Angiotensin I-Converting Enzyme Inhibition but Not Angiotensin II Suppression Alters Angiotensin I-Converting Enzyme Gene Expression in Vessels and Epithelia

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ABSTRACT

The concentration of angiotensin-converting enzyme (ACE) increases during chronic treatment with ACE inhibitors for unknown reasons. We investigated whether alterations in ACE mRNA and ACE concentration occur in the different tissues during ACE inhibition and the role of angiotensins in these regulations by comparing ACE inhibitors with other blockers of the renin-angiotensin system. Enalapril, an ACE inhibitor, in the range of 0.3 to 10 mg/kg/day in rats induced dose- and time-dependent increases in plasma ACE up to two to three times control values. There were significant increases in the steady state ACE mRNA in the lung (32%), duodenum (64%) and aorta (324%) and 40% to 140% increases in membrane-bound enzyme concentration in these tissues and in the heart and kidney. The ACE content of purified duodenal brush border was increased by 80%, but the enzyme and its mRNA in the testis were not altered. The angiotensin II receptor antagonist losartan at several regimens of up to 30 mg/kg twice a day for 14 days produced no change in plasma ACE level or lung ACE mRNA. The human renin inhibitor ciprokiren was tested in guinea pigs, a species sensitive to this compound. Both enalapril and ciprokiren induced 2-fold increases in plasma ACE, but ciprokiren (24 mg/kg/day for 12 days) had no effect. Enalapril treatment of BN/Kat rats (lacking circulating kininogens) caused a similar increase in ACE as in other rats. This study documents a general increase in ACE gene expression and enzyme concentration in tissues during ACE inhibition, with the exception of the testis, most probably reflecting an activation of the 5’, so-called somatic promoter of the ACE gene. Angiotensins are not involved in this regulation and do not seem to control ACE gene expression in normal rodents.

ACE (dipeptidyl carboxypeptidase I, kininase II; EC 3.4.15.1) is an ectoenzyme of vascular cells that is secreted into the plasma. It plays a major role in cardiovascular homeostasis by converting Ang I into the potent vasopressor peptide Ang II and inactivating the vasodilatory peptide bradykinin (Erdos, 1990). ACE also is a widely distributed transmembrane enzyme, found in abundance in extravascular tissues, especially in intestinal and renal tubular epithelial cells, neuroepithelial cells in the central nervous system, mononuclear cells, and male germinal cells. The physiological role of ACE in these extravascular localizations has yet to be elucidated.

This enzyme is important mainly because of its role in regulating vascular tone and blood pressure and its possible involvement in the pathogenesis of degenerative cardiovascular and renal diseases (Cambien et al., 1992, 1994, Marre et al., 1994). The pharmacological inhibition of ACE is widely used as a therapeutic approach, especially in the treatment of hypertension and congestive heart failure (Cushman and Ondetti, 1980; Lonn et al., 1994). It also seems to increase survival after myocardial infarction (Pfeffer et al., 1992; Swedberg et al., 1992). ACE inhibition is used to protect against degradation of renal function in type I diabetic patients with incipient nephropathy, and there is growing evidence that it can be beneficial in patients with established renal insufficiency of several origins (Marre et al., 1987; Lewis et al., 1993; Maschio et al., 1996).

The ACE inhibition triggers hormonal mechanisms related to Ang II suppression, such as a rise in renin synthesis and secretion with subsequent angiotensinogen consumption and a transient decrease in aldosterone secretion (Cushman and Ondetti, 1980). Kinin levels increase in kidney and perhaps also in plasma and the heart as a consequence of diminished inactivation (Margolius, 1995). An elevation in plasma ACE concentration has also been documented in humans and rats during treatment with ACE inhibitors (Larochelle et al., 1993; Lewis et al., 1993).

ABBREVIATIONS: ACE, Ang I-converting enzyme; Ang, Angiotensin; AT1 receptor, type I angiotensin II receptor.
and Sacchi, 1987), and the ACE mRNA content was determined through solution hybridization and ribonuclease protection assay using a rat ACE cRNA probe as described previously (Costerousse et al., 1994). This assay is more sensitive than Northern blot hybridization and quantifies even the small amounts of ACE mRNA present in the kidney (Costerousse et al., 1994). It also has the advantage of avoiding separation and transfer of RNA before hybridization, and results for a given cRNA probe depend directly on the quantity of input RNA without the requirement for normalization with reference mRNAs, which can themselves undergo specific alterations (Durnam and Palmer, 1983). For preparation of the single-stranded cRNA probe complementary to rat ACE mRNA, the recombinant plasmid pBTR3/PsIP/sf1 (Costerousse et al., 1994), containing a 365-bp cDNA fragment corresponding to the 3′ portion of rat ACE cDNA common to the somatic and germinal forms, was linearized by digestion with BamHI, and the 32P-labeled antisense cRNA probe was synthesized using a transcriptional protocol with T3 RNA polymerase and [α-32P]UTP (RNA transcription kit; Stratagene, Heidelberg, Germany). Solution hybridization and ribonuclease protection assay were performed with total RNA (2.5–20 μg) from rat tissues as described previously (Costerousse et al., 1994). The cRNA probe consisted of 446 bases, and its protected fragment consisted of 365 bases.

**Analyses** were also performed with Northern blot hybridization, with a rat ACE cDNA and normalized with reference to the 28S rRNA content through oligonucleotide hybridization (Barbu and Dautry, 1989) as described previously (Costerousse et al., 1994). In brief, total cellular RNA samples (10–40 μg/lane) were denatured, electrophoresed on a 1.2% agarose gel and transferred to Hybond-N membranes (Amersham, Braunschweig, Germany). The RNA blots were hybridized with 32P-labeled XTR1 rat ACE cDNA (Costerousse et al., 1994) for 16 to 18 hr at 42°C with 2 × 106 cpm/ml labeled probe added. Blots were washed first in 2× standard saline citrate/0.1% sodium dodecyl sulfate for 30 min at room temperature and then under high-stringency conditions in 0.1× standard saline citrate/0.1% sodium dodecyl sulfate for 20 min at 60°C. After exposure, the blots were reprobed with a synthesized 26-mer oligonucleotide (EugeneGen, Seraing, Belgium) complementary to a region of the 28S rRNA sequence and labeled with [γ-32P]ATP (DuPont-New England Nuclear, Boston, MA) as described previously (Barbu and Dautry, 1989). Relative changes in ACE mRNA levels were determined through laser densitometry of autoradiographs and were normalized for 28S rRNA. The advantages of using the 28S rRNA estimated by oligonucleotide hybridization, as a reference for ACE mRNA were discussed previously (Barbu and Dautry, 1989; Costerousse et al., 1994). For each organ studied, samples corresponding to control and treated animals were processed together. Results are expressed in arbitrary units corresponding to the absorbance measured with laser densitometry of autoradiography films.

Both ACE mRNA assays gave results linear with the amount of input RNA in the range tested (10–40 μg for Northern blot, 2.5–20 μg for the ribonuclease protection assay) under the present assay conditions. The two methods were further validated by comparing the results obtained for the same samples of total RNA from rat lung and duodenum from control and enalapril-treated animals; a very significant correlation was observed between results of both methods (duodenum: r = 0.88, n = 10; lung: r = 0.80, n = 10; results not shown).

**Preparation of tissue membranes and measurement of ACE activity.** All extractions were done on the organs from individual animals. Membrane fraction were prepared by homogenizing the organ at 4°C in a 20-fold excess (w/v) of 20 mM sodium phosphate buffer, pH 7.5, 0.25 M sucrose and 5 mM MgCl2. The crude homogenate was centrifuged at 600 g for 10 min at 4°C. The resulting supernatant was centrifuged at 10,000 g for 10 min at 4°C, and the supernatant was then centrifuged at 105,000 g for 1 hr at 4°C to yield the microsomal pellet containing cell membranes. This pellet was resuspended in phosphate-buffered saline containing 8 mM 3-[(3-cholamidopropyl)dimethylammonio] propane sulfonate

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**Materials and Methods**

**Animals and physiological studies.** Male Wistar rats (weight, 250–300 g) were purchased from IFFA-CREDO Breeding Laboratories (Larbresse, France). All animal procedures were conducted according to the guidelines for the care and use of experimental animals. ACE inhibitor enalapril maleate (MK421; Merck, Sharp and Dohme, Rahway, NJ) (Patchett et al., 1980) and AT1 receptor antagonist, or a renin inhibitor. The up-regulation of ACE gene expression during inhibition of the enzyme is independent of Ang II suppression because the ACE mRNA or enzyme levels were not altered by the other blockers of the renin-angiotensin system. These results suggest that angiotensins do not control ACE gene expression in normal rodents.
(CHAPS) at 4°C, sonicated (twice for 30 sec) and incubated for 1 hr at 4°C to complete membrane solubilization (Costerousse et al., 1994). Preparations were then centrifuged at 900 × g for 10 min, and the supernatants, containing the solubilized membranes, were diazoyzed to dissociate and eliminate the ACE inhibitor (see below).

The tunicae of the rat thoracic aorta were separated as described previously (Arnal et al., 1994), and membranes were prepared from the isolated media or from the media plus endothelium. Intestinal brush border membranes were prepared according to the method of Kessler et al. (1978) by scraping of the intestinal mucosa, followed by homogenization in 2 mM TrisHCl buffer, pH 7.1, and 50 mM mannitol and precipitation through treatment for 15 min at 4°C with CaCl2 (10 mM final concentration). The precipitate was centrifuged at 3,000 × g for 15 min at 4°C, and the resulting supernatant was centrifuged at 27,000 × g for 30 min. The pellet containing purified brush border membranes was resuspended in phosphate-buffered saline containing 8 mM 3-(3-cholamidopropyl)dimethylammonio)propanesulfonate as described above and then diazoyzed to eliminate the ACE inhibitor.

Plasma and membrane preparations from control and ACE inhibitor-treated rats were diazoyzed in buffer without chloride to dissociate the enzyme/inhibitor complex and eliminate the inhibitor before measurement of ACE activity. Samples were diazoyzed against 10 mM potassium phosphate buffer, pH 8.0, and 10 μM EDTA for 24 hr and against 10 mM potassium phosphate buffer, pH 8.0, for 36 hr. This procedure completely dissociates the enzyme/inhibitor complex, which has a very short half-life in the absence of chloride ions (Wei et al., 1992). The EDTA prevents reassociation of the inhibitor before its elimination through dialysis. The ACE activity in plasma and membrane preparations from control and losartan-treated rats remained stable during the dialysis procedure. Plasma ACE activity in some rats treated with enalapril was also measured before dialysis to assess the extent of ACE inhibition.

The ACE activity in plasma and membrane samples was measured through hydrolysis of the synthetic substrate p-benzoyl-glycyl-l-histidyl-l-leucine (Hip-His-Leu; Bachem A.Gs, Bubendorf, Switzerland) according to the assay conditions described by Cushman and Cheung (1971a). Substrate hydrolysis was quantified by high performance liquid chromatography (Costerousse et al., 1993). One unit of activity is defined as the amount of enzyme catalyzing the release of 1 μmol of hippuric acid/min (Cushman and Cheung, 1971a). Substrate consumption was maintained at ≤5% by using diluted samples, and activity was determined under initial velocity conditions.

Other measurements. Systolic arterial pressure was measured in conscious rats under standardized conditions routinely used in our laboratory with the use of a tail cuff and pulse transducer (BP recorder 8006; Appelex, Paris, France) after 20 min under a heating ramp at 32°C with four to six consecutive recordings. Blood pressure was measured every week 6 hr after the morning gavage. Results are presented for the day before the animals were killed. In the guinea pig, mean aortic pressure was measured with the animal under anesthesia through an aortic catheter connected to a pressure transducer just before death as reported previously (Clozel et al., 1994).

The plasma renin concentration was determined with radioimmunoassay of the Ang I generated during incubation of plasma in vitro with added rat angiotensinogen, as described previously (Ménard and Catt, 1972).

Protein concentration was measured according to the method of Bradford (1976) with bovine serum albumin as the standard.

Statistical analysis. Values are expressed as mean ± S.D. Multiple comparisons were performed by one- or two-way analysis of variance followed by Fisher’s test. The .05 level of probability was used as the criterion of significance.

Results

Effect of ACE inhibition on ACE gene expression and plasma and tissue ACE concentrations. Enalapril (0.3–10 mg/kg/day administered to Wistar rats via daily gavage for 14 days) induced a dose-related increase in plasma ACE concentration (table 1), as assessed by the enzymatic activity after removal of the inhibitor by dialysis. This increase was detected with the lowest dose of the inhibitor administered (0.3 mg/kg/day) (P < .01) and reached a maximum of more than two times the control level with doses of 3 and 10 mg/kg/day (P < .001). There was no significant difference between the effects of 3 and 10 mg/kg/day. Enalapril (5 mg/kg/day) significantly increased plasma ACE levels on the second day of treatment (to 2-fold the control level) (P < .001). Plasma ACE levels continued to increase until the seventh day of treatment (table 2). No significant difference was detected between 7 and 14 days of treatment. The effect of the inhibitor was dose dependent throughout the treatment. The plasma ACE level increased faster with the higher dose of inhibitor than with the low dose. Rats treated for 60 days with 1 mg/kg/day enalapril had higher plasma ACE levels (212.6 ± 39.1 mU/ml) than rats treated for 14 days (177.2 ± 36.7 mU/ml; n = 8 rats/group) (P < .01), whereas there was no significant difference between rats treated for 14 and 60 days with a dose of 5 mg/kg/day (14 days: 245.2 ± 52.9 mU/ml; 60 days: 235.7 ± 49.0 mU/ml; n = 8 rats/group).

Enalapril (5 mg/kg/day for 14 days) administered to BN/Kat rats (which lack low- and high-molecular-weight kininogens) also induced a large rise in plasma ACE levels (P < .001) (controls: 131.2 ± 16.1 mU/ml, n = 5; enalapril-treated rats: 357.2 ± 48.0 mU/ml, n = 5), similar to the increase in Wistar rats treated in the same way.

Wistar rats were treated with 5 mg/kg/day enalapril for 14 days to further examine the effect of ACE inhibitors on membrane-bound ACE and ACE mRNA levels in vascular and extravascular rat tissues. The pharmacological effect of the inhibitor was confirmed by a drop in the blood pressure (109 ± 12 mm Hg) below that of control animals (136 ± 4 mm Hg; n = 5) (P < .01). At the time of death 6 hr after the last gavage, the plasma ACE activity, measured before enalapril removal, was inhibited by 80% in treated animals (23.6 ± 10.5 mU/ml) compared with controls (124.4 ± 12.2 mU/ml, n = 5). However, the ACE activity measured after dialysis indicated that concentration of the enzyme was more than doubled in treated animals (P < .001) (table 3). The ACE concentration in tissue microsomal fractions, measured after dissociation of the enzyme/inhibitor complex, was significantly increased in the lung (P < .001), the richest source of vascular endothelial ACE, and in the heart (P < .05) (table 3). The increase in ACE in the aorta, after dissection of the
TABLE 2
Time-dependent effect of enalapril treatment on plasma ACE level
Wistar rats were treated with 5 mg/kg/day enalapril (n = 5 animals/group). Plasma ACE level was determined by measuring enzymic activity after dissociation of the enzyme-inhibitor complex as described in Materials and Methods. Controls vs. enalapril, P < .001. Length of the treatment significantly influenced the effect of the treatment, P < .05 (two-factor analysis of variance).

<table>
<thead>
<tr>
<th>Plasma ACE activity (mU/ml)</th>
<th>Days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td>118 ± 30</td>
</tr>
<tr>
<td>Enalapril</td>
<td>234 ± 38</td>
</tr>
</tbody>
</table>

ACE and Angiotensin Suppression

Fig. 1. Effect of enalapril treatment on ACE mRNA. Wistar rats were treated with enalapril (5 mg/kg/day) for 14 days. ACE mRNA levels were assessed by solution hybridization and ribonuclease protection assay, except in the testis, where ACE mRNA was quantified by Northern blot (see Materials and Methods). Results for enalapril-treated rats (right bar in data for each tissue) are expressed as percentage changes from the controls (left bar) and are given as mean ± S.D. * , P < .05; **, P < .001, compared with controls (n = 5 animals per group, except for aorta (n = 3)).

Fig. 2. Ribonuclease protection assay for ACE mRNA in the aorta and intestine of control Wistar rats and rats treated with enalapril. Rats were administered enalapril (5 mg/kg/day) for 14 days. The size of the labeled cRNA probe (C-) is 446 bases and that of the protected fragment 365 bases in duodenum.

Effect of an Ang II receptor antagonist. The AT1 receptor antagonist losartan was administered at doses of 1, 3, 10 or 30 mg/kg/day for 14 days by daily gavage (n = 8 animals/group) and 10 and 30 mg/kg twice a day (n = 4). No dose induced a significant change in plasma ACE level compared with controls (controls: 127.4 ± 13.6 mU/ml; 10 mg/kg losartan twice a day: 144.0 ± 9.5 mU/ml; 30 mg/kg losartan twice a day: 139.6 ± 27.6 mU/ml), whereas blood pressure was significantly lowered (P < .001) (controls: 131 ± 12 mm Hg; 10 mg/kg losartan twice a day: 105 ± 11 mm Hg; 30 mg/kg losartan twice a day: 99 ± 8 mm Hg; n = 8 animals/group). In the same experiments, the plasma ACE level of enalapril-treated animals (10 mg/kg/day) was more than doubled (fig. 3). Losartan induced a significant increase in plasma renin concentration (P < .05) (controls: 77 ± 37 ng of Ang I/ml/hr; 1 mg/kg losartan: 209 ± 147; 5 mg/kg losartan: 246 ± 142 ng of Ang I/ml/hr; n = 8 animals per group), similar to the increase in animals treated with 5 mg/kg/day enalapril (224 ± 55 ng of Ang I/ml/hr).

Prolonged administration of losartan (5 mg/kg/day for 28 days) induced no significant change in plasma ACE level (controls: 102.4 ± 18.3 mU/ml; losartan: 110.5 ± 26.6 mU/ml; n = 8), whereas the plasma renin concentration was 3-fold higher than in controls (P < .01) (controls: 59 ± 23 ng of Ang I/ml/hr; losartan: 173 ± 81 ng of Ang I/ml/hr; n = 8).

The lung ACE mRNA levels in losartan-treated animals (5 mg/kg/day for 14 days) were similar to those of controls (n = 5 animals/group) (results not shown).

TABLE 3
Plasma and tissue ACE levels during enalapril treatment
Results are expressed in mUnits/mg of protein. ACE activity in plasma and membrane preparations (microsomes or purified brush border membranes (BBM)) was determined after dissociation of the inhibitor (see Materials and Methods). Animals were treated for 14 days with enalapril (5 mg/kg/day) (n = 5 animals/group).

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Lung</th>
<th>Duodenum</th>
<th>Kidney</th>
<th>Heart</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole</td>
<td>BBM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.36 ± 0.25</td>
<td>230 ± 25</td>
<td>72 ± 21</td>
<td>210 ± 54</td>
<td>17.0 ± 1.7</td>
</tr>
<tr>
<td>Enalapril</td>
<td>4.00 ± 0.41</td>
<td>317 ± 24</td>
<td>170 ± 39</td>
<td>371 ± 93</td>
<td>18.7 ± 1.7</td>
</tr>
<tr>
<td>P</td>
<td>.0001</td>
<td>.0005</td>
<td>.001</td>
<td>.01</td>
<td>.0001</td>
</tr>
</tbody>
</table>

N.S., not significant.
Effects of enalapril and losartan on plasma ACE concentration in Wistar rats. Rats were treated with losartan (3 or 30 mg/kg/day) or enalapril (10 mg/kg/day) for 14 days. ACE concentration was assessed by measuring ACE activity after dialysis of the samples to dissociate and eliminate the inhibitor (see Materials and Methods). Results are given as mean ± S.D. (n = 8), * P < .001.

Effect of a renin inhibitor. The effect of the human renin inhibitor ciprokiren was tested on guinea pigs, a species whose renin is inhibited by this compound (Clozel et al., 1994), because no orally active rat renin inhibitor is available.

The guinea pig plasma concentration of ACE is much higher than that of other species, but it can still be induced by ACE inhibitors. Thus, a daily dose of 1 mg/kg enalapril for 14 days increased the plasma ACE level 2.5-fold (P < .001), from 1274 ± 158 mU/ml in controls to 3175 ± 284 mU/ml after treatment (n = 5). Plasma renin activity was also increased 2.3-fold (P < .01) from 6.5 ± .6 ng of Ang I/ml/hr in controls to 14.7 ± 3.9 ng of Ang I/ml/hr after treatment (n = 5).

We compared the effects of 12-day treatment with the renin inhibitor ciprokiren (24 mg/kg/day) and the ACE inhibitor cilazapril (30 mg/kg/day) both produced decreases in blood pressure (P < .05), from 54 ± 2 mm Hg (n = 21) in controls to 39 ± 2 mm Hg (cilazapril, n = 11) or 47 ± 1 mm Hg (ciprokiren, n = 24) (Clozel et al., 1994). However, guinea pigs treated with the renin inhibitor showed no change in plasma ACE (controls: 1572 ± 266 mU/ml, n = 11; ciprokiren: 1535 ± 200 mU/ml, n = 9), whereas the plasma ACE level of cilazapril-treated animals was doubled (P < .001) (cilazapril: 2990 ± 368 mU/ml, n = 9) (fig. 4).

Discussion

The plasma ACE concentrations of rat or guinea pig increase in response to ACE inhibitors. This effect depends on the dose of inhibitor and duration of treatment, and the maximum increase can be two to three times the basal value. This magnitude is comparable to the increase in humans with therapeutic doses of inhibitor (Larochelle et al., 1979; Boomsma et al., 1981; Sassano et al., 1987; Cambien et al., 1994). The cellular source or sources of the plasma ACE secretion during ACE inhibitor treatment remain unknown, but the results, and those of others (Fyhrquist et al., 1980; King and Oparil 1992; Fyhrquist et al., 1982), suggest that the ACE biosynthesis in vascular endothelial cells, which secretes ACE, is increased after inhibition of the enzyme, and these cells may therefore participate in the increase in plasma ACE. In the lung, a rich and physiologically important source of ACE in capillary endothelial cells, the ACE mRNA level was increased together with an increase in membrane ACE level. A similar increase in ACE lung content was documented by in vitro autoradiography with radiolabeled inhibitors after lisinopril treatment (Kohzuki et al., 1991) and a rise in lung ACE mRNA was observed after 3 days of quinapril treatment (Schunkert et al., 1993). The ACE mRNA level in the aorta was also increased, and although ACE in this vessel is synthesized by both the endothelium and smooth muscle (Andre et al., 1990; Arnal et al., 1994), ACE seemed induced mainly in the endothelium. The discrepancy between a large increase in aortic ACE mRNA and a modest alteration in enzymatic activity in this vessel is probably explained by the fact that although there are equivalent amounts of ACE activity in the media and endothelium, ACE mRNA content is largely higher in the endothelium (which produces a membrane-bound ACE and the plasma-secreted enzyme) than in the media (Arnal et al., 1994, present study). Because up-regulation of ACE gene expression occurs mainly or exclusively in the endothelium, measurements of ACE mRNA are a more sensitive estimation of the phenomenon. In addition, the membrane-bound endothelial ACE is continuously released by proteolysis into the plasma, and this can minimize the consequences of variations in ACE gene expression on the level of membrane bound ACE.

This study shows that the effect of ACE inhibition on the ACE gene expression is not restricted to endothelial cells. It affects absorptive epithelial cells, which also have a high level of ACE gene expression (Sibony et al., 1993). ACE inhibitor treatment induced a large increase in membrane ACE level in the duodenum. Capillary endothelial cells of the vessels of the musculoconnective layer may be partly responsible for this increase, but there also was a large increase in the ACE content of purified duodenal brush border membranes. This was associated with an increase in the steady state level of ACE mRNA, most probably reflecting an increase in ACE gene transcription in epithelial cells, the major site of ACE gene expression in the intestine (Sibony et al., 1993). The ACE level in the rat kidney was much lower than that in lung and duodenum, as reported previously (Cushman and Cheung, 1971b; Costerousse et al., 1994). Renal
ACE was increased by ACE inhibition, but there was no significant change in the ACE mRNA level in the kidney. The kidney is a heterogeneous organ composed of many different cell types, and cortex and medulla have different sensitivities to ACE inhibitors (Song et al., 1988). There is much less ACE gene transcription in kidney compared with in the duodenum, and ACE mRNA was accurately quantified only with the ribonuclease protection assay. The low ACE mRNA concentration in the rat kidney is probably linked to both the low ACE content of the proximal tubule in this species and the slow turnover rate of the brush border membrane (Cushman and Cheung, 1971b; Costerousse et al., 1994). Because ACE mRNA represents only a very low fraction of the kidney mRNA, the lack of a detectable change in ACE mRNA does not rule out an increase in ACE gene transcription and ACE synthesis in the kidney, which is suggested by microsomal measurements.

In the heart, ACE gene expression is restricted to the coronary endothelial cells and valvular endocardium (Johnston, 1992), and ACE level is very low. Heart ACE level increased 55% during enalapril treatment, probably reflecting induction of ACE synthesis at these sites.

The testis produces the germinal form of the enzyme, translated from a shorter mRNA transcribed from a germinal specific intragenic promoter (Soubrier et al., 1988; Lattion et al., 1989; Kumar et al., 1989; Ehlers et al., 1989; Howard et al., 1990). There was no significant change in ACE mRNA or in membrane ACE during ACE inhibitor treatment. This may be because the germinal promoter of the ACE gene is not sensitive to the unknown regulatory factor or factors that activate the somatic promoter during inhibition of the enzyme. Alternately, the induction mechanism may be a local process, determined by an autocrine or a paracrine mechanism (King and Oparil, 1992; Fyhrquist et al., 1982), which is not triggered in the testis because enalapril does not readily cross the blood-testis barrier (Kohzuki et al., 1991).

Our results suggest that there is a generalized increase in ACE gene transcription and ACE synthesis in somatic cells during ACE inhibitor treatment. The physiological mechanism responsible for the upregulation of ACE gene expression during inhibition of the enzyme is unknown, but this phenomenon occurs with inhibitors of different chemical structures and in different cell types and may be an adaptive response to inhibition of the enzyme. Thus, an increase in ACE synthesis may depend on the disappearance of a product of the enzymatic reaction that normally has a negative feedback effect on ACE gene expression or on the accumulation of a substrate of the enzyme that could activate its synthesis. Because Ang II is known to down-regulate the expression of some genes (Johns et al., 1990; Lassgue et al., 1995), we hypothesized that it may down-regulate ACE gene expression and that its suppression during treatment with ACE inhibitor stimulates ACE gene transcription. Recent studies on rats showed that chronic Ang II infusion has a weak inhibitory effect on ACE gene expression in the lung, testis or brain (Berecek et al., 1992; Kohara et al., 1992; Schunkert et al., 1993), but our results with an AT1 receptor antagonist and a renin inhibitor indicate that Ang II is not involved in the physiological control of ACE gene expression and that blockade of its biological effects does not account for overexpression of the ACE gene in response to ACE inhibitor. The AT1 receptor antagonist losartan blocks the effects of Ang II, as indicated by the lowering of blood pressure and the increase in renin secretion due to the lack of negative feedback on the juxtaglomerular cells. It did not affect the plasma ACE concentration or the ACE mRNA level in tissues. The doses and regimen of losartan used in our experiments had maximal effects on blood pressure and renin secretion. It is unlikely that the increase in ACE gene expression during ACE inhibition is mediated by another type of Ang II receptor because there was no increase in plasma ACE level during renin inhibition that blocked all the biological effects of angiotensin. A number of other observations also indicate that Ang II does not regulate ACE synthesis and secretion. Plasma and tissue ACE levels are not altered in response to variations in sodium intake that induce large alterations in Ang II levels (Jackson et al., 1986). There also is no change in ACE content in endothelial cells treated with Ang I or II (Fyhrquist et al., 1982). Vascular endothelial cells bear very few Ang II receptors compared with smooth muscle cells. It has recently been observed that smooth muscle cells, which are very sensitive to Ang II, synthesize ACE, which is especially abundant in the smooth muscle of the rat aorta (Andre et al., 1990; Arnal et al., 1994). The observation that ACE is induced almost exclusively in the endothelial layer of the aorta during inhibitor treatment also argues against a role for Ang II. Thus, Ang II is not involved in the overexpression of the ACE gene induced by ACE inhibitors, and this peptide does not seem to regulate ACE gene expression and plasma levels in normal rats.

The accumulation of Ang I in response to ACE inhibitor is also probably not involved in the overexpression of the ACE gene. First, Ang I has no demonstrated specific biological effect or identified receptor. Second, treatment with the Ang II receptor antagonist losartan produced an increase in circulating Ang I much like that produced by ACE inhibition, but it did not induce ACE production.

The accumulation of another substrate of ACE, bradykinin, may have an effect. The release and autocrine action of bradykinin in vascular endothelial cells cultivated in the absence of serum have been postulated (Wiemer et al., 1991). However, as evidence against a role of bradykinin accumulation, there was no significant difference in ACE induction when Wistar and BN/Kat rats, which lack plasma high- and low-molecular-weight kininogens (Damas and Adam, 1980; Reis et al., 1985), were treated with an ACE inhibitor. These BN/Kat rats normally synthesize kininogens in the liver but are unable to secrete it because of a point mutation in the 3' portion of the mRNA sequence, affecting the sorting of the protein (Hayashi et al., 1993). High-molecular-weight kininogen binds to vascular endothelium but may also be synthesized in endothelial cells (Schmaier et al., 1988). Our results with BN/Kat rats indicate that bradykinin produced from circulating kininogen is not involved in ACE induction, but they do not completely exclude effects of the putative autocrine kallikrein-kinin system.

Finally, ACE has a wide substrate specificity and can hydrolyze many biological peptides in vitro and perhaps in vivo (Erdos, 1990; Azizi et al., 1996). Other ACE substrates or products, perhaps some still unknown, may be involved in the control of ACE gene expression. The results of the present study indicate that hemodynamic factors per se that alter shear stress and can influence the expression of a number of genes in endothelial cells probably are not involved in ACE...
induction because losartan is a renin inhibitor (and hydralazine; unpublished observations) can induce the same lowering of blood pressure as the ACE inhibitors but without altering ACE concentration or ACE gene expression. Moreover, ACE is also induced in epithelial cells, which are not sensitive like vascular endothelial cells to these hemodynamic alterations.

The physiological and pharmacological consequences of ACE induction during inhibition of the enzyme are unknown. The increase in ACE in the intestinal brush border membrane of these pigs is not seen to accompany a significant reduction in the bioavailability of inhibitors administered orally as active forms. The general increase in ACE synthesis in these cells may reduce the therapeutic effect of these drugs. In humans, in whom the plasma ACE levels are under the control of a genetic polymorphism, the rise in these levels during ACE inhibitor treatment is influenced by the ACE genotype (Cambièn et al., 1994). However, the total quantity of ACE in the body probably does not increase more than 2- to 3-fold during treatment, whereas the doses of ACE inhibitor are a large molecular excess. The increases in plasma and tissue ACE, coupled with the reduced affinity of ACE inhibitors for one of the two active sites of the enzyme (Wei et al., 1992), can nevertheless contribute to the well-documented observation of nearly normal circulating Ang II levels during chronic treatment with ACE inhibitors at distance from drug intake, when the residual circulating inhibitor level is low and Ang I levels are high (Juillerat et al., 1990).

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