Chronic administration of furosemide augments renal weight and glomerular capillary pressure in normal rats

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Lane, Pascale H., Larry D. Tyler, and Paul G. Schmitz. Chronic administration of furosemide augments renal weight and glomerular capillary pressure in normal rats. Am. J. Physiol. 275 (Renal Physiol. 44): F230–F234, 1998.—Angiotensin II (ANG II) is believed to promote progressive renal injury via augmented glomerular capillary hydraulic pressure (PGC). Acute volume reduction secondary to diuretic administration increases circulating ANG II and augments PGC, yet the hemodynamic effects of sustained diuretic administration are unknown. Therefore, glomerular micropuncture studies were performed in male Munich-Wistar rats after 6–8 wk of treatment with daily furosemide (F, 40 mg/day), furosemide plus the AT1 receptor antagonist, losartan (F+L, 5 mg/day), or no therapy (C, control). Renal weight was increased in F rats (1.23 ± 0.7 g) vs. C (1.00 ± 0.06 g) or F+L (0.97 ± 0.01 g). In addition, PGC was elevated in F animals (52.1 ± 1.5 mmHg) vs. C (43.7 ± 1.5) or F+L-treated rats (41.3 ± 1.7). F-treated rats were also characterized by a relative increase in efferent arteriolar resistance and filtration fraction. The latter was markedly attenuated in F+L-treated animals. Collectively, these findings are consistent with an ANG II-mediated alteration in intrarenal hemodynamics. In contrast to acute volume manipulations, however, chronic furosemide augmented renal growth, whereas losartan administration completely arrested this phenomenon. Further studies are warranted to determine whether the hemodynamic and growth adaptations elicited by chronic F administration induce or accelerate renal injury.

glomerular hypertension; angiotensin II; renal hypertrophy; losartan

THE ROLE OF ANGIOTENSIN II (ANG II) in the pathogenesis of glomerular hypertrophy and progressive renal injury is amply established in experimental and clinical renal disease (13). Accordingly, activation of the renin-angiotensin system (RAS) is believed to promote progressive renal scarring. Furthermore, therapeutic agents that attenuate the activity of the RAS offer promise in the prevention of progressive renal dysfunction (8, 10, 11, 17). In contrast, agents that promote activation of the RAS may have a deleterious effect on progressive renal injury. For example, diuretics have been widely used to manage hypertension and edema in the setting of renal disease; however, the potential adverse consequences of chronic volume manipulations coupled with the expected directional changes in the RAS have only recently been appreciated. Our laboratory recently demonstrated that chronic administration of oral furosemide increases glomerular volume and plasma renin activity in the normal rat (16). Importantly, glomerular enlargement was attenuated by simultaneous inhibition of ANG II converting enzyme (ACE), implicating the RAS in the pathogenesis of glomerular hypertrophy in this setting. These findings raise the disturbing possibility that chronic diuretic administration in vivo may promote progression of renal disease by activation of the RAS. Indeed, several clinical trials have suggested that diuretic monotherapy is associated with acceleration of renal dysfunction (9, 25).

Acute (<7 days) activation of the endogenous RAS induced via salt restriction or administration of exogenous ANG II raises glomerular capillary hydraulic pressure (PGC) and arteriolar resistance in normal rats and rats with renal disease (14, 19, 22). Importantly, augmented PGC, if sustained, has proven to be deleterious in the progression of renal disease (15). Nonetheless, the effects of chronic administration (>7 days) of diuretic monotherapy on glomerular hemodynamics have yet to be explored. The studies presented herein demonstrate that daily oral furosemide for more than 42 days results in an increase in PGC and renal weight. We then examined the effects of selective antagonism of the ANG II type 1 (AT1) receptor on glomerular hemodynamics to determine the potential contribution of ANG II to the pathogenesis of glomerular hypertension and renal hypertrophy in this setting. These latter studies suggest that the basis for our findings was secondary to an increase in ANG II activity.

METHODS

Experimental design. All experiments were carried out in weanling male Munich-Wistar rats. Animals were pair fed a paste of Purina rat chow for 6–8 wk. Animals had free access to tap water. One group of animals received furosemide (n = 8), 40 mg per day, mixed into the chow; a second group received furosemide plus losartan, 5 mg per day, mixed into the chow (n = 9); and a third group received standard chow alone (n = 9). Glomerular micropuncture studies were performed after 6–8 wk of treatment as described below.

Micropuncture studies. Rats were anesthetized with pentobarbital sodium (50 mg/kg body wt), placed on a temperature-controlled operating table, and prepared for micropuncture as previously described (21). Briefly, a tracheostomy was performed, the left femoral vein was cannulated (PE-50), and a bolus of Ringer solution (0.5% body wt) was slowly administered over 15 min. Ringer solution containing 25 µCi/ml [3H]inulin was then infused at a rate of 0.5 ml·h⁻¹·100 g body wt⁻¹ for the remainder of the experiment. The femoral artery was cannulated (PE-50), and mean arterial pressure was monitored with a digital display pressure transducer. A bladder catheter (PE-50) was placed suprapubically. The left kidney was exposed by a subcostal incision, dissected free of perinephric tissue, immobilized in a plastic holder, and continuously bathed in mineral oil at 37.5°C. After a 45-min stabilization period, urine was collected in preweighed tubes for 30 min. During this interval, three to four timed (3–4 min)
proximal tubular fluid collections were obtained from randomly selected superficial nephrons to determine single-nephron glomerular filtration rate (SNGFR). Samples of urine, plasma, and tubular fluid were added to a scintillation cocktail, and radioactivities of the samples were measured in a liquid scintillation spectrometer (model LS 230; Beckman Instruments, Fullerton, CA).

Intratubular hydraulic pressures were measured under free-flow conditions in one group of tubules. Proximal tubular stop-flow pressures were obtained in the first surface convolutions distal to Bowman’s space, after blockage of the tubular lumina with Sudan Black mineral oil. Pressures were also determined in randomly selected efferent vascular wellings points. All pressure measurements were performed with a servo-null micropressure system (World Precision Instruments, New Haven, CT). PGC was calculated as the sum of the proximal tubular stop-flow pressure and arterial colloid osmotic pressure (COP). Plasma COP was determined directly utilizing a colloid osmometer (model 4401; Wescor, Logan, UT). Blood samples were taken from efferent vascular welling points and analyzed, together with an arterial sample, for afferent (CA) and efferent (CE) arterial protein concentration using the Micro-Lowry technique. Single-nephron filtration fraction (SNFF) and single-nephron plasma flow (QA) were calculated as follows

\[ SNFF = 1 - \frac{CA}{CE} \]

\[ QA = \frac{SNFF}{SGFR} \]

The glomerular ultrafiltration coefficient (Kf) was calculated using an iterative method as previously described (21). Renal afferent (RA) and efferent (RE) arterial resistances were calculated using the following relationships

\[ RA = 7.692 \times 10^{10} \times (MAP - PGC) \times (1 - Hct) + QA \]

\[ RE = 7.692 \times 10^{10} \times (PGC - P_E) \times \left( \frac{QA}{1 - Hct} \right) - SNFF \]

where P_E is efferent arterial pressure, MAP is mean arterial pressure, and Hct is hematocrit.

Statistics. The three treatment groups were compared using one-way analysis of variance followed by post hoc Scheffé testing. P < 0.05 was considered significant. All analysis was performed using the Statview package of statistical software (Abacus Concepts, Berkeley, CA).

RESULTS

Glomerular hemodynamic studies and laboratory data in rats subjected to micropuncture experiments are summarized in Table 1. Animals weighed less after treatment with furosemide, with or without losartan, compared with control animals (Table 1). In contrast, kidney weight was increased in the furosemide group compared with control rats or rats receiving the combination of furosemide and losartan (Table 1). Therefore, chronic administration of furosemide promotes renal hypertrophy in the normal rat. Moreover, these effects were attenuated by the simultaneous administration of the AT1 receptor antagonist, losartan. These findings parallel those observed in our previous investigation (16), with the notable exception that the reduction in renal hypertrophy was minimal in rats receiving enalapril and furosemide compared with these experiments in which losartan was utilized.

Renal micropuncture studies revealed an increase in PGC in furosemide-treated animals (52.1 ± 1.5 mmHg) compared with control rats (43.7 ± 1.5 mmHg) or rats receiving the combination of furosemide and losartan (41.3 ± 1.7 mmHg) (Table 1). These changes were accompanied by a relative increase in RA compared with RE (Table 1), a decrease in Kf, and an increase in SNFF (Table 1). Collectively, these hemodynamic findings are reminiscent of those obtained with salt restriction or exogenous administration of ANG II (14, 22). However, MAP at the time of micropuncture was significantly less in rats maintained on losartan and furosemide versus furosemide alone or controls (Table 1). Thus the decrease in PGC in rats maintained on furosemide plus losartan was secondary to a fall in MAP as well as a relative decrease in RE. Since there were no significant differences in proximal free-flow tubular pressure among these groups, the transcapillary hydraulic pressure determinations paralleled the changes in PGC (Table 1). Unexpectedly, SNFF and GFR were highest in rats receiving furosemide alone, although these findings failed to reach statistical significance (Table 1). Whether these latter observations represent adaptive changes in glomerular function induced by glomerular hypertrophy or were secondary

Table 1. Summary of renal micropuncture studies

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>BW, g</th>
<th>LKW, %</th>
<th>Hct, %</th>
<th>MAP, mmHg</th>
<th>WKGF, ml/min</th>
<th>SGFR, ml/min</th>
<th>QA, nl/min</th>
<th>FF</th>
<th>PGC, mmHg</th>
<th>Pr, mmHg</th>
<th>TCP, dyn·s·cm⁻²</th>
<th>Rₓ, ×10⁻⁵</th>
<th>Rₑ, ×10⁻⁵</th>
<th>Kf, nl·s⁻¹·mmHg⁻¹</th>
<th>Uᵥ, ml/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>294</td>
<td>0.99</td>
<td>54.3</td>
<td>141d</td>
<td>1.35</td>
<td>36.1</td>
<td>113</td>
<td>0.29</td>
<td>43.7</td>
<td>10.5</td>
<td>33.2</td>
<td>2.22</td>
<td>1.00</td>
<td>0.038</td>
<td>11.3</td>
</tr>
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<td></td>
<td></td>
<td>±5</td>
<td>±0.02</td>
<td>±1.1</td>
<td>±3.3</td>
<td>±0.43</td>
<td>±4.4</td>
<td>±16</td>
<td>±0.02</td>
<td>±1.5</td>
<td>±0.7</td>
<td>±1.6</td>
<td>±0.4</td>
<td>±0.1</td>
<td>±0.007</td>
<td>±3.1</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>256a</td>
<td>0.23</td>
<td>53.9</td>
<td>139d</td>
<td>1.63</td>
<td>40.3</td>
<td>108</td>
<td>0.33a</td>
<td>52.0abcd</td>
<td>11.0</td>
<td>40.9abcd</td>
<td>2.11</td>
<td>1.12abcd</td>
<td>0.026</td>
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<tr>
<td></td>
<td></td>
<td>±11</td>
<td>±0.03</td>
<td>±1.9</td>
<td>±2.4</td>
<td>±0.27</td>
<td>±11.5</td>
<td>±35</td>
<td>±0.00</td>
<td>±1.5</td>
<td>±0.7</td>
<td>±1.5</td>
<td>±0.3</td>
<td>±0.22</td>
<td>±0.009</td>
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<tr>
<td>F+L</td>
<td>9</td>
<td>233</td>
<td>0.97</td>
<td>49.8</td>
<td>95</td>
<td>1.26</td>
<td>30.7</td>
<td>149</td>
<td>0.29a</td>
<td>41.3</td>
<td>12.5</td>
<td>28.8</td>
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<td>0.88</td>
<td>0.073</td>
<td>19.2</td>
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<td></td>
<td></td>
<td>±7</td>
<td>±0.00</td>
<td>±0.2</td>
<td>±2.3</td>
<td>±0.55</td>
<td>±4.6</td>
<td>±20</td>
<td>±0.01</td>
<td>±1.7</td>
<td>±0.6</td>
<td>±1.8</td>
<td>±0.4</td>
<td>±0.14</td>
<td>±0.027</td>
<td>±4.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. F, furosemide; F + L, furosemide + losartan; BW, body weight; LKW, left kidney weight; Hct, hematocrit; MAP, mean arterial pressure; WKGF, whole kidney glomerular filtration rate; SGFR, single-nephron glomerular filtration rate; QA, single-nephron plasma flow; FF, filtration fraction; PGC, glomerular capillary hydraulic pressure; Pr, free-flow tubular pressure; TCP, transcapillary hydraulic pressure; Rₓ, afferent arterial resistance; Rₑ, efferent arterial resistance; Kf, glomerular ultrafiltration coefficient; Uᵥ, urine volume. The control group was compared with the furosemide group using one-way analysis of variance followed by post hoc Scheffé testing. Statistical significance is indicated by asterisks: *P < 0.05 vs. control. **P < 0.0001 vs. control. ***P < 0.0001 vs. furosemide. ****P < 0.0001 vs. furosemide + losartan. &lt; P < 0.01 vs. furosemide.
to the rise in $P_{GC}$ can only be inferred from these studies. The filtration fraction was highest in rats receiving furosemide alone and lowest in those animals maintained on combination therapy (Table 1). As anticipated, the ratio of $R_E$ to $R_A$ was highest in rats receiving diuretic alone (0.53 ± 0.08) and was lowest in rats maintained on combination therapy (0.38 ± 0.04). The latter may play an essential role in the attenuation of $P_{GC}$ when coupled with the observed fall in systemic pressure in this group. There was a tendency for 24-h urine volume to be greater in either group of diuretic-treated animals; however, the results failed to achieve statistical significance ($P = 0.08$ vs. controls) (Table 1).

**DISCUSSION**

An important finding in these investigations was the presence of increased $P_{GC}$ during chronic administration of furosemide. Indeed, although several investigators have demonstrated an increase in $P_{GC}$ following acute volume contraction, the present studies are unique in the demonstration of augmented $P_{GC}$ after chronic administration (6 wk) of diuretic monotherapy. In addition, we observed an increase in filtration fraction, a decrease in $K_r$, and a relative increase in $R_E$ versus $R_A$ in rats receiving furosemide. Collectively, these hemodynamic changes parallel those observed after acute administration of ANG II or salt deprivation for short periods of time (<7 days), a manipulation that stimulates endogenous ANG II production (14, 22). An important finding in these studies was the attenuation of the glomerular hemodynamic changes induced by furosemide via concurrent treatment with the AT$_1$ receptor antagonist, losartan. The mechanism responsible for the attenuation of augmented $P_{GC}$ included a fall in MAP coupled with a relative decrease in $R_E$. However, MAP in this group was occasionally below the range of autoregulation, which may lower $P_{GC}$ independent of changes in arteriolar resistance. Thus these experiments do not prove conclusively that ANG II is mediating the intrarenal changes in hemodynamics observed in rats receiving chronic furosemide. Nevertheless, the observation that increases in $P_{GC}$ occur with chronic administration of diuretic monotherapy is unique and raises the disconcerting possibility that chronic diuretic administration may engender a deleterious effect on progression of renal disease in vivo by unfavorably altering glomerular hemodynamics. Clearly, further studies in experimental renal disease are essential to investigate this hypothesis.

An important additional finding in these experiments was the induction of renal hypertrophy in rats maintained on oral furosemide for 6 wk. Previous studies from our laboratory revealed an increase in renal weight and glomerulomegaly in normal rats maintained on oral furosemide for a similar period of time (16). However, in those experiments, pretreatment with enalapril attenuated glomerular enlargement but did not abrogate renal hypertrophy. In contrast, in the present experiments the AT$_1$ receptor antagonist, losartan, completely arrested renal hypertrophy in rats maintained on diuretic therapy. Therefore, these studies suggest that endogenous activation of the AT$_1$ receptor is an essential step in the development of renal hypertrophy after chronic administration of oral furosemide and, accordingly, implies that AT$_1$ receptor antagonists may offer a therapeutic advantage in arresting renal hypertrophy compared with sustained inhibition of ACE. The potential mechanism underlying these differences may be inferred from emerging data which indicate that ACE-independent synthesis of ANG II represents a prominent synthetic pathway of ANG II production in rat and humans (12, 24). Thus AT$_1$ receptor antagonists should completely antagonize the effects of ANG II on the AT$_1$ receptor, whereas sustained inhibition of ACE would not. Other pharmacodynamic differences between ACE inhibition and antagonism of AT$_1$ receptors include augmented synthesis of bradykinin in the former and activation of ANG II type 2 (AT$_2$) receptors in the latter (23). Whether these actions also contribute to the disparate findings we observed in enalapril-treated animals (16) compared with losartan treatment cannot be answered by the present investigations. Nonetheless, the implications of this observation in the context of the treatment of progressive renal insufficiency are intriguing, since renal hypertrophy frequently accompanies progression of renal disease (2, 16).

Interestingly, several recent clinical investigations have suggested that diuretic monotherapy may accelerate progressive renal injury in the setting of hypertension (7, 9). For example, thiazide diuretics have been found to accelerate nephrosclerosis produced by nitro-L-arginine methyl ester (i.e., L-NAME) in spontaneously hypertensive rats (18). The adverse structural and functional consequences of thiazide treatment correlated with elevated $P_{GC}$. No evidence of glomerulosclerosis or tubulointerstitial fibrosis has been demonstrated in our experiments thus far; however, we have limited our studies to a relatively short time period (6–8 wk) in animals without coexisting renal disease or loss of renal mass.

Interestingly, there was a trend for an increase in SNGFR in rats receiving furosemide alone, despite the fact that these findings failed to achieve statistical significance. Statistics notwithstanding, this ostensibly paradoxical trend could, in part, be ancillary to an alteration in tubuloglomerular feedback. For example, furosemide interrupts the sensing step at the macula densa, engendering a fall in $R_A$, which in turn would augment $Q_A$ and SNGFR. Although there was a slight fall in $R_A$ in the furosemide-treated animals, total renal vascular resistance increased slightly and $Q_A$ fell accordingly. In contrast, the trend toward an increase in SNGFR could be incident to the rise in $P_{GC}$ observed under these conditions. Interestingly, chronic but not acute diuretic monotherapy elicits an increase in renal plasma flow and GFR in humans (25). A biphasic response has generally been observed, e.g., initiation of diuretic therapy is accompanied by an early reduction in renal plasma flow and GFR; however, after several months of therapy, renal plasma flow and GFR either increase or return to baseline (25).
Diuretics have been included in multiple drug regimens to lower blood pressure in the rat model of renal ablation (27). Although such regimens typically lower systemic blood pressure, they do not consistently lower P\text{GC} or alter progression of renal disease (1, 4). In contrast, similar degrees of blood pressure reduction with sustained inhibition of ACE ameliorates progressive renal dysfunction and lowers P\text{GC} (2–4). These observations raise the attractive possibility that multidrug therapy that includes a diuretic agent may blunt the expected decrease in P\text{GC} otherwise elicited by a fall in systemic blood pressure unless these regimens are accompanied by an agent that antagonizes the synthesis or action of ANG II. Admittedly, the results of other investigators would dispute this hypothesis. For example, Yoshida et al. (27) demonstrated a salutary effect of reserpine/hydralazine/thiazide on glomerular morphology in rats with renal ablation, which parallels the findings obtained in experiments using ACE inhibitors. Nevertheless, most studies have been unable to demonstrate a beneficial effect of multiple drug combinations unless accompanied by an agent that blocks ANG II action or synthesis (2, 3, 5, 27). It is plausible that the net effect of unique antihypertensive regimens on the behavior or synthesis of ANG II may underlie the potential of these regimens to alter progressive renal dysfunction in evolving chronic renal disease. In summary, chronic administration of furosemide was accompanied by an increase in P\text{GC} in the normal rat, most likely via activation of the RAS. The hemodynamic pattern observed in rats receiving sustained administration of oral furosemide was reminiscent of the effects of acute infusion of ANG II or salt deprivation for short periods of time. Although the AT\text{1} receptor antagonist, losartan, abrogated the hemodynamic changes accompanying furosemide administration, we cannot conclusively determine that these actions are secondary to intrinsic blockade of the AT\text{1} receptor. Nevertheless, the attenuation of P\text{GC} was likely incident to a fall in MAP coupled with a relative reduction in R\text{E} since a relative decrease in R\text{E} is essential to elicit a fall in P\text{GC} when MAP is reduced within the range of autoregulation (1, 4). These hemodynamic effects are reminiscent of those obtained following blockade of the synthesis of ANG II. In addition, losartan completely arrested the development of renal hypertrophy induced by diuretic monotherapy. This contrasts with previous studies from our laboratory utilizing concurrent treatment with furosemide and the ACE inhibitor, enalapril (16). Although these contrasting findings could be related to variances in dose response, an alternative hypothesis is that the AT\text{1} receptor antagonists offer a selective advantage in arresting renal hypertrophy, perhaps by antagonizing ACE-independent mediated synthesis of ANG II (12). Regardless, persistent or intermittent activation of the RAS via diuretic monotherapy may prove to be deleterious in the treatment of progressive renal disease. Accordingly, it will be necessary to define the effects of diuretic monotherapy on glomerular hemodynamics, glomerular growth, and glomerulosclerosis in experimental models of renal disease.

These studies were presented at the Annual Meeting of the American Society of Nephrology, 1995, and have been published in abstract form (J. Am. Soc. Nephrol. 6: 681, 1995).

These experiments were supported by grants from the Fleur de Lis Foundation of Cardinal Glennon Children’s Hospital (to P. H. Lane) and the Missouri Affiliate of the American Heart Association (to P. H. Lane) and by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-52039 (to P. G. Schmitz).

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Received 24 July 1997; accepted in final form 27 April 1998.

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