Video-microscopic Assessment of the Role of Tissue Angiotensin-converting Enzyme in the Control of the Renal Microcirculation

P. M. TER WEE, H. G. FORSTER and M. EPSTEIN
Nephrology Section, Department of Veterans Affairs Medical Center, and Division of Nephrology, University of Miami School of Medicine, Miami, Florida
Accepted for publication December 20, 1996

ABSTRACT
In the present study, we assessed the role of tissue angiotensin-converting enzyme as a determinant of intrarenal hemodynamics by using the angiotensin-converting enzyme inhibitor trandolaprilat and the angiotensin II receptor antagonist losartan. Afferent and efferent arteriolar diameters were measured with computer-assisted vessel imaging in isolated perfused hydronephrotic rat kidneys. In response to the addition of 1.0 nM angiotensin I, afferent arterioles constricted by 27.3 ± 2.4% and efferent arterioles by 20.9 ± 2.4%. These constrictions were similar to those observed after the administration of 0.3 nM angiotensin II (33.7 ± 2.3% and 20.9 ± 2.4% in afferent and efferent arterioles, respectively). Pretreatment with the angiotensin-converting enzyme inhibitor trandolaprilat (0.1–10 μM) blunted the angiotensin I-induced constriction of afferent arterioles (12.7 ± 1.4%) and completely abolished the angiotensin I-induced constriction of efferent arterioles. Subsequent addition of angiotensin II to the perfusate resulted in a marked decrease of afferent (39.9 ± 1.8%) and efferent (27.8 ± 3.3%) arteriolar diameters. Pretreatment with the angiotensin II receptor antagonist losartan completely blocked the angiotensin I-induced constriction of both afferent and efferent arterioles. Collectively, these data suggest that angiotensin I affects renal microvessels through its conversion to angiotensin II, mediated by locally available tissue angiotensin-converting enzyme, which subserves the local control of the renal microcirculation.

It has become increasingly apparent that the renin-angiotensin system acts not only as a circulating hormone system but also as a local endocrine/paracrine system participating in the control of renal function (Rosivall, 1995). ACE has been localized in the microvilli of the proximal tubule and in the vascular endothelium of the kidney (van Sande et al., 1985; Johnston and Kohsuki, 1989; Zhou and Mendelsohn, 1992), as well as in other tissue beds, most notably the vascular endothelium of the lung (Ryan et al., 1975). The de novo generation of AII by renal tissue has been demonstrated in the isolated perfused kidney model (Schmidt et al., 1986), as well as by infusions of AI directly into the renal artery (Aiken and Vane, 1972). To date, however, the effects of ACE-mediated conversion of AI to AII on AA and EA responsiveness have not been investigated directly.

As detailed previously (Hayashi et al., 1989), the isolated perfused rat kidney model facilitates direct examination of the renal microvascular behavior in a controlled in vitro setting. Thus, this experimental model offers distinct experimental advantages for evaluating the intrarenal conversion of AI to AII, in which the factors influencing the renal microvasculature are carefully defined and controlled (Hayashi et al., 1989). In the experiments described in the present study, we used the isolated, perfused, hydronephrotic kidney model to characterize the effects of AI on rat AA and EA. In addition, the AI-induced vascular responses were further characterized by studying the effects of the ACE inhibitor trandolaprilat and the AII type 1 (AT1) receptor antagonist losartan on the renal microcirculation.

Methods
Preparation of Donor Animals
In 6-week-old male Sprague-Dawley rats (Charles River Co.), the right ureter was ligated under methoxyflurane anesthesia (Metofane; Pittman-Moore, Mundelein, IL) to establish unilateral hydronephrosis. After 4 to 8 weeks, the hydronephrotic kidneys were excised and perfused in vitro as described below. At that point, renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of renal microvessels (Steinhausen et al., 1983; Loutzenhiser et al., 1990).

Received for publication May 29, 1996.

1 Supported in part by a grant from the Department of Veterans Affairs (2456) and by Knoll Pharmaceutical Co.
2 Present address: Free University Hospital, Dept. of Nephrology, Amsterdam, The Netherlands.

ABBREVIATIONS: AA, afferent arteriole(s); ACE, angiotensin-converting enzyme; AI, angiotensin I; AII, angiotensin II; EA, efferent arteriole(s).
Perfusion of Hydronephrotic Kidneys

After the rats were anesthetized with methoxyflurane, the kidneys were exposed through a midline incision. The renal artery of the hydronephrotic kidney was cannulated by introducing the perfusion catheter through the mesenteric artery and across the aorta, as previously described (Epstein et al., 1980). Perfusion with warm oxygenated medium (pH 7.4) was initiated in situ during the cannulation procedure, to avoid ischemia of the perfused kidney. Subsequently, the kidney was excised and placed on the stage of an inverted microscope (model K; Nikon, Tokyo, Japan), modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom (Loutzenhiser et al., 1988).

The perfusion medium consisted of Krebs-Ringer bicarbonate buffer, containing 6.5 g/100 ml bovine serum albumin (Bovimari; Intergen Co., Purchase, NY), 5 mM d-glucose and a complement of amino acids (Epstein et al., 1980). Perfusate was provided to the kidney at a constant pressure from a pressurized medium reservoir. The reservoir pressure was maintained by the inflow of warm hydrated gas (95% O<sub>2</sub>/5% CO<sub>2</sub>), which exited through an adjustable back-pressure regulator (model 10 BP; Fairchild Industrial Products, Winston Salem, NC). The perfusion pressure was monitored at the level of the renal artery through an indwelling catheter and was maintained constant at 80 mm Hg throughout the experiments, to avoid pressure-induced vasoconstriction. The effluent was returned to the pressurized chamber by two rolling pumps (Masterflex, Chicago, IL).

**Determination of Vessel Diameters**

Video images of renal microvessels were obtained using an Ikegami video camera (model ITQ-47; Ikegami, Tokyo, Japan) and recorded with a videocassette recorder (model 8290; Panasonic). Vessels were selected for study on the basis of adequate flow, as estimated by the response to temporary (2-sec) occlusion of the perfusion line. Vessels that exhibited a sluggish or blunted response were considered to be perfused inadequately and excluded. To determine vessel diameters, the video recording was transmitted to an IBM-AT computer equipped with a video acquisition board (model IVG-128; Datacube, Peabody, MA). Vessel diameters were determined with an automated program custom-designed to measure the mean distance between parallel edges. At the magnification used, the vertical and horizontal resolutions were 0.23 and 0.42 μm/pixel, respectively. Vessels were aligned so that diameter measurements were obtained vertically, and individual measurements were taken at each pixel point (i.e., horizontally) to obtain the mean diameter over the vessel segment being scanned. The renal microvessel diameters were measured during the plateau of the response. A segment of AA or EA approximately 10 μm in length was scanned at 2- to 5-sec intervals.

**Perfusion Experiment Protocols**

After placement on the stage of the microscope, the kidneys were allowed to equilibrate for at least 30 min before basal measurements were obtained in each experiment. Investigated drugs were added directly to the perfusate. Sufficient time was allowed (15 min) for mixing and equilibration before re-evaluation of vessel diameters after the addition of the drugs. In preliminary dose-ranging studies, it was established that 1.0 nM AII induced AA and EA vasoconstriction similar to those that had been observed in previous studies from our laboratory after the administration of 0.3 nM AII (Loutzenhiser et al., 1991).

AI and AII. In the first experimental protocol, the constrictor effect of 1.0 nM AII on AA and EA (n = 4 kidneys) was compared with that observed in kidneys (n = 5) exposed to 0.3 nM AII. ACE inhibition. To assess the efficacy of ACE inhibition in attenuating the AII-induced constriction, trandolaprilat (10 μM; n = 4 kidneys) was added to the perfusate after basal measurements had been obtained. Subsequently, AII (1.0 nM) was added to the perfusate. Finally, at the end of the study, AII (0.3 nM) was added to the perfusate.

In an additional set of experiments, the potency of trandolaprilat was further characterized in the renal microvasculature. In three kidneys, the effect of pretreatment with a low dose of trandolaprilat (0.1 μM) on AI-induced constrictions of AA and EA was investigated. In another three kidneys, the ability of a high dose of trandolaprilat (10 μM) to block AII was assessed. Both studies were conducted using 1.0 nM AII.

In four other kidneys, the efficacy of the oral prodrug trandolapril in inhibiting AI-induced constrictions of AA and EA was investigated and compared with that of its diacid trandolaprilat. After determination of base-line diameters, 10 μM trandolapril was administered to the perfusate. Subsequently, 1.0 nM AII was added to the medium.

**All receptor blockade.** To determine whether the effect of AI on the renal microvessels is established by its ACE-mediated conversion to AII, the effects of pretreatment with the AT<sub>1</sub> receptor antagonist losartan was studied (n = 3 kidneys). After the determination of base-line diameters, losartan (10 μM) was added to the perfusate at a dose previously shown in our model to block the effect of AII on AA and EA constriction (Loutzenhiser et al., 1991). Subsequently, 1.0 nM AII was added to the perfusate and vessel diameters were re-evaluated. To further assess the efficacy of this AII receptor antagonist, endothelin-1 (0.3 nM) was added to the perfusate at the end of this protocol.

**Drugs**

AI and AII were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions of these peptides were dissolved in water, aliquoted and kept frozen until use. Trandolapril and its orally active, diacid form of ACE inhibitor (i.e., trandolaprilat) were kindly provided by Knoll Pharmaceutical Co. (Whippany, NJ). Stock solutions of the ACE inhibitors were prepared on the day of experiments by being dissolved in 1.0 N NaOH. Sodium lighting and yellow filters were used throughout the experiments to avoid photodegradation. Losartan was provided by DuPont-Merck (Wilmington, DE), and endothelin-1 was obtained from Peninsula Laboratories Inc. (Belmont, CA).

**Statistics**

All data are expressed as the mean ± S.E. Unless otherwise stated, the n values refer to the number of vessels studied. Data were analyzed by one-way analysis of variance, followed by Student’s t test. Changes within the experimental groups were subjected to a paired Student t analysis; a value of (P < .05) was chosen as the level of significance after correction by the Bonferroni method, with N being the number of interventions in the particular experiment.

**Results**

AI vs. AII. The addition of 1.0 nM AII elicited a constrictor response of the AA of 27.3 ± 2.4% (reduction in vessel diameter from 19.3 ± 0.6 to 14.0 ± 0.6 μm, P < .001 vs. base line; n = 24 vessels). This AII-induced AA constriction did not differ from that observed after administration of 0.3 nM AII alone (33.7 ± 2.3%; reduction in vessel diameter from 17.2 ± 0.5 to 11.4 ± 0.5 μm, P < .001 vs. base line, N.S. vs. AI; n = 17). Likewise, the AI-induced constriction of EA (20.9 ± 2.4%; from 18.13 ± 0.67 to 14.4 ± 0.75 μm, P < .001 vs. base line; n = 19) did not differ from that of AII (27.1 ± 3.1%; reduction in vessel diameter from 17.5 ± 1.1 to 12.5 ± 0.5 μm, P < .001 vs. base line, N.S. vs. AI; n = 11).

ACE inhibition. The addition of the ACE inhibitor trandolaprilat to the perfusate had no effect on either AA or EA base-line diameters. Pretreatment with trandolapril resulted in attenuation of the response of the AA to the addition...
of 1.0 nM AI (fig. 1, upper). AI-induced AA constriction after pretreatment with trandolaprilat averaged 12.7 ± 1.4% (reduction in vessel diameter from 19.3 ± 0.5 to 16.8 ± 0.4 μm, P < .001 vs. baseline and P < .001 vs. 1.0 nM AI alone; n = 23). Subsequent addition of 0.3 nM AII resulted in a brisk AA constriction of 39.9 ± 1.8% (reduction in vessel diameter to 11.6 ± 0.4 μm, P < .001 vs. both AI and control). In contrast to the observation in AA, pretreatment with trandolaprilat completely prevented the vasoconstrictor effects of AI on EA (fig. 1, lower). At medium concentrations of 0.1 nM AI, pretreated EA did not constrict (reduction in vessel diameter from 19.0 ± 1.2 to 18.8 ± 1.1 μm, P = .2 vs. base line, P < .001 vs. 1.0 nM AI alone; n = 13). The subsequent addition of AII resulted in a marked EA constriction of 27.8 ± 3.3% (reduction in vessel diameter to 13.5 ± 0.8 μm, P < .001 vs. both AI and control).

In the studies assessing the potency of trandolaprilat, no significant differences in the inhibitory effects of 0.1 μM vs. 10 μM trandolaprilat could be discerned. Pretreatment of the kidneys with either 0.1 or 10 μM trandolaprilat had no effect on basal AA or EA diameter (fig. 2, upper). Upon the addition of 1.0 nM AI to the perfusate, the AA of the kidneys treated with 0.1 μM trandolaprilat constricted by 7.8 ± 1.6% (reduction in vessel diameter from 19.0 ± 0.7 to 17.5 ± 0.7 μm, P < .001 vs. baseline; n = 18). The AA of kidneys pretreated with the higher dose of trandolaprilat (10 μM) constricted by 13.9 ± 2.8% (reduction in vessel diameter from 19.8 ± 0.4 to 17.1 ± 0.8 μm, P < .001 vs. base line, N.S. vs. 0.1 μM trandolaprilat; n = 16). In the same kidneys, pretreatment with either dose of trandolaprilat (fig. 2, lower) completely blocked the constrictor effects of AI on the EA when it was added to the perfusate (0.1 μM trandolaprilat, reduction in vessel diameter from 20.5 ± 1.0 to 19.3 ± 0.6 μm, N.S. vs. base line; n = 12; 10 μM trandolaprilat, from 21.3 ± 1.9 to 20.9 ± 1.8 μm, N.S. vs. base line, n = 11). Thus, the ability of either dose of trandolaprilat to prevent AI-induced constriction was greater for the EA than the AA.

Surprisingly, the oral prodrug trandolapril appeared to be as effective as the ACE inhibitor trandolaprilat. Pretreatment with trandolapril (10 μM) did not affect base-line AA or EA diameters. Subsequent addition of 1.0 nM AI to the perfusate caused an AA constriction of 15.7 ± 2.1% (reduction in vessel diameter from 21.0 ± 0.5 to 17.6 ± 0.6 μm; n = 25), a value significantly different from base line (P < .001) as well as from that of 1.0 nM AI alone (P < .001) but not significantly different from pretreatment with 10 μM trandolaprilat (fig. 3, upper). In analogy with the results from pretreatment with trandolaprilat, the pretreatment of kidneys with trandolapril completely prevented the vasoconstrictor effects
of AI on the EA (fig. 3, lower). With the addition of 1.0 nM AI, pretreated EA failed to constrict (reduction in vessel diameter from 19.4 ± 0.7 to 19.3 ± 0.7 μm, P < .001 vs. baseline and P < .001 vs. AI alone; n = 18).

**AII receptor blockade.** The addition of the AT₁ receptor antagonist losartan to the perfusate did not affect baseline AA or EA diameters. The AI-induced constriction of both AA and EA was completely prevented after pretreatment with losartan (fig. 4). Subsequent addition of endothelin-1 resulted in a significant constriction of AA by 21.4 ± 1.3% (P < .001 vs. base line) and of EA by 20.3 ± 3.3% (P < .001 vs. base line).

**Discussion**

Previous studies using pharmacological agents that interrupt the renin-angiotensin axis have been undertaken to define the role of AII as a determinant of the vasomotor tone of the renal vasculature. It was demonstrated in isolated perfused rat kidneys (Schmidt et al., 1986) that the ACE inhibitors captopril and ramipril partially inhibited the vasocostrictor responses to an infusion of AI. This response was completely inhibited by the AII antagonist saralasin, indicating conversion of AI to AII in this kidney preparation. Similarly, the infusion of AI into the renal artery of dogs in vivo was associated with a dose-dependent reduction in renal blood flow (Aiken and Vane, 1972) and could be attributed to a conversion of AI to AII. The renal blood flow of the contralateral kidney was not affected. The magnitude of the conversion of AI to AII has been estimated to approximate 20% (Rosivall and Navar, 1983). Unfortunately, it cannot be discerned from these studies whether such a conversion occurs uniformly in all parts of the renal vascular tree. Consequently, we undertook the present study using the isolated perfused kidney model. This model confers discrete experimental advantages to study the local effects of components of the renin-angiotensin system on the renal microcirculation. First, renal perfusion pressure can be kept constant, thus eliminating reflex and autoregulatory responses. Second, the influence of extrarenal factors such as volume status, circulating levels of related vasoactive hormones, renal neural stimulation and anesthetic agents is eliminated. Finally, one has the capability of directly visualizing the AA and EA by establishing hydronephrosis before the study, thereby offering a rigorous approach to assess the effects of diverse pharmacological and physiological interventions in the renal microcirculation.

In the present study, we demonstrated that administration of AI evoked AA and EA vasoconstriction in isolated perfused hydronephrotic kidneys. To assess whether the vasoconstric-
tor effects of AI were mediated by the conversion of AI to AII, in a second set of experiments we investigated the efficacy of an ACE inhibitor. The AI-induced constriction of AA was partially inhibited and that of the EA completely prevented by pretreatment with the ACE inhibitor trandolaprilat. Subsequent exposure of the hydronephrotic kidneys to AII resulted in a marked vasoconstriction of both AA and EA. From these findings it may be concluded that the AI-induced vasoconstriction of AA and EA is mediated through the conversion of AI to AII by locally derived tissue ACE, which then mediates vasoconstriction by binding to its specific receptor. We conducted a third set of experiments to validate our hypothesis. We demonstrated that pretreatment with an AT₁ receptor antagonist completely blocked the AI-induced constriction of both AA and EA. The subsequent addition of endothelin in these experiments produced marked AA and EA constriction, thereby delineating the specificity of the AII receptor activation after AI administration. Collectively, our observations are in accord with the notion that the effect of AI is the result of its conversion to AII, thus confirming the existence of local tissue ACE, which presumably participates in the local control of the renal microcirculation.

Local conversion of AI to AII has been demonstrated in several vascular beds, particularly in the pulmonary vascular bed (Ryan et al., 1975; Johnston and Kohsuki, 1989; Zhou and Mendelsohn, 1992). In the present study we could demonstrate that differences may exist in conversion within the vascular bed of a single organ. Whereas pretreatment with an AT₁ receptor antagonist completely blocked the vasoconstriction of both AA and EA, pretreatment with an ACE inhibitor completely prevented the AI-induced constriction only in EA. In AA, pretreatment with an ACE inhibitor prevented the AI-induced constriction only partially (figs. 1–3). Several possible mechanisms might account for the different responsiveness to AI after pretreatment with trandolaprilat of AA, compared with EA.

ACE inhibitors may differ in their efficacy in blocking tissue ACE. Thus, Allan et al. (1994) compared the effects of enalapril vs. ramipril on AI and AII levels in plasma and renal tissue. They demonstrated that pretreatment with the ACE inhibitors resulted in an essentially identical fall in plasma AII concentrations at every dosage used, without a difference between enalapril and ramipril. In contrast, however, a dose-dependent decline in AII concentrations was observed in renal tissue. In addition, at lower dosages, ramipril appeared to be more effective in suppressing AII than was enalapril, but at higher dosages this difference disappeared. Consequently, these investigators concluded that ACE inhibitors possess different efficacies in blocking tissue ACE. Such a phenomenon was also postulated by Mitchell and Navar (1991), who demonstrated that vasoconstrictor responses of peritubularly administered AI were only partially prevented by either local or systemic pretreatment with an ACE inhibitor. Mitchell and Navar (1991) also demonstrated that ACE inhibition abolished the conversion of AI to AII in plasma completely but in the kidney only partially. Again this was explained by less effective blockade of tissue ACE by ACE inhibition.

In the present study we demonstrated that, at two different dosages, trandolaprilat blocked the AI-induced EA vasoconstriction to the same extent, i.e., completely (fig. 2). In AA, however, the AI-induced constrictions were only partially blocked (fig. 2). Thus, it is possible, albeit unlikely, that local tissue ACE is more difficult to block at the afferent site than at the efferent site and higher dosages are needed to achieve complete blockade of AI-induced vasoconstriction. Alternatively, it is possible that the distribution of the drugs may be different in AA vs. EA and that access of the drug to cellular ACE may be lower in AA. Finally, it is possible that the AA may respond somewhat to AI with a modest vasoconstriction, even in the absence of conversion to AII. To our knowledge, however, data to support such speculation are not available.

An alternative explanation for the inability to completely block the AI-induced AA constriction with trandolaprilat pretreatment is the existence of a non-ACE pathway. It has been demonstrated that the human heart contains a dual enzymatic pathway for AII generation (Urata et al., 1990, 1993). Indeed, it was demonstrated that ACE-dependent AII generation was minor, compared with chymase-dependent AII formation (Urata et al., 1993). Likewise, several investigators, using isolated kidney models, have suggested the possibility of endorenal generation of AII from perfused AI by the existence of a peptidyl dipeptidase other than ACE (Hofbauer et al., 1973; Itskovitz and McGiff, 1974). In addition, such an enzyme capable of converting AI to AII, but insensitive to captopril, has been identified in mouse brain cytosol and cultured bovine pulmonary endothelial cells (Neidle and Kelly, 1984; Lanzillo et al., 1986). Thus, our observation that the AI-induced AA vasoconstriction is partially blocked by the ACE inhibitor trandolaprilat but completely blocked by the AII receptor antagonist losartan might be explained by the conversion of AI to AII through a non-ACE pathway at the afferent site. Nevertheless, because the AI-induced constriction was equally effectively blocked by trandolaprilat and losartan at the efferent site, it is unlikely that a non-ACE pathway would be present in EA.

Apart from mediating the conversion of AI to AII, ACE mediates the breakdown of bradykininin, a vasodilator (Erdos, 1976). Therefore, ACE inhibition hampers the elimination of bradykininin. Kon et al. (1993) demonstrated that, in volume-depleted rats, bradykininin caused selective EA dilation during ACE inhibition (Kon et al., 1993). In addition, Komers and Cooper (1995) suggested that kinins play an important role in mediating the acute renal hemodynamic effect of ACE inhibition in experimental diabetes. Thus, it is possible that our observation of the complete blockade of the AI-induced vasoconstriction of the EA is attributable in part to the vasodilating effect of locally present bradykininin. The lack of a vasodilatory effect of ACE inhibition alone on EA diameters, however, argues against such an interpretation.

In the current study, we have shown that the prodrug trandolapril and the active drug trandolaprilat have similar protective effects against AI, which is consistent with previous suggestions. Several lines of evidence have provided a theoretic framework for anticipating such findings. In a recent review, Cong Duc and Brunner (1992) observed that the prodrug trandolapril might be capable of producing direct ACE inhibition, because the IC₅₀ of unchanged trandolapril for human serum ACE is only 7-fold higher than that of trandolaprilat. This is a modest difference, compared with the inhibitory effects of enalapril and its diacid enalaprilat (i.e., 200-fold) (Chevillard et al., 1988). In addition, it was demonstrated that in rats trandolapril and trandolaprilat reduced serum ACE activity equally effectively, because the
two agents appeared to have identical IC\textsubscript{50} values (Cong Duc and Brunner, 1992). Likewise, Chevillard et al. (1994) demonstrated in aortic tissue and atrial tissue that trandolapril had IC\textsubscript{50} values for inhibition of ACE activity that were close to that of trandolapril. In addition, those investigators showed that the serum ACE-inhibitory effect of trandolapril was similar to that of trandolapril; in human serum, IC\textsubscript{50} values of enalapril for tissue and serum ACE were both close to those of trandolapril, whereas the IC\textsubscript{50} values of enalapril were >100 times higher. Thus, those investigators suggested that hydrolysis of the produg trandolapril into its active metabolite trandolaprilat is not required for achieving ACE inhibition in either the in vitro or in vivo studies (Chevillard et al., 1994).

In summary, we have demonstrated in the present study that administration of AI produces AA and EA vasoconstriction of hydronephrotic kidneys. The AI-induced AA constriction is partially prevented by pretreatment with an ACE inhibitor but completely prevented by pretreatment with an AI receptor antagonist. The effect of AI administration on EA is completely offset by pretreatment with either the ACE inhibitor or the AI receptor antagonist. We interpret these findings as indicating that the effects of AI on the renal microcirculation are caused by its local conversion to AII by renal tissue peptidases, including ACE. In addition, the differences between AA and EA responsiveness after pretreatment with an ACE inhibitor or an AI receptor antagonist raise the possibility of non-ACE pathways and/or bradykinin-mediated control of the local renal microcirculation.

References


Send reprint requests to: Murray Epstein, M.D., Nephrology Section, VA Medical Center, 1201 NW 16th St., Miami, FL 33125.