Differential Effects of Angiotensin Converting Enzyme Inhibitors on the Vasodepressor and Prostacyclin Responses to Bradykinin

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ABSTRACT
Angiotensin converting enzyme (ACE) inhibitors block degradation of bradykinin and bradykinin stimulates prostacyclin production. ACE inhibitors are reported to increase prostaglandins. Therefore, we set out to determine 1) the contribution of prostacyclin to the bradykinin-mediated vasodepressor effects of ACE inhibitors, 2) whether ACE inhibitors alter the effect of bradykinin on prostacyclin, and 3) whether the effects of ACE inhibitors on bradykinin and prostaglandins are class effects or dependent on ACE inhibitor structure. To address these questions, we compared the effects of captopril, quinapril and placebo on blood pressure, urinary excretion of 2,3-dinor-6-keto-PGF₁α, and the vasodepressor response to i.v. bradykinin in 21 salt-replete normal-to-high renin hypertensive patients. Captopril and quinapril doses were titrated to lower pressure similarly. Captopril, but not quinapril, increased excretion of prostacyclin metabolite (217 ± 50 vs. 135 ± 21 pg/mg Cr base line, P < .05).

ACEI are widely used in the treatment of hypertension, congestive heart failure and diabetic nephropathy. There are currently seven ACEI on the market in the United States. Despite the widespread use of angiotensin converting enzyme inhibitors, their effects on bradykinin and prostaglandins are controversial.

ACE inhibitors block the conversion of Ang I to Ang II, a potent vasoconstrictor. However, the clinical observation that ACE inhibitors lower blood pressure even under low renin conditions (Brunner et al., 1979; Gavras et al., 1978), suggests an additional non-renin-angiotensin-dependent mechanism(s) for their action. One possibility is raised by the finding that ACE (kininase II) not only catalyzes the conversion of Ang I to Ang II but also the degradation of bradykinin (Erdos and Skidgel, 1987), a potent vasodilator. Bradykinin itself activates bradykinin subtype B₂ receptors on endothelial cells to stimulate release of nitric oxide (Cherry et al., 1982), prostacyclin (Barrow et al., 1986) and P450 metabolites of arachidonic acid (Fulton et al., 1992).

ACE inhibitors have been reported to increase vasodilatory prostaglandins both in vitro and in vivo (Swartz et al., 1980; Abe et al., 1980; Moore et al., 1981; Hornych et al., 1982; Stanek and Silberbauer, 1986; Oparil et al., 1987; Mittman et al., 1986; Quillely et al., 1987; Omata et al., 1987). Because ACE inhibitors decrease bradykinin degradation (Erdos and Skidge, 1987) and bradykinin stimulates prostacyclin secretion (Barrow et al., 1986), it is not clear whether this effect on prostaglandins is a direct effect of converting enzyme inhibition or an indirect effect, mediated through bradykinin. In vitro studies, in which investigators have measured the effect of bradykinin receptor antagonists (Beierwaltes and Carretro, 1989; Wiener et al., 1991) or kallikrein antagonists (Scherf et al., 1986; Galler et al., 1982) on the prostaglandin response to ACE inhibitors, have both supported and refuted

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ABBREVIATIONS: ACEI, angiotensin converting enzyme inhibitors; Ang I, angiotensin I; Ang II, angiotensin II; PRA, plasma renin activity; bpm, beat per minute; MAP, mean arterial pressure.
a bradykinin-mediated effect. Moreover, although potentiality of bradykinin by ACE inhibitors would be expected to increase prostaglandin levels, suppression of Ang II synthesis would be expected to decrease prostaglandin synthesis. Ang II has been shown to stimulate prostaglandin synthesis in isolated tissues and cells (Mullane and Moncada, 1980a; Grodzinska and Gryglewski, 1980; Shebuski and Aiken, 1980; Woodman et al., 1983; Hura and Kunau, 1988; Char- donnens et al., 1989), and both ACE inhibitors (Yanagisawa et al., 1990) and AT1 receptor antagonists (Leung et al., 1992) block Ang II-stimulated prostaglandin secretion. Finally ACE inhibitors may favor increased production of Ang I-7 from Ang I (Lawrence et al., 1990) and Ang I-7 stimulates prostaglandin synthesis with a potency equal or greater to that of Ang II (Tallant et al., 1991). Thus, the effect of ACE inhibitors on vasodilatory prostaglandins may depend on the balance between bradykinin, Ang I-7 and Ang II.

The effect of ACE inhibition on bradykinin and vasodilatory prostaglandins may also depend on the chemical structure of the ACEI. For example, van Gilst et al. (1991) have reported that captopril and zofenopril, sulhydryl-containing ACE inhibitors, are more potent in vitro than nonsulphydryl containing ACEI in enhancing bradykinin-mediated coronary vasodilation. Cysteine, a sulhydryl-containing compound that lacks converting enzyme inhibitor activity, also potentiates the vasodilatory effects of bradykinin (van Gilst et al., 1991). Similarly, Zusman (1987) has proposed that the sulhydryl group present in captopril confers prostaglandin stimulatory activity independent of its effect on converting enzyme. Other studies suggest that potentiation of bradykinin and increased prostaglandin production may contribute to the hemodynamic effects of nonsulphydryl-, as well as sulphydryl-containing ACEI (Beierwaltes and Carretero, 1989; Wiemer et al., 1991; Prostran et al., 1991).

In this study, we set out to determine whether an increase in prostacyclin contributes to the bradykinin-mediated vasoconstrictor effects of ACE inhibitors, to determine whether ACE inhibitors alter prostaglandin production independently of their effect on bradykinin, and to determine whether the effects of ACE inhibitors on bradykinin and prostaglandins are common to all drugs of the class or dependent on the structure of the ACE inhibitor. To do this, we compared the effects of treatment with captopril (a sulphydryl-containing ACEI), quinapril (a nonsulphydryl-containing, carboxyl-containing ACEI), or placebo on ambulatory 24-hr blood pressure and urinary excretion of a stable prostacyclin metabolite, 2,3-dinor-6-keto PGF1α, and on the vasodressor and prostacyclin responses to i.v. bradykinin in salt-replete patients with normal-to-high renin essential hypertension. We measured urinary 2,3-dinor-6-keto PGF1α because this metabolite has been shown to accurately reflect systemic prostacyclin production (FitzGerald et al., 1983, 1987) whereas 6-keto-PGF1α, a nonenzymatic metabolite of prostacyclin, largely reflects renal prostacyclin production (Patrignani et al., 1989).

Methods

Subjects. Men and women, age 18 to 65 yr, with normal-to-high renin essential hypertension were eligible for study. All subjects had an untreated diastolic blood pressure (mean of three measurements) of 90 mm Hg or more while seated and had had documented hypertensive of at least 6 mo duration. All subjects had normal to high renin levels, measured before study entry and defined as an upright PRA of 2.4 ng/ml/hr or greater although they were off medications and ingesting a low salt (10 mmol/day) diet. Secondary causes of hypertension were excluded by history, physical examination and appropriate laboratory testing (including renal arteriogram, if indicated). Subjects with significant concurrent disease were excluded, as were subjects who were not able to discontinue use of nonsteroidal antiinflammatory drugs. Subjects with a seated, untreated diastolic blood pressure of 115 mm Hg or greater were excluded from participation. Women of child-bearing potential were excluded from the study because of the known risks of angiotensin converting enzyme inhibitors for the newborns (Shotan et al., 1994). Smokers (1 subject) were not excluded but were asked to maintain their cigarette consumption constant throughout the study. All subjects gave written informed consent and the study protocol was approved by the Institutional Review Board of Vanderbilt University.

Protocol. Figure 1 illustrates the randomized, placebo-controlled, single-blind study design. Subjects were asked to discontinue their antihypertensive medicines and any drugs affecting prostaglandins (e.g., nonsteroidal antiinflammatory drugs) on day −15. (Beta-)Blockers and centrally acting sympatholytics were tapered rather than discontinued abruptly. During the 2-wk washout period, subjects underwent regular blood pressure monitoring. At the end of the washout period, each subject underwent baseline 24-hr blood pressure monitoring (Accutracker II ABPM device, Suntech Medical Instruments, Raleigh, NC) and collected a base-line 24-hr urine for measurement of electrolytes and the stable prostacyclin metabolite, 2,3-dinor-6-keto PGF1α. Each subject was then randomized to treatment with captopril (Bristol-Meyers Squibb, Princeton, NJ), quinapril (Parke-Davis, Morris Plains, NJ), or placebo, administered in identical appearing capsules. During the next 6 days, the dose of medication was increased to achieve a sitting diastolic pressure less than 85 mm Hg, or until the highest allowable dose had been reached. In the case of captopril this was 100 mg three times a day; for quinapril, this was 40 mg twice a day. Subjects who had been randomized to placebo received a new set of placebo pills if their diastolic pressure was greater than 85 mm Hg during the dose escalation phase of the study. Subjects with a diastolic blood pressure of 115 mm Hg or greater were discontinued from the study. Subjects were then continued on this effective dose for the remainder of the study.

Starting on day 15, subjects were given a 200 mmol sodium, 80 mmol potassium, 2500 ml fluid per day xanthine-free diet, supplied by the Vanderbilt Clinical Research Center. They collected their urine daily for measurement of urine electrolytes. On the evening of day 18, subjects were admitted to the Clinical Research Center at Vanderbilt University. Three weeks after they had been randomized (study day 21), they again underwent 24-hr ambulatory blood pressure monitoring and collected their urine for measurement of prostacyclin metabolite. On the morning of day 22, subjects were given i.v. bradykinin in graded doses according to the protocol below. If there was no response to lower doses of bradykinin, the protocol was repeated the next morning. The next day, subjects were begun on indomethacin, 50 mg per os tid. Ambulatory blood pressure monitoring, urine collection for prostacyclin metabolite and bradykinin infusion were repeated after 4 days of indomethacin. (Only one bradykinin infusion was performed in the first three patients randomized.)

Bradykinin infusion. Subjects were studied fasting and in the supine position. They were given their antihypertensive medicine (and indomethacin) 1.5 hr before the bradykinin infusion (fig. 1, inset). Subjects were given 1000 ml D5W i.v. over 1 hr before bradykinin infusion to facilitate urine production. Blood pressure and pulse were measured every 1 to 2 min using an automated, nonvasive blood pressure cuff (Critikon Dinamap 1846 SXLP version 085, Critikon Inc., Tampa, FL). Beginning at time 0, bradykinin was infused i.v. in graded doses, increased at 15- to 30-min intervals, until one of the following endpoints was obtained: a 15 mm Hg decrease in
mean arterial pressure, a 20 bpm increase in pulse, or symptoms of dyspnea. All subjects experienced flushing. A majority experienced dysgeusia and conjunctival erythema. Urine was collected at time 0 and then hourly for 4 hr for measurement of 2,3-dinor-6-keto-PGF₁α.

On the first day of bradykinin infusion, the bradykinin doses administered were 0.5, 1, 3, 10 and, if tolerated, 25 ng/kg/min. Subjects who had been pretreated with placebo did not respond to these low doses of bradykinin. In these subjects, bradykinin doses of 25, 100, 200, 400 and, if tolerated, 800 ng/kg/min, were administered on a second day. The volume of fluid infused with bradykinin ranged from 30 ml (low dose day) to 60 ml (high dose day). Only the high dose infusion day was repeated after indomethacin. The first subject randomized to ACE inhibitor received a bradykinin dose of 100 ng/kg/min. This resulted in a rapid 58% decrease in his MAP. The bradykinin infusion was immediately discontinued and his MAP returned to base line within 1 min.

Analytical methods. During 24-hr ambulatory blood pressure monitoring, blood pressure and pulse were measured every twenty minutes from 6 A.M. to 11 P.M. and every hour from 11 P.M. to 6 A.M.

Data were edited and meaned by computer.

Blood samples for plasma renin activity were collected on ice in tubes containing 0.3 ml of 10% EDTA, centrifuged at -4°C for 20 min and separated immediately. PRA was measured by radioimmunoassay for angiotensin I (Workman et al., 1979). Urine electrolytes were measured by flame photometry. Urinary 2,3-dinor-6-keto PGF₁α was measured by gas chromatography-mass spectrometry after lactonization, reverse phase column extraction and derivatization (Daniel et al., 1994).

Data are presented as means ± S.E.M. Statistical analysis was performed using SPSS for Windows Release 6.0 (SPSS Inc., Chicago, IL). Comparisons of the effects of treatment with captopril, quinapril or placebo, with indomethacin, and with bradykinin were made using 2 or 3 factor ANOVA, with repeated measures where appropriate. Post hoc comparisons between treatment groups were made using a two-tailed unpaired Student's t test, although those within groups were made using a two-tailed paired Student's t test or Wilcoxon signed ranks test when appropriate.

Results

Twenty-one hypertensive subjects were randomized to therapy, seven to each treatment arm. One patient randomized to placebo developed hypertensive headaches and was discontinued from the protocol 1 wk after randomization. One patient randomized to captopril developed urticaria that did not respond to a reduction in his dose. He was withdrawn the morning of his first bradykinin infusion, but completed the 24-hr ambulatory blood pressure monitor and urine collection for 2,3-dinor-6-keto PGF₁α although taking captopril. A second patient randomized to captopril developed profound nausea after taking indomethacin and did not complete the second bradykinin infusion.

Table 1 shows the base-line characteristics of the patients. There were no differences in age, gender, race, height, weight, PRA in the salt-deplete state, base-line systolic and diastolic blood pressure, urinary sodium excretion or urinary 2,3-dinor-6-keto-PGF₁α among the three treatment groups.

Effect of treatment on urinary 2,3-dinor-6-keto-PGF₁α excretion, and ambulatory blood pressure and pulse. The effect of treatment on 24-ambulatory MAP is
presented in Figure 2. Repeated measure ANOVA indicated that treatment (F = 13.1, P = .006) affected mean arterial pressure. Treatment with captopril and quinapril lowered the MAP 10.9 ± 2.5 mm Hg and 13.9 ± 3.5 mm Hg (N.S.), respectively. The mean dose of captopril required to achieve this antihypertensive effect was 203 ± 36 mg/day. The mean dose of quinapril required to achieve the same antihypertensive effect was 31.4 ± 8.6 mg/day. The addition of indomethacin significantly attenuated the antihypertensive effect of ACE inhibition (decrease in MAP -10.0 ± 2.2 vs. -12.4 ± 2.1 mm Hg for ACEI alone (captopril and quinapril combined) P < .03). Indomethacin transiently lowered both urinary volume and sodium excretion and increased weight (table 2). The effect of indomethacin on blood pressure was similar in the captopril- and quinapril-treated patients, although the change was statistically significant only in the quinapril group. Indomethacin also transiently decreased urine volume and sodium excretion in the placebo-treated subjects (data not shown). However, the addition of indomethacin lowered mean arterial pressure in the placebo-treated patients (fig. 2). By ANOVA, treatment also affected pulse (F = 8.98, P = .001) but there was no interaction with treatment group. Treatment with quinapril, but not with captopril or placebo, significantly lowered the 24-hr ambulatory pulse (71 vs. 79 bpm, P < .05).

Figure 3 illustrates the effect of treatment on the urinary excretion of 2,3-dinor-6-keto-PGF\textsubscript{1α}. By ANOVA, treatment affected urinary excretion of 2,3-dinor 6-keto PGF\textsubscript{1α} (F = 31, P < .001). At equidosepressor doses, treatment with captopril significantly increased the urinary excretion of this prostacyclin metabolite (217 ± 50 vs. 135 ± 21 pg/mg Cr, P < .05), although treatment with quinapril did not. There was no correlation between base-line urinary prostacyclin metabolite excretion and the increase in prostacyclin metabolite excretion after ACE inhibition. As expected, the addition of indomethacin significantly lowered the excretion of 2,3-dinor-6-keto-PGF\textsubscript{1α} in all three treatment groups (fig. 3). Although the magnitude of decrease in 2,3-dinor-6-keto-PGF\textsubscript{1α} after indomethacin was similar in the three groups, the 24-hour excretion of this metabolite was higher in the captopril-treated group than in the quinapril-treated group (81 ± 20 vs. 28 ± 5 pg/mg Cr, P < .02).

The effect of ACE inhibition on the hemodynamic and prostacyclin responses to i.v. bradykinin. Intravenous infusion of bradykinin lowered blood pressure (ANOVA: F = 17.4, P < .001) and caused a compensatory tachycardia (ANOVA: F = 45, P < .001) in a dose-dependent manner (fig. 4). Repeated measures ANOVA indicated that pretreatment with ACE inhibitor significantly shifted both response curves to the left (F = 4.65, P < .05 for MAP; F = 7.56, P = .014 for pulse). There was no difference in either the MAP response (F = .67, P = .437) or pulse response (F = .19, P = .674) to bradykinin between those subjects treated with captopril and those treated with quinapril. Thus, the dose of bradykinin required to achieve a 15 mm Hg decrease in MAP or a 20 bpm increase in pulse was 50-fold lower in the captopril- and quinapril-treated patients (10 ± 0 and 12.1 ± 2.1 ng/kg/min, respectively) compared to the placebo-treated subjects (567 ± 109 ng/kg/min, P < .005 vs. either ACEI-treated group).

Table 3 shows hourly urine output during and after i.v. bradykinin infusion. Urine output tended to be highest during the infusion hour and decrease over time after infusion. There was no effect of either ACE inhibition or indomethacin on urine volume. Urinary 2,3-dinor-6-keto PGF\textsubscript{1α} excretion increased significantly after i.v. administration of bradykinin (P = 6.55, P = .013) (Fig. 5). There was no difference between the effect of captopril and quinapril (F = 1.07, P = .323). Because ACEI-treated patients tolerated lower bradykinin doses than did the placebo-pretreated patients (see "Methods"), a direct comparison of the effect of bradykinin on urinary prostaglandin excretion in the presence of placebo or ACE inhibitor is difficult to make. Urinary 2,3-dinor-6-keto-PGF\textsubscript{1α} increased from 156 ± 16 to a peak of 596 ± 146 pg/mg Cr after a mean cumulative bradykinin dose of 190 ± 40 ng/kg in the ACEI-treated subjects and from 174 ± 31 to a peak of 380 ± 156 pg/mg Cr and from 184 ± 28 to 2857 ± 825 pg/mg Cr after mean cumulative bradykinin doses of 592 ± 0 and 10,400 ± 2529 ng/kg bradykinin, respectively, in the placebo-treated subjects. The relationship between the cumulative dose of bradykinin infused and the cumulative urinary excretion of prostacyclin metabolite from time 0 to 4 hr (expressed as the area-under-the-concentration curve) is shown in Figure 6. Again pretreatment with an ACE inhibitor potentiated the effect of bradykinin.

To determine whether ACE inhibition potentiated the prostacyclin response to bradykinin to the same degree as the hypotensive response, we plotted the maximal change in urinary prostacyclin after bradykinin vs. the maximal decrease in blood pressure in subjects treated with either placebo or ACE inhibitor (fig. 7). If ACE inhibition potentiated each of these effects equally, then the slope of this line would be the same in patients treated either placebo or ACEI. However, as illustrated in Figure 7, ACE inhibition markedly altered the relationship between the pressure response to bradykinin and the prostacyclin response (P < .002).

The effect of indomethacin on the response to i.v. bradykinin. Indomethacin increased the supine resting MAP, measured before i.v. infusion of bradykinin (105.6 ± 6 mm Hg vs. 99.7 ± 4.4 mm Hg, P < .03). Supine pulse was significantly lowered in the presence of indomethacin (66.5 ± 2.44 vs. 72.4 ± 2.7 bpm, P < .03). Significantly, pretreatment with indomethacin abolished the effect of bradykinin on pros-
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>ACEI</th>
<th>ACEI + INDO Day 2</th>
<th>ACEI + INDO Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hr Na excretion (mEq)</td>
<td>168 ± 10.5</td>
<td>138 ± 11*</td>
<td>170 ± 11.4</td>
</tr>
<tr>
<td>24-hr urine volume (cc)</td>
<td>1949 ± 162</td>
<td>1747 ± 150*</td>
<td>1995 ± 167</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.2 ± 5.22</td>
<td>88.0 ± 5.31*</td>
<td></td>
</tr>
</tbody>
</table>

ACEI, ACE inhibitor; INDO, indomethacin.
* P < .05 vs. ACEI alone.

arm, and found no difference between these two structurally different ACE inhibitors (Li Kam et al., 1993) Bonner et al. have previously shown that acute captopril administration (50 mg) reduced the i.v. dose of bradykinin required to lower blood pressure in hypertensive subjects 20- to 50-fold (Bonner et al., 1990). Acute doses of lisinopril (20 mg) and ramipril (5 mg) appeared to exert a similar effect in salt-deplete normal controls, but the number of subjects studied and the lack of titration of ACE inhibitor dose did not permit the investigators to compare agents. The effect of ACE inhibition on prostacyclin was not measured in this study.

In our study, chronic pretreatment with an ACE inhibitor significantly potentiated both the vasodepressor and prostaglandin response to bradykinin. At equipotent doses, although captopril selectively increased urinary prostacyclin excretion, captopril and quinapril equally potentiated the response to bradykinin. This suggests that potentiation of bradykinin, in contrast to increased prostacyclin production, is a class effect and does not require the presence of a sulfhydryl group.

ACE inhibitors alter the vasodepressor response to bradykinin by blocking the degradation of this endogenous vasodilator. Because safety considerations precluded intraaortic injection of bradykinin we were not able to assess to what extent potentiation of the vasodepressor response by ACE inhibitor resulted from inhibition of pulmonary ACE or kininase. Although the measurement of bradykinin levels is fraught with methodological problems, Bonner et al. (1990) have reported that the fall in systolic blood pressure after i.v. injection of bradykinin correlates with the increase in arterial kinin. To the extent that the decrease in blood pressure after bradykinin does reflect bradykinin levels, data from our study suggest that, although ACE inhibitors increase bradykinin levels by approximately 50-fold, they also attenuate the effect of bradykinin on prostacyclin production. Thus, for a given hypotensive response to bradykinin the prostacyclin response was decreased in the ACE treated subjects (fig. 7). The mechanism through which ACE inhibitors might attenuate the prostacyclin response to bradykinin is not known; however, ACE inhibitors decrease Ang II levels and, as outlined in the introduction, Ang II increases prostacyclin pro-
duction (Mullane and Moncada, 1980a; Grodzinska and Gryglewski, 1980; Shebuski and Aiken, 1980; Woodman et al., 1983; Hura and Kunau, 1988; Chardonnens et al., 1989). Interestingly, more than one group has found that ACE inhibition decreases prostaglandin production in vitro (Yanagisawa et al., 1990; Kobayashi et al., 1991). Our study suggests that in the absence of ACE inhibition there may be synergy between the effects of bradykinin and Ang II on prostacyclin production.

The effect of indomethacin on the response to intravenous bradykinin. The addition of indomethacin abolished the prostacyclin response to bradykinin, but did not alter the vasodepressor response. This conflicts with a study by Mullane and Moncada (1980b) in which indomethacin partially blocked the potentiation of both the blood pressure and the renal vasodilator responses to bradykinin by captopril. Although regional blood flow, cardiac output and systemic vascular resistance were not measured in our study, the observed lack of effect of indomethacin on the blood pressure response to bradykinin is consistent with the results of Bonner et al. (1990) who showed that acute indomethacin did not alter the vasodilator response to bradykinin in normal controls. Recent in vitro and in vivo studies suggest that the vasodilator response to bradykinin is mediated through nitric oxide and, possibly, the P450 metabolite of arachadonic acid. Thus, Vallance et al. (1989) have shown that the nitric oxide synthesis inhibitor, L-NMMA, blocks the venodilator response to bradykinin in healthy subjects, although Quilley et al. (1993) have demonstrated a P450-dependent, nitric-oxide-independent coronary and renal (Fulton et al., 1992) vasodilator effect of bradykinin. Further studies are needed to delineate the relative importance of these mediators in the vasodilator response to ACE inhibition in humans. The disassociation between the effect of indomethacin on the prostacyclin response to bradykinin and its effect on the vasodepressor response to bradykinin suggests that prostacyclin plays a relatively minor role in the antihypertensive effects of ACE inhibitors.

Effect of treatment on urinary 2,3-dinor-6-keto-PGF₁α excretion. Treatment with captopril, titrated to maintain a diastolic blood pressure <85 mm Hg, increased urinary excretion of 2,3-dinor-6-keto-PGF₁α, whereas treatment with quinapril, a carboxyl-containing ACE inhibitor, did not. Importantly, the doses used were equipotent in lowering blood pressure. Although many previously published studies have also shown that captopril increases prostaglandin production (Swartz et al., 1980; Moore et al., 1981; Hornych et al., 1982; Stanek and Silberbauer, 1986; Quilley et al., 1987; Omata et al., 1987), this has not been true universally in humans (Ogihara et al., 1981; Witzgall et al., 1982; Vlasses et al., 1983; Gerber et al., 1993). Studies of the effects of enalapril, a carboxyl-containing ACEI, on prostaglandin production in humans have yielded similarly conflicting results (Oparil et al., 1987; Mittman et al., 1986; Shoback et al., 1983; Gerber et al., 1993). One possible explanation for the divergent findings regarding the effect of ACE inhibitors on prostaglandin production is that the effect of captopril on prostaglandin production appears to be dose-dependent in vitro (Zusman, 1983). In this regard, because we did not measure a dose-urinary prostaglandin response curve for each ACE inhibitor we cannot exclude the possibility that, at higher doses, quinapril also increases excretion of prostacyclin metabolite. However, the finding that quinapril did not increase the urinary excretion of this prostacyclin metabolite is compatible with the results of an earlier study by Säynävälammi et al. (1988), in which the investigators found that quinapril did not alter plasma or urinary concentrations or of PGE₂, 6-keto-PGF₁α, and thromboxane B₂. Whether the increases in urinary excretion of 2,3-dinor 6-keto-PGF₁α, in response to captopril measured in this study are of therapeutic importance remains to be determined.

The effect of treatment on ambulatory blood pressure. In the salt-replete normal-to-high renin patients studied here, indomethacin attenuated the effects of ACE inhibition on 24-hr ambulatory mean arterial blood pressure, as well as on the supine mean arterial pressure, measured...
before bradykinin infusion. This effect cannot be ascribed to a nonspecific effect of indomethacin, because indomethacin did not similarly raise 24-hr blood pressure in the placebo control group.

Although the effect of indomethacin on 24-hr ambulatory mean arterial pressure appears to confirm other studies that have shown indomethacin to raise casual blood pressure measurement in ACEI-treated subjects (Moore et al., 1981; Quilley et al., 1987; Omata et al., 1987; Oghara et al., 1981; Witzgall et al., 1982, Goldstone et al., 1981; Salvetti et al., 1982), the data also raise inconsistencies that must be addressed. The first is that, although only captopril increased the urinary excretion of prostacyclin metabolite, indomethacin significantly attenuated the antihypertensive effect only of quinapril. The failure of indomethacin to significantly attenuate the antihypertensive effect of captopril may reflect a type II error, as two patients did not complete captopril plus indomethacin treatment (see "Methods"). Nevertheless, the mechanism through which indomethacin raised blood pressure in the quinapril-treated patients, who did not have increased urinary excretion of prostacyclin metabolite, should be further explored. Additional studies are needed to clarify the role of prostacyclin in the antihypertensive effect of angiotensin-converting enzyme inhibitors.

**Table 3: Hourly Urine Volume (ml) during and after i.v. bradykinin**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Captopril</th>
<th>Quinapril</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>0</td>
<td>315 ± 60</td>
<td>325 ± 35</td>
<td>293 ± 30</td>
</tr>
<tr>
<td>1</td>
<td>475 ± 56</td>
<td>467 ± 79</td>
<td>465 ± 82</td>
</tr>
<tr>
<td>2</td>
<td>203 ± 72</td>
<td>138 ± 59</td>
<td>134 ± 67</td>
</tr>
<tr>
<td>3</td>
<td>169 ± 54</td>
<td>118 ± 48</td>
<td>99 ± 45**</td>
</tr>
<tr>
<td>4</td>
<td>144 ± 60*</td>
<td>210 ± 60</td>
<td>91 ± 34***</td>
</tr>
</tbody>
</table>

**Legend:** *P < .05, **P < .01, ***P < .005 vs. baseline. +P < .02, ++P < .005 vs. placebo before bradykinin infusion. There were no significant differences in urine volume between groups or with and without indomethacin.
2,3-dinor-6 keto PGF$_{1\alpha}$, is not clear. Indomethacin transiently decreased measured urinary sodium excretion and volume in the ACE inhibitor-treated subjects such that body weight increased significantly during indomethacin therapy. Indomethacin causes sodium retention by blocking the formation of renal PGE$_2$ (Dunn and Hood, 1977). The instability of the PGE$_2$ metabolite and the lack of commercially available mass spectrometry standards precluded our measuring urinary excretion of PGE$_2$ in this study and it is possible that both quinapril and captopril could have increased renal PGE$_2$ production. It is difficult to attribute the attenuation of the antihypertensive effect of ACE inhibition by indomethacin to sodium retention alone, because indomethacin also caused sodium retention in the placebo-treated patients but appeared to lower blood pressure in this group. The hypotensive effect of indomethacin in the placebo group may relate to the fact that only normal-to-high renin subjects were studied. Indomethacin has been reported to lower blood pressure in renin-dependent animals and patients (Oates, 1988). Given these inconsistencies, it is likely that the small effect of indomethacin on blood pressure in the ACEI-pretreated patients does not represent a clinically significant finding. Moreover, the dissociation of the vasodepressor effect of bradykinin from the excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$ also contradicts the idea that increases in prostacyclin contribute to the hypotensive effects of ACE inhibitors.

**Conclusion**

In summary, at equipotent doses, captopril and quinapril dramatically potentiated the vasodepressor response to bradykinin. ACE inhibition altered the relationship between the vasodepressor response to bradykinin and the prostacyclin response to bradykinin. Indomethacin abolished the prostacyclin response to bradykinin but did not alter the vasodepressor response. These data confirm a prostaglandin-inde-
Fig. 8. The effect of indomethacin on (A) the mean arterial pressure (MAP) response and (B) the pulse response in ACEI-pretreated patients. *P < .05, **P < .001 vs. baseline.


