Effect of Intrarenal Angiotensin II Blockade on Renal Function in Conscious Dogs

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SUMMARY The effects of intrarenal infusion of l-sar-8-ala angiotensin II (P 113) and a converting enzyme inhibitor, SQ 20881, at doses that did not affect systemic blood pressure (2.0 μg/kg per min) were studied in conscious, uninephrectomized dogs. In dogs receiving =5 mEq/day of sodium, intrarenal infusion of P 113 increased renal blood flow (RBF) from 219.8 ± 32.3 to 283.7 ± 20.0 ml/min (P < 0.004), and with intrarenal SQ 20881 infusion from 215.3 ± 14.2 to 278.0 ± 22.2 ml/min (P < 0.005). In sodium-restricted dogs (=5 μEq/day), glomerular filtration rate (GFR) also increased with intrarenal P 113 infusion from 57.0 ± 5.7 to 66.3 ± 6.6 ml/min (P < 0.05), and with SQ 20881 infusion from 43.1 ± 2.1 to 55.7 ± 4.5 ml/min (P < 0.01). Dogs on =5 mEq/day of sodium showed significant increases in plasma renin activity (PRA) with intrarenal infusion of the peptides, unmasking a negative feedback inhibition of renin release by angiotensin II. No increases in RBF, GFR, or PRA were seen with infusion without inhibitors, or in dogs given P 113 or SQ 20881 while on =80 mEq/day of sodium. In addition, angiotensin II inhibition increased sodium excretion during sodium restriction. These findings suggest that intrarenal angiotensin II is intimately involved in renal responses to sodium restriction which result in conservation of sodium and water.

THE renin-angiotensin-aldosterone system is a delicately balanced hormonal cascade which regulates blood pressure and sodium and potassium balance primarily through the coordinated action of the effector compounds, angiotensin II and aldosterone.1 The system is activated by renin secretion in response to a variety of stimuli which affect renal perfusion.2 Renin secretion then is suppressed as a consequence of increased angiotensin II and aldosterone concentration, either by systemic mechanisms mediated through volume expansion or intrarenal feedback mechanisms mediated by local angiotensin II generation.3 The normal animal appears dependent on the system during sodium deprivation when plasma concentrations of both angiotensin II and aldosterone are increased and renal cortical blood flow is reduced.4 Hence, the parenteral administration of competitive antagonists of angiotensin II induces a reduction in blood pressure in conscious, sodium-depleted rats5 and rabbits,6 and anesthetized, sodium-restricted dogs.7 Moreover, intrarenal angiotensin II modulates intrarenal blood flow without a change in perfusion pressure in anesthetized, sodium-restricted dogs but not in sodium-repleted dogs.8

On the basis of these findings, we hypothesized that during sodium restriction the renin-angiotensin-aldosterone system maintains sodium and volume homeostasis via modulation of renal plasma flow and glomerular filtration as well as aldosterone biosynthesis. The generation of angiotensin II within the kidney may be important for the control of renal hemodynamics, glomerular filtration, sodium, and water excretion, and renin release. The responses to intrarenal infusion of angiotensin II may not accurately reproduce the physiological actions of the endogenous peptide generated intrarenally. Therefore, we used sodium depletion to activate physiologically the renin-angiotensin-aldosterone system, and deduced the import of the system via selective blockade of angiotensin II. Intrarenal infusion of l-sar-cine-8-alanine (l-sar-8-ala) angiotensin II and an angiotensin I-converting enzyme inhibitor, SQ 20881, was given at doses that did not affect systemic blood pressure in conscious, sodium-depleted, and sodium-repleted dogs. The effects of the inhibition of the renin-angiotensin-aldosterone system on renal blood flow (RBF), glomerular filtration rate (GFR), sodium excretion, and renin release were determined and the results are described.

Methods

Female foxhounds weighing 16–25 kg were anesthetized with sodium pentobarbital (20 mg/kg, iv) the trachea was intubated, and a midline celiotomy was performed. A right nephrectomy was performed, and Teflon catheters with outer diameter (o.d.) of 0.075 cm were inserted into the inferior vena cava and aorta. Using a steel guidewire, a smaller Teflon catheter (o.d. = 0.025 cm) was inserted into the left renal artery by puncturing the aortic wall opposite the aortic and left renal arterial bifurcation, and the catheter was guided into the left renal artery. The guidewire was removed and the catheter was anchored in place by a suture in the aorta. The three catheters then were exteriorized through a separate flank stab wound near the costovertebral angle, and the midline incision was
The dogs were uninephrectomized so that the total renal mass could be perfused with one intrarenal arterial catheter, and a split bladder preparation for urine collection was not necessary. After a 7-day recovery period, the dogs were placed on a controlled intake of sodium. Dogs on a low sodium diet received a standard chow with less than 5 mEq of sodium/day. To maintain standard sodium repletion, dogs were supplemented with daily intravenous infusions of 75 mEq of sodium as isotonic sodium chloride. Three groups of dogs were studied under each sodium intake after at least 5 days of equilibrium on the respective diets.

On the day of the study a Foley catheter was inserted for collection of urine, and the dogs were placed in a canvas sling with their feet touching the floor. This enabled the dog to lie down or stand. During the experimental period the mean aortic pressure was monitored continuously and the renal arterial pressure monitored intermittently with a P23D Statham strain gauge and Sanborn recorder. Clearances were measured using 14C-inulin to determine GFR and tritiated p-aminohippuric acid (PAH) to determine effective renal plasma flow in a vehicle of 0.5 N sodium chloride. After an initial bolus of 150 ml, a constant infusion of 14C-inulin and tritiated PAH was sustained at 4 ml/min for 30 minutes. Ten ml of blood then were obtained for control plasma renin activity (PRA), and three 10-minute control renal clearances were measured (15 ml of blood).

In eight dogs the converting enzyme inhibitor, SQ 20881, was infused into the renal artery in a vehicle of 0.5 N sodium chloride at 2.0 /ug/kg per min for 20 minutes. Ten ml of blood then were obtained for control plasma renin activity (PRA), and three 10-minute control renal clearances were measured (15 ml of blood). In eight dogs the converting enzyme inhibitor, SQ 20881, was infused into the renal artery in a vehicle of 0.5 N sodium chloride at 2.0 /ug/kg per min for 20 minutes. Ten ml of blood then were obtained for control plasma renin activity (PRA), and three 10-minute control renal clearances were measured (15 ml of blood). In eight dogs the converting enzyme inhibitor, SQ 20881, was infused into the renal artery in a vehicle of 0.5 N sodium chloride at 2.0 /ug/kg per min for 20 minutes. Ten ml of blood then were obtained for control plasma renin activity (PRA), and three 10-minute control renal clearances were measured (15 ml of blood).

FIGURE 1 Fall in renal blood flow (RBF) with blockade of the renin-angiotensin system with SQ 20881 in sodium-replete dogs (=80 mEq/day). Results for all figures are given as mean ± SE; n = number of dogs in each group.

Results

In the three studies with the dogs receiving =80 mEq sodium/day, mean aortic pressure (MAP) was: study 1, 105.0 ± 4.1 mm Hg, control, and with the vehicle alone, 104.0 ± 3.8 mm Hg; study 2, 103.8 ± 2.0 mm Hg, control, and with P 113 infusion, 104.8 ± 0.5 mm Hg; and study 3, 117.8 ± 3.4 mm Hg, control, and with SQ 20881 infusion, 118.0 ± 3.4 mm Hg. In dogs receiving =5 mEq sodium/day, MAP was: study 1, 108.8 ± 1.2 mm Hg, control, and with P 113 infusion, 110.3 ± 3.2 mm Hg; and study 3, 111.6 ± 3.4 mm Hg, control, and with SQ 20881 infusion. None of the changes in MAP with infusion were significant. Figure 1 shows the change in RBF with blockade of the renin-angiotensin system in dogs receiving =80 mEq of sodium/day. There was no significant change in RBF either with the sham experiments or with intrarenal infusion of P 113. With the administration of SQ 20881, however, there was a small but significant decrease in RBF. Figure 2 shows changes in the RBF in dogs on sodium restriction. Intrarenal infusions of SQ 20881 resulted in a highly significant increase in RBF. Similar results were obtained with P 113. RBF in the sham dogs did not change.

There were no significant changes in GFR in dogs receiving =80 mEq of sodium/day during the sham experiments (GFR = 60.3 ± 10.8 to 58.7 ± 9.6 ml/min), during intrarenal infusion of SQ 20881 (GFR = 61.3 ± 8.7 to 61.2 ± 8.0 ml/min), or during intrarenal infusion of P 113 (GFR = 48.7 ± 9.3 to 54.0 ± 12.4 ml/min). In contrast, in sodium-restricted dogs (Fig. 3) there were significant increases in GFR with intrarenal administration of SQ
FIGURE 3 Increase in glomerular filtration rate (GFR) with blockade of the renin-angiotensin system with intrarenal P 113 in sodium-restricted dogs (≈5 mEq/day).

20881 as well as following P 113 infusion. The GFR in the sham dogs remained stable. The increase in GFR in the sodium-restricted dogs paralleled the increase in RBF, thus, the filtration fraction did not change significantly.

Sodium excretion in dogs receiving ≈80 mEq of sodium/day did not significantly change during the sham experiments (57.5 ± 12.1 to 69.8 ± 15.8 µEq/min), during intrarenal SQ 20881 infusion (124.2 ± 29.8 to 111.2 ± 20.1 µEq/min), or during intrarenal P 113 infusion (63.4 ± 17.5 to 82.9 ± 33.3 µEq/min). Figure 4 depicts sodium excretion during sodium restriction. Although the sham dogs had a slight increase in sodium excretion, much larger changes were seen with the administration of both SQ 20881 and P 113.

Figure 5 depicts the changes in PRA with blockade of the renin-angiotensin system in dogs receiving ≈80 mEq of sodium/day. The sham dogs showed no change in PRA, whereas in the dogs infused with the angiotensin-converting enzyme inhibitor, SQ 20881, PRA increased from a control value of 2.05 ± 0.36 to 4.57 ± 1.53 ng/ml per hour (P < 0.05). Intrarenal infusion of P 113 induced a statistically insignificant increase in PRA. The changes in PRA in sodium-restricted dogs are presented in Figure 6. In the sham dogs, there was no change in PRA, whereas both intrarenal SQ 20881 infusion and intrarenal infusion of P 113 increased PRA significantly. Thus, in sodium-depleted dogs, the increase in PRA with angiotensin II and/or converting enzyme blockade unmasked the suppressive effect of angiotensin II on renin release.

Discussion

Previous studies of the effects of angiotensin II on renal function based on intravenous infusion of suppressor quantities of the peptide have generally resulted in a decrease in RBF, GFR, urine flow, and sodium excretion, and an inhibition of renin release. These findings have been interpreted as resulting from angiotensin-induced renal vasoconstriction. A more physiological activation of the renin-angiotensin system may occur with sodium depletion. In this setting, endogenous angiotensin II levels are elevated, more vascular smooth muscle receptor sites are occupied, and there is reduced pressor responsiveness to exogenous angiotensin II. Moreover, there is physiological conservation of sodium and decreased renal cortical blood flow. Specific blockade of the renin-angiotensin system indicates that peripheral resistance is dependent on angiotensin II, since blockade causes a fall in blood pressure and a rise in RBF.

The present study demonstrates that in conscious, uni-
nephrectomized dogs the renin-angiotensin system plays a physiological intrarenal role in the modification of renal function during sodium restriction. Intrarenal infusion of the competitive antagonist of angiotensin II, 1-sar-8-ala angiotensin II (P 113), and the converting enzyme inhibitor, SQ 20881, in doses which did not affect systemic arterial pressure, consistently increased RBF and GFR. The results are in agreement with the work of Freeman et al. in anesthetized dogs with respect to RBF. Contrasting with some of the results of Freeman's studies, there were no changes in systemic blood pressure with the intrarenal dose used in any of our experiments. The similar responses obtained following the administration of either P 113 or SQ 20881 suggest that the common mechanism is antagonism of angiotensin II-mediated vasoconstriction, rather than any response that SQ 20881 might produce which is dependent on inhibition of the degradation of bradykinin.

The filtration fraction in response to inhibition of the renin-angiotensin system did not change significantly, suggesting that both pre- and postglomerular arterioles were being influenced equally by angiotensin II. Alternatively, it is possible that blockade influenced the characteristics of glomerular filtration directly, since angiotensin II localizes to the glomerular mesangial cells and affects glomerular morphology.

In the sodium-replete dogs with preexisting angiotensin II-mediated vasoconstriction, the fall in RBF subsequent to intrarenal SQ 20881 infusion may reflect a direct action of angiotensin I. Keim et al. have shown that the pressor effect of angiotensin I was eliminated by preventing its conversion to angiotensin II by SQ 20881. However, SQ 20881 did not completely abolish the angiotensin I-initiated depression of RBF, suggesting a direct effect of angiotensin I. In contrast, P 113 blocks the actions of both angiotensin I and angiotensin II within the kidney.

In sodium-restricted animals the enhancement of sodium excretion (above that seen with vehicle alone) after intrarenal SQ 20881 infusion may reflect a direct action of angiotensin I. Keim et al. have shown that the pressor effect of angiotensin I was eliminated by preventing its conversion to angiotensin II by SQ 20881. However, SQ 20881 did not completely abolish the angiotensin I-initiated depression of RBF, suggesting a direct effect of angiotensin I. In contrast, P 113 blocks the actions of both angiotensin I and angiotensin II within the kidney.

In sodium-restricted animals the enhancement of sodium excretion (above that seen with vehicle alone) after angiotensin II blockade with either agent suggests that intrarenal angiotensin II regulates sodium excretion. These increments in sodium excretion may be attributable solely to the increase in filtered load of sodium accompanying the rise in GFR. However, a selective redistribution of blood flow to the cortex following inhibition of angiotensin II-mediated cortical vasoconstriction or inhibition of a direct effect of angiotensin II on the renal tubule cannot be excluded. The limited time of infusion and the 30-minute half-life of plasma aldosterone would make a fall in aldosterone secretion with a resultant natriuresis unlikely.

Several investigators have postulated an intrarenal negative feedback of angiotensin II on renin release. Intrarenal infusion of angiotensin II at physiological concentrations into dogs or sheep decreases renin release without affecting sodium excretion or blood pressure. Ayers et al. have shown that in acute experimental renovascular hypertension, renal vasoconstriction is mediated by the renin-angiotensin system. In these experiments, intrarenal infusion of SQ 20881 or P 113 was associated with an increase in PRA, thus demonstrating a negative intrarenal feedback mechanism for renin release mediated by angiotensin II. The mechanism probably is due to changes in afferent arteriolar pressure or flow, or both, but a direct effect on juxtapaglomerular cells has not been excluded. The present work confirms the presence of the negative intrarenal feedback loop of angiotensin II on renin release in sodium-restricted dogs. In sodium-replete dogs, the control renin level was normal as a result of systemic volume-mediated feedback. Hence, the intrarenal angiotensin II-induced feedback mechanism was less apparent, as shown by a smaller rise in renin.

Taken altogether, these findings in the conscious, un-nephrectomized dog suggest that intrarenal angiotensin II is intimately involved in renal responses to sodium restriction. Whether the observed rises in RBF, GFR, and sodium excretion are due to inhibition of the direct renal actions of angiotensin II, or are partly due to the inhibition of the effects of angiotensin II that are mediated indirectly through the actions of prostaglandins or catecholamines, remains to be defined.

References

Cardiac Output and Renal Blood Flow in Glycerol-Induced Acute Renal Failure in the Rat

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SUMMARY Cardiac output (CO) and renal blood flow (RBF) were simultaneously evaluated by the microsphere method in water-drinking and chronic saline-drinking rats at 3, 12, and 24 hours after induction of acute renal failure by glycerol injection. Three hours after glycerol injection CO and RBF decreased to 36% and 20% of the respective controls in water-drinking rats and to 41% and 24% of the controls in saline-drinking rats. Renal vascular resistance (RVR) increased significantly in both groups at this time. Isosonotic plasma expansion (3% of body weight) restored the RBF and RVR to normal in water-drinking rats 3 hours post-glycerol injection, although CO increased to only 70% of the control. Twelve hours after glycerol injection, CO and RBF returned to normal in saline-drinking rats, whereas they remained lower than controls in water-drinking rats. Twenty-four hours post-glycerol injection, when acute renal failure was evident as indicated by blood urea nitrogen (BUN) values of 116.9 and 63.8 mg/100 ml in water- and saline-drinking rats, respectively, CO and RBF returned to normal, except that the CO of water-drinking rats was slightly higher than control. Thus, we conclude that decreased CO is an important determinant of the early decrease of renal perfusion in glycerol-induced acute renal failure. Furthermore, the observed earlier return of CO and RBF to normal in saline-drinking rats may be partly responsible for reducing the severity of acute renal failure.

RECENT STUDIES have demonstrated that renal cortical ischemia is not responsible for maintenance of impaired glomerular filtration in experimental acute renal failure. These findings are contrary to earlier reports that indicated a primary role for decreased renal perfusion in the severely decreased glomerular filtration rate (GFR) that occurs in acute renal failure. However, a severe reduction of renal blood flow (RBF) during the initial hours of glycerol-induced acute renal failure has been consistently demonstrated and such a reduction of RBF undoubtedly contributes to the early decrease of GFR seen in this model. The mechanisms for this reduction of RBF are not entirely clear, but it has been suggested that the renin-angiotensin axis and increased intrarenal vascular resistance may be involved. This study was designed to investigate the mechanism of decreased RBF in the early stage of glycerol-induced acute renal failure in water-drinking rats and chronic saline-drinking rats.

**Methods**

Experiments were performed on male Sprague-Dawley rats weighing 180–300 g. Group I contained rats maintained on Purina laboratory chow and tap water. In group II, rats were given 1% NaCl instead of tap water to drink for 4–6 weeks prior to experimentation.

Cardiac output (CO) and RBF were determined simultaneously by modified microsphere methods previously described. Measurements were made in awake rats at 3, 12, and 24 hours after intramuscular injection of 50% glycerol, 1 ml/100 g of body weight. All rats were dehydrated for 15 hours prior to injection of glycerol. Rats evaluated 12 and 24 hours post-glycerol were allowed to drink water or saline during the interim period. Control water- and saline-drinking rats not injected with glycerol also were studied. Controls corresponding to the 3-hour post-glycerol experimental groups were dehydrated for 18 hours before measurement of CO and RBF, whereas those corresponding to the 12- and 24-hour post-glycerol groups were not.

Rats were weighed, anesthetized with ether, and cannulated with polyethylene tubing (no. 10) through the femoral artery for blood collection and through the right carotid artery into the left ventricle for injection of microspheres. Following surgery, rats were placed in restraining cages and allowed to recover for at least 1 hour prior to injection. Radioactive microspheres, 15 ± 5 μm in diameter, were used to measure RBF and CO simultaneously. Two different nuclides were used, 85Sr and 141Ce, for separate measurements taken approximately 15–30 minutes apart. For each determination, slightly less than 0.1 ml of a concentration of microspheres (2-3 mg/ml in 10% dextan solution) was injected through the carotid catheter within 15–20 seconds. Prior to filling the syringe, the solution...
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