Journal of Cellular Biochemistry 53:352–359 (1993)

Regulation of Angiotensin I-Converting Enzyme in Cultured Bovine Bronchial Epithelial Cells

Günther Müns, Jamboor K. Vishwanatha, and Israel Rubinstein

Department of Internal Medicine, Pulmonary and Critical Care Medicine Section, University of Nebraska Medical Center, Omaha, Nebraska 68198 (G.M., I.R.); Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska 68198 (J.K.V.); and Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, Nebraska 68198 (G.M., I.R.)

Abstract The purpose of this study was to determine whether angiotensin I-converting enzyme (ACE) is present in cultured bovine bronchial epithelial cells (BBECs) and whether its activity can be modulated. We found that extracts of confluent monolayers of cultured BBECs degraded [glycine-1-^{14}C]hippuryl-L-histidyl-L-leucine at a rate of 843 ± 66 pmol/hr/mg protein (mean ± SEM, n = 5). In addition, we found that the enzyme was shed into the culture medium. ACE activity in BBECs was inhibited by three selective, but structurally different, ACE inhibitors (captopril, quinapril, and cisalaprilat) with an IC_{50} of approximately 2 nM. Increasing chloride concentration in the assay buffer resulted in an increase in BBECs ACE activity of 63%. Enzyme activity was also modulated by the presence of zinc cation in the assay buffer. Addition of dexamethasone to the culture medium was associated with a significant increase in BBECs ACE activity ($P < 0.05$), which was inhibited by the steroid receptor antagonist RU 38486. Western blot analysis of BBECs, tracheal and bronchial mucosal strips utilizing a cross-reacting rabbit anti-mouse ACE antibody, showed a faint 175 kDa band and additional strong 52 kDa and 47 kDa band. The mechanism of generation of the low M.W. bands is unknown. Our data indicate the presence of ACE in cultured BBECs and that enzyme activity can be modulated.

Key words: airway, bronchi, peptidases, proteinase inhibitors, dexamethasone, inflammation, zinc, chloride, RU 38486

The glycosylated, zinc-containing, membrane-bound angiotensin I-converting enzyme (ACE, EC 3.4.15.1, Kininase II) is a chloride-dependent peptidyl dipeptidase that has been localized in endothelial cells and macrophages [Ryan et al., 1976; Sugiyama et al., 1988]. It is postulated that the major physiological role of ACE in the lung is to hydrolyze circulating bradykinin, a potent proinflammatory mediator [Bhoola et al., 1992].

Angiotensin I-converting enzyme activity has also been detected in bronchoalveolar lavage fluid (BALF) obtained from laboratory animals and humans [Specks et al., 1990]. However, the cellular origin of ACE in BALF as well as its physiological significance are currently unknown. It is conceivable that epithelial cells that line the airway mucosa express ACE, which then can be retrieved by BALF. Thus, the purpose of this study was to determine whether ACE is present in cultured bovine bronchial epithelial cells and whether its activity can be modulated.

Received April 12, 1993; revised and accepted August 2, 1993.

Address reprint requests to Günther Müns, University of Nebraska Medical Center, 600 South 42nd Street, Omaha, NE 68198.

METHODS

ACE Activity in Cultured Bovine Bronchial Epithelial Cells (BBECs)

Lungs from cows were obtained from a local slaughterhouse. Bovine epithelial cells were harvested freshly from subsegment bronchi by protease digestion as previously described [Wu et al., 1982; Takizawa et al., 1990]. Contamination with endothelial cells has been found to be nonexistent with this technique. The cells were seeded in 35 mm 6-well tissue culture plates (Becton Dickinson, Lincoln Park, NJ) at a density of 1×10^6/dish in serum-free LHC-9/RPMI 1640 medium (1:1) containing 50 U/ml penicillin, 50 μg/ml streptomycin, and 1.25 μg/ml fungizone [Lechner and LaVeck, 1985]. The cells were incubated at 37°C and 5% CO_2: 95% air and

cultured to different stages of confluence (3 to 7 days). Then the cells were scraped in phosphate-buffered saline (PBS) and centrifuged at 1,500 rpm for 10 min. The cell pellet was resuspended in homogenization buffer consisting of 50 mM HEPES, 0.3 M NaCl, 0.5% Triton X-100, and 1 μM $ZnCl_2$ (pH = 7.4) and homogenized using a Branson sonifier five times for 10 s at 4°C. The homogenates were centrifuged at 3,500 rpm for 10 min in an Eppendorf microcentrifuge. The supernatant was then subjected to ultracentrifugation in a Beckman type 65 rotor at 34,000 rpm for 30 min at 4°C. The resulting supernatant was collected to measure tissue and BBECs ACE. Culture medium was aspirated at different days of culture, centrifuged at 1,500 rpm to remove cell debris, resuspended, precipitated with $(NH_4)_2SO_4$ at 60% saturation, and dialyzed against the assay buffer.

In another series of experiments, bovine tracheas and bronchi (mainstem to 4th generation) were dissected free from the lungs, and adherent connective and alveolar tissues were removed. Small airway mucosal strips were peeled off with a forceps. The tissues were then rinsed with PBS and minced in the homogenization buffer, sonicated, and further processed as described above.

Protein concentration in tissue and cell homogenates was determined as previously described [Smith et al., 1985] utilizing a bicinchoninic acid assay (BCA). ACE activity in the supernatants was measured using a modification of a radiometric assay published elsewhere [Rohrbach, 1978]. Briefly, [glycine-1-^{14}C]hippuryl-L-histidyl-L-leucine (specific activity: 3.0 mCi/mmol; Amersham, Arlington Heights, IL) was used as substrate. All reagents were made up in the assay buffer consisting of 50 mM HEPES, 0.3 M NaCl, and 1 μM $ZnCl_2$ (pH = 7.4). The assays were performed in duplicate, using 50 μl of cell or tissue extracts and 1 μl of substrate. To ensure good reproducibility of the ACE activity, the protein concentration in the assay was maintained at <2 mg/ml. Reaction mixtures were incubated for up to 720 min at 37°C; then the reaction was stopped by adding 50 μl of 2 N HCl. To extract the cleaved substrate, [gly-1-^{14}C]hippuric acid, ethyl acetate (500 μl) was added to each tube, and the tubes were vortexed and then centrifuged for 5 min at 1,500 rpm. The radioactivity in the ethyl acetate layer (200 μl) containing the cleaved [gly-1-^{14}C]hippuric acid was then measured by a scintillation counter. Reaction tubes without cell or tissue extracts served as controls, and the background radioactivity in these tubes was subtracted from test values. Background radioactivity was around 160 cpm in all our experiments. Specific ACE activity was determined from the rate of formation of [gly-1-^{14}C]hippuric acid under conditions of initial velocity ($<5\%$ of substrate hydrolysed) and was expressed as pmol of [gly-1-^{14}C]hippuric acid released per hour per mg of protein.

Effects of Selective ACE Inhibitors on Enzyme Activity

To determine the inhibitor sensitivity of ACE activity in cultured BBECs and bovine tracheal or bronchial mucosa, we compared the effect of captopril (Sigma Chemical Co., St. Louis, MO), quinapril (Parker-Davis, Ann Arbor, MI), and cisalaprilat (Hoffmann-LaRoche, Basel, Switzerland) at concentrations of 10^{-12}, 10^{-9}, and 10^{-6} M. The inhibitors were prepared in the assay buffer each day and added 10 min prior to the substrate.

Effects of Chloride Anion Concentration on ACE Activity

To determine the effects of chloride anion concentration in the assay buffer on degradation of [Gly-1-^{14}C]Hip-L-His-L-Leu by cultured BBECs and bovine tracheal or bronchial mucosa, enzymatic assays were carried out in both the absence and presence of sodium chloride (0.15, 0.3, 0.6, 0.9, and 1.2 M).

Effects of Zinc Concentration on ACE Activity

The presence of zinc (Zn) at the two active sites of the enzyme is a salient characteristic of ACE. To determine the effect of Zn on ACE activity, cells were grown in Zn-free LHC-9/RPMI 1640 medium (1:1), and ACE activity was compared to that of cells grown in medium supplemented with 1.5 μM $ZnSO_4$. ACE activity was determined in Zn-free and Zn-containing assay buffer (1 μM $ZnCl_2$).

Effects of Steroids on ACE Activity

Steroids are known to upregulate ACE activity in endothelial cells. We incubated BBECs from the first day of culture with increasing concentrations of dexamethasone (0.1 nM, 0.5 nM, 1 nM, 10 nM, 1 μM, and 100 μM).

Dexamethasone (Sigma Chemical Co., St. Louis, MO) was dissolved in absolute ethanol

and LHC-9/RPMI 1640 (1:1) culture medium depleted of hydrocortisone (0.1 μM) and triiodothyronine (5 nM) to yield a stock solution containing 2% ethanol. The stock solution was diluted to a final concentration of 1:10. In preliminary experiments we determined that ethanol at a concentration of 0.2% had no effect on cell viability and ACE activity. Cells cultured without dexamethasone served as control.

In a second series of experiments, BBECs were grown to confluency in the same hydrocortisone- and triiodothyronine-depleted medium. Then we added either dexamethasone (1 μM), 1,3,5(10)-estratrien-3,17β-diol (1 μM; Steraloids Inc., Wilton, NH), or the steroid receptor antagonist RU 38486 (0.5, 2.5, and 5.0 μg/ml; Roussel-UCLAF, Paris, France) together with dexamethasone.

Western Blot Analysis of Cell and Tissue Extracts

Partially purified extracts were used for the Western blot analysis. Bovine tracheal mucosa and lungs were cut into small pieces and suspended overnight in a protein extraction buffer at 4°C as previously described [Kumble et al., 1992]. BBECs were scraped in PBS and subjected to the same extraction conditions.

The crude extract was centrifuged at 1,500 rpm for 10 min to remove cell debris. The protein that precipitated from the resulting supernatant between 40% and 70% saturation with $(NH_4)_2SO_4$ was resuspended in PBS and dialyzed against several changes of this buffer. Protein concentration and ACE activity were measured, and the enzyme solutions were used for Western blot analysis (SDS-PAGE, 3% stacking gel, 10% running gel). Polyclonal rabbit anti-mouse ACE antiserum was kindly donated by Dr. Bernstein, Emory University, Atlanta, GA, and has previously shown to crossreact with bovine ACE [Shai et al., 1992]. Dilutions of 1:100 were used. ^{125}I-labeled Protein A (NEN, Boston, MA) served as the secondary antibody.

In a second series of experiments, eight proteinase inhibitors were added to all buffers to prevent degradation of ACE: Bestatin (10^{-5} M; Peninsula Laboratories, Belmont, CA), leupeptin (10^{-5} M; Peninsula Laboratories, Belmont, CA), phosphoramidon (10^{-6} M; Peninsula Laboratories, Belmont, CA), soybean trypsin inhibitor (100 μg/ml; Sigma Chemical Co., St. Louis, MO), aprotinin 30 μg/ml; Sigma Chemical Co., St. Louis, MO), chymostatin (50 μg/ml; Sigma Chemical Co., St. Louis, MO), bacitracin (100 μg/ml; Sigma Chemical Co., St. Louis, MO), and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (10^{-5} M, Calbiochem; La Jolla, CA).

TABLE I. Angiotensin I-Converting Enzyme Activity in Cultured BBECs and in Culture Medium (in Parenthesis) at Different Days of Culture

Day	ACE-activity pmol/hr/mg protein[a]	N
0[b]	120 ± 29* not done	5
3	695 ± 45 (281 ± 23)	9 (3)
5	843 ± 66 (334 ± 35)	15 (3)
7	760 ± 63 (301 ± 29)	9 (3)

[a]ACE activity in culture medium is based on protein concentration of serum-free medium
[b]After protease digestion, before plating. Values are means ± SEM.
*$P < 0.01$.

TABLE II. Angiotensin I-Converting Enzyme Activity in Bovine Tracheal and Bronchial Mucosal Strips at Different Locations and in Bovine Lung

Location	ACE-activity pmol/hr/mg protein[a]	N
Trachea	245 ± 60	5
Bronchus		
mainstem	197 ± 38	5
first division	280 ± 65	3
second division	235 ± 55	3
third division	277 ± 70	3
fourth division	297 ± 83	3
Lung	2,347 ± 357	5

[a]Values are means ± SEM.

Statistical Analysis

All data are expressed as means ± SEM. Statistical analysis was performed using one-way analysis of variance and Newman-Keuls test for multiple comparisons. A P value less than 0.05 was considered significant.

RESULTS

ACE Activity in Cultured BBECs

[Gly-1-^{14}C]Hip-L-His-L-Leu was hydrolyzed by extracts from cultured BBECs at different stages of confluence, from culture medium, and from bovine tracheal and bronchial mucosa hydrolysates (Tables I and II). ACE activity was highest when cells reached confluency, usually between days 4 and 5, and lowest immediately after the protease digestion on day 0. ACE activity was

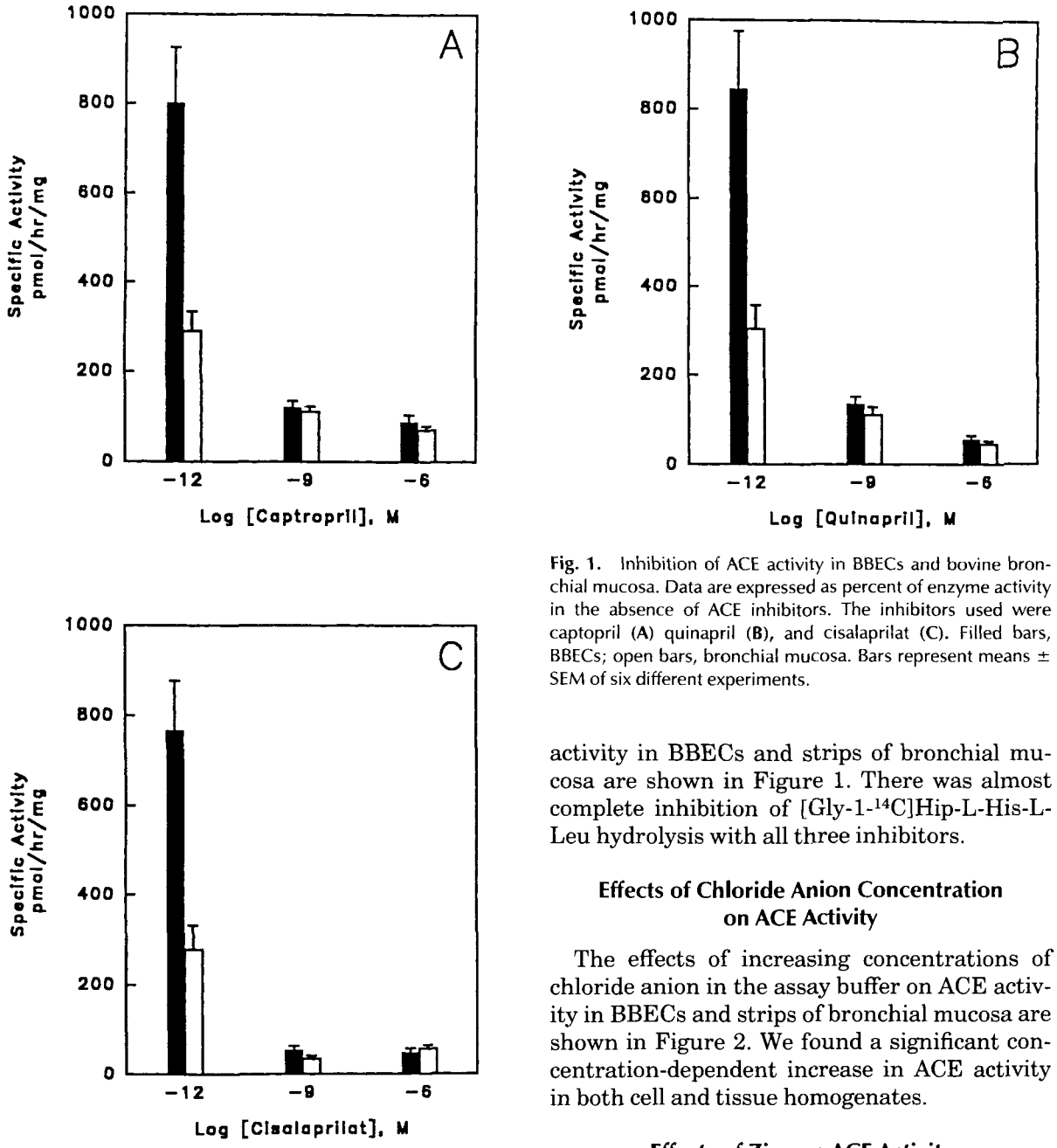

Fig. 1. Inhibition of ACE activity in BBECs and bovine bronchial mucosa. Data are expressed as percent of enzyme activity in the absence of ACE inhibitors. The inhibitors used were captopril (A) quinapril (B), and cisalaprilat (C). Filled bars, BBECs; open bars, bronchial mucosa. Bars represent means ± SEM of six different experiments.

detected in the culture medium (Tables I and II). However, it was only approximately 40% of the activity found in BBECs in any corresponding day of culture. ACE activity was similar in various segments of the tracheobronchial tree studied and approximately 10% of those of bovine lung (Table II).

Effects of Selective ACE Inhibitors on Enzyme Activity

The effects of the selective ACE inhibitors, captopril, quinapril, and cisalaprilat on ACE activity in BBECs and strips of bronchial mucosa are shown in Figure 1. There was almost complete inhibition of [Gly-1-^{14}C]Hip-L-His-L-Leu hydrolysis with all three inhibitors.

Effects of Chloride Anion Concentration on ACE Activity

The effects of increasing concentrations of chloride anion in the assay buffer on ACE activity in BBECs and strips of bronchial mucosa are shown in Figure 2. We found a significant concentration-dependent increase in ACE activity in both cell and tissue homogenates.

Effects of Zinc on ACE Activity

The effects of zinc in the assay buffer on ACE activity in cultured BBECs is shown in Figure 3. We found that BBECs cultured in zinc-free medium exhibited a three-fold decrease in ACE activity in comparison to control when the assay was performed in the absence of zinc in the buffer. However, in the presence of zinc in the assay buffer, ACE activity in these cells was similar to that of control. Finally, ACE activity in BBECs cultured in zinc-free medium and assayed in zinc-supplemented buffer was similar to that of control.

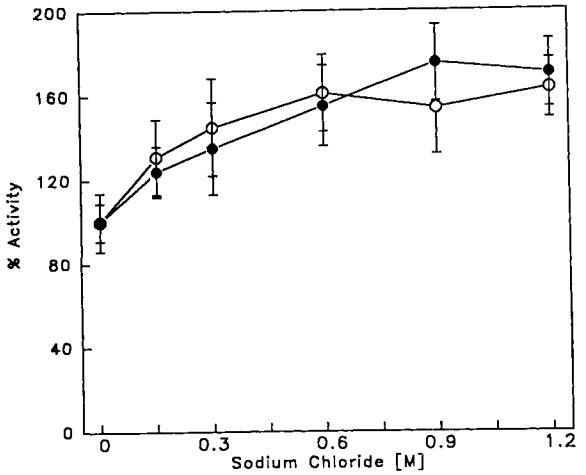

Fig. 2. Effects of chloride anion concentration in the assay buffer on BBECs and bovine bronchial mucosal ACE activity. Data are expressed as percent of enzyme activity in the absence of sodium chloride. Open symbols, BBECs; filled symbols, bronchial mucosa. Symbols represent means ± SEM of six different experiments.

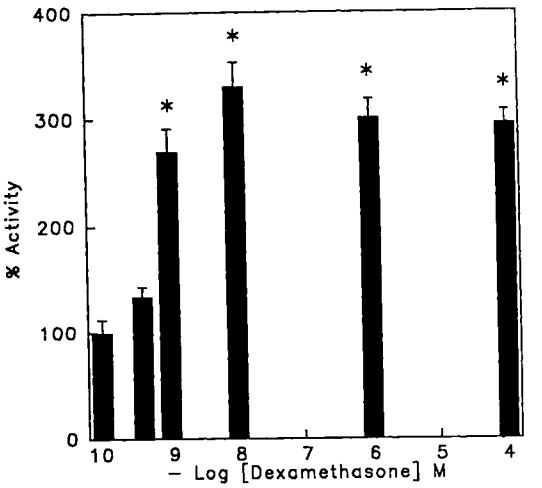

Fig. 4. Effects of dexamethasone on ACE activity. BBECs were cultured in medium supplemented with increasing concentrations of dexamethasone to confluency (day 5). ACE activity and total protein concentration were then determined. Results are expressed as percent of enzyme activity in the absence of dexamethasone (control). The bars represent means ± SEM of five different experiments (*$P < 0.05$).

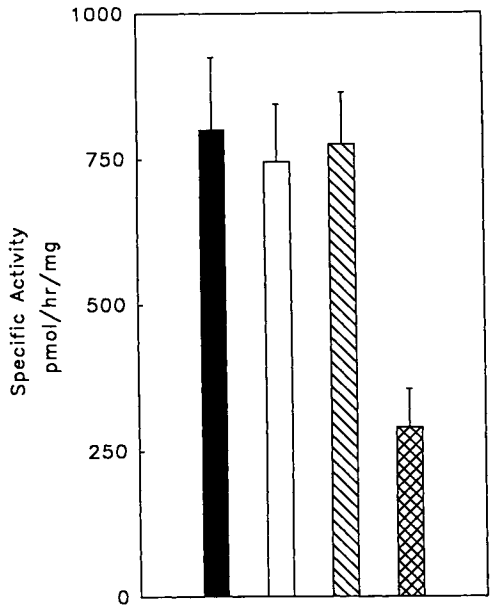

Fig. 3. Effects of zinc concentration on ACE activity of cultured BBECs. (See text for explanations.) The bars represent the means ± SEM of three different experiments. Solid bar, cells cultured in zinc-supplemented medium and ACE assay performed in zinc-containing buffer; open bar, cells cultured with zinc, ACE activity determined in zinc-depleted buffer; hatched bar, cells grown in zinc-free medium, assay buffer contained zinc; crosshatched bar, culture medium and assay buffer free of supplemental zinc.

Effects of Steroids on ACE Activity

The effects of dexamethasone on ACE activity in BBECs is shown in Figure 4. We found that dexamethasone induced a significant, concentration dependent increase in ACE activity in cultured BBECs ($P < 0.05$). The maximal effect was observed at a concentration of 10^{-8} M.

The steroid receptor antagonist, RU 38486, inhibited dexamethasone-induced responses in a concentration-dependent fashion (Fig. 5). The estrogen, 1,3,5(10)-estratrien-3,17β-diol, had no significant effect on ACE activity in cultured BBECs (data not shown).

Western Blot Analysis of Tissue and Cell Extracts

Western blot analysis of partially purified tissue extracts showed a weakly cross-reacting protein in the 175 kDa range with all examined extracts (Fig. 6). While the appearance of the 175 kDa band is variable in different tissues as well as in different preparations from the same tissue, we always see strongly cross-reactive 52 and 47 kDa bands. This was not due to unspecific IgG binding proteins in the crude extract, for incubation with non-immune rabbit antiserum (25 μg) instead of primary antibody failed to show these bands, as did incubation with ^{125}I-labeled protein A in the absence of primary antibody (not shown).

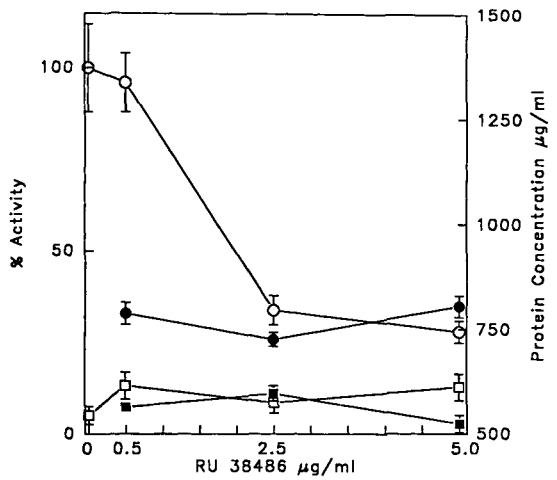

Fig. 5. Effects of RU 38486 on dexamethasone induction of ACE activity. Confluent BBECs were treated with increasing concentrations of RU 38486 in presence and absence of 1 μM dexamethasone. After 48 h, the cells were washed and sonicated and ACE activity and total protein concentration were determined. Results are expressed as percent of enzyme activity in the absence of RU 38486 and represent means ± SEM of five different experiments. Filled circles, RU 38486 alone; open circles, RU 38486 and dexamethasone. Filled and open squares are the protein concentrations of BBECs exposed to RU 38486 alone and to RU 38486 together with dexamethasone, respectively.

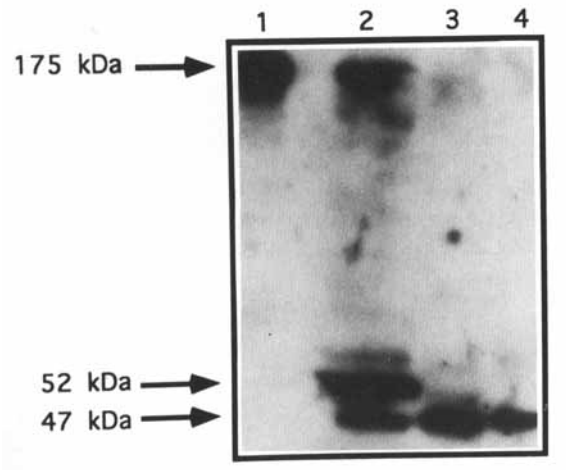

Fig. 6. Western blot analysis of protein extract of cultured BBECs and bovine tracheal mucosal strips. Rabbit anti-mouse ACE antiserum (1:100) was used as the primary antibody. Lane 1, purified rabbit ACE (5 μg; Sigma Chemical Co., St. Louis, MO); lane 2, bovine lung extract; lane 3, BBECs extract; lane 4, tracheal mucosal strip extract. Lanes were loaded with 200 μg protein.

The addition of a cocktail of proteinase inhibitors to all buffers did not result in the disappearance of the 52 and 47 kDa bands, indicating that the production of these bands is a physiological event and not one of non-specific proteolysis

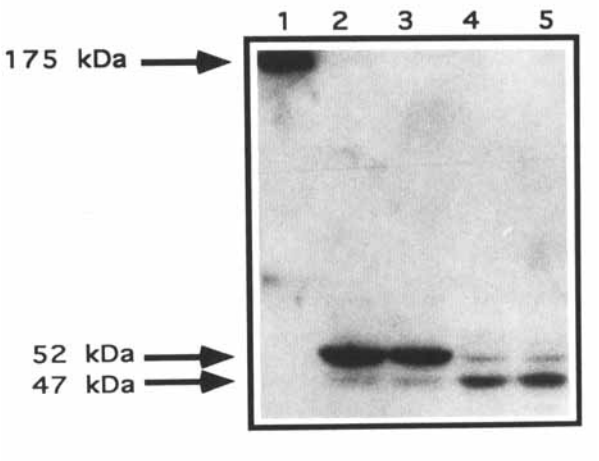

Fig. 7. Western blot analysis of protein extracts from BBECs and bovine lung in the presence of proteinase inhibitors. Lane 1 (control), rabbit ACE (5 μg); lane 2, lung tissue; lane 3, lung tissue with proteinase inhibitors; lane 4, BBECs; lane 5, BBECs with proteinase inhibitors (see text for proteinase inhibitors used in these experiments). The 175 kDa band is almost not detectable. Lanes were loaded with 200 μg protein.

during tissue handling and protein extraction (Fig. 7). The mechanism how these low M.W. bands are generated is not known.

DISCUSSION

The results of the present study show that cultured BBECs and strips of bovine bronchial and tracheal mucosa express ACE activity. In addition, we found that cultured BBECs release ACE into the culture medium. Enzyme activity was upregulated by dexamethasone and modulated by chloride and zinc in the assay buffer. Finally, Western blot analysis of BBECs and tracheal and bronchial mucosal strip homogenates confirmed the presence of ACE in bovine airway epithelial cells. Collectively, these data indicate that ACE is present in cultured BBECs and that its activity can be modulated.

ACE activity in tissue homogenates of human fetal airway was found in a previous study [Johnson et al., 1985]. However, this group did not localize ACE activity to the airway mucosa. It is well established that significant ACE activity is present in endothelial cells and tissue macrophages. It is conceivable, therefore, that ACE activity measured in the airway in their investigation was partially derived from these cells. The results of the present study clearly indicate the presence of ACE in cultured BBECs. In addition, we found that cultured BBECs shed ACE into the culture media. The latter observation may explain, in part, the source of ACE

activity in BALF obtained from laboratory animals and humans. The results of this study do not allow us to examine the mechanisms that mediate shedding of ACE from cultured BBECs.

It is well established that ACE activity is modulated by chloride anion concentration. Human ACE enzyme contains two independent catalytic domains, N and C, that have different chloride activation profiles [Wei et al., 1991]. The N domain is much less chloride dependent than the C domain. These observations suggest that the N and C domains may also have different substrate specificities and sensitivities to ACE inhibitors, for hydrolysis of bradykinin is known to be less chloride dependent than that of angiotensin I [Skidgel and Erdös, 1993]. The results of the present study clearly show that ACE activity in cultured BBECs is also modulated by chloride anion. Whether chloride anion also modulates ACE activity in airway epithelial cells in situ remains to be established.

The results of the present study confirm previous observations that zinc is required for ACE activity [Henning et al., 1992]. In addition, we found that the biologic expression of ACE activity in cultered BBECs does not require the presence of zinc in the culture media.

Previous studies showed that corticosteroids upregulate ACE activity in cultured endothelial cells [Mendelsohn et al., 1982; Dasarthy et al., 1992]. The results of the present study confirm these observations in cultured BBECs. In addition, we found that the effects of dexamethasone were specific, for they were inhibited by the steroid receptor antagonist, RU 38486, and that an estrogen derivative had no effect on BBECs ACE activity. The physiological significance of dexamethasone-induced upregulation of ACE activity in cultured BBECs is unclear at this stage. However, this may serve to protect airway epithelial cells from the deleterious effects of bradykinin when local concentrations of the peptide are increased [Christiansen et al., 1992].

The physiological role of ACE in airway epithelial cells was not investigated in the present study. It is conceivable that ACE may regulate the kallikrein-kinin system in the airway mucosa by hydrolysing bradykinin. Bradykinin receptors have been localized in airway epithelial cells [Denning and Welsh, 1991; Rangachari et al., 1988]. The peptide has been shown to regulate airway epithelial cell function, such as chloride transport [Denning and Welsh, 1991], and to exert potent proinflammatory and mitogenic effects [Woll and Rosengurt, 1988; Vicentini and Villereal, 1984]. Thus, ACE may have an autocrine role in modulating airway epithelial cell function by regulating local concentrations of bradykinin. Whether airway epithelial cell ACE is also involved in regulating the metabolism of other peptides released during airway injury and inflammation, such as substance P and angiotensin I is unknown [Skidgel and Erdös, 1987, 1993].

In summary, our results show that ACE is present in cultured BBECs. In addition, we found that ACE activity in cultured BBECs is modulated by chloride, zinc, and dexamethasone. We suggest that airway epithelial ACE plays an important role in regulating cell function.

ACKNOWLEDGMENTS

We thank Leroy Tate, Randy Davis, and Mary Illig for technical assistance and Sandy Benson for editorial assistance. This study was supported, in part, by NIH grants DE-10347 and GM-46459, grant-in-aid from the American Heart Association-Nebraska Affiliate (NE-91-G-16 and NE-92-GS-6) and the Milheim Foundation.

REFERENCES

Bhoola KD, Figueroa CD, Worthy K (1992): Bioregulation of kinins: Kallikreins, kininogens, and kininases. Pharmacol Rev 44:1–80.

Christiansen SC, Proud D, Sarnoff RB, Juergens U, Cochrane CG, Zuraw BL (1992): Elevation of tissue kallikrein and kinin in the airways of asthmatic subjects after endobronchial allergen challenge. Am Rev Resp Dis 145:51–58.

Dasarthy Y, Lanzillo JJ, Fanburg BL (1992): Stimulation of bovine pulmonary artery endothelial cell ACE by dexamethasone: Involvement of steroid receptors. Am J Physiol 263:L645–L649.

Denning GM, Welsh MJ (1991): Polarized distribution of bradykinin receptors on airway epithelial cells and independent coupling to second messenger pathways. J Biol Chem 266:12932–12938.

Henning B, Wang Y, Ramasamy S, McClain CJ (1992): Zinc deficiency alters barrier function of cultured porcine endothelial cells. J Nutr 122:1242–1247.

Johnson AR, Ashton J, Schulz WW, Erdös EG (1985): Neutral metalloendopeptidase in human lung tissue and cultured cells. Am Rev Respir Dis 132:564–568.

Kumble KD, Hirota M, Pour PM, Vishwanatha JK (1992): Enhanced levels of annexins in pancreatic carcinoma cells of Syrian hamsters and their intrapancreatic allografts. Cancer Res 52:163–167.

Lechner JF, LaVeck MA (1985): A serum-free method for culturing normal human bronchial epithelial cells at clonal density. J Tissue Cult Methods 9:43–48.

Mendelsohn FAO, Lloyd CJ, Kachel C, Funder JW (1982):

Induction by glucocorticoids of angiotensin converting enzyme production from bovine endothelial cells in culture and rat lung in vivo. J Clin Invest 70:684–692.

Rangachari PK, McWade D, Donoff B (1988): Luminal receptors for bradykinin on the canine tracheal epithelium: Functional subtyping. Regulatory Peptides 21:237–244.

Rohrbach MS (1978): [Glycine-1-^{14}C]hippuryl-L-histidyl-L-leucine: A substrate for the radiochemical assay of angiotensin converting enzyme. Anal Biochem 84:272–276.

Ryan US, Ryan JW, Whitaker C, Chiu A (1976): Localization of angiotensin converting enzyme (kininase II). II. Immunocytochemistry and immunofluorescence. Tissue Cell 8:125–145.

Shai S-Y, Fishel RS, Berk BC, Bernstein KE (1992): Bovine angiotensin converting enzyme cDNA cloning and regulation. Circ Res 70:1274–1281.

Skidgel RA, Erdös EG (1987): The broad substrate specificity of human angiotensin I converting enzyme. Clin Exper Hypertension A9:243–259.

Skidgel RA, Erdös EG (1993): Biochemistry of angiotensin converting enzyme. In Robertson JIS, Nicholls MG (eds): "The Renin Angiotensin System." London: Gower Medical Publishing, pp 10.1–10.10.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985): Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85.

Specks U, Martin II, WJ, Rohrbach MS (1990): Bronchoalveolar lavage fluid angiotensin-converting enzyme in interstitial lung diseases. Am Rev Resp Dis 141:117–123.

Sugiyama Y, Yotsumoto H, Okabe T, Takaku F (1988): Measurement of angiotensin-converting enzyme activity in intact human alveolar macrophages and effect of smoking. Respiration 53:153–157.

Takizawa H, Beckmann JD, Shoji S, Claasen LR, Ertl RF, Linder J, Rennard SI (1990): Pulmonary macrophages can stimulate cell growth of bovine bronchial epithelial cells. Am J Respir Cell Mol Biol 2:245–255.

Vicentini LM, Villereal ML (1984): Serum, bradykinin and vasopressin stimulate release of inositol phosphates from human fibroblasts. Biochem Biophys Res Comm 123:663–670.

Wei L, Alhenc-Gelas A, Corvol P, Clauser P (1991): The two homologous domains of human angiotensin I-converting enzyme are both catalytically active. J Biol Chem 266: 9002–9008.

Woll PJ, Rosengurt E (1988): Two classes of antagonist interact with receptors for the mitogenic neuropeptides bombesin, bradykinin, and vasopressin. Growth Factors 1:75–83.

Wu R, Groelke JW, Chang LY, Porter ME, Smith D, Nettesheim P (1982): Effects of hormones on the multiplication and differentiation of tracheal epithelial cells in culture. In Sirbasku D, Sato GH, Pardee A (eds): "Growth of Cells in Hormonally Defined Media." New York: Cold Spring Harbor, pp 641–656.