Determinants of Angiotensin II Generation During Converting Enzyme Inhibition

Lucienne Juillerat, Jürg Nussberger, Joël Ménard, Vincent Mooser, Yves Christen, Bernard Waeber, Peter Graf, and Hans R. Brunner

The reaction of the renin-angiotensin system to acute angiotensin converting enzyme inhibition was investigated in a single-blind, crossover study in nine normal volunteers receiving two out of three regimens in random order: the new converting enzyme inhibitor benazepril (20 mg once or 5 mg four times at 6-hour intervals) or enalapril (20 mg). Plasma converting enzyme activity, drug levels, angiotensin I and angiotensin II, active renin, and aldosterone were measured before and 1–4 hours and 14–30 hours after drug intake. Baseline in vitro plasma converting enzyme activity was 97±15 nmol/ml/min (mean±SD) when Hip-Gly-Gly was used as substrate, but with carbobenzoxy-Phe-His-Leu (Z-Phe-His-Leu) or angiotensin I as substrate it was only 20±4 and 1.7±0.3 nmol/ml/min, respectively. Discriminating power at peak converting enzyme inhibition was enhanced with the two latter substrates. In vivo converting enzyme activity was estimated by the plasma angiotensin II/angiotensin I ratio, which correlated well with in vitro converting enzyme activity using Z-Phe-His-Leu as substrate (r=0.76, n=252). Angiotensin II levels returned to baseline less than 24 hours after drug administration, whereas in vitro and in vivo converting enzyme activity remained considerably inhibited and active renin together with angiotensin I levels were still elevated. A close linear relation was found between plasma angiotensin II and the angiotensin I/drug level ratio (r=0.91 for benazepril and r=0.88 for enalapril, p<0.001). Thus, plasma angiotensin II truly reflects the resetting of the renin-angiotensin system at any degree of converting enzyme inhibition. The ratio of plasma angiotensin II to angiotensin I represents converting enzyme inhibition more accurately than in vitro assays, which vary considerably depending on substrates and assay conditions used. (Hypertension 1990;16:564–572)

Angiotensin converting enzyme (ACE) is a carboxypeptidase of broad specificity that is able to split various natural and synthetic substrates. Such substrates have been used for the in vitro measurement of ACE activity, but the low sensitivity of the detection of the enzymatic reaction products makes it necessary to use substrates at concentrations much higher than that of the natural substrate angiotensin I (Ang I) in plasma, and their affinities toward the enzyme vary from less than 3 μM to 16 mM. In addition, the use of buffers in vitro modifies the conditions encountered in vivo. Methods capable of assessing the inhibitory effect of ACE inhibitors not only in vitro but also in vivo are clearly needed. So far, no systematic study has been performed to compare the effects of ACE inhibitors on the plasma ACE activity estimation in vitro using different substrates with the in vivo ACE activity reflected by the angiotensin II (Ang II)/Ang I ratio. Furthermore, ACE inhibition as assessed by either in vitro or in vivo methods should be related to the final product of the enzymatic reaction (i.e., plasma Ang II). It is the purpose of this report to compare well established in vitro methods of measurement with the in vivo blockade of converting enzyme activity (reflected by the Ang II/Ang I ratio) and the status of the renin-angiotensin system (reflected by plasma Ang II).

In addition to the radioactive substrate [3H]benzoyl-Gly-Gly (Hip-Gly-Gly), which has been generally used in this and other laboratories, we used the fluorescent determination with o-phthalaldehyde of His-Leu released by ACE from either Ang I or the N-terminal-protected peptide carbobenzoxy-Phe-His-Leu (Z-Phe-His-Leu), which represents the C-terminal sequence of Ang I. Then we compared in
vitro ACE activity determinations with these three substrates with the ratio of plasma Ang II to Ang I in blood samples of normal volunteers after the oral administration of two different ACE inhibitors: a single dose (20 mg) of enalapril, a single dose (20 mg) of benazepril, and four doses (4×5 mg) of benazepril at 6-hour intervals.

Because during ACE inhibition the generation rate of Ang II theoretically is largely determined by two variables, the concentration of the substrate of the enzymatic reaction (i.e., Ang I) and that of the inhibitor, the plasma Ang I/inhibitor ratio was related to measured plasma Ang II. The resulting close correlation provides strong evidence that even during ACE inhibition plasma Ang II levels are not only determined by the degree of ACE inhibition or circulating drug levels but also by the circulating Ang I concentration reflecting the compensatory renin response to the inhibition.

Methods

Design of the Study

This single-blind study included nine normotensive male volunteers who received, according to a randomized, crossover design with a 1-week washout interval, two of three medications (20 mg enalapril, 20 mg benazepril, or 4×5 mg benazepril). Enalapril tablets were provided by the pharmacy of the hospital. Benazepril was provided by Ciba-Geigy, Basel, Switzerland.

The protocol was approved by the Hospital Ethics Committee; it was explained to the volunteers and written consent was obtained. All subjects underwent a complete physical exam and routine laboratory workup to document their good health and in particular to exclude liver disease or sarcoidosis. No medication other than the study drug was allowed during the period beginning 7 days before the study until the end of the second study phase. Alcohol consumption was not permitted during the same period, and no caffeine-containing foods or beverages were allowed on the day of investigation. The volunteers maintained their usual sodium intake.

The subjects came to the investigational ward at 2:30 PM where they were installed in a supine position. The single or the initial doses were given at 4 PM to allow for investigation of the changes in the renin-angiotensin system during the initial 4 hours of ACE inhibition (between 4 PM and 8 PM) and from 14 to 30 hours after drug ingestion (between 6 AM and 10 PM) on the next day. For the multiple administration of benazepril, 5 mg tablets were ingested at 4 PM, 10 PM, 4 AM, and 10 AM.

All blood samples were drawn from the cubital vein with the subjects in a supine position for at least 1 hour. After the 4-hour blood sample, the volunteers were allowed to leave the hospital; they returned the following morning at 5 AM. Drug levels (active metabolite), plasma ACE activity, Ang I, Ang II, active renin, and aldosterone were measured at times 0, 1, 2, 3, 4 and 14, 16, 18, 20, 22, 24, 26, 28, and 30 hours after first drug intake. All blood was drawn on ice and immediately centrifuged at 4°C. To avoid in vitro generation of Ang II after blood sampling, the renin inhibitor CGP 29287 (1 μM final concentration) was added to the peptidase inhibitors of the collecting tube. The plasma aliquots were shock frozen in liquid nitrogen and stored at −70°C until analyzed. Urinary aldosterone was measured in urine collected every 6 hours the day before drug administration and for 48 hours thereafter.

Angiotensin Converting Enzyme Activity Determinations

ACE activity was measured with [³H]Hip-Gly-Gly as described previously, using a commercially available kit (Ventrex Labs, Portland, Me.).

ACE activity determination with Ang I or Z-Phe-His-Leu was done by a modification of the method of Piquilloud et al, minimizing the dissociation of the enzyme-inhibitor complex. Substrate solutions were prepared as follows: 15 mg Ang I (Bachem, Bubendorf, Switzerland) was dissolved in 1 ml normal saline or 6 mg Z-Phe-His-Leu (Bachem) was suspended in 1 ml saline, then 100 μl NaOH 0.1N was added. On ice, 10 μl substance solution (11 mM) was added to 50 μl heparinized plasma, and the mixture was incubated for 15 minutes (Z-Phe-His-Leu) or 60 minutes (Ang I) at 37°C. Then, 1 ml NaOH 0.1N, 400 μl phosphate buffer 0.1 M, pH 8.0, containing 0.3 M NaCl, and 50 μl of a solution of o-phthalaldehyde (Serva-Brunschwig, Basel, Switzerland) 20 mg/ml in dimethylsulfoxide were added. The reaction was stopped after 10 minutes at room temperature by adding 1.5 ml HCl 0.8N. After centrifugation, the fluorescence was read on a Perkin-Elmer LS5 spectrophuorimeter (Perkin Elmer, Zurich, Switzerland) at 365/495 nm (slits 2.5/5 nm). Blanks, if necessary, were obtained by adding 1 ml NaOH 0.1N before the substrate. In control experiments, we did not find it necessary to run blanks for each sample; the fluorescence of blanks was always smaller than 1% of that of samples. Enzymatic units (nmol/ml/min) were calculated by comparing them with the fluorescence of His-Leu (Bachem) at a final concentration of 0.7 μg/ml under the same conditions as the samples.

Statistics

The results are presented as mean±SEM. For analysis of plasma Ang I and Ang II levels and the Ang II/Ang I ratio, data of each subject were averaged for four time periods (period A: 2, 3, and 4 hours after first drug intake; period B: 14, 16, and 18 hours; period C: 20, 22, and 24 hours; and period D: 26, 28, and 30 hours). An analysis of covariance, with baseline as covariate, was carried out at each of the four periods. Regression lines were calculated according to the least-squares method.
TABLE 1. Kinetic Characteristics of the Three Substrates In Vitro

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Concentration in assay</th>
<th>Benazeprilat $K_i$ (nM)</th>
<th>Enalaprilat $K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip-Gly-Gly</td>
<td>8 mM</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Z-Phe-His-Leu</td>
<td>1.8 mM</td>
<td>0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Ang I</td>
<td>1.8 mM</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Plasma Ang I</td>
<td>10–1,000 pM</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

ND, not determined; Hip-Gly-Gly, benzoyl-Gly-Gly-Gly; Z-Phe-His-Leu, carbobenzoxy-Phe-His-Leu; Ang I, angiotensin I.

**Results**

**Measurement of Plasma Angiotensin Converting Enzyme Activity In Vitro During Converting Enzyme Inhibition**

The kinetic characteristics of the reaction between plasma ACE and the three substrates are summarized in Table 1, which also depicts the in vitro concentration of each of the three substrates.

Table 2 depicts the initial values of in vitro plasma ACE activity before drug intake, using the three methods of measurement.

The time course of ACE activity inhibition measured with the three different substrates is shown on Figure 1. With the substrate Hip-Gly-Gly, enalapril reduced ACE activity to 29.8±5.9% of its control value 1 hour after drug intake. Peak suppression at 1.9±1.0% was observed 4 hours after drug administration. At 24 and 30 hours, ACE activity was 17.1±3.8% and 25.8±4.0%, respectively. Benazepril (20 mg) suppressed ACE activity more rapidly to 3.3±1.3% of the baseline value at 1 hour after drug intake and longer than enalapril to 5.2±2.1%, 6.0±1.5%, and 13.7±4.3% at times 4, 24, and 30 hours, respectively. Benazepril (5 mg) was initially equally effective as benazepril 20 mg. Plasma ACE activity decreased to 3.6±2.3% from baseline at 1 hour after drug intake and to 5.2±3.3 at 4 hours, but at 24 and 30 hours, plasma ACE activity was more reduced after the repeated administration of 5 mg benazepril (3.0±2.0% and 4.6±3.5%) than after the single administration of 20 mg benazepril.

With the substrate Z-Phe-His-Leu, ACE activity was found to be decreased at times 1, 4, 24, and 30 hours after enalapril intake to, respectively, 70.0±8.2%, 64.8±4.1%, and 74.3±3.6% of its initial value. The corresponding values after 20 mg benazepril were 4.0±0.5%, 4.1±0.3%, 50.8±1.9%, and 59.5±0.9%, and after benazepril (5 mg four times), 15.6±8.6%, 15.3±4.1%, 13.7±2.2%, and 32.2±6.8%.

With the substrate Ang I, ACE activity was decreased at times 1, 4, 24, and 30 hours after enalapril intake to 95.8±3%, 61.3±1.1%, 93.5±2.9%, and 98.2±2.6%, respectively. The corresponding values after benazepril (20 mg) were 45.2±3%, 52.5±4.5%, 93.5±2.5%, and 89.3±1.8%, and after benazepril (5 mg four times) were 76.3±2.8%, 72.6±3.5%, 77.3±2.0%, and 88.8±30%.

Major differences were found in the discriminating power of the three methods of plasma ACE activity measurement as shown in Figure 2. With Hip-Gly-Gly, all values after drug intake were below 40% of the initial value, and most of them were below 15% residual activity. With Z-Phe-His-Leu, all values spread between 2% and 86% residual activity; this range was between 35% and 112% when Ang I was the substrate. All three methods provided similar results when comparing benazepril (20 mg) and enalapril (20 mg); benazepril showed faster onset of action and a tendency toward longer duration of plasma ACE inhibition.

In contrast, the Hip-Gly-Gly method was unable to discriminate between the initial effects of the two doses of benazepril (5 and 20 mg) because an almost complete inhibition was already obtained with 5 mg. The Z-Phe-His-Leu or Ang I methods
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discriminated better the effects on the intensity and on the duration of plasma ACE inhibition of 20 mg benazepril given either as a single dose or as four consecutive doses of 5 mg.

**Plasma Angiotensin I and Angiotensin II during Angiotensin Converting Enzyme Inhibition**

As shown in Figure 3, 20 mg benazepril decreased plasma Ang II more rapidly than 20 mg enalapril, which had its maximal effect 4 hours after drug intake. From 14 to 30 hours after administration of each of the two drugs, plasma Ang II was comparable and returned toward its baseline level. The initial fall in plasma Ang II was accompanied by a rise in active renin that persisted from 14 to 30 hours after drug intake despite the fact that plasma Ang II had returned to baseline. Plasma Ang I correlated very well with active renin \((r=0.82, n=252, p<0.001)\) (Figure 4) and remained elevated also from 14 to 30 hours after drug intake.

The comparison of the effects of benazepril (5 mg four times) with those of a single dose of benazepril (20 mg) shows several interesting trends. Initially, 20 mg benazepril was more effective than 5 mg, but between 14 and 30 hours, the repeated intake of 5 mg benazepril, by increasing the circulating level of the active drug, maintained a more pronounced converting enzyme inhibition. There was a trend for a slightly lower plasma Ang II and a slightly higher plasma Ang I concentration after the repeated administration of 5 mg benazepril. The value of plasma Ang II adjusted for differences in baseline levels was

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**FIGURE 2.** Scatterplots showing comparison of discriminating power of three methods of in vitro estimation of plasma angiotensin converting enzyme (ACE) activity during ACE inhibition. Results are expressed as fractions of pretreatment ACE activity (A/Ao).

**FIGURE 3.** Line graphs showing plasma renin-angiotensin system (RAS) during angiotensin converting enzyme (ACE) inhibition: angiotensin II (Ang II) levels returned to baseline between 14 and 30 hours after initial drug intake. Arrows indicate intake of a light meal (which stimulated the RAS). Ang I, angiotensin I.

**FIGURE 4.** Scatterplot showing correlation between plasma angiotensin I and plasma active renin \((n=252, r=0.82)\).
slightly lower after benazepril (5 mg four times) than after the single dose of benazepril (20 mg) at the second period (2.1 versus 3.8 fmol/ml, \( p=0.07 \)) and the third period (2.8 versus 5.2 fmol/ml, \( p=0.06 \)). The adjusted value of the plasma Ang I was higher at the third period (72.8 versus 25.4 fmol/ml, \( p<0.01 \)) and still at the fourth period (59.4 versus 22.8 fmol/ml, \( p<0.05 \)).

As shown in Table 3, plasma aldosterone fell during the first 4 hours after first drug intake, but returned to baseline during the hours 14 to 30. Urinary aldosterone excretion behaved similarly.

### Relations Between the Plasma Angiotensin II

#### Angiotensin I Ratio and Plasma Angiotensin II

Table 4 summarizes the results of the plasma levels of active drug, Ang I, Ang II, the ratio of plasma Ang II to Ang I, and the ratio of Ang II to drug level.

The effect of 20 mg enalapril or 20 mg benazepril on the time course of the plasma Ang II/Ang I ratio used as an index of the in vivo enzyme activity is shown in Figure 5. The figure compares this in vivo evaluation of ACE inhibition with the in vitro ACE inhibition estimated by the Hip-Gly-Gly and the Z-Phe-His-Leu methods. After enalapril the Hip-Gly-Gly method reflects more closely than the Z-Phe-His-Leu method the in vivo inhibition, whereas this ratio was higher after benazepril (5 mg four times) at the first period (0.055 versus 0.017, \( p<0.05 \)) and lower at the third period (0.049 versus 0.179, \( p<0.01 \)) and the last period (0.126 versus 0.217, \( p=0.065 \)).

#### Relations Between the Plasma Angiotensin II/Drug Level Ratio and Plasma Angiotensin II

Assuming that the product (Ang II) of the enzymatic reaction (ACE) is mainly determined by the concentrations of the substrate (Ang I) and of the inhibitor (drug level), the measured plasma Ang II concentrations \( y \) were related to the calculated ratio \( x \) of plasma Ang I to drug level (Figure 6). Highly significant correlations were found both after enalapril (\( y=1.0x+1.6, r_{2}=0.88, n=12 \)) and benazepril (\( y=3.0x+1.1, r_{2}=0.91, n=26 \)) (Figure 6, upper panel) and benazepril (\( y=3.0x+1.1, r_{2}=0.91, n=26 \)) (Figure 6, lower panel) when mean values for each time point were compared (\( p<0.001 \)).

### Discussion

Angiotensin converting enzyme activity is frequently measured to assess the effects of ACE inhibitors in plasma\(^{14,15} \) and in tissues.\(^{16} \) Results obtained by such measurements are used to define the efficacy and the duration of ACE inhibition. Changes in blood pressure of hypertensive patients or animals are often compared with ACE activity measured in plasma in an attempt to better understand the mechanisms of action of ACE inhibiting drugs. Obviously, both clinical testing of ACE inhibitors and research on their mechanisms of action depend on appropriate methodology used for ACE activity measurement.
We have tested three possible substrates of ACE for the estimation of the degree of inhibition induced by two different ACE inhibitors, enalapril and benazepril. The structure of the most widely used substrate, Hip-Gly-Gly, differs from that of Ang I, and its $K_M$ (5 mM) is much less favorable than that determined for Ang I (70 $\mu$M). The second substrate used in this study, Z-Phe-His-Leu, represents the C-terminal sequence of Ang I. Its N-terminal is protected against nonspecific hydrolysis. Its $K_M$ (60 $\mu$M) is close to that of Ang I. The third substrate is Ang I itself. The problem with ACE activity measurements, particularly using the two latter substrates, is the need for substrate concentrations of 30- and 25-fold $K_M$ conditions, which tends to produce results that underestimate the inhibition occurring in vivo.
In vivo (Ang II / Ang I) and In vitro ACE Inhibition

In contrast, the Hip-Gly-Gly assay is performed at $K_M$ conditions and the poor substrate Hip-Gly-Gly (compared with the natural substrate Ang I) is more sensitive to the presence of ACE inhibitors of nanomolar $K_i$.

A striking result is the fact that the three in vitro methods provided different information (Figure 1). The Hip-Gly-Gly method showed a long and intense inhibition of plasma converting enzyme and did not detect any major difference between enalapril (20 mg), benazepril (20 mg), and benazepril (5 mg) during the first hours after drug intake, as an almost complete inhibition was obtained with the three treatments. In contrast, the Z-Phe-His-Leu method discriminated between 5 and 20 mg benazepril during the first hours after drug administration, and demonstrated better the faster onset of the action of benazepril compared with enalapril, which takes more time to be converted from the pro-drug to the active drug. Similar observations were made when Ang I instead of Z-Phe-His-Leu was used as substrate in vitro. Several hours after drug intake, the three in vitro measurements still provided different information. When Ang I was the substrate, plasma converting enzyme inhibition appeared to be minimal between 14 and 30 hours after drug intake, whereas it seemed to be still extremely effective when Hip-Gly-Gly was used for measurement of ACE activity. Z-Phe-His-Leu provided intermediate results. With the latter substrate, 20 mg benazepril seemed to be more potent than 20 mg enalapril, and 5 mg four times a day benazepril appeared to be more effective than a single dose of 20 mg benazepril. The Z-Phe-His-Leu and Ang I substrates were appropriate to demonstrate a reinforcement of ACE inhibition after the fourth ingestion of 5 mg benazepril at 18 hours, which was not detected with Hip-Gly-Gly.

Our results show that there is no absolute information in a result of an in vitro-measured plasma converting enzyme activity, and that a 50% or 90% inhibition is only meaningful within the assay system that has been selected. The relation $K_M/K_i$ or substrate concentration must be taken into account when comparing results. It is extremely difficult to correlate these in vitro data to the in vivo situation, and the in vitro measurements can hardly be considered as an appropriate evaluation of the in vivo situation as they essentially depend on the method used. Ang I is the product of the enzymatic reaction of renin with its substrate angiotensinogen. The conversion of Ang I to Ang II is a function of the decapeptide's concentration in plasma, and of the available active ACE. The plasma Ang II/Ang I ratio can be used as an index of the in vivo enzyme activity. Twenty-four hours after enalapril intake, the residual Ang II/Ang I ratio was close to the residual activity measured with Hip-Gly-Gly, whereas 24 hours after benazepril intake the residual Ang II/Ang I ratio followed more closely the residual activity measured with Z-Phe-His-Leu.
These observations also suggest that different methods for estimation of plasma ACE activity should be selected according to the objectives of the measurement. The Hip-Gly-Gly substrate could be more appropriate to check a patient’s drug intake (compliance) during the 24 to 48 hours preceding the visit at the doctor’s office. The Z-Phe-His-Leu substrate (we do not use the Ang I substrate routinely in our laboratory) could be more useful for the investigation of the peak effect and duration of plasma ACE inhibition during a dose-finding study for a new converting enzyme inhibitor. Similar limitations may also be encountered when these methods are used to investigate ACE activity in various tissues, and pathophysiological conclusions derived from such measurements should be interpreted cautiously.18

In normal volunteers, plasma Ang II is the most appropriate end point to investigate the degree of the blockade of the renin-angiotensin system during ACE inhibition. Blood pressure changes, which in hypertensive patients are difficult to measure accurately, are minimal or absent in recumbent normal volunteers on a normal sodium diet.19 Less than 24 hours after a single dose of either benazepril or enalapril, plasma Ang II had returned toward its initial value, even though the in vitro measurements of plasma ACE activity indicated a persistent inhibition that was more pronounced with benazepril than with enalapril. The high-performance liquid chromatography separation used for the Ang II measurement excludes angiotensin fragments that would cross-react with the assay antiserum. The results obtained with this very accurate method nevertheless confirm previous observations made with enalapril, lisinopril,20 and ramipril21 where only immunoreactive Ang II was quantitated. The time course of plasma and urinary aldosterone was similar to that of plasma Ang II. The rise in the converting enzyme substrate Ang I is the key to this Ang II escape 24 hours after drug administration.

Active renin increases immediately after the ingestion of an orally active converting enzyme inhibitor in parallel with the fall in plasma Ang II. This is thought to reflect the acute interruption of the permanent negative feedback effect of Ang II on renin release.22 The extent of the later renin and Ang I increase (fivefold) 14 hours after a single dose of the ACE inhibitors is clearly more pronounced. For an explanation, several factors have to be considered: First, the fall in aldosterone levels may have caused some natriuresis. Second, the circadian rhythm may play a role (the acute phase was tested in the evening and the 14-hour values are from 6 AM). Third, the total renin concentration measured concomitantly in the same plasma samples (data not shown) decreased also after the initial rise in parallel with active renin, and twenty-four hours after drug intake, total renin also was considerably higher. Were stores of active renin temporarily depleted after acute ACE inhibition? The rise in renin persisted 24 hours after drug intake, despite plasma levels of Ang II that had returned toward their initial values. These apparently normal circulating Ang II levels 24 hours after single or repeated doses of an ACE inhibitor have led to the hypothesis that a tissular renin-angiotensin system24 or Ang II–forming enzymes independent of the renin-angiotensin system determine circulating Ang II.25

To elucidate the influence of the reactive increase in circulating substrate (Ang I) on the product formation (Ang II) in the presence of ACE inhibitor (drug), we have calculated the plasma Ang I/drug level ratio and compared it with the plasma levels of Ang II, which represent at a given time the in vivo production of the active peptide of the renin-angiotensin system. The powerful correlations between plasma Ang II and the Ang I/drug level ratio found with both enalapril and benazepril strongly suggest that the return to baseline of Ang II in plasma can be entirely explained by the simultaneous disappearance of the ACE inhibition and the rise in its substrate plasma Ang I. The latter is the consequence of the increased renin release, secondary to the initial effect of the ACE inhibitors on plasma Ang II. Thus, even in the presence of an ACE inhibitor the closed cybernetic loop regulating plasma Ang II levels is operating normally and tends to attenuate the efficacy of ACE inhibitors in blocking the renin-angiotensin system. Clearly, Ang II is the pivotal parameter in the closed loop, and the system works to compensate any interference by adjusting renin secretion to restore as rapidly and completely as possible normal Ang II levels. Although they did not perform such calculations, Manhém et al21 observed that the administration to normal volunteers of ramipril at doses varying from 5 to 50 mg did not influence plasma Ang II 24 hours after drug intake, whereas a dose-dependent rise in plasma renin and Ang I was described. A dose-dependent increase in Ang I levels and plasma active renin was also found during a 10-day administration of trandolapril to normal volunteers: on day 10, plasma immunoreactive Ang II was equally reduced over the 16-fold dose range of drug.26 Thus, already in that study it was postulated that the reactive dose-dependent renin response to ACE inhibition may be the reason for the very shallow dose–response curve of ACE inhibitors in reducing plasma Ang II and blood pressure. The present data demonstrate that plasma drug levels are directly related to these adjustments of the cybernetic loop regulating Ang II levels via renin secretion. Even if the small number of subjects included in this study does not allow a definite conclusion, there was a trend for plasma Ang II to be lower at 24 hours when a converting enzyme inhibitor was administered in a fractionated rather than a single dose. All methods of plasma converting enzyme activity measurement, in vitro as well as in vivo, have shown that after the fourth 5 mg dose of benazepril, ACE inhibition was more pronounced than at the corresponding time interval after a single 20 mg dose. This reinforcement of ACE inhibition may decrease plasma Ang II. Shortening the dose interval might be
more effective to block the renin-angiotensin system than increasing the dose, but this remains to be seen with longer testing, because in the end effect this will again depend on the capacity of the system to compensate for the higher drug levels. The lack of a further fall in blood pressure reported after increasing the doses of converting enzyme inhibitors with lisinopril between 20 and 80 mg once daily27 and enalapril between 20 and 60 mg once daily has already been reported.28

In conclusion, different information is provided by different in vitro methods of measurement of plasma ACE activity. The in vivo assessment of ACE inhibition by the simultaneous estimation of plasma Ang I, Ang II, and inhibitor levels demonstrates that the blockade of the renin-angiotensin system is self-limiting. This self-limitation is due to the closed cybernetic loop regulating Ang II levels, which is fully operative even in the presence of the ACE inhibitor.

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References


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