Jpn. J. Pharmacol. 81, 346–352 (1999)

# The Lipophilic Properties of Angiotensin I-Converting Enzyme Inhibitors Do Not Influence Their Diffusion Through Cultured Endothelium

Walter Raasch, Andreas Dendorfer, Benedikt Ball and Peter Dominiak

*Institute of Experimental and Clinical Pharmacology and Toxicology, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany*

*Received June 28, 1999 Accepted September 14, 1999*

ABSTRACT—The background for these investigations was the discovery that formation of angiotensin II by the renin angiotensin system can take place in extravascular tissues (e.g., cardiomyocytes and neurons) and within single cells. Consequently, the question arose about whether such tissue-based systems might be differentially influenced by angiotensin I-converting enzyme (ACE) inhibitors with distinct physicochemical properties. Therefore, the aim of this study was to investigate how the membrane penetration of various ACE inhibitors depends on their lipophilia. All diacid forms of ACE inhibitors are dissociated at a pH of 7.4 and scarcely extractable into octanol (extraction coefficient $<10\%$). In contrast, the extraction coefficients of the parent substances showed marked differences in the following order of increasing lipophilia: enalapril=perindopril<captopril=ceranapril<ramipril<quinapril<HOE288=zofenopril<fosinopril<HOE065. For selected substances, the kinetics of diffusion through a monolayer of cultured bovine aortic endothelium were determined. The diffusion rates (expressed as half lives) of captopril (59.6 min), enalapril (53.4 min), enalaprilat (50.8 min), ramipril (56.9 min) and ramiprilat (51.1 min) are similar indicating: 1) that penetration is independent on lipophilia and 2) that endothelium constitutes no specific barrier for the passage of ACE inhibitors into the vessel wall.

*Keywords*: Lipophilic property, Angiotensin I-converting enzyme (ACE), ACE inhibitor, Membrane penetration, Endothelium

It is well documented that the angiotensin I-converting enzyme (ACE) inhibitors differ only moderately with respect to their pharmacodynamic efficacies (for a review, see 1). They reduce blood pressure by inhibiting ACE, which metabolizes angiotensin I (Ang I) to angiotensin II (Ang II) and bradykinin to inactive fragments (2). Ang II increases blood pressure 10 times more compared to noradrenaline (3). Both mechanisms contribute to the hypotensive actions of ACE inhibitors. Initially, it was assumed that Ang II is generated only in the circulation after liberation of renin from the juxtaglomerular cells of the kidneys (4). Recent studies have localized renin angiotensin systems in a variety of organs, such as lung, heart, blood vessels, adrenal gland and brain. Most protective actions of ACE inhibitors were suggested to be due to an inhibition of ACE in specific tissues (5). The efficacy of ACE inhibitors depends on various pharmacokinetic properties such as the capability of the prodrugs or the active metabolites to penetrate through the endothelium. This property is related to the size of the molecules and their lipophilia. Because the molecular weights of all the ACE inhibitors are rather similar, lipophilia may be a key parameter for tissue penetration. Therefore, we characterized various ACE inhibitors with respect to their lipophilia and correlated this property with their ability to penetrate an endothelial monolayer. ACE inhibitors included in this study differ in their chemical structure (see Fig. 1) which binds to the zinc-containing active site of ACE: these are the sulfhydryl compounds captopril and zofenopril; the carboxyalkyldipeptides enalapril, ramipril, perindopril, quinapril, HOE065 and HOE288; and the fosfinic acids fosinopril and ceranapril as well as their active metabolites.

## MATERIALS AND METHODS

### *Reagents and chemicals*

Captopril, ceranapril, fosinopril, fosinoprilat, zofenopril and zofenoprilat were kind gifts from Squibb-Heyden (München, Germany). Enalapril and enalaprilat,

perindopril and perindoprilat, and quinapril and quinaprilat were generously put at our disposal by Merck, Sharp & Dohme (München, Germany), Servier (Orleans, France) and Goedecke/Park-Davis (Freiburg, Germany), respectively. Ramipril, ramiprilat as well as the experimental substances HOE065, HOE288 and HOE288-diacid were all donated from Hoechst-Marion-Roussel (Frankfurt, Germany). All other chemicals (HPLC or analytical grade) were obtained either from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

*Determination of extraction coefficients of ACE inhibitors*

The octanol-water distribution is the common standard to assess lipophilic and hydrophilic characteristics of drugs (6). In the present experiments we have expressed the distribution properties as octanol-water extraction coefficients because of methodical reasons. Small negative extraction coefficients have been calculated in a few cases which reflect a certain uptake of water into the octanol layer leading to increased concentrations of ACE inhibitors in the hydrophilic phase (7).

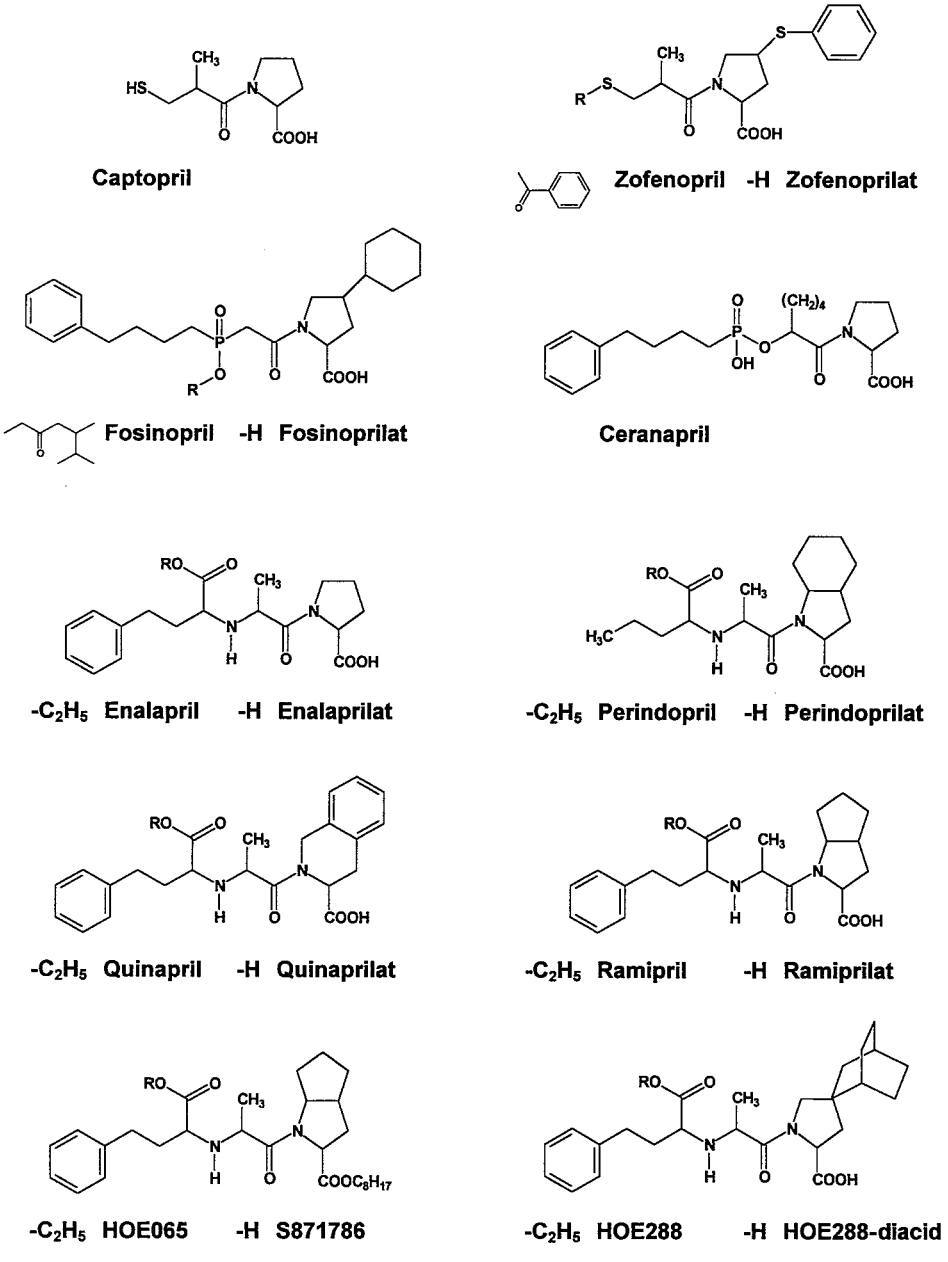


**Fig. 1.** Chemical structures of various ACE inhibitors.

Stock-solutions (1 mg/ml) of captopril, ceranapril, enalapril, enalaprilat, fosinopril, perindopril, perindoprilat, quinapril, ramipril and zofenoprilat were prepared by dissolving them in water. Fosinoprilat and quinaprilat were dissolved in sodium hydroxide (0.1 N). Zofenopril was dissolved in acidified acetonitrile (hydrochloric acid, pH 2.0). HOE065, S871786, HOE288 and HOE288-diacid were dissolved in phosphoric acid (50 mM), acetonitrile, water and acetonitrile (pH 2, HCl), respectively. A 100-$\mu$l aliquot of a stock solution was mixed with 900 $\mu$l phosphate buffer (50 mM, pH 2.0–12.0). After addition of 1 ml octanol, the samples were vortexed (30 min) and centrifuged (10 min, 350 × $g$). A 20-$\mu$l sample of the hydrophilic layer was analyzed by HPLC and UV detection ($\lambda$=214 nm; solid phase: Nucleosil C18 stainless steel column (5 $\mu$m 100 × 4.6 mm; Machery & Nagel, Düren, Germany) 60 ℃; mobile phase: mixtures of sodium phosphate (50 mM, pH 2) and acetonitrile (0–55 vol-%). The relative loss of substance from the hydrophilic layer was defined as the extraction coefficient.

*Cell culture*

Bovine aortas were obtained from a local slaughter house. Aortas were cut longitudinally and the luminal side was incubated with trypsin (0.05% in EDTA 0.05%, at pH 7.4). Thereafter, cells were removed with Locke solution (154 mMNaCl, 5.6 mM KCl, 5.0 mM HEPES and 5.0 mM glucose, pH 7.4) and centrifuged (10 min 1700 × $g$). The medium was removed, and the cells were washed twice. Cells were kept under standard conditions and used for experiments in the 2nd up to the 4th passage.

*Passage of ACE inhibitors through endothelium*

According to a slightly modified method of Borchardt (8) bovine endothelial cells (see above) were grown on polycarbonate filters (No: 3412; Costar, Cambridge, MA, USA). After 10–14 days, the cells reached confluence. These monolayers were used for passage studies. ACE inhibitors (captopril, enalapril, enalaprilat, ramipril and ramiprilat) were diluted with HEPES-Earle solution containing 0.2% albumin to a concentration of 500 $\mu$M. Aliquots (1.5 ml) of these mixtures were pipetted onto the apical side of the filter. Filter plates were filled with 2.6 ml HEPES-Earle solution enriched with 0.2% albumin. At certain time points, aliquots (100 $\mu$l) were taken out of the apical and basal compartment of the filters. The removed volume was replaced by HEPES-Earle buffer. The sample was deproteinized with perchloric acid (final concentration of 1.6 M). Afterwards, 400 $\mu$l of the HPLC eluent was added to 100 $\mu$l of the supernatant, and an aliquot was analyzed by HPLC/UV (see above). At the end of each incubation, filters were examined for integrity of the endothelial cell layers with Trypan Blue (9). For quantification, ACE inhibitor concentrations in each compartment were corrected with respect to the dilution caused by the buffer replacement. Control experiments were carried out in the same way, but using polycarbonate filters without endothelium monolayers. Half times of the diffusion rates were determined by non-linear regression of the concentration kinetics using a mono-exponential curve fitting (Prism®; Graph Pad Software, Inc., San Diego, USA). Data (MW±S.E.M., n=6) were statistically analyzed using ANOVA and Bonferoni's multiple comparison test, respectively.

## RESULTS

Since most ACE inhibitors are applied as prodrugs, we studied the lipophilic properties of both, the prodrugs and their corresponding active metabolites (for the chemical structures, see Fig. 1). A common property of all tested compounds is that the prodrugs are more lipophilic, resulting in a better solubility in the organic layer within the whole pH range when compared to their corresponding active substances (Table 1 and Fig. 2). However, we found substantial differences, namely a different extractability dependent on the pH of the buffer system. Therefore, the substances tested could be divided into two groups.

In the first group, the prodrugs are almost completely extracted within the whole pH range. This pattern could be observed for fosinopril (Fig. 2A), zofenopril (Fig. 2B), HOE065 (Fig. 2C) and HOE288 (Fig. 2D). The extraction coefficients of their corresponding active metabolites are 100% at pH 2, except for HOE288-diacid (Fig 2D). By increasing the pH to less acidic conditions, the extraction coefficients decrease and at pH >7, none of those substances is extractable into the organic layer anymore (Fig.

**Table 1.** Extraction coefficients (E; mean±S.E.M., n=6) of various structurally different ACE inhibitors and their active metabolites determined in an octanol-buffer system (ratio 1:1) at physiological pH of 7.4

| ACE inhibitor prodrugs | E (%) | | Active substances |
|---|---|---|---|
| | | 9±1.1 | Captopril |
| | | 10±4.0 | Ceranapril |
| Enalapril | 1±0.7 | 0±2.3 | Enalaprilat |
| Fosinopril | 100±1.7 | 1±3.4 | Fosinoprilat |
| Perindopril | 2±0.8 | 0±2.8 | Perindoprilat |
| Quinapril | 34±1.6 | 0±3.7 | Quinaprilat |
| Ramipril | 23±2.9 | 0±3.1 | Ramiprilat |
| Zofenopril | 92±3.8 | 7±2.4 | Zofenoprilat |
| HOE065 | 100±0.3 | 4±1.8 | S871786 |
| HOE288 | 82±3.9 | 4±1.2 | HOE288-diacid |

2: A and B) with the exception of zofenoprilat, which showed an increased extraction coefficient at pH 12 (Fig. 2C). The extractability of HOE288-diacid is nearly independent of changes of the pH. The lipophilic properties of ceranapril are comparable to those of fosinoprilat.

In order to differentiate the lipophilic properties of

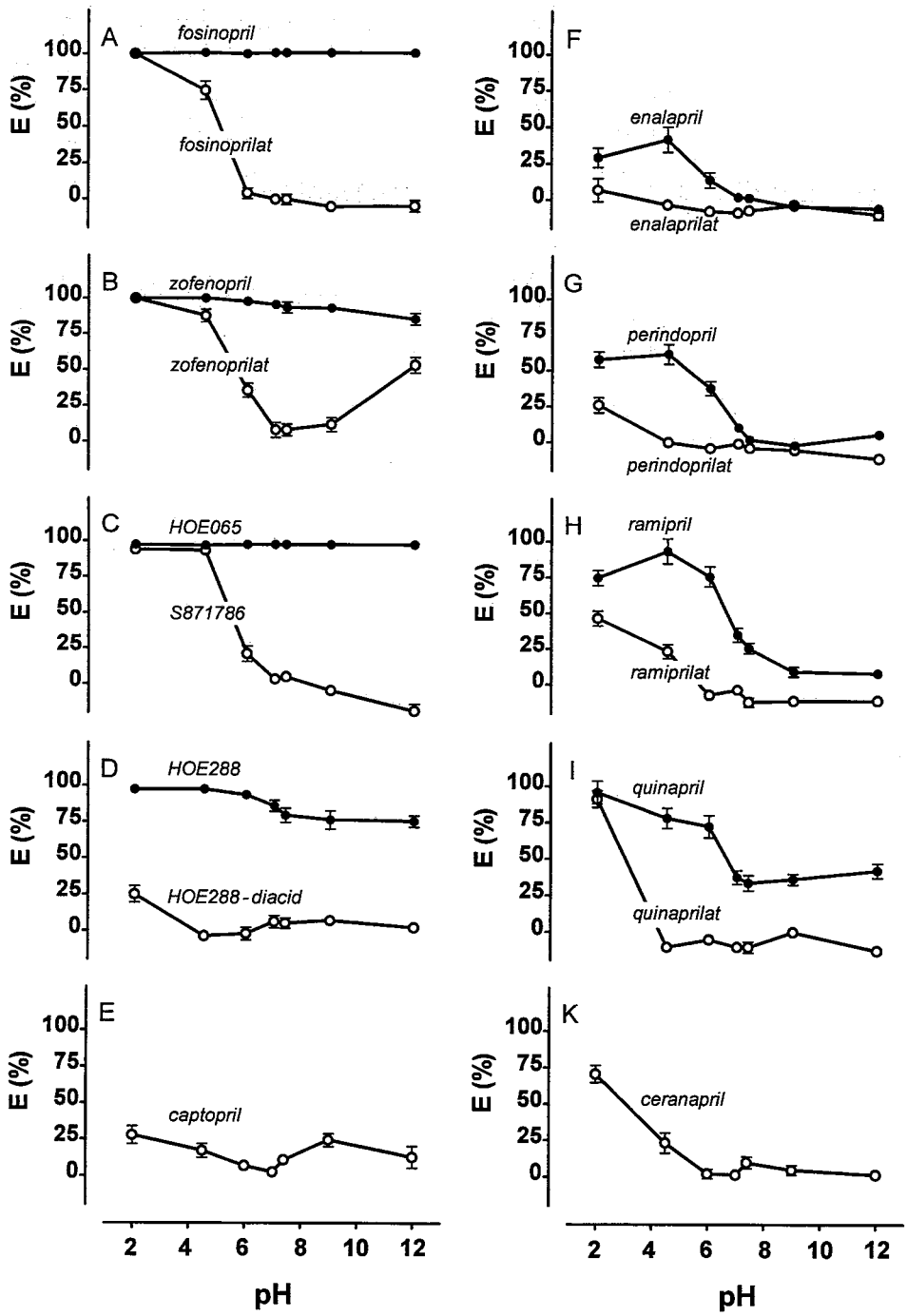


**Fig. 2.** pH dependency of extraction coefficients (E; means±S.E.M., n=6) of various structurally different ACE inhibitors and their active metabolites. ACE inhibitors differ in their chemical structure: these are the carboxyalkyldipeptides (prodrug/active metabolite) enalapril/enalaprilat (F), perindopril/perindoprilat (G), quinapril/quinaprilat (I), ramipril/ramiprilat (H), HOE065/S871786 (C) and HOE288/HOE288-diacid (D); the sulfhydryl compounds zofenopril/zofenoprilat (B) and captopril (E); and the fosfinic acids fosinopril/fosinoprilat (A) and ceranapril (K). E were determined by extraction in an octanol-buffer system.

fosinopril, zofenopril, HOE065 and HOE288, even those ACE inhibitors that were not extractable within the whole pH range, substances were extracted from buffer at pH 7.4 into smaller volumes of octanol. Even at an octanol/buffer ratio of 0.01 (v/v), HOE065 could be almost completely extracted (Fig. 3). Moreover, a rank order of lipophilia for these ACE inhibitors could be established: HOE065 > fosinopril > HOE288 = zofenopril.

ACE inhibitors, which belong to group 2 are characterized by a reduced extractability of their prodrugs (enalapril, perindopril ramipril and quinapril; Fig. 2: F–I). Moreover, the extraction coefficient is decreased by increasing the pH and is always less when compared to all prodrugs of the first group at pH 2. Among these compounds, quinapril has the highest lipophilic properties at pH 2 (E = 96%, Fig. 2I) followed by ramipril (E = 74%, Fig. 2H), perindopril (E = 57%, Fig. 2G) and enalapril (E = 19%, Fig. 2F). Focusing on the active metabolites, the dependency of extractability on pH is less distinct since the extraction coefficient of the prodrugs at pH 2 is reduced when compared to the corresponding prodrug. Only quinaprilat has a similar extraction coefficient at pH 2 compared to quinapril. The extraction coefficient is markedly diminished in the case of ramiprilat (−39% vs ramipril, Fig. 2H), perindoprilat (−55% vs perindopril, Fig. 2G) and enalaprilat (−77% vs enalapril, Fig. 2F). The extraction coefficient of enalaprilat and perindoprilat is unchanged within the pH range. These lipophilic properties are similar to those of HOE288-diacid and captopril (Fig. 2E).

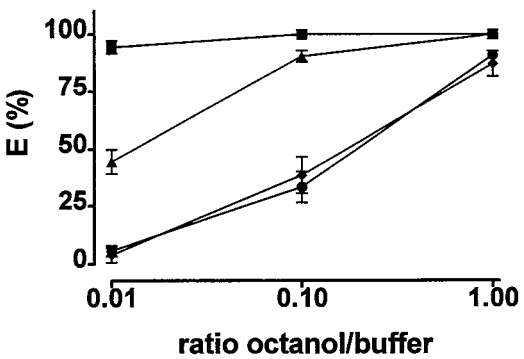


**Fig. 3.** Extraction coefficients (E; means ± S.E.M., n = 6) of some lipophilic ACE inhibitors (E > 80% at pH 7.4: ■ HOE065, ▲ fosinopril, ◆ zofenopril, ● HOE288). E were determined in an octanol-buffer system that was adapted by reducing the volumes of octanol.

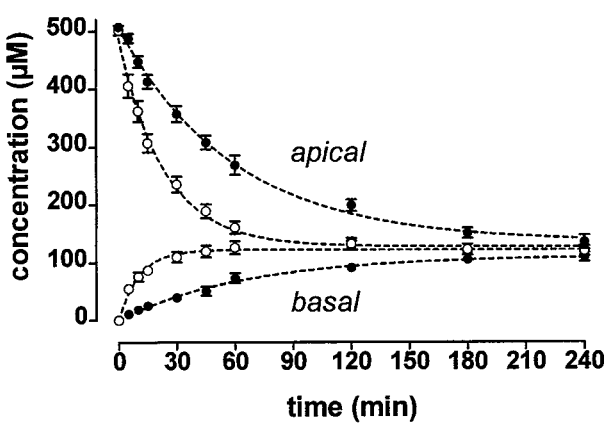


**Fig. 4.** Equilibration of ramipril concentrations in the apical and basal compartment, respectively of polycarbonate filters with (●) or without (○) a confluent monolayer of endothelial cells. Values are expressed as means ± S.E.M., n = 6.

The passage of ACE inhibitors through polycarbonate filters was tested with and without a monolayer of endothelial cells. Substance concentrations were determined up to 4 h in the apical and basal compartment of the filters. Within this time period, ACE inhibitor concentrations at both sides of the filters were almost equilibrated (e.g. ramipril, Fig. 4). In the absence of endothelium (controls), diffusion rates of captopril ramipril, ramiprilat, enalapril and enalaprilat were similar. The half life of exchange ranged from 11.9 to 14.5 min (Table 2). When compared, the presence of an endothelial monolayer leads to a significant decrease (approximately 4 times) of diffusion rates. All ACE-inhibitors investigated passed through the endothelial layer with identical velocities (Table 2).

## DISCUSSION

Independent of the ACE binding moiety, all parent substances are more soluble in the organic layer. Diminished extraction coefficients are particularly observed under neutral or alkaline conditions in which the carboxyl groups of the ACE inhibitors (prodrugs or active metabolites) are mainly dissociated. Therefore, the

**Table 2.** Passage of various ACE inhibitors through an endothelial cell monolayer of bovine aorta

| | Without cell monolayer half life (min) | With cell monolayer half life (min) | |
|---|---|---|---|
| Captopril | 11.9 ± 0.29 | 59.6 ± 3.0 | * |
| Enalapril | 13.3 ± 0.27 | 53.4 ± 0.6 | * |
| Enalaprilat | 13.6 ± 0.51 | 50.8 ± 1.5 | * |
| Ramipril | 13.4 ± 0.19 | 56.9 ± 3.2 | * |
| Ramiprilat | 14.5 ± 0.53 | 51.1 ± 1.6 | * |

ACE inhibitors are compared with respect to exchange rates through polycarbonate filters with and without endothelial cells. Half lives (means ± S.E.M., n = 6) were calculated by monoexponential fits of apical and basal concentration time curves. No differences between the substances were detected. *$P < 0.05$, Student's *t*-test.

solubility of the substances in the lipophilic environment is consecutively reduced. The observation that all prodrugs were shown to be significantly more lipophilic when compared to their corresponding parent drugs is not surprising since the prodrugs are esters of the free acids which have been introduced to increase bioavailability. Ramipril, quinapril or HOE288 could be extracted to a higher extent at pH 7.4 (Table 1) in comparison to enalapril which could be due to a substitution of enalapril with lipophilic moieties such as cyclopentane (=ramipril), benzene (=quinapril) or bicyclo[2.2.2]octan (=HOE288, see Fig. 1). Trandolapril, which is an enalapril-like compound and substituted with cyclohexene, has been demonstrated to be more lipophilic, like enalapril is (10). The pattern confirms our observation that substitution of enalapril with lipophilic moieties leads to an enhanced extraction. The extraction coefficient is even more increased if the pyrrolidine ring of enalapril is substituted as observed with benazepril (E=41%, data on file; Novartis, Nürnberg, Germany) and cilazapril (E=45%, data on file; Merck). The ramipril derivative HOE065 possesses a significantly higher lipophilia when compared to ramipril due to the esterification of the pyrrolidine ring substituted carboxyl group with octanyl alcohol. Since the pyrrolidine ring is substituted with a second carboxyl group that is dissociated at pH >5, it is comprehensible that the extraction coefficient is reduced under neutral and alkaline conditions.

Similar mechanisms have been observed when compounds with sulfhydryl groups or fosfinic acids were extracted. Modifications of captopril such as substitution of the sulfhydryl group with benzoic acid and of the pyrrolidine ring with thiophenol (= zofenopril) resulted in an almost complete extraction within the pH range tested. In contrast, captopril could only be extracted to less than 30% at pH 2. The lipophilia of ceranapril under acidic conditions has been minimized when compared to fosinoprilat due to the positively charged amino group of ceranapril.

Although we were not able to demonstrate substantial differences of lipophilia between the active metabolites of the ACE inhibitors (Table 1), modification of the prolyl residue seems to be highly important for their inhibitory activity against ACE (11). It could be demonstrated that ramiprilat ($K_i$=7 pmol/l) inhibits in vitro enzyme activity more potentially when compared to enalapril ($K_i$=50 pmol/l) or captopril ($K_i$=330 pmol/l). These differences appear to be due to the binding to a hydrophobic pocket of the angiotensin I-converting enzyme (11). Similar effects could be observed when an influence on enzyme activity was determined of various structurally diverse ACE inhibitors ex vivo and in vivo (12).

Since high levels of ACE are present in plasma or at the surface of vascular endothelium (12, 13), the intravascular sites were frequently considered as the main targets of ACE inhibitors. Nowadays, the generation of Ang II in the myocardium has been recognized and ACE has been found in non-vascular tissues under physological and pathophysiological conditions (e.g., fibrosis or hypertrophy) (14, 15). Several important effects of ACE inhibitors have been related to the blockade of local renin angiotensin systems (RAS) (16–18). Especially, low doses of ACE inhibitors that do not significantly reduce blood pressure seem to influence selectively local RASs and can specifically prevent hypertrophy of vascular smooth muscle cells in hypertension (19). Penetration of ACE inhibitors through the endothelium is a prerequisite for the action of ACE inhibitors on tissue ACE. As this property might be decisively related to the lipophilic properties of different ACE inhibitors, we examined the capabilities of various ACE inhibitors to penetrate through cultured endothelium. Since we did not find any differences in penetration of ACE inhibitors and their active metabolites through an endothelial cell monolayer, it could be assumed that all ACE inhibitors can penetrate through the endothelium and could therefore interact with the converting enzyme of local RASs. Therefore, we were able to confirm the findings of Gohlke et al. (20), who demonstrated in isolated rat aorta using just only one ACE inhibitor that endothelium does nor act as a barrier to the penetration of ramipril. After luminal application of radiolabeled ramipril, the substance appeared rapidly in the media and adventitia of the vessel wall (20). Since no differences of penetration rates were observed between parent substances and active metabolites, it is rather unlikely that lipophilic properties are essential for the penetration of endothelium. It is suggested that ACE-inhibitors pass through intercellular gaps that are known to determine the solute permeability of endothelial layers (21). The size of those intercellular gaps would be sufficient to render the lipophilic properties irrelevant. Moreover, from our data, there is no evidence that ACE inhibitors might underlie any additional mechanisms of transcellular transport.

In summary, it is concluded that lipophilic properties do not influence the penetration of ACE inhibitors through the endothelium of blood vessels.

REFERENCES

1 Salvetti A: Newer ACE inhibitors. A look at the future. Drugs **40**, 800–828 (1990)

2 Pellacani A, Brunner HR and Nussberger J: Plasma kinins increase after angiotensin-converting enzyme inhibition in human subjects. J Clin Sci **87**, 567–574 (1994)

3 Distler A, Liebau H and Wolff HP: Action of angiotensin on

sympathetic nerve endings in isolated blood vessels. Nature **207**, 764–765 (1965)
4 Inagami T, Okamura T, Clemens D, Celio MR, Naruse K and Naruse M: Local generation of angiotensin in the kidney and in tissue culture. Clin Exp Hypertens [A] **5(7–8)**, 1137–1149 (1983)
5 Dzau VJ: Circulating versus local renin-angiotensin system in cardiovascular homeostasis. Circulation **77**, Suppl **1**, 4–13 (1988)
6 Hansch C and Fujita T: $\rho$-$\sigma$-$\pi$ analysis: a method for the correlation of biological activity and their chemical structure. J Am Chem Soc **86**, 1616–1626 (1964)
7 Leo A, Hansch C and Elkins D: Partion coefficients and their uses. Chem Rev **71**, 525–616 (1971)
8 Borchardt RT: Assessment of transport barriers using cell and tissue culture systems. Drug Dev Int **16**, 2595–2612 (1990)
9 Gudgeon JR and Martin W: Modulation of arterial endothelial permeability: studies on an in vitro model. Br J Pharmacol **98**, 1267–1274 (1989)
10 Duc LNC and Brunner HR: Trandolapril in hypertension: overview of a new angiotensin-converting enzyme inhibitor. Am J Cardiol **70**, 27D–34D (1992)
11 Bünning P: Inhibition of angiotensin converting enzyme by 2-[*N*-(*S*)-1-carboxy-3-phenylpropyl]-L-alanyl]-(1*S*,3*S*,5*S*)-2-azabicyclo[3.3.0]octane-3-carboxylic acid (HOE498 diacid): comparison with captopril and enalaprilat. Drug Res **34**, 1406–1410 (1984)
12 Cushman DW, Wang FL, Fung WC, Grover GJ, Harvey CM, Scalese RJ, Mitch SL and DeForrest JM: Comparisons in vitro, ex vivo, and in vivo of the actions of seven structurally diverse inhibitors of angiotensin converting enzyme (ACE). Br J Clin Pharmacol **28** Suppl **2**, 115S–130S (1989)
13 Lilly LS, Pratt RE, Alexander RW, Larson DM, Ellison KE, Gimbrone MA and Dzau VJ: Renin expression by vascular endothelial cells in culture. Circ Res **57**, 312–318 (1984)
14 Hirsch AT, Talsness CE, Schunkert H, Paul M and Dzau VJ: Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. Circ Res **69**, 475–482 (1991)
15 Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS and Lorell BH: Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. J Clin Invest **86**, 1913–1920 (1990)
16 Dzau VJ: Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am J Cardiol **59**, 59A–65A (1987)
17 Pipilli E, Manolopoulus VG, Catravas JD, Maragoudakis ME: Angiotensin converting enzyme activity is present in the endothelium-denuded aorta. Br J Pharmacol **98**, 333–335 (1989)
18 Lindpaintner K and Ganten D: The cardiac renin-angiotensin system. An appraisal of present experimental and clinical evidence. Circ Res **68**, 905–921 (1991)
19 Raasch W, Häuser W, Dendorfer A, Schlecht T, Schwartz C, Fitschen M and Dominiak P: Modulation of myocardial and vascular hypertrophy of SHR by various ACE-inhibitors. Naunyn Schmiedebergs Arch Pharmacol **355**, Suppl R76 (1997)
20 Gohlke P, Unger T and Bünning P: Distribution of the angiotensin converting enzyme inhibitor ramiprilat in the blood cell wall. Pharm Pharmacol Lett **2**, 66–69 (1992)
21 Dejana E, Corada M and Lampugnani MG: Endothelial cell-to-cell junctions. FASEB J **9**, 910–918 (1995)