Effect of an Angiotensin II and a Kinin Receptor Antagonist on the Renal Hemodynamic Response to Captopril

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The role of angiotensin II and kinins on the renal cortical and papillary hemodynamic and on the sodium and water excretory responses to converting enzyme inhibition with captopril was examined in euvolemic Munich-Wistar rats. Cortical and papillary blood flows were measured using a laser Doppler flowmeter. Cortical blood flow increased 28% after blockade of angiotensin II receptors with DuP 753 (2 mg/kg i.v., n=6). Captopril (2 mg/kg i.v., n=6) had no effect on cortical blood flow in rats pretreated with the angiotensin II antagonist. DuP 753 had no effect on papillary blood flow, nor did it prevent the rise in papillary blood flow produced by captopril (2 mg/kg, n=6). Infusion of a kinin receptor antagonist, d-Arg, [Hyp, Thi, D-Phe]-bradykinin (2.5 μg/min i.v.), reduced basal papillary blood flow by 15% and blocked the rise in papillary blood flow produced by captopril. 

Renal blood flow rose by 11% after DuP 753, but captopril produced additional increases in urine flow and sodium excretion of 68% and 46%, respectively. Fractional sodium excretion rose from 0.85±0.15% to 1.56±0.14% after captopril. Infusion of the kinin antagonist returned sodium and water excretion to control levels, but fractional sodium excretion was not significantly altered. Glomerular filtration rate was not altered by DuP 753 or captopril; however, it fell from 1.6±0.1 to 1.2±0.1 ml/min/g kidney wt during infusion of the kinin antagonist. This fall in glomerular filtration rate was associated with a 30% fall in the ultrafiltration coefficient. These results indicate that captopril alters renal hemodynamics through mechanisms other than blockade of the renin-angiotensin system. In particular, its effects on papillary blood flow and glomerular dynamics may be due to alterations in the intrarenal levels of kinins. (Hypertension 1991;17:1038–1044)

Converting enzyme inhibitors (CEIs) are antihypertensive agents that promote sodium excretion at lower levels of arterial pressure. However, the mechanisms underlying the natriuretic and antihypertensive actions of CEIs remain uncertain. It generally is thought that these drugs act by blocking the renin-angiotensin system in the kidney and the peripheral vasculature. However, CEIs lower arterial pressure in nephrectomized animals, as well as in rats pretreated with angiotensin (Ang) II antagonists. In addition, CEIs are effective antihypertensive agents in low-renin models of hypertension, for example, the spontaneously hypertensive rat or the deoxycorticosterone acetate–salt hypertensive rat.

Some of the actions of CEIs may be mediated by kinins. Plasma and urine kinin levels increase after administration of CEIs. Blockade of the kallikrein-kinin system attenuates the antihypertensive response to CEIs. However, little is known with certainty about the role of kinins in the renal hemodynamic and natriuretic actions of CEIs. Acute administration of CEI has been reported to increase total renal blood flow (RBF) and papillary blood flow. The increase in RBF induced by CEIs cannot be blocked completely by saralasin. The rise in papillary blood flow after administration of CEIs can be reversed by a kinin-receptor antagonist. Intrarenal infusion of bradykinin elevates RBF and medullary blood flow, and these effects are potentiated by CEIs. These data suggest that some of the renal hemodynamic effects of CEIs may be mediated in part by kinins.
The renal responses to CEIs and Ang II antagonists also are somewhat different. Administration of a CEI increases sodium and water excretion, but blockade of Ang II receptors with saralasin usually has little effect on renal function unless the renin-angiotensin system is activated first by sodium depletion. This latter result generally has been attributed to the agonist action of saralasin.

Recently, a new class of nonpeptide Ang II receptor antagonists has been described. These compounds block the actions of Ang II and have little agonist activity. In the present study, rats were pretreated with the nonpeptide Ang II antagonist DuP 753 to determine if captopril alters renal function through actions other than blockade of the renin-angiotensin system. In addition, we studied whether the kinin receptor antagonist D-Arg, [Hyp3,Thi5,8,D-Phe7]-bradykinin could reverse the renal effects of CEIs in rats pretreated with DuP 753.

**Methods**

Experiments were performed on 35 euvolemic Munich-Wistar rats (175–200 g body wt) (Harlan Laboratories, Madison, Wis.). The rats were anesthetized with ketamine (30 mg/kg i.m.) and Inactin (50 mg/kg i.p.) and were placed on a heated table to maintain body temperature at 36.5°C. Cannulas were placed in the jugular vein for infusions and in the femoral artery for measurement of arterial pressure. The rats received an intravenous infusion of a 0.9% sodium chloride solution containing 1% bovine serum albumin at a rate of 15 μl/min/100 g throughout the experiment. After surgery, 1–2 ml 6% albumin in a 0.9% sodium chloride solution was administered intravenously to replace fluid losses.

**Protocol 1: Laser Doppler Blood Flow Measurements**

These rats were prepared for measurement of cortical and papillary blood flows by laser Doppler flowmetry (model PF3, Perimed KB, Stockholm, Sweden) as described previously. The papilla was exposed by excising the ureter. An adjustable clamp was placed on the aorta above the renal arteries so that renal perfusion pressure (RPP) could be controlled.

After surgery and a 50-minute equilibration period, RPP was lowered to 110 mm Hg and maintained at this level by loosening the aortic clamp if arterial pressure fell. After a 10-minute stabilization period, cortical and papillary blood flows were measured using a laser Doppler flowmeter during a 15-minute control period. The rats received a dose of DuP 753 (2 mg/kg i.v., n=6) that reduced the pressor response to a 100 ng/kg bolus of Ang II by 96% (from 30±4 to 1.2±1 mm Hg). After a 15-minute stabilization period, cortical and papillary blood flows were recorded during a 15-minute experimental period. The rats were given captopril (2 mg/kg i.v.), and after 15 minutes, cortical and papillary blood flows were recorded during another 15-minute period. In a second group of rats (n=4), the effects of a kinin antagonist on the cortical and papillary blood flow responses to DuP 753 and captopril were examined. Cortical and papillary blood flows were measured during a control period, 15 minutes after initiation of an intravenous infusion of the kinin antagonist (2.5 μg/min), and 15 minutes after sequential intravenous administration of DuP 753 (2 mg/kg) and captopril (2 mg/kg).

**Protocol 2: Clearance Experiments**

These rats were surgically prepared as described above, except that cannulas were placed in both ureters for collections of urine, and a 1.5-mm probe was placed on the left renal artery for measurement of RBF using an electromagnetic flowmeter (model 501, Carolina Medical Instruments, Inc., King, N.C.). [H]Inulin (1 μCi/ml) was added to the infusion solution to allow for measurement of glomerular filtration rate (GFR). Renal hydrostatic interstitial pressure was measured using an acutely implanted capsule, as described previously.

After a 1-hour equilibration period, urine flow, sodium excretion, RBF, GFR, arterial pressure, and renal interstitial pressure were measured during a 30-minute control period (n=6). Then, DuP 753 (2 mg/kg i.v.) was given, and after a 15-minute equilibration, urine and midpoint plasma samples were collected during a 15-minute period. Captopril (2 mg/kg i.v.) then was administered, and after a 15-minute equilibration, urine and plasma samples were obtained during a second 15-minute period. An infusion of the kinin receptor antagonist D-Arg, [Hyp3,Thi5,8,D-Phe7]-bradykinin (2.5 μg/min i.v.) was initiated, and 15 minutes later, urine and plasma samples were collected during a final 15-minute experimental period. Time-control experiments were performed following the same protocol in a separate group of rats (n=4) treated with vehicle.

**Protocol 3: Micropuncture Experiments**

These rats were surgically prepared as described in protocol 2, and the left kidney was placed in a holder and covered with saline. Experiments were performed in two groups of rats because of the limited number of glomeruli available per animal. In group 1 (n=7), glomerular, tubular, and capillary pressures were measured during a control period, 15 minutes after DuP 753 (2 mg/kg i.v.), and 15 minutes after administration of captopril (2 mg/kg i.v.). In group 2 (n=7), pressures were measured after the rats received both DuP 753 and captopril and then during the infusion of the kinin antagonist.

During each period, pressures were measured in three to five proximal tubules (Pm), star vessels (Pm, and in the capillaries of superficial glomeruli (Pf) using micropipettes (3–5 μm) and a servo-null micropressure device (model 900, World Precision Instruments, Quinipac, Conn.). Pf measurements were accepted if the recording was steady for 30 seconds and synchronous with arterial pressure. Filtration fraction (FF) was determined by the formula...
FF = GFR/RBF(1 - hematocrit)

Preglomerular vascular resistance ($R_{\text{pre}}$) was calculated by the formula

$$R_{\text{pre}} = \frac{(RPP - P_\gamma)}{RBF}$$

Efferent arteriolar resistance ($R_e$) was calculated as

$$R_e = \frac{(P_e - P_{\text{in}})}{(RBF - GFR)}$$

Renal venous resistance ($R_v$) was calculated as

$$R_v = \frac{P_{\text{out}} - P_{\text{ven}}}{RBF}$$

assuming that venous pressure ($P_{\text{ven}}$) was constant and equal to 4 mm Hg. Plasma protein concentration ($C_p$) was measured using a clinical refractometer (Schuco, Carle Place, N.Y.). Efferent arterial protein concentration ($C_e$) was calculated by the formula $C_e = C_p/(1 - FF)$. Oncotic pressure ($P_\gamma$) of arterial plasma ($P_a$) and efferent arterial plasma ($P_e$) were calculated as

$$P = 0.009C_\gamma + 0.16C_p + 2.1C_\gamma$$

Glomerular ultrafiltration pressure ($P_{uf}$) was calculated as

$$P_{uf} = P_e - P_{\text{in}} - \frac{(P_e - P_a)}{2}$$

When this value was negative, indicating the existence of filtration equilibrium, the equation

$$P_{uf} = P_e - P_{\text{in}} - \frac{(P_a)}{2}$$

was used. A minimal whole-kidney glomerular ultrafiltration coefficient ($K_f$) was calculated as

$$K_f = \frac{GFR}{P_{uf}}$$

Statistical Methods

Data are presented as mean ±1 SEM. The significance of differences between and within groups was evaluated using an analysis of variance for repeated measures followed by a Duncan multiple range test. A value of $p<0.05$ was considered significant.

Results

Protocol 1: Laser Doppler Blood Flow Measurements

The laser Doppler blood flow data are presented in Figure 1. Cortical blood flow increased 30% after DuP 753 (from 3.2±0.3 to 4.1±0.3 V), and it did not increase further after administration of captopril (4.8±0.49 V). DuP 753 had no effect on papillary blood flow; however, it increased by 70% after captopril (from 2.0±0.1 to 3.6±0.4 V). Control arterial pressure and RPP averaged 125±4 and 108±5 mm Hg, respectively. Because papillary blood flow is dependent on the level of RPP,24 RPP was maintained at the control level throughout these experiments by loosening the aortic clamp as needed.

Administration of kinin antagonist had no effect on cortical blood flow, but it blunted by 50% the increase in cortical blood flow produced by captopril. Papillary blood flow decreased by 15% after the administration of the kinin antagonist (from 2.2±0.2 to 1.9±0.2 V), and it remained at this level after DuP 753. Infusion of the kinin antagonist blocked the increase in papillary blood flow produced by captopril. Control RPP averaged 109±5 mm Hg in this group and was maintained at this level throughout the experiment.

Protocol 2: Clearance Experiments

In time-control rats, urine flow, sodium excretion, RBF, GFR, and blood pressure were not significantly altered over the course of the experiment (Figures 2 and 3). Mean arterial pressure decreased slightly during the experiment in rats given DuP 753 and captopril (Figure 2), but the fall in pressure was not different from that seen in the time-control rats. RBF increased 11% after DuP 753, and it was not altered by subsequent administration of captopril or the kinin antagonist. Control RPP averaged 125±4 and 108±5 mm Hg, respectively. Because papillary blood flow is dependent on the level of RPP,24 RPP was maintained at the control level throughout these experiments by loosening the aortic clamp as needed.

Administration of kinin antagonist had no effect on cortical blood flow, but it blunted by 50% the increase in cortical blood flow produced by capto-
Protocol 3: Micropuncture Experiments

Star vessel and tubular pressures were not significantly altered by treatment with DuP 753, captopril, or the kinin antagonist (Figure 4). Glomerular capillary pressure fell, and net ultrafiltration pressure decreased from 21.5±2.2 to 17.2±2.0 mm Hg after DuP 753. Subsequent administration of captopril or kinin antagonist had no additional effect on glomerular capillary pressures (Figure 4). Control mean arterial pressure was 131±3 mm Hg and was not significantly altered by DuP 753 (126±2 mm Hg) or captopril (125±2 mm Hg). Similarly, infusion of kinin antagonist had little effect on arterial pressure in the rats (group 2) pretreated with DuP 753 and captopril (140±2 versus 137±2 mm Hg).

DuP 753 reduced preglomerular, efferent arteriolar, and renal venous resistances by 16%, 24%, and 13%, respectively (Figure 4). Subsequent administration of captopril had no further effect on renal vascular resistances. Infusion of kinin antagonist in rats treated with DuP 753 and captopril (group 2) decreased preglomerular resistance by 9%, but it had no effect on efferent arteriolar or renal venous resistances. The ultrafiltration coefficient \( K_t \) tended to increase from 0.06±0.01 to 0.08±0.01 and to 0.10±0.03 ml/min-mm Hg after DuP 753 and captopril, but these changes were not significant. Infusion of the kinin antagonist, however, reduced \( K_t \) from 0.10±0.01 to 0.07±0.01 ml/min-mm Hg in rats pretreated with DuP 753 and captopril.
Discussion

In the present study, the new nonpeptide Ang II receptor antagonist DuP 753 was used to determine the contribution of Ang II to the renal actions of captopril. DuP 753 increased cortical blood flow and total RBF, but it had no effect on papillary blood flow. Captopril had no additional effect on cortical blood flow or RBF in rats given DuP 753, but papillary blood flow still increased by 70%. The increase in papillary blood flow after captopril was blocked by a kinin receptor antagonist. These results suggest that the renal hemodynamic actions of CEIs have two components. The increase in RBF after captopril appears to be due to renal cortical vasodilation secondary to the suppression of Ang II synthesis; however, the increase in papillary blood flow appears to be mediated by kinins. We cannot exclude the possibility that exposure of the papilla may increase intrarenal generation of kinins and exaggerate the response to kinin receptor blockade in this study. Our previous finding that the papillary blood flow response to a kinin antagonist was similar in rats with an intact ureter versus an exposed papilla, however, argues against this possibility.

The selective increase in papillary blood flow seen after captopril is not unique but has been seen after administration of other vasoactive agents. Atrial natriuretic peptide and the calcium channel blocker nisoldipine can increase papillary blood flow in rats without altering cortical blood flow. In addition, cyclooxygenase inhibitors and a kinin antagonist have been reported to reduce papillary blood flow preferentially.

The contribution of Ang II to the papillary blood flow response to captopril remains uncertain. Previous observations that intrarenal infusion of Ang II reduces medullary blood flow in dogs and that captopril and saralasin increase papillary blood flow in hydropenic rats suggest that Ang II exerts some influence on the medullary circulation. However, we have reported recently that the increase in papillary blood flow after captopril in hydropenic rats in which plasma renin activity was elevated was similar to the increases in cortical blood flow and RBF observed. Moreover, the present finding that DuP 753 does not increase papillary blood flow or block the effects of captopril on papillary blood flow and previous results indicating that infusion of Ang II does not reduce papillary blood flow in rats given captopril argue against a role for Ang II in this response. Finally, the present observation that a kinin antagonist can block the rise in papillary blood flow produced by captopril indicates that this effect is largely mediated by kinins and not by Ang II.

Blockade of Ang II receptors with DuP 753 increased sodium and water excretion and lowered urinary osmolality. The further increase in sodium and water excretion after administration of captopril in rats given DuP 753 indicates that the natriuretic actions of CEIs are related to both blockade of the production of Ang II and breakdown of kinins. This is consistent with previous findings that intrarenal infusion of bradykinin increases RBF and papillary blood flow and washes out the medullary solute gradient. The fall in filtration fraction during infusion of bradykinin may increase sodium excretion by changing renal interstitial pressure or by washout of the medullary solute gradient. Our results indicating that captopril increases papillary blood flow and the fractional excretion of sodium and water and reduces urinary osmolality, and that a kinin antagonist blocks these effects, are compatible with this view. There is some evidence indicating that kinins directly inhibit sodium reabsorption in the distal nephron. Thus, direct effects of kinins on tubular transport also may contribute to the natriuretic response to CEIs.

GFR fell after the administration of the kinin antagonist to rats given DuP 753 and captopril, in the absence of changes in RBF. This suggests that changes in renal vascular resistance or glomerular...
capillary pressure could not explain the fall in GFR. The micropuncture study indicated that the fall in GFR produced by the kinin antagonist was associated with a 30% fall in $K_f$. Kinins may affect $K_f$ by affecting contraction of mesangial cells. The recent finding that bradykinin increases cyclic GMP and inhibits the contractile response of mesangial cells to Ang II when added to coinjections of mesangial and endothelial cells supports this possibility. The fall in preglomerular resistance seen during the infusion of the kinin antagonist may be secondary to the fall in $K_f$ and partial compensation via the tubuloglomerular feedback mechanism.

Blockade of Ang II receptors with DuP 753 decreased preglomerular, efferent, and renal venous resistances, indicating that Ang II increases preglomerular and postglomerular vascular resistances in euvolemic surgically stressed rats in which the renin-Ang II system is activated. However, our finding that glomerular capillary and net ultrafiltration pressures decreased after DuP 753 suggest that Ang II has a greater influence on the efferent arteriole in these animals. Captopril had no effect on glomerular, tubular, or peritubular capillary pressures or renal vascular resistances after blockade of Ang II receptors with DuP 753. This finding indicates that the cortical vascular actions of CEIs were mediated primarily by changes in Ang II levels. However, pretreatment of the rats with the kinin antagonist blunted by 50% the increase in cortical blood flow seen after the administration of DuP 753, indicating that kinins may contribute to the magnitude of renal cortical vasodilation after blockade of the renin–Ang II system. This conclusion is in agreement with previous results indicating that kinins and Ang II both contribute to the increase in RBF produced by enalaprilat.

$K_f$ values tended to increase after DuP 753 and captopril, although this change was not significant. Previous investigators have reported that high doses of Ang II constrict mesangial cells and reduce $K_f$ in Munich-Wistar rats. However, the role of endogenous Ang II in the regulation of $K_f$ remains unclear. Our results indicate that, in euvolemic rats in which plasma levels and renin and Ang II are elevated, blockade of the renin-angiotensin system has little effect on $K_f$. Rather, our results suggest that kinins may help preserve GFR in face of a fall in glomerular capillary pressure after CEIs by increasing $K_f$.

References


KEY WORDS • kidney • glomerular function • hemodynamics • angiotensin II • captopril • kallikrein • kinins
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