Decreased Vascular Responsiveness Produced by Angiotensin-Converting Enzyme Inhibitors in the Rat Isolated Kidney

SATORU CHIBA, M.D., CAROLINE P. QUILLEY, B.SC., AND JOHN C. MCGIFF, M.D.

SUMMARY The effects of three angiotensin converting enzyme (ACE) inhibitors — captopril, MK-421 diacid, and teprotide — on renal vascular responses to graded (50, 100, 200 ng) injections of norepinephrine (NE) were examined in rat isolated perfused kidneys, bearing a mean basal perfusion pressure of 78 ± 10 mm Hg. The minimum dose of captopril (0.05 μg/ml, low dose) that abolished the vasoconstrictor responses to 100 and 200 ng angiotensin I did not affect NE-induced renal vasoconstriction, whereas a dose 100 times greater (high-dose captopril, 5 μg/ml) reduced the vasoconstrictor action of NE. MK-421 diacid also at high dose (1 μg/ml), caused similar reductions in renal vasoconstrictor responses to NE. In contrast, a high dose of teprotide (50 μg/ml) did not affect renal vascular responsiveness to NE.

The threshold dose of NE that released prostaglandins, measured by bioassay, was 50 ng. Indomethacin (1 μg/ml) prevented NE-induced release of prostaglandins but did not affect the ability of captopril to attenuate NE-induced vasoconstriction. We conclude that captopril and MK-421 diacid decreases vascular reactivity in the rat isolated kidney by a mechanism independent of ACE inhibition and unrelated to a prostaglandin-dependent vascular mechanism. Moreover, the presence of a mercapto function in the ACE inhibitor is not essential since captopril, which has a sulfhydryl group, and MK-421 diacid, which lacks this group, have similar effects on renal vascular responsiveness. (Hypertension 4 (suppl II): II-80-II-85, 1982)

KEY WORDS • captopril • teprotide • MK-421 • norepinephrine • rat isolated kidney • renal prostaglandins • vascular reactivity

The introduction of clinically effective inhibitors of angiotensin-converting enzyme (ACE) has resulted in reexamination of the importance of the renin-angiotensin system in the initiation and maintenance of hypertension in humans and in experimental animals. The first inhibitor of ACE to become available, the nonapeptide teprotide (SQ 20,881),1 was inactive when taken orally. The antihypertensive effectiveness of teprotide led to the development of captopril (SQ 14,225), an orally active ACE inhibitor. Captopril, a sulfhydryl-containing drug, has been intensively studied in primary and secondary forms of hypertension since its introduction in 1977.2 Because of the possible relationship of the sulfhydryl group of captopril to toxic manifestations associated with its use, an orally active ACE inhibitor lacking a mercapto function was sought. This led to the recent discovery of MK-4213 which has been reported to be about eightfold more potent than captopril in reducing rat blood pressure.4 MK-421 is a prodrug that must be deesterified to the active diacid form, after oral ingestion, to be effective. MK-421,5 like captopril,6 reduces blood pressure in some forms of experimental and genetic hypertension in which involvement of the renin-angiotensin system is not thought to be primary.

To circumvent conceptual difficulties arising from the need to ascribe all of the antihypertensive effects of ACE inhibitors to the prevention of the transformation of angiotensin I (AI) to angiotensin II (AII), other effects of these drugs have been examined, such as those related to the stimulation of prostaglandin-dependent mechanisms, as well as to increasing levels of kinins since ACE also inactivates kinins.7

In the present study, we have compared the effects of teprotide, captopril, and the parent diacid form of MK-421 (MK-421 diacid), which is active in vitro on the vascular responsiveness of the isolated kidney of the rat, to the effects of norepinephrine (NE). As only captopril possesses a free sulfhydryl group, these experiments should clarify the relationship of the mer-
capto function to any effects of ACE inhibitors on vascular responsiveness. We also examined the possible participation of prostaglandin-dependent mechanisms in determining the renal vascular effects of ACE inhibitors.

Methods

Isolation and Perfusion of Kidneys

Pairs of kidneys from male Wistar rats (body weight 240–280 g; age 8–10 weeks) were prepared by a modification of the methods described by Armstrong et al. The animals were anesthetized with Inactin 150 mg/kg i.p., the abdominal cavity exposed, and the kidneys flushed with warmed perfusate through a polyethylene cannula (PE 190) inserted into the lower abdominal aorta. The kidneys were rapidly removed from the animal and perfused in a water-jacketed container with Krebs-Henseleit solution at 37°C and gassed with 95% O₂, 5% CO₂. The perfusion rate was fixed at 20 ml/min/2 kidneys using a Harvard peristaltic pump. Changes in perfusion pressure were measured by means of a Gould Statham P23 1D pressure transducer placed proximally to the kidneys.

Assays

Renal prostaglandin release was measured by directing the renal effluent so as to superfuse continuously a rat stomach strip (fig. 1). A mixture of pharmacological antagonists and indomethacin (1 μg/ml) was added to the perfusion fluid before it superfused the tissue. Musculotropic activity of the rat stomach strip was detected with a Harvard transducer (Type 386) and recorded by a Soltec KA-41 pen

![Figure 1](http://hyper.ahajournals.org/doi/abs/10.1161/01.hyp.70.2.128.png)

**Figure 1.** Two pairs of kidneys were perfused with Krebs-Henseleit medium containing an inhibitor of converting enzyme. In some experiments, indomethacin (1 μg/ml) was added to the perfusion fluid. Changes in renal perfusion pressure and release of prostaglandin-like material were recorded simultaneously from the paired kidneys. Norepinephrine was injected into the perfusion system, and the effects were recorded as indicated on the displayed traces.
At the end of each experiment, contractile responses of the stomach strip to known amounts of standard PGF$_2\alpha$ were obtained, and the amount of prostaglandin-like material released from the kidney was estimated and expressed as PGF$_2\alpha$ equivalents. The sensitivity of the tissue was 1–2 ng for the PGF$_2\alpha$ standard. Captopril (5 µg/ml) and MK 421 diacid (1 µg/ml) did not affect the contractile responses of the stomach strip to standard PGF$_2\alpha$ and PGF$_2\alpha$.

In some experiments, immunoreactive PGF$_2\alpha$ and PGF$_{2\alpha}$ were measured by radioimmunoassay (RIA). Samples were acidified to pH 3.0 with formic acid and extracted with ethyl acetate and cyclohexane (1:1). PGF$_2\alpha$ and PGF$_{2\alpha}$ antisera were obtained from the Institut Pasteur Production, Paris, France. The cross-reactivity of PGF$_{2\alpha}$ antiserum with PGF$_2\alpha$ was 0.15% and that of PGF$_2\alpha$ antiserum with PGF$_{2\alpha}$ was 0.06%. Assays were carried out in duplicate. The lower limits of sensitivity of these RIA's were 7–10 pg for PGF$_2\alpha$ and PGF$_{2\alpha}$ as determined by 10% displacement of radioactivity from the maximum binding value.

**Experimental Protocol**

Agents and drugs were injected or infused into a port proximal to the perfusion pump (fig. 1). The volumes used for bolus injections were less than 50 µl, and the infusion rate of drugs was fixed at 0.2 ml/min by a Watson-Marlow roller pump (MHRE7). The effects of three different ACE inhibitors, captopril, MK-421 diacid, and teprotide, on renal vascular responsiveness to NE were studied separately. The minimal concentration of captopril and MK-421 required to inhibit ACE activity maximally in the isolated perfused rat kidney was determined by measuring drug-induced changes in vasoconstrictor responses to AI. In preliminary experiments, we observed that a 100-fold increase in the concentration of captopril that caused maximal inhibition of ACE was required to influence consistently renal responsiveness to NE. These concentrations of captopril and MK-421 diacid required to inhibit ACE activity maximally in the isolated perfused rat kidney were measured simultaneously. The effectiveness of the ACE inhibitor was tested at the end of each experiment by determining the degree of suppression of the renal vasoconstrictor responses to either 100 or 200 ng of AI. The effect of a cyclooxygenase inhibitor, indomethacin (1 µg/ml), on the vascular action of high-dose captopril was also studied in similar parallel experiments.

**Drugs**

Captopril (Squibb), teprotide (Squibb), or MK-421 diacid (Merck) were dissolved in Krebs-Henseleit solution just before infusion. Indomethacin (Sigma) was first dissolved in 4.2% of NaHCO$_3$ and then diluted 100-fold with distilled water. Norepinephrine (dl-arterenol-HCl, Sigma) was dissolved in 0.9% saline containing 5 µg/ml of citric acid, and the dose of NE was expressed in terms of the salt. Angiotensin I (Sigma) and All (Sigma) were dissolved in 0.9% saline just before use. The Krebs-Henseleit solution had the following composition (mM): NaCl, 118.4; KCl, 4.7; CaCl$_2$, 2.5; KH$_2$PO$_4$, 1.2; MgSO$_4$, 7H$_2$O, 1.2; NaHCO$_3$, 25.0; and dextrose 5.6.

**Statistics**

Results are expressed as means ± standard error of the mean (SEM). The statistical significance of the differences between mean values were estimated by Students' paired t tests where pairing was based on similar but not identical animals in which obser-
observations were obtained on the same day. However, the two pairs of controls in figure 2 were analyzed separately since the experiments were performed several months apart. A p value less than 0.05 was considered to be significant.

An additional analysis was performed which included in a single analysis all four treatments in figure 2 as well as the three doses of NE received by each kidney but which ignored the pairing of animals. The analysis is that of a repeated measurement analysis of variance in which the repeat measurements referred to doses of NE. The data analyzed were square roots of the observations since inspection of the data indicated an increase in variability as the mean increased. Because the analysis indicated that differences between treatments (on a square root scale) were constant for the different doses of NE, an overall test for each dose and its control was done, and a Bonferroni adjustment was performed for the multiple tests of active against control.

Results

There were no significant differences among groups in body weight, kidney weight (range, 2.13 ± 0.03 g to 2.20 ± 0.09 g) and basal renal perfusion pressures. Within 40 minutes after removal of the kidneys, renal perfusion pressure averaged 78 ± 10 mm Hg. Changes in basal perfusion pressure during the experimental period, an index of the stability of the preparation, was usually less than 10 mm Hg and rarely exceeded 15 mm Hg. Administration of ACE inhibitors did not modify renal perfusion pressure.

Captopril caused a dose-related inhibition of Al-induced vasoconstriction. Maximal inhibition was obtained with 50 ng/ml of captopril, the low dose. MK-421 diacid was at least 10 times more potent than captopril, causing maximal inhibition of Al-induced renal vasoconstriction at 5 ng/ml. The possibility that captopril, in doses greater than those required for ACE inhibition, could diminish vascular reactivity to AlI was examined by assessing the effect of captopril on renal vascular responsiveness to 5 ng AlI, a dose that had an equipotent effect from renal vasoconstrictor 200 ng NE. Administration of high-dose captopril resulted in attenuation of the renal vasoconstrictor action of AlI as indicated by a reduction of AlI-induced elevation of perfusion pressure from 93 ± 9 to 43 ± 6 mm Hg (p < 0.001).

Although both doses of captopril caused maximal inhibition of Al-induced renal vasoconstriction, they differed in their effects on the vasoconstrictor responses to NE (fig. 2). Low-dose captopril did not change the vasoconstrictor response to NE significantly, although the dose response curve tended to shift to the left. However, in the presence of high-dose captopril, renal vasoconstrictor responses to NE were reduced significantly; perfusion pressure responses to 50 ng NE decreased from 15 ± 4 to 3 ± 1 mm Hg and from 86 ± 7 to 33 ± 10 mm Hg to 200 ng NE.

MK-421 diacid in a concentration of 1 μg/ml had effects similar to high-dose captopril on renal vascular responsiveness to NE; e.g., perfusion pressure increments to 50 and 200 ng of NE were decreased from control values of 13 ± 3 and 102 ± 11 mm Hg to 5 ± 1 and 60 ± 13 mm Hg, respectively (fig. 3). In contrast,
trepotide infused at a concentration of 50 μg/ml did not affect the vasoconstrictor responses to NE. This concentration of trepotide, tenfold that of high-dose captopril, was determined from the reported difference in its potency, calculated on a weight basis, as an inhibitor of ACE. AI-induced renal vasoconstriction was inhibited maximally in kidneys treated with either trepotide or MK-421 diacid, as indicated by suppression of the vasoconstrictor action of AI (fig. 3).

NE caused a dose-related increase in renal efflux of prostaglandins measured by bioassay (table 1). High-dose captopril and MK-421 diacid (1 μg/ml) tended to decrease the release of prostaglandins evoked by NE, although this effect was significant only at the 100 ng dose of NE for kidneys treated with captopril. As the bioassay-superfusion method does not permit assay of basal levels of prostaglandins, but rather changes in release elicited by a stimulus above basal efflux, RIA was used to determine possible changes in concentrations of PGE$_{2}$ and PGF$_{2}$a, induced by inhibition of ACE. In five control experiments, PGE$_{2}$ and PGF$_{2}$a concentrations in renal venous effluent were 99 ± 28 and 89 ± 24 pg/ml, respectively, when measured by RIA within 40 minutes after beginning the experiment. Inhibition of ACE with high-dose captopril or MK-421 diacid did not change these levels significantly, viz., PGE$_{2}$ and PGF$_{2}$a concentrations were 110 ± 33 and 94 ± 21 pg/ml, respectively, when measured within 40 minutes after starting infusion of the ACE inhibitor.

Indomethacin, 1 μg/ml, suppressed cyclooxygenase activity as it prevented NE-induced release of immunoreactive and bioassayable prostaglandin-like material, the latter indicated by the absence of contraction of the stomach strip (fig. 1). Moreover, indomethacin caused reduction of basal release of prostaglandins as indicated by the decline in basal tone of the stomach strip (fig. 1). Elimination of prostaglandin-dependent vascular mechanisms with indomethacin did not affect the capacity of high-dose captopril to reduce the renal vascular responsiveness to NE. In kidneys infused simultaneously with either high-dose captopril or both high-dose captopril and indomethacin, the vasoconstrictor effect of NE was not modified: perfusion pressure increased by 6 ± 1 vs 5 ± 2 mm Hg, respectively, to 50 ng NE and by 59 ± 13 and 58 ± 19 mm Hg, respectively, to 200 ng NE.

**Discussion**

The present study addressed changes in vascular reactivity in the rat isolated kidney induced by inhibitors of ACE. We demonstrated that the ACE inhibitors differ in their ability to reduce vascular reactivity, an effect that appeared to be independent of inhibition of converting enzyme. Further, the presence of a mercapto function in captopril and its absence in MK-421, each of which diminished vascular reactivity, indicated that a sulfhydryl group was not essential for this effect. This finding is of some importance to the understanding of the action of ACE inhibitors, as sulfhydryl-containing compounds have the capacity to affect the activity of some vasoactive hormones as well as proteins.

The doses of captopril and MK-421 that attenuated vascular reactivity were approximately 100-times greater than those doses that maximally suppressed ACE activity in the isolated kidney of the rat, the latter indicated by abolition of changes in renal perfusion pressure to injected AI. This suggests that effects other than ACE inhibition contribute to some of the vascular actions of captopril and MK-421 diacid. As the ACE inhibitors did not affect renal perfusion pressure, changes in the intrarenal levels of AI and AII caused by ACE inhibitors are assumed to be without direct effects on the renal vasculature. This consideration applies even for the isolated kidney, in view of the demonstration of AII in the vascular pole of the glomerulus. Although there is evidence that changes in the AII levels within the vascular wall can influence adrenergic neurotransmission, and that captopril is capable of decreasing adrenergic nervous activity in blood vessels through a prejunctional action, the effects of captopril and MK-421 on injected NE in the present study are best explained by a post-junctional site of action, i.e., effects independent of inhibition of ACE. Moreover, high-dose captopril was also shown to decrease renal vascular responsiveness to AII, an effect that cannot readily be related to ACE inhibition. Nonetheless, it is difficult to interpret those vascular actions of captopril presumed to be independent of ACE inhibition because of the complicated interactions between the renin-angiotensin system and other hormonal and neural systems; e.g., locally generated AII in blood vessels enhances noradrenergic nervous activity.

Two recent studies have supported the view that captopril can affect vascular reactivity "directly" as it has been shown to attenuate the vasoconstrictor effect of NE in the isolated kidney and in the isolated mesenteric vascular bed of the rat. Moreover, in each study, trepotide, in contrast to captopril, did not attenuate NE-induced vasoconstriction in these vascular beds. We have also shown the absence of an effect of trepotide on vascular responsiveness, even

---

**Table 1. NE-Induced Release from the Rat Isolated Kidney of Prostaglandins, Measured by Bioassay as ng PGE$_{2}$ Equivalents (Mean ± SEM)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (μg/ml)</th>
<th>n</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0</td>
<td>7</td>
<td>6 ± 2</td>
<td>21 ± 6</td>
<td>48 ± 12</td>
</tr>
<tr>
<td>Captopril</td>
<td>1.0</td>
<td>3</td>
<td>3 ± 1</td>
<td>8 ± 4</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>Control</td>
<td>3.0</td>
<td>7</td>
<td>2 ± 1</td>
<td>7 ± 2*</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>MK 421, diacid</td>
<td>5.0</td>
<td>7</td>
<td>1 ± 1</td>
<td>14 ± 6</td>
<td>45 ± 13</td>
</tr>
</tbody>
</table>

* p < 0.05.
when teprotide was administered in doses many fold the dose required to produce maximal inhibition of ACE. This differential effect of three ACE inhibitors on vascular reactivity is additional evidence for a dose-related effect of captopril and MK-421 on vascular reactivity that is independent of converting enzyme inhibition. If this were not the case, then teprotide should also have inhibited renal vascular responsiveness to NE. Diminished renal vascular reactivity to NE caused by high-dose captopril appears to be relatively nonspecific since the renal vasoconstrictor effects of AII were also attenuated. The direct effects of ACE inhibitors on blood vessels may contribute to our understanding of their antihypertensive actions in various forms of experimental and genetic hypertension in which the renin-angiotensin system is apparently not primarily responsible for blood pressure elevation.6-8

The hypotensive effect of captopril has been suggested to be mediated in part by increased production of prostaglandins.9,10 We have shown that treatment with either captopril or MK-421 did not cause enhanced release of bioassayable prostaglandins in response to NE in the rat isolated kidney and did not affect basal release of PGE\(_2\) and PGF\(_2\alpha\), when measured by RIA. Rather, there was diminished release of prostaglandins in response to NE, probably a secondary effect of captopril and MK-421 diazid resulting from the diminished vasoconstrictor action of the pressor hormone. Further, inhibition of prostaglandin synthesis with indomethacin did not affect the capacity of high-dose captopril to diminish vascular responsiveness to NE. However, it is entirely possible that in the intact animal or in hypertensive humans other factors come into play whereby inhibition of ACE results in augmentation of prostaglandin-dependent mechanisms.10

Whether the effects of ACE inhibitors, similar to those described in this study, also occur in vivo is unknown, and statements based on translation of the findings of the present study to conscious animals and humans must be made cautiously, if at all. Thus, the relatively high concentration of ACE inhibitors used in the present study, and the importance of extrarenal sites of action that respond to the effects of ACE inhibitors, such as the central nervous system, must be considered when interpreting these findings. We conclude that high-dose captopril and MK-421 can modify vascular reactivity and this effect may contribute to the blood pressure lowering action of ACE inhibitors in low-renin and, perhaps, normal-renin forms of experimental and human hypertension.

References


Decreased vascular responsiveness produced by angiotensin-converting enzyme inhibitors in the rat isolated kidney.
S Chiba, C P Quilley and J C McGiff

*Hypertension*. 1982;4:80-85
doi: 10.1161/01.HYP.4.3_Pt_2.80

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/4/3_Pt_2/80

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org/subscriptions/