Angiotensin II and Renal Functional Reserve in Rats With Goldblatt Hypertension

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We have previously demonstrated that loss of renal functional reserve (renal response to protein loading) in two-kidney, one clip Goldblatt hypertension is characterized by no change in glomerular filtration rate or single nephron glomerular filtration rate and decreased absolute proximal tubular reabsorption during glycine administration. Captopril restores proximal reabsorption and renal functional reserve in this condition. Because captopril suppresses angiotensin II generation and increases bradykinin, prostaglandins, and potentially nitric oxide, we have investigated the role of angiotensin II blockade in restoring proximal reabsorption and renal functional reserve by comparing captopril with DuP 753, an angiotensin II receptor antagonist, in Goldblatt rats. One month after clipping, two period micropuncture studies (control and glycine) were performed on the unclipped kidney. Normal rats and three groups of clipped rats were studied: an untreated group (HYP), a group treated with captopril (CEI), and a group treated with DuP 753 (DuP) 5 days before micropuncture. Glycine increased glomerular filtration rate, nephron plasma flow, and single nephron glomerular filtration rate in normal rats. Systemic and glomerular hypertension in HYP rats was associated with loss of renal functional reserve and a decrease in absolute proximal reabsorption during glycine. Captopril and DuP 753 normalized systemic and glomerular capillary pressure and prevented the decrease in proximal reabsorption during glycine; however, only CEI rats increased single nephron glomerular filtration rate and glomerular filtration rate after glycine. In conclusion, abnormal responses of both glomerular and tubular function are responsible for the loss of renal functional reserve in Goldblatt rats. Inhibitory angiotensin II activity is responsible for decreasing proximal reabsorption during glycine; however, factors other than angiotensin II limit the glomerular response to glycine. Other, angiotensin II-independent, effects of captopril (i.e., bradykinin and nitric oxide) are important in restoring a normal glomerular response to glycine. (Hypertension 1992;19:790–794)

KEY WORDS • angiotensin II • captopril • DuP 753 • Goldblatt hypertension • glycine • glomerular function

Since the early studies of Pitts,1 it has been recognized that protein intake or intravenous infusion of amino acids is associated with increases in glomerular filtration rate (GFR). The normal vasodilator response to proteins (renal functional reserve) is lost in the presence of renal disease.2,3 It has been suggested that loss of renal functional reserve can be used as an important marker for the presence of risk factors leading to progression of renal disease.2,3 Previous studies in our laboratory have established the loss of renal functional reserve in different experimental models, such as Goldblatt hypertension and recent onset experimental diabetes.4,5 These studies have recognized two important factors in the abnormal renal response: first is the presence of significant decreases in proximal tubular reabsorption during glycine infusion, and second is the capacity of converting enzyme inhibitors to restore the increase in GFR during glycine infusion. Because converting enzyme inhibitors suppress angiotensin II (Ang II) generation, we have speculated that the decrease in tubular reabsorption may be secondary to an unmasked inhibitory Ang II activity. Converting enzyme inhibitors not only suppress Ang II but also increase bradykinin and prostaglandins; therefore, it is difficult to establish what the role of Ang II is in limiting the normal response to glycine infusion under differing disease conditions.6 The discovery of the orally active nonpeptide Ang II receptor antagonist 2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)bi-phenyl-4-yl)methyl]imidazole (DuP 753) provides an excellent tool to define the contribution of Ang II suppression in the specific effect of converting enzyme inhibition.7 The purpose of this study is to compare the effects of DuP 753 and a converting enzyme inhibitor on the renal response to glycine infusion in rats with Goldblatt hypertension to answer the following questions: Is an increase in Ang II activity solely responsible for limiting the response to glycine infusion in this experimental model? Is there any evidence that the non-Ang II effects of converting enzyme inhibition may be important to the restoration of a normal response to glycine infusion?
Methods

Studies were performed in 27 male Munich-Wistar rats (180–200 g) obtained from Simonsen Laboratories, Gilroy, Calif. One group of normotensive control rats (NOR, n=6) and three groups of two-kidney, one clip (2K1C) Goldblatt model rats were studied. The latter three groups were prepared with rats under methohexital sodium anesthesia by placement of a 0.2-mm slit width silver clip on the right renal artery. Systolic blood pressure was measured weekly in awake clipped animals via the tail-cuff method with an electrosphygmomanometer (Narco BioSystems, Austin, Tex.) and a heated animal restraining cage. Clipped rats with systolic blood pressure less than 140 mm Hg were excluded from the study.

One month after clipping, 2K1C rats were divided into hypertensive untreated rats (HYP group, n=8) and hypertensive rats that received either captopril (500 mg/l in drinking water, CEI group, n=7) or DuP 753 (125 mg/l in drinking water, DuP group, n=6) 5 days before micropuncture.

Micropuncture Studies

Rats were anesthetized with Inactin (BKY, Konstanz, FRG) (10 mg/100 g body wt i.p.), and surgical preparata included tracheostomy and cannulation of the left jugular vein, left femoral artery, left ureter, and bladder.8 The left unclipped kidney was exposed and placed in a Lucite cup, and the surface was covered with heated (37°C) NaCl-NaHCO3 solution. All studies were performed with rats in a euvoletic state by infusion of 1% body wt donor plasma over a 1-hour period followed thereafter by 0.15% body wt donor plasma per hour. All rats received two additional infusions of NaCl-NaHCO3 solutions, one containing [3H]inulin at a rate of 110 μCi/hr in a volume of 0.8 ml/hr and the other one (1.4 ml/hr) serving as control for glycine infused during the second period. Both solutions were initiated 60 minutes before the first micropuncture measurement and were maintained throughout the experiment.

Two period studies were conducted in each rat, euvoletic control and glycine infusion. Hydrostatic pressures in the glomerular capillaries, urinary space, and efferent arterioles were measured with a glass micropipette using a servo-nulling pressure measurement device (IPM, San Diego, Calif.) as previously described.8 Glass pipettes of 13–16 μm were used to collect at least three efferent arteriole blood samples for determination of efferent protein concentration, and these were bracketed by two collections from femoral artery for measurement of protein concentration that is assumed equal to afferent arteriole protein concentration. Lowering mean arterial pressure normalized glomerular hydrostatic pressure and the transcapillary hydrostatic pressure gradient; the lowest values for the latter were found in CEI rats. The ultrafiltration coefficient was not modified significantly in HYP, CEI, or DuP rats.

Analytical Methods and Calculations

[3H]inulin activity in plasma, urine, and tubular fluid was monitored on a model B4530 Tri/Carb Packard liquid scintillation counter (Packard Instrument Co., Downers Grove, III.). Single nephron GFR (SNGFR), GFR, fractional reabsorption, and absolute proximal reabsorption (APR) were determined as previously described.8 Single nephron filtration fraction, single nephron plasma flow, transcapillary hydrostatic pressure gradient, afferent and efferent arteriolar resistance, oncotic pressure, and the ultrafiltration coefficient were calculated as previously described.8 Protein concentration was measured by a microadaptation of the Lowry protein method.10

Statistics

Statistical significance of measurements between the two periods was evaluated by either unpaired or paired Student's t test, depending on whether there were multiple or single values obtained per period.11 Tukey multiple comparison analysis was also performed to obtain statistical significance among groups of animals. The level of statistical significance was defined at a value of p<0.05. All results are expressed as mean±SEM.

Results

Systolic blood pressure before initiation of treatment did not differ among the three groups of clipped rats (162.5±5.7 mm Hg in HYP, 157.1±6.5 mm Hg in CEI, and 171.7±4.6 mm Hg in DuP). Body weight was 251±6 g in NOR rats, 250±6 g in HYP rats, 256±5 g in CEI rats, and 275±6 g in DuP rats (p<0.05 versus other three groups).

Glomerular Hemodynamics During Control Period

Table 1 presents micropuncture data. HYP rats were associated with a significant increase in left kidney GFR in comparison to DuP rats. SNGFR was increased in HYP rats when compared with NOR, CEI, and DuP rats. The increase in SNGFR was a result of a significant elevation in glomerular hydrostatic pressure, the transcapillary hydrostatic pressure gradient, and single nephron plasma flow. Both captopril and DuP 753 were equally effective in lowering mean arterial pressure to values not different from those of NOR rats. Control of blood pressure restored GFR and SNGFR to normal. Single nephron plasma flow was consistently higher in both DuP and CEI rats when compared with NOR rats because of significant decreases in both afferent and efferent arteriolar resistance. Lowering mean arterial pressure normalized glomerular hydrostatic pressure and the transcapillary hydrostatic pressure gradient; the lowest values for the latter were found in CEI rats. The ultrafiltration coefficient was not modified significantly in HYP, CEI, or DuP rats.

No differences were observed for single nephron filtration fraction, hematocrit (51±0.01%, 51±0.01%, 50.6±0.7%, 49.5±1%), and afferent oncotic pressure (πa) (16.4±1.1, 15.54±0.9, 13.8±0.4, 14.6±0.8 mm Hg) among the four groups. Efferent oncotic pressure (πe) was significantly higher in NOR and HYP rats (31.5±1 and 31.4±0.8 mm Hg) when compared with CEI and DuP rats (24.2±1.6 and 24±2.5 mm Hg).
Effects of Glycine Infusion on Glomerular Hemodynamics

Glycine infusion produced significant increases in GFR and SNGFR in NOR rats. The increase in SNGFR was due to a significant increase in single nephron plasma flow dependent on both afferent and efferent arteriolar vasodilation. In contrast with NOR rats, HYP rats were associated with lack of response to glycine infusion, because neither GFR nor SNGFR changed during glycine infusion.

Although captopril and DuP 753 were effective in normalizing mean arterial pressure, SNGFR, and the transcapillary hydrostatic pressure gradient, the response to glycine infusion was quite different in the two groups, as GFR and SNGFR increased only in CEI rats. The increase in SNGFR in these rats was due to an increase in the transcapillary hydrostatic pressure gradient rather than to changes in single nephron plasma flow, as was the case in NOR rats.

Hematocrit was slightly decreased during glycine infusion in both HYP rats (51 ±0.01% versus 48.4 ±0.01%, p<0.05) and DuP rats (49.5 ±1% versus 48.2 ±0.9%, p<0.05). Similar decreases in \( \pi_t \) were found in CEI rats (13.8 ±0.4 versus 12.2 ±0.26 mm Hg, p<0.05) and DuP rats (14.6 ±0.85 versus 12.6 ±0.65 mm Hg, p<0.05). Values for \( \pi_t \) in NOR rats (14.9 ±0.9 mm Hg) and HYP rats (13.9 ±0.75 mm Hg) remained unchanged. No significant differences were found for \( \iota_A \) among the four groups (29 ±1.6, 27.1 ±1.5, 23.1 ±1.7, 22.5 ±2 mm Hg).

As depicted in Figure 1, the increase in SNGFR during glycine infusion in NOR rats was associated with a parallel increase in APR such that fractional reabsorption remained constant. HYP rats were not only characterized by absence of a response to glycine infusion, but interestingly, glycine infusion was associated with a decrease in both APR and fractional reabsorption (0.36 versus 0.16, p<0.05). Although the response in SNGFR during glycine infusion differed significantly between CEI and DuP rats, tubular reabsorption was well maintained in both groups, as glycine infusion did not modify fractional reabsorption in these two groups.

Discussion

The results of this study suggest provocative conclusions regarding 1) the role of Ang II as a limiting factor in the normal increase in GFR during amino acid infusion and 2) significant differences in the overall renal response between two agents that suppress Ang II activity by different mechanisms. We have previously demonstrated that decreases in APR during glycine infusion are associated with loss of the normal vasodilator effect of glycine infusion. Indeed, studies in Goldblatt hypertension, recent onset experimental diabetes, and more recently in chronic glomerulonephritis have all demonstrated an association between decreases in APR and absence of a SNGFR response to glycine infusion. Administration of various converting enzyme inhibitors in these three conditions has been found to restore APR to normal during glycine infusion, suggesting some role for Ang II as a mediator of the decrease in APR. The results of the present study further strengthen this observation, because both captopril and DuP 753, agents that suppress Ang II action...
Although it has been established that Ang II increases absolute proximal reabsorption (APR) and fractional reabsorption (FR) in the four groups of rats. NOR, normal group; HYP, hypertensive untreated group; CEI, captopril-treated group; DUP, DuP 753-treated group. *p<0.05 vs. control; +p<0.05 vs. other groups, same period.

**Graphs showing effect of glycine infusion on absolute proximal reabsorption (APR) and fractional reabsorption (FR) in the four groups of rats.**

**FIGURE 1.**

by different mechanisms, were equally effective in restoring normal tubular function during glycine infusion. Although it has been established that Ang II increases APR, studies have demonstrated that Ang II can either increase or decrease tubular reabsorption, probably through different Ang II receptors with different signal transduction pathways, depending on the specific Ang II concentration.13,14 The mechanism by which glycine infusion has unmasked this inhibitory reabsorptive effect of Ang II is not clear. Studies from our laboratory using the nitric oxide synthase inhibitor Nω-monomethyl-L-arginine have demonstrated the presence of an interaction between Ang II and nitric oxide in the control of proximal tubular reabsorption. Administration of Nω-monomethyl-L-arginine to a normal rat decreases APR, and reabsorptive rate can be restored toward normal with concurrent administration of DuP 753.15 It is therefore possible that in the presence of different pathophysiological conditions, glycine infusion unmask the presence of a complex interaction between nitric oxide and Ang II, which leads to reductions in APR through activity of the inhibitory Ang II mechanism. Nitric oxide and Ang II activity both may normally increase during glycine infusion, but under conditions of endothelial dysfunction, i.e., hypertension, the nitric oxide response may be diminished, permitting an inhibitory effect of Ang II on proximal reabsorption to dominate unopposed.

Another finding of this study is the observation that although both DuP 753 and captopril restored tubular reabsorption to normal, only captopril reestablished the increase in GFR during glycine infusion. We have previously speculated that the decrease in APR during glycine infusion in pathophysiological conditions should increase distal delivery of sodium chloride. As a consequence of the increase in distal delivery, the tubuloglomerular feedback system may be activated, thereby limiting vasodilation and preventing an increase in GFR during glycine infusion.4 Supporting the importance of maintenance of tubular reabsorption in the renal response associated with amino acids, recent data by Woods and Young16 demonstrated that inhibition of proximal tubular reabsorption with maleic acid prevented the vasodilator response to amino acid infusion. The results of our study have demonstrated that restoring tubular function to normal with DuP 753 was not sufficient to restore the increase in GFR during glycine infusion. These findings suggest that activation of the tubuloglomerular feedback system may not be the only reason for loss of the GFR response to glycine infusion. It is possible that two components (glomerular and tubular) interact in the normal response to glycine infusion. Non-Ang II-dependent effects of converting enzyme inhibitors may contribute to the glomerular component and restore the filtration rate response to glycine infusion under pathophysiological conditions, possibly by enhancing bradykinin or nitric oxide activity. Several pieces of evidence have linked bradykinin to the increase in GFR observed during amino acid infusion. Bradykinin has been shown to stimulate nitric oxide.17 Nitric oxide represents an important mediator of the increase in GFR during amino acid infusion.18,19 Administration of bradykinin receptor antagonist prevents the increase in GFR during amino acid infusion.20 However, preliminary studies from our laboratory using a bradykinin receptor antagonist have pointed out the complexity of this analysis.21 The bradykinin receptor antagonist prevents the normal glycine filtration response, not only by limiting the glomerular response, as previously postulated, but by decreasing APR. Our preliminary findings suggest that bradykinin or nitric oxide may also play a critical role in the maintenance of APR during glycine infusion.

In summary, the results of this study demonstrate that in the presence of systemic hypertension, the loss of the normal GFR response to amino acid infusion depends not only on a decrease in proximal tubular reabsorption but also on an abnormal glomerular response. Stimulation of the inhibitory proximal tubular receptor by Ang II appears responsible for the reduction in proximal reabsorption. Although captopril and DuP 753 are equally effective in correcting systemic hypertension and tubular function, other Ang II-independent effects of converting enzyme inhibition are critical to full restoration of normal increase in GFR during glycine infusion, suggesting that Ang II alone is not the sole cause of loss of renal functional reserve in pathophysiological conditions.

**References**


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