had dissociated from the culture plate. A 200-μL sample or ET standard (range, 0.5 to 500 fmol per tube) was incubated overnight at 4°C with 100 μL of antibody raised against ET at 1:10 000-fold dilution.26 125I–ET-1 (15 000 cpm/100 μL) was added to the supernatants, and these were incubated overnight at 4°C. Amerlex-M reagent was added (1 hour, 20°C), and the tubes were placed in a magnetic rack. Supernatants were discarded, and bound radioactivity in the precipitates was counted.

**Confocal Microscopy**

HUVECs were grown on glass coverslips in synthetic culture medium 199 (n = 5). The cells were rinsed in 0.1 mol/L PBS, fixed, and permeabilized by incubation with methanol/acetone (1:1 [vol/vol], 10 minutes, −20°C), rinsed in PBS, and blocked in 10% FCS for 30 minutes at 23°C. The cells were labeled with mouse anti-human vWF (1:25), rabbit anti-human big ET-1 (1:100), and rabbit anti-human ECE-1α (1:200) and ECE-1β (1:500) antisera or a combination of vWF and big ET-1, ECE-1α, or ECE-1β antisera (18 hours, 4°C). Other cells were doubly labeled with rabbit anti-human ECE-1α (1:200) and mouse anti-human P-selectin (1:5). Cells were washed in PBS (6 × 5 minutes, 4°C). Immunoreactivity was detected by using fluoresceinated anti-rabbit IgG (20 μg/mL), Texas red anti-mouse IgG (20 μg/mL), or both. Coverslips were washed in PBS (6 × 5 minutes, 4°C), dipped in distilled water, and mounted onto glass microscope slides with glycerol. Cells were viewed with a Leica TCS 4D confocal laser scanning microscope (Leica) equipped with an Ar-Kr laser source, Leica 63× and 40× oil-immersion objectives, and dual-channel photodetectors. Optical sections were collected at excitation wavelengths of 488 and 560 nm to image the distribution of FITC- and Texas red-labeled antisera, respectively. Images were scanned and line averaged 16 times to obtain a high signal-to-noise ratio. Colocalization of double-labeled antisera was determined by electronic overlay of signals obtained from channels 1 and 2.

**Immunoelectron Microscopy in Human Coronary Artery ECs**

Coronary arteries (n = 5) were opened by a single longitudinal incision and then immersed briefly in 15% gelatin at 30°C to prevent endothelial detachment from the intima. The gelatin was allowed to gel at 4°C, and the preparation was fixed for an additional 30 minutes in 0.5% formaldehyde. Vessels were incubated in sucrose solution (2.1 mol/L sucrose and 0.1 mol/L...
PIPES buffer; 4 to 12 hours, 4°C) and snap-frozen on aluminum pins by rapid immersion in LN2. Specimen blocks were mounted in a Reichert Ultracut FCS ultramicrotome (Leica), and ultrathin sections (0.14 μm) were cut with a glass knife at −90°C. Sections were collected on a film of sucrose supported in a platinum loop, and these were transferred to carbon Formvar-coated nickel grids. Sucrose was removed by washing the grids in Tris buffer (Tris HCl 10 mmol/L, KCl 2.7 mmol/L, NaCl 148 mmol/L, and Tween 0.01%; 10 × 2 minutes, 4°C), and the tissue was blocked with 10% FCS (10 minutes, 4°C). Sections were incubated with antisera raised against human ECE-1α, ECE-1β, or preimmune serum at 1:100-fold dilution (18 hours, 4°C) or monoclonal mouse anti-human vWF (1:25; 18 hours, 4°C). Colocalization of ECE and vWF immunoreactivity was determined by using goat anti-rabbit IgG/5-nm gold conjugate and goat anti-mouse IgG (H+L)/10-nm gold conjugate, respectively. Sections were counterstained with a mixture of methyl cellulose (1.8%) and uranyl acetate (0.25%) (10 minutes, 4°C), air dried, and viewed with a CM100 transmission electron microscope.

Stimulated Release of Peptide From HUVECs
Cultured HUVECs were grown to confluence in medium 199 containing 30% FCS. This medium was aspirated from the culture plates, replaced with fresh medium 199 containing 10% FCS, and incubated for 5 hours at 37°C. This medium was then aspirated from the culture plates, and cells (n=7, 2 replicates per treatment) were incubated in HEPES buffer (HEPES 50 mmol/L, MgCl2 5 mmol/L, and BSA 0.3%, pH 7.4) containing 1 mmol/L CaCl2 in the absence (control) or the presence of the calcium ionophore A23187 (500 μmol/L) (30 minutes, 37°C) to stimulate the mobilization and degranulation of cell storage granules. ET-like immunoreactivity was measured in the culture supernatants by radioimmunoassay, as described above.

Results
Cell Surface Detection of ECE on Human ECs
Scanning electron microscopy was used to examine possible cell surface expression of ECE on human ECs. Cultured

Figure 1. Representative scanning electron microscope images of HUVECs (A to D) and en face preparations of human coronary artery ECs (E and F) labeled with antisera raised against vWF and the C-terminus of the bovine ECE-1 sequence. Filamentous immunoreactive staining to vWF was observed on the surface of HUVECs by using the secondary electron detector (A) and the backscatter detector (B) and on the surface of human coronary artery ECs by using the secondary electron detector (E) (arrows). Only a moderate level of ECE-like immunoreactive staining was detected on the plasma membrane of HUVECs (C) and coronary artery ECs (F). HUVECs (D) and coronary artery ECs (not shown) labeled with preimmune serum displayed negligible staining. Bars = 5 μm (A and B) and 2 μm (C to F).
Evidence for an Intracellular Converting Enzyme in HUVECs

Conversion of big ET-1, which was added exogenously to permeabilized and nonpermeabilized HUVECs, was measured by detection of ET in culture supernatants by radioimmunoassay. Basal release of ET from cells treated for 2 hours with or without Triton X-100 was low (Figure 2). Conversion of exogenously added big ET to ET was significantly higher with or without Triton X-100 was low (Figure 2). Conversion in permeabilized cells was inhibited by phosphoramidon (100 μmol/L; \( P = 0.025, n = 5 \)) but not by thiorphan (10 μmol/L) or chloroquine (30 μmol/L). A similar level of inhibition was observed for cells coincubated with phosphoramidon and chloroquine and cells treated only with phosphoramidon. Values are expressed as mean ± SEM. Significance defined by \( P < 0.05 \) was determined with a 2-sided, paired t test; \( \delta P < 0.05 \) vs big ET-1 in nonpermeabilized cells; \( P < 0.05 \) vs big ET-1 in permeabilized cells.

HUVECs and en face preparations of human coronary artery ECs were labeled with antisera raised against human vWF to ensure that the expected filamentous pattern of labeling could be detected by the silver-enhanced immunogold labeling method. Positive immunoreactivity was detected on processes on the surfaces of both EC types (Figure 1). Cells labeled with the general ECE antisera showed only a moderate level of staining, which was uniform over the entire cell. Staining with the preimmune serum was negligible. X-ray analysis of the labeled sites confirmed the presence of silver aggregates on the plasma membrane (not shown).

Determination of Subcellular Big ET-1 and ECE Expression in HUVECs

HUVECs were permeabilized to allow access of antibodies to subcellular structures. The cells were optically sectioned by using confocal microscopy to reveal the intracellular localization of ECE-1α–, ECE-1β–, and big ET-1–like immunoreactivity. Only a moderate level of ECE-1α–like immunofluorescence staining was observed over the plasma membrane (Figure 3), consistent with the findings obtained by scanning electron microscopy. A diffuse, punctate pattern of staining was detected within the cytoplasm. Intense labeling was also observed in the perinuclear region and in Weibel-Palade bodies located in the cytoplasm beneath the plasma membrane. Cells doubly labeled with antisera to ECE-1α or ECE-1β and vWF showed colocalization of immunoreactive staining within Weibel-Palade bodies. Optical sectioning through the cell nucleus revealed no positive staining in this region. Big ET-1–like immunoreactivity was detected in the perinuclear region and within some but not all Weibel-Palade bodies. Cells labeled with the preimmune serum showed negligible staining. When cells were labeled with ECE-1α antisera/FITC and viewed by using the Texas red channel, no breakthrough was detected. Similarly, no breakthrough into the FITC channel was detected when cells were labeled with vWF antiserum/Texas red (not shown).

To further characterize ECE-1α staining in the perinuclear region, cells were doubly labeled with antisera raised against ECE-1α and P-selectin, a cell adhesion molecule that is released by both constitutive and regulated pathways and is expressed in the endoplasmic reticulum and Golgi complex. ECE-1α and P-selectin were colocalized in the perinuclear region, indicating expression of ECE-1 in the endoplasmic reticulum/Golgi complex, and in Weibel-Palade bodies and cytosolic granules, consistent with the dual-pathway transport for the converting enzyme (Figure 4).

Localization of ECE in Weibel-Palade Bodies in the Human Coronary Artery Endothelium by Immunoelectron Microscopy

Ultrathin sections of human coronary artery were labeled with antisera raised against the N-termini of the ECE-1α and ECE-1β sequences. Weibel-Palade bodies were positively identified by profile size (usually ~0.2 μm in diameter and 2 to 3 μm long), shape (round or rodlike, depending on the plane of section), the presence of a single limiting membrane, and positive immunoreactive staining to antisera raised against human vWF. Occasionally, distinct tubules were apparent within the dense matrix of the storage granules. Double immunolabeling showed colocalization of antisera against the ECE isoforms and vWF in some but not all Weibel-Palade body profiles (Figure 5).

Evidence for the Release of ET via the Regulated Secretory Pathway

We tested the possibility that ET is released via the regulated secretory pathway. Cultured HUVECs treated with the cal-
Cadmium ionophore A23187 showed a marked increase in culture supernatant ET levels as measured by radioimmunoassay (basal release of ET, 44.1 ± 4.67 pmol/L; stimulated release of ET, 194.3 ± 50.2 pmol/L; mean ± SEM; n = 7, P = 0.03; 2-sided, paired t test). Neither ECE-1α nor ECE-1β was detected in the supernatant from untreated cells or from cells treated with the ionophore as measured by ELISA (not shown). In contrast to the stimulatory effect of A23187, no increase in ET release was observed after exposure of the cells to compound 48/80, ATP, or thrombin.

Figure 3. Confocal microscopy of permeabilized HUVECs immunolabeled with antisera raised against vWF, big ET-1, and the N termini of the ECE-1α and ECE-1β sequences. Cells labeled with the ECE-1α antibody and a secondary fluoresceinated goat anti-rabbit antibody showed positive immunofluorescence staining over the perinuclear region (PN) and Weibel-Palade bodies (WP) (A). Only a moderate level of immunoreactive staining was detected over the plasma membrane (PM). Cells incubated with preimmune serum showed negligible staining (B). Cells were doubly labeled with antisera to ECE-1α (C), ECE-1β (F), big ET-1 (I), and vWF (D, G, and J). Electronic overlay of images to vWF and ECE-1α (E), ECE-1β (H), or big ET-1 (K) revealed colocalization in WP (yellow). Bar = 70 μm (A and B) and 40 μm (C to K).
Discussion

In the present study we examined the ultrastructural localization of ECE in human ECs. The possibility that the regulated secretory pathway might be involved in the processing of big ET-1 to ET-1 and in the subsequent stimulated release of the mature peptide was investigated.

ECE-like immunoreactive staining was detected over the EC plasma membrane, suggesting that ECE may in part act as an ectoenzyme to cleave circulating big ET-1. This is supported by in vivo experiments showing production of a small, phosphoramidon-sensitive increase in plasma immunoreactive ET levels after infusion of big ET-1 into the human brachial artery. However, staining over the plasma membrane was weak, and most immunoreactivity appeared to be associated with intracellular compartments. Conversion of exogenously added big ET-1 to the mature peptide in cultured HUVECs was markedly enhanced when the cells were permeabilized with Triton X-100. This enzymatic activity was inhibited by phosphoramidon, an inhibitor of ECE and NEP, but was not inhibited by thiorphan, an NEP-selective inhibitor, or chloroquine, a lysosomal inhibitor. These findings indicate the involvement of an intracellular ECE.

Whereas we found predominant association of ECE-1-like immunoreactivity within intracellular compartments, converting enzyme activity has been attributed primarily to the expression of a plasma membrane–bound enzyme. This discrepancy is unlikely to be explained by low expression of a high-efficiency converting enzyme on the plasma membrane, since cell surface conversion of exogenous big ET-1 has been reported to be less efficient than intracellular conversion of endogenous big ET-1.

Interestingly, the plasma membrane fraction that displayed high ECE activity contained galactosyltransferase activity, a marker for the secretory (trans) side of the Golgi complex. Weibel-Palade bodies are derived from the trans-Golgi network, and it is therefore conceivable that some of the enzymatic activity attributed to plasma membrane–bound ECE could result from a converting enzyme that is associated with the storage granules.

Secretory proteins are sorted at the trans-Golgi network for intracellular transport to various post-Golgi destinations. The regulated secretory pathway involves translocation of proteins to storage granules that are mobilized to the cell surface after an appropriate stimulus. The localization of ECE and big ET-1 in EC-specific storage granules called Weibel-Palade bodies indicates that the regulated pathway may be important in the processing of big ET-1. The pH optimum for ECE-1 is between 6.7 and 6.9, indicating that the storage granules should be weakly acidic if this site is involved in the enzymatic conversion of the peptide precursor. Although the internal pH of Weibel-Palade bodies has not been determined to our knowledge, other storage granules, e.g., granules in parotid acinar cells, have previously been characterized and found to be weakly acidic (internal pH of 6.8). We therefore speculate that big ET-1 is translocated from the trans-Golgi network into cell storage granules and is converted to ET-1 by both ECE-1α and ECE-1β. This hypothesis is supported by the localization of big ET-1–like immunoreactive staining in HUVEC Weibel-Palade bodies (present study) and by our recent findings showing the localization of ET-like immunoreactivity within storage granules in human coronary artery endothelium.

We examined whether ET was released from the storage granules via the regulated secretory pathway. HUVECs were stimulated with A23187, a calcium ionophore that causes degranulation of EC storage granules by mediating an influx of extracellular calcium. ET was detected in the culture supernatants after stimulation with the ionophore, thus indicating rapid release of the peptide from storage granules. However, the concentration of ionophore used in these experiments was high, and we cannot exclude the possibility that this is a nonspecific effect. Indeed, other stimuli reported to degranulate Weibel-Palade bodies, such as compound 48/80 and thrombin, did not increase ET levels above those in nonstimulated controls. The nonresponsive nature of the cells to these compounds is unclear, although variability has been reported in release of vWF from Weibel-Palade bodies after short-term exposure of HUVECs to ATP. The maximal response to ATP varies from only 24% to 938% above nonstimulated controls. In addition, although compound 48/80 was shown to stimulate degranulation of toad aortic EC storage granules, no such degranulation was detected in HUVECs.

In the present study ECE-1α was colocalized with P-selectin in the perinuclear region of HUVECs. P-Selectin is a well-characterized cell adhesion molecule that is located in the endoplasmic reticulum and Golgi cisternae and is released via the constitutive and regulated secretory path-
ways. The colocalization of ECE-1α and P-selectin in small, punctate vesicles and in larger, rod-shaped storage granules indicates that processing of big ET-1 may occur via both the constitutive and regulated pathways. Transport of ET via the constitutive pathway is supported by the detection of immunoreactive ET, big ET-1, and ECE activity in bovine aortic EC secretory vesicles.7,15

Under normal physiological conditions, ET and NO are constitutively released by the endothelium and provide a balance between vasoconstrictor and vasodilator activity. However, vascular injury can compromise EC integrity and cause reduced NO synthesis and overproduction of ET-1. Release of ET and vWF36 via the regulated secretory pathway may therefore provide an initial hemostatic response to vascular EC damage.

ET-1 is a potent vasoconstrictor and comitogen and is therefore a potential therapeutic target in the effective management of cardiovascular diseases, including atherosclerosis, coronary vasospasm, and congestive heart disease.37–39 The development of selective inhibitors of ECE may be beneficial in reducing adverse hemodynamic effects and the migration and proliferation of vascular smooth muscle cells by inhibiting conversion of big ET-1 to the vasoactive peptide. We conclude that both ECE-1α and ECE-1β are located in the human endothelium within the perinuclear region and in EC-specific storage granules.

Figure 5. Representative electron photomicrographs showing double immunolabeling in Weibel-Palade body (WP) profiles from human coronary artery ECs. Anti-vWF and ECE-1α-like immunoreactivity (arrowheads; A and B) or ECE-1β-like immunoreactivity (arrowheads; C) were colocalized in some but not all WP profiles. Primary antibodies were detected with goat anti-mouse IgG/10-nm gold (vWF) and goat anti-rabbit IgG/5-nm gold (ECE). N indicates nucleus; PM, plasmalemmal membrane. Bar=0.2 μm.
called Weibel-Palade bodies, and so an effective inhibitor of ECE will be one that can readily penetrate the EC plasma membrane and access these intracellular compartmants.

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