Impairment of pressure-natriuresis and renal autoregulation in ANG II-infused hypertensive rats

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Wang, Chi-Tarng, So Yeon Chin, and L. Gabriel Navar. Impairment of pressure-natriuresis and renal autoregulation in ANG II-infused hypertensive rats. Am J Physiol Renal Physiol 279: F319–F325, 2000.—Chronic infusions of initially subpressor doses of angiotensin II (ANG II) lead to progressive hypertension over a 2-wk period and to augmented intrarenal ANG II levels. The present study was performed to investigate total renal blood flow (RBF) and medullary blood flow (MBF) autoregulatory behavior and pressure-natriuresis in ANG II-infused hypertensive rats and how these are modified by concomitant treatment with an ANG II AT1 receptor antagonist. ANG II-infused rats (n = 27) were prepared by administration of ANG II at 60 ng/min via osmotic minipump for 13 days. Twelve of the ANG II-infused hypertensive rats were treated with losartan in the drinking water (30 mg·kg−1·day−1). Rats were anesthetized with pentobarbital sodium (50 mg/kg, ip) and prepared for renal function measurements. An aortic clamp was placed above the junction of the left renal artery to reduce renal arterial pressure. Autoregulatory responses for renal plasma flow, overall RBF, and glomerular filtration rate were impaired in ANG II-infused hypertensive rats; however, MBF autoregulation was not disrupted. Most strikingly, pressure-natriuresis was markedly suppressed in ANG II-infused hypertensive rats. Chronic treatment with losartan prevented the impairment of the pressure-natriuresis relationship caused by chronic ANG II infusion. These findings demonstrate that chronic ANG II infusion leads to marked impairment of sodium excretion and suppression of the pressure-natriuresis relationship, which may contribute to the progressive hypertension that occurs in this model. These renal effects are prevented by simultaneous treatment with an AT1 receptor blocker.

autoregulation; glomerular filtration rate; renal blood flow; medullary blood flow; sodium excretion; angiotensin II

IT IS RECOGNIZED THAT ELEVATED angiotensin II (ANG II) levels play an important role in the development of hypertension in 2-kidney, 1 clip (2K1C) Goldblatt hypertension (11, 29, 30, 37). An influence of ANG II on the nonclipped kidney is supported by experiments using angiotensin-converting enzyme inhibitors or ANG II receptor antagonists which prevent the development of hypertension and increase hemodynamic and excretory function of the nonclipped kidney (13, 14, 16, 30, 40). Chronic ANG II-infused hypertensive models have been used to mimic 2K1C hypertension by replacing release of renin from the stenotic kidney with a chronic ANG II infusion at a rate that elicits a similar onset, temporal pattern, and magnitude of blood pressure responses observed in the 2K1C model (12, 15, 17, 29, 35, 36, 37, 38, 41).

Several studies have demonstrated an abnormal pressure-natriuresis relationship in different types of hypertension (9, 10, 20, 34, 36). Ploth et al. (31) found a reduced efficiency of renal blood flow (RBF) and glomerular filtration rate (GFR) autoregulation in response to reductions in renal arterial pressure (RAP) in the nonclipped kidney of 2K1C Goldblatt hypertensive rats and suggested that the impaired autoregulatory capacity might contribute to reduced sodium excretion capability and to the maintenance phase of hypertension. Both acute ANG II and chronic ANG II intravenous infusions shift the pressure-natriuresis response toward higher arterial pressures (19, 36). Kline and Liu (18) demonstrated that chronic but not acute treatment with the AT1 receptor antagonist losartan restored the pressure-natriuresis relationship in spontaneously hypertensive rats, indicating long term actions of either elevated ANG II levels or arterial pressure on pressure-natriuresis. Our previous study demonstrated that ANG II-induced hypertension in uninephrectomized rats was associated with reduced GFR which was not fully restored by acute treatment with losartan (38). In contrast, chronic treatment with losartan prevented the decreases in GFR along with the increases in arterial pressure. The ANG II-infused model has also been shown to exhibit an impaired capability to adjust afferent arteriolar diameter in response to changes in perfusion pressure (17).

The changes in sodium excretion in response to changes in arterial pressure are likely due to changes in renal hemodynamics as well as in tubular transport function (18, 19, 33, 34). Impairment of renal autoregulation contributes to the impairment of sodium excretion at reduced arterial pressures. Some studies have also suggested that an alteration in renal medullary...
hemodynamics during changes in RAP may be responsible for pressure-natriuresis (5, 33). Other studies have demonstrated that blood flow to the medulla of the kidney is reduced by ANG II infusion (4, 8). This reduced MBF may influence the pressure-natriuresis relationship and contribute to the development of hypertension.

In a study by van der Mark and Kline (36), it was shown that 7–10 days of ANG II infusion in rats unilaterally adrenalectomized and nephrectomized, and drinking a 1% NaCl solution, led to a blunting of the pressure-natriuresis relationship with a rightward shift in the pressure-natriuresis curve. Zou et al. (41) showed that there was a marked increase in intrarenal ANG II content after 13 days of ANG II infusion. The present study was performed to characterize the renal alterations that might be responsible for the suppressed pressure-natriuresis and in particular to determine if the impairment in pressure-natriuresis is associated more with alterations in whole kidney autoregulation or with alterations in MBF (37, 41). The use of single fiber laser-Doppler flow probes allowed us to determine if there were associated MBF changes that could contribute to the reduced sodium excretory capability. Furthermore, we wanted to determine if the effects elicited by chronic ANG II infusion could be prevented by concomitant treatment with an AT1 receptor blocker (38).

METHODS

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were housed in a temperature- and light-controlled room. The rats had free access to standard rat chow (Ralston-Purina, St Louis, MO) and tap water throughout the experiments. Rats with body wt from 170–230 gm were divided into three groups. Rats in group I (n = 14) were not treated or given ANG II before the acute experiment and provided control data. Rats in groups II and III (n = 27) were anesthetized with pentobarbital sodium (50 mg/kg ip), and osmotic minipumps (Model 2002, Alza, Palo Alto, CA) containing ANG II (Calbiochem, San Diego, CA) at concentrations sufficient to allow an infusion rate of 60 ng/min were implanted subcutaneously in the back. After recovery, rats in group III (n = 12) were treated chronically with losartan (Du Pont-Merck Pharmaceutical, Wilmington DE) in the drinking water (30 mg . kg⁻¹. day⁻¹). Rats were housed in the vivarium for 13 days before the acute experiment.

Experimental Protocol

**Series I study: Responses of renal plasma flow (RPF), GFR, and sodium excretion to decreases in RAP.** Thirteen days after osmotic minipump implantation, rats were prepared for acute clearance experiments. Rats were anesthetized with pentobarbital sodium (50 mg/kg, ip) and placed on a thermoregulated surgical table to maintain body temperature at 37°C. After tracheostomy, a PE-240 tube was inserted to maintain a patent airway. The left jugular vein was catheterized for fluid infusion and anesthetic administration as needed. A catheter was inserted via the left femoral artery to measure arterial pressure (Grass Instrument, Quincy, MA). As previously described (38), a left flank incision was made and the kidney was isolated and placed in a plastic cup. A ureteral catheter was inserted for urine collections, and an aortic clamp was placed above the junction of the left renal artery to regulate RAP.

During surgery, a isotonic saline solution containing albumin (6 g/dl) was infused at 20 μl/min for 30 min. The rats were then infused with an isotonic saline solution containing albumin (1 g/dl), p-aminohippurate sodium (PAH, 1.5 g/dl) and inulin (Inutest, 2 g/dl) at the same infusion rate. After a stabilization period of about 1 h, 30-min urine samples were collected at different RAP levels. In group I (normal control rats), urine collections were made at RAP levels of 130, 110, and 90 mmHg. In group II (ANG II-infused hypertensive rats), RAP levels of 150, 130, 110, and 90 mmHg were selected. In group III (ANG II-infused rats treated with losartan), the systemic arterial pressures were lower and clearance measurements were made only at RAP levels of 110 and 90 mmHg. Ten minutes of equilibration time were allowed after each reduction in RAP. Blood samples were collected at the midpoint of each urine collection period. After plasma separation, red blood cells were reinfused. Urine and plasma samples were assessed for inulin and PAH concentrations.

**Series 2: Assessment of RBF and MBF autoregulation efficiency.** Thirteen days after osmotic minipump implantation, rats were prepared for assessment of renal hemodynamic responses to reductions in perfusion pressure as described for the first series with the following additional procedures. The carotid arteries were isolated, and a suture was placed around each artery. The left renal artery was separated from its surrounding tissue and instrumented with an electromagnetic flow probe of 2-mm diameter (Carolina Medical Electronics, King, NC). A needle laser-Doppler flow probe (Periflex 4001, Perimed, Stockholm, Sweden) was inserted about 3 mm deep from the kidney surface for measurement of MBF. After a 1-h equilibration period, the responsiveness of RBF and MBF signals was checked by giving bolus injections of a vasodilator (bradykinin, 10 μg) and a vasoconstrictor (angiotensin II, 30 ng). RBF and MBF measurements were taken at spontaneous RAP and after reduction of RAP in steps of about 20 mmHg down to 90 mmHg, allowing a stabilization period of 3 min. After measurements at 90 mmHg, the aortic clamp was released and control arterial pressure was reestablished. The carotid arteries were constricted to raise systemic arterial pressure in group I (normal control) and III (ANG II-infused rats treated with losartan) rats, and measurements of RBF and MBF were recorded at the elevated arterial pressures and following reduced RAP. At the end of the experiment, zero values were established by complete occlusion of the aorta. Upon completion of the experiment, the location of the tip of the laser-Doppler flow probe was examined, and only studies where the tip was in the outer medulla were included in the analysis.

**Analyses and Statistics**

Blood and urine samples were analyzed for inulin, PAH, sodium, and potassium. Inulin and PAH concentrations were measured colorimetrically. Sodium and potassium concentrations were determined by flame photometry. GFR was calculated from urine inulin and plasma inulin concentrations and urine flow. PAH clearance was used as an index of RPF. Differences between different groups were analyzed with an unpaired t-test. Autoregulation capability for RBF and MBF responses to reductions in RAP was analyzed using repeated measures analysis of variance. Results are expressed as means ± SE. Values exceeding the 95% critical values (P < 0.05) are considered to be statistically significant.
RESULTS

Arterial Pressures

As previously reported, rats infused with ANG II developed hypertension and administration of losartan in the drinking water prevented the rise of arterial pressure (17, 37, 38, 41). Mean arterial pressure in normal rats under anesthesia averaged 127 ± 2 mmHg. The ANG II-infused rats had a mean arterial pressure of 163 ± 7 mmHg. In contrast, the ANG II-infused rats receiving losartan in the drinking water had a mean arterial pressure of 110 ± 2 mmHg.

RPF and GFR Responses to Reduced RAP

As shown in Fig. 1, GFR values at the respective control pressures were similar in normal and ANG II-infused rats with and without losartan treatment. When compared at equivalent levels of RAP, however, GFR values in the ANG II-infused hypertensive rats were significantly lower than in the normotensive control rats (0.5 ± 0.08 vs. 0.73 ± 0.03 ml/min at 130 mmHg RAP, P < 0.05). These differences in GFR at the reduced pressures are due, in large part, to the clear impairment of GFR autoregulation efficiency in the ANG II-infused hypertensive rats compared with control rats. In contrast, the rats treated with losartan in the drinking water did not develop hypertension nor the decreases in GFR and had higher GFR values than the ANG II-infused rats before and after reduction of RAP as shown in Fig. 1.

When compared at equivalent pressures, RPF values were not significantly different between the control and ANG II-infused rats. The autoregulatory pattern for RPF based on PAH clearances was equivocal because it exhibited low autoregulatory efficiency in response to RAP reduction to 130 mmHg but RPF was not significantly diminished upon further reduction of RAP to 110 mmHg and then decreased substantially upon further reduction in RAP. The control rats exhibited high-efficiency autoregulation of both GFR and RPF between 130 and 110 mmHg but also had decreases with reductions in RAP to 90 mmHg. RPF values in the rats treated with losartan were significantly higher than the RPF values in either the normotensive control group or the ANG II-infused hypertensive group.

Sodium Excretion and Urine Flow Responses to Reduced RAP

As shown in Fig. 2, sodium excretion was markedly reduced in the ANG II-infused rats, and the pressure-natriuresis relationship in the hypertensive rats exhib-
ITED a very flat slope compared with that in the normal control rats. In contrast, the sodium excretion responses in the ANG II-infused hypertensive rats treated chronically with losartan were similar to those in the control rats. Concomitant treatment with losartan prevented the suppression of the pressure-natriuresis curve caused by chronic ANG II infusions. A similar pattern was observed in the fractional sodium excretion responses to decreases in RAP. Thus there was a markedly higher fractional sodium reabsorption rate in the ANG II-infused rats, reflecting the direct effects of ANG II to enhance tubular reabsorption. The urine flow curve (not shown) was also shifted but did not show the same degree of impairment as the sodium excretion curve. Thus the impairment of sodium excretion was due to both lower urine flows and markedly lower urine sodium concentrations.

**Autoregulation of RBF and MBF**

To obtain a more reliable assessment of autoregulatory efficiency of total RBF and of MBF, the RBF and MBF responses were evaluated using electromagnetic and single fiber laser-Doppler flow probes, respectively. The total kidney blood flow responses are shown in Fig. 3. The normal control rats exhibited high efficiency of autoregulation of RBF at RAP values above 100 mmHg. Autoregulation efficiency of RBF in response to reductions of RAP was reduced in ANG II-infused hypertensive rats (slope 0.40 ± 0.02 vs. 0.14 ± 0.03% change of RBF/mmHg at RAP above 100 mmHg for control, \( P < 0.05 \)). Autoregulatory efficiency after losartan also remained intact. However, with reduction in RAP to 90 mmHg, the RBF decrease was less than observed in either control or ANG II-infused rats, suggesting that the RBF autoregulation plateau was extended to lower pressures by losartan. In response to the increase in systemic arterial pressure caused by carotid occlusion, RBF increased only slightly. As shown in Fig. 4, MBF was well autoregulated in normal rats and ANG II-infused rats, suggesting that MBF autoregulation is preserved in the ANG II-infused hypertensive rats. MBF in ANG II-infused rats receiving losartan was decreased slightly upon reduction of RAP to 90 mmHg but exhibited similar autoregulation with increases in systemic arterial pressure following carotid occlusion. In absolute perfusion units, MBF values were not significantly different among the three groups.

**DISCUSSION**

The major objectives of this study were to examine the influence of chronic ANG II-infusion on autoregulatory ability of RBF, MBF, and GFR and on the pressure-natriuresis relationship. As previously reported, rats infused with ANG II develop elevated arterial pressures and the concomitant administration of AT1 receptor blockers in the drinking water prevents the hypertension (35, 38, 41). These previous studies were conducted in uninephrectomized rats, which may exacerbate the hypertension-induced renal injury. Nevertheless, the arterial pressures generated in the two-kidney model are similar to those previously reported for the uninephrectomized model although higher ANG II-infusion rates were required. The use of the two-kidney chronic ANG II-infused hypertensive model in the present study provides the advantage that there were no concomitant growth stimuli related to the compensatory hypertrophy response that occurs following uninephrectomy. Thus the changes observed could be more directly associated with the specific consequences of chronic elevations in ANG II concentrations. In addition, we felt that it was essential to avoid extensive surgical procedures, extracellular fluid volume expansion, or infusion of exogenous hormones such as vasopressin or norepinephrine since these could modify the alterations in renal function caused specifically by the chronic ANG II infusion (36). In the present study, the ANG II-infused rats exhibited a marked suppression of the pressure-natriuresis rela-

Fig. 3. Relationships between RAP and relative renal blood flow (RBF) in normal rats (white triangle; \( n = 7 \)), ANG II-infused rats (black circle; \( n - 6 \)), and ANG II-infused rats treated with losartan (gray triangle). Points designated with * indicate that they are significantly different from their respective control value set at 100%. *\( P < 0.05 \).

Fig. 4. Relationships between RAP and relative medullary blood flow (MBF) in normal rats (white triangle; \( n = 7 \)), ANG II-infused rats (black circle; \( n - 6 \)), and ANG II-infused rats receiving losartan (gray triangle).
tionship that was not observed in the previous volume-expanded rats.

Although GFR values were similar in the three groups when measured at their respective control pressures, when the GFR values were compared at equivalent arterial pressures, GFR values in the ANG II-infused hypertensive rats were significantly lower than in the normotensive control rats. Chronic losartan treatment prevented the decreases in GFR and allowed better maintenance of GFR for any given level of RAP. RPF values, based on PAH clearance data, were similar in the control and ANG II-infused rats at their respective arterial pressures while the losartan-treated rats exhibited significantly higher RPF values. The greater RPF values observed in the ANG II-infused rats treated with losartan suggest that some of the actions of chronic ANG II infusions include effects not blocked by losartan. It is possible that the stimulation of intrarenal nitric oxide production by chronic ANG II is not affected by AT1 receptor blockade and that when the AT1 receptor-mediated vasoconstriction is blocked, an AT2 receptor-mediated vasodilation becomes apparent (3).

When all values for RPF above 110 mmHg were evaluated, the ANG II-infused rats had a greater slope than the control rats. These data suggest a greater effect of chronic ANG II infusions to impair GFR autoregulation than RBF autoregulation. The effects of GFR may be due to the direct effects of ANG II on glomerular ANG II binding sites and the glomerular filtration coefficient (1, 2, 7) as well as the effects to reduce glomerular pressure and effective filtration pressure for any given level of RAP (26, 28). The failure to see equivalent decreases in RPF may be due to chronic compensatory renoprotective mechanisms such as nitric oxide that are activated by chronic ANG II infusions so that RPF is preserved (3). Nevertheless, the compensatory mechanisms that are activated along with the consequences of sustained elevations in systemic arterial pressure result in compromised renal autoregulatory capability. The whole kidney RBF data, based on electromagnetic flowmeter measurements, more clearly reflect the reduced RBF autoregulatory efficiency in the ANG II-infused rats. This impairment of autoregulatory efficiency is similar to that found in the nonclipped kidney of 2K1C Goldblatt rats (30, 31) and is consistent with the recent study evaluating afferent arteriolar responses to changes in perfusion pressure in ANG II-dependent hypertension (17). While systematic histological evaluation was not formally performed, the tissue sections failed to reveal obvious signs of vascular or glomerular injury as seen with the uninephrectomized preparation (41). Nevertheless, it is possible that more subtle microvascular injury, which was prevented by concomitant losartan treatment, could have contributed to the impairment in whole kidney autoregulation and sodium excretion.

Interestingly, the MBF responses to changes in RAP exhibited high-efficiency autoregulatory capability in the ANG II-infused rats as well as in the control rats. Thus there was a dissociation between MBF autoregulation and whole kidney blood flow autoregulation, supporting the concept that additional mechanisms participate in MBF autoregulation. Nevertheless, the finding that there were no major differences in MBF autoregulation between the control and hypertensive rats indicates that alterations in medullary hemodynamics were not of major significance in the suppression of pressure-natriuresis that was observed. This is consistent with the previous reports that renal interstitial pressure was not significantly altered by chronic ANG II infusions and appeared not to be a contributing factor to the increased fractional reabsorption rate seen in the ANG II-infused rats (36). Similarly, the present data indicate that MBF regulatory behavior was not altered by the chronic ANG II infusions or by the concomitant treatment with losartan.

Previous studies in SHR or rats infused with ANG II for 7–10 days showed rightward shifts of the pressure-natriuresis curve (18, 36). In the previous study, the rats received several hormones and were volume expanded, which led to preparations with elevated fractional sodium excretion. In addition, the ANG II infusions were for 7–10 days, which are not sufficient to maximally increase intrarenal ANG II levels (41). In the present study, the reduction in the slope of the pressure-natriuresis relationship in the ANG II-infused rats was much more marked than previously reported. Many studies have shown that while ANG II is not a mediator of pressure-natriuresis it is a powerful modulator of the slope of the pressure-natriuresis curve (22, 23, 27). ANG II alters the magnitude of the sodium excretory response to changes in RAP by exerting multiple actions on intrarenal hemodynamics as well as on tubular transport (23, 24). Because the rats infused with ANG II have lower GFR than normal control rats for any given RAP, the reductions in filtered load could clearly contribute to an enhanced fractional sodium reabsorption and a reduced sodium excretion. In addition, there is now clear evidence for powerful actions of ANG II to enhance tubular reabsorption rate at both proximal and distal tubular segments (24, 25, 32, 39). It is likely that the combined actions of reduced filtered sodium load and enhanced ANG II-dependent sodium reabsorption led to marked decreases in distal nephron sodium delivery such that the effects of alterations in RAP could not exert any substantial alterations to the already near maximal fractional sodium reabsorption seen at the higher arterial pressures. Thus, in the ANG II-infused rats, the strong intrarenal action of ANG II to increase tubular sodium reabsorption chronically impaired the pressure-natriuresis response.

As already mentioned, autoregulation of MBF in chronic ANG II-infused hypertensive rats was well maintained and was not significantly altered by concomitant losartan treatment. Cupples et al. (6), using a dual-slit technique in descending and ascending vasa recta of the exposed renal papillae of antidiuretic rats, demonstrated that captopril had no effect on autoregulation of blood flow in either descending or ascending vasa recta. This observation suggests that the blood
flow in the vasa recta of renal medulla is efficiently autoregulated and that this autoregulation is independent of ANG II. Mattson et al. (19) also found that infusion of ANG II had no effect on the autoregulation of papillary blood flow.

Although the circulating ANG II levels are increased as a consequence of the chronic ANG II-infusions, these concentrations approach a plateau within a few days, but the hypertension develops more slowly over a period of 10–14 days (21, 35, 41). Recent studies have demonstrated that these chronic infusions of ANG II lead to progressive augmentation of intrarenal ANG II that appears to be closely associated with the development of hypertension. Consequently, it has been postulated that the multiple synergistic actions of increased intrarenal ANG II enhance sodium reabsorption and reduce sodium excretion, leading to the progressive hypertensinogenic process (22, 23). These sodium-retaining effects coupled with the direct vascular effects of ANG II may serve as powerful progressive stimuli for hypertension (23).

In summary, chronic low-dose infusions of ANG II lead to marked impairment of sodium excretion and suppression of the pressure-natriuresis relationship as well as reduced RBF and GFR autoregulatory efficiency. It is likely that this impairment is due to the progressive intrarenal augmentation of ANG II, which contributes to the progressive development of hypertension in the chronic ANG II-infused hypertensive model. Losartan treatment prevented the suppression of pressure-natriuresis and the hypertension, indicating that the actions of ANG II to modulate pressure-natriuresis is primarily mediated by the AT1 receptor.

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