Maintenance of Baseline Angiotensin II Potentiates Insulin Hypertension in Rats

Henry L. Keen, Michael W. Brands, Manis J. Smith, Jr, John E. Hall

Abstract—Chronic insulin infusion in rats increases mean arterial pressure (MAP) by a mechanism dependent on angiotensin II (Ang II). However, the fact that plasma renin activity (PRA) decreases with insulin infusion suggests that Ang II sensitivity is increased and that the parallel reduction in Ang II may partly counteract any hypertensive action of insulin. This study tested that hypothesis by clamping Ang II at baseline levels during chronic insulin infusion. Sprague-Dawley rats were instrumented with artery and vein catheters, and MAP was measured 24 hours per day. In seven angiotensin clamped rats (AC rats), renin–angiotensin II system activity was clamped at normal levels throughout the study by continuous intravenous infusion of the angiotensin-converting enzyme inhibitor benazepril at 5 mg/kg per day (which decreased MAP by 18±2 mm Hg) together with intravenous Ang II at 5 ng/kg per minute. Control MAP in AC rats after clamping averaged 99±1 mm Hg. which was not different from the 101±2 mm Hg measured before clamping Ang II levels. Control MAP in the 8 vehicle-infused rats averaged 105±2 mm Hg. A 7-day infusion of insulin (1.5 mU/kg per minute IV) plus glucose (20 mg/kg per minute IV) increased MAP in both groups of rats; however, the increase in MAP was significantly greater in AC rats (12±1 versus 5±1 mm Hg). This enhanced hypertensive response to insulin in AC rats was associated with a greater increase in renal vascular resistance (153±10% versus 119±6% of control) and a significant increase in renal formation of thromboxane (149±11% of control). Thus, decreased Ang II during insulin infusion limits the renal vasoconstrictor and hypertensive actions of insulin, and this may be caused, at least in part, by attenuation of renal thromboxane production. (Hypertension. 1998;31:637–642.)

Key Words: angiotensin • insulin • blood pressure • thromboxane

Chronic hyperinsulinemia increases blood pressure in rats by mechanisms that are not completely understood.1–6 The hypertension likely is not volume mediated because there is no significant increase in cumulative sodium balance1–5 or cardiac output.1 However, the fact that the rats maintain sodium balance at a significantly elevated blood pressure is indicative of an insulin-induced antinatriuretic shift in the pressure-natriuresis relationship.7 Moreover, our finding of decreased GFR on day 3 of insulin infusion1 suggests that renal vasoconstriction might be partly responsible for this shift in pressure natriuresis. This is supported by our recent findings that chronic α1- and β-adrenergic receptor blockade8 attenuated neither the decrease in GFR nor the increase in blood pressure, whereas thromboxane synthase inhibition9 and ACE inhibition10 significantly attenuated both the renal vasoconstrictor and the hypertensive responses to insulin infusion.

Because PRA decreases during insulin infusion, insulin hypertension probably does not depend on increased Ang II. The attenuating effect of ACE inhibition on insulin hypertension suggests therefore that Ang II sensitivity is increased or that some permissive level of Ang II is required for insulin to increase blood pressure. In addition, because Ang II–dependent increases in renal vascular resistance and blood pressure are dependent partly on increased thromboxane formation,8,10 and because of the important role of thromboxane in insulin hypertension, reduced Ang II during insulin infusion might counteract the hypertensive action of insulin both by a direct action of reduced Ang II per se and by an indirect action through decreased renal thromboxane production. The goal of this study, therefore, was to test the hypothesis that prevention of the insulin-induced fall in Ang II, by clamping Ang II at baseline levels, would potentiate the renal vasoconstrictor and hypertensive actions of chronic insulin infusion.

Methods

Male Sprague-Dawley rats weighing approximately 350 g were used for all experiments, and surgery and care of the rats were conducted in accordance with National Institutes of Health guidelines using protocols approved by the Animal Care and Use Committee of the University of Mississippi Medical Center. Under pentobarbital sodium anesthesia and aseptic conditions, a laparotomy was performed and a nonocclusive polyvinyl catheter was inserted into the abdominal aorta, distal to the kidneys, through a puncture made with an 18-gauge needle tip. The insertion point was sealed with cyanoacrylate adhesive, and the catheter was exteriorized through the lateral abdominal wall. A femoral vein catheter was implanted through a separate incision, and the tip was maneuvered into the inferior vena cava distal to the kidneys. Incisions were infiltrated with penicillin G procaine and...
Sensory nerve blockade, and both catheters were routed subcutaneously to the scapular region and exteriorized through a stainless steel button that was implanted subcutaneously.

Rats were allowed to recover from surgery in a warmed cage for 1 to 2 hours. Thereafter, rats were placed in individual metabolic cages in a quiet, air-conditioned room with a 12-hour light/dark cycle. The catheters were connected to a dual-channel infusion swivel (Instech) mounted above the cage and were protected by a stainless steel spring. The arterial catheter was filled with heparin solution (1000 U/mL) and connected, via the swivel, to a pressure transducer (Cobe) mounted on the cage exterior at the level of the rat. Pulsatile arterial pressure signals were sent to an analog-to-digital converter and analyzed by computer using customized software. The analog signal was sampled 4 seconds each minute, 24 hours per day.

The rats received food and water ad libitum throughout the study. An intravenous infusion of 18 mL of sterile 0.9% saline per day containing 18.6 mg/mL KCl, combined with sodium- and potassium-deficient rat chow, allowed sodium and potassium intakes to be clamped at ∼2.8 and 4.5 mmol/d, respectively, independent of food intake. In addition, sterile water was infused as vehicle for the insulin and glucose infusion during the experimental period, yielding a total infusion of 41 mL/d. This infusion was started immediately after placement of rats in their cages, and ∼1 week was allowed for recovery and acclimation before baseline measurements were made. All solutions contained antibiotic (30 000 U/d penicillin G potassium and 27 mg/d Mezlocillin) and were infused intravenously with a syringe pump (Harvard Apparatus) through a filter (22 μm, Millipore).

Experimental Protocol

After baseline measurements were made, endogenous Ang II formation was suppressed for the remainder of the experiment in 7 rats (AC rats) by continuous intravenous infusion of the ACEI benazepril at a rate of 1.5 mU/kg per minute and continued for 7 days. The water vehicle was substituted for a 50% dextrose solution containing 18.6 mg/mL KCl, combined with sodium- and potassium-deficient rat chow, allowed sodium and potassium intakes to be clamped at ∼2.8 and 4.5 mmol/d, respectively, independent of food intake. Six days of recovery and acclimation before baseline measurements were made. All solutions contained antibiotic (30 000 U/d penicillin G potassium and 27 mg/d Mezlocillin) and were infused intravenously with a syringe pump (Harvard Apparatus) through a filter (22 μm, Millipore).

Analytical Methods

Concentrations for plasma insulin and urinary thromboxane B2 and 6-keto-PGF1α were measured by radioimmunoassay. Samples for measurement of thromboxane B2 and 6-keto-PGF1α concentrations were extracted on the day taken and were stored at −30°C until assayed. PRA was measured by radioimmunoassay using the method of Haber et al.11 Plasma glucose was determined by an automated analyzer using the glucose oxidase method (YSI Scientific), and urinary sodium concentrations were determined using flame photometry (Instrumentation Laboratories). GFR and ERPF were measured using a 4-hour fasted plasma sample after a 24-hour intravenous infusion of [125I]iothalamate (Glofil) and [131I]iodohippuran (both at 0.015 μC/kg per minute). Because steady state is achieved during the 24-hour infusion, an infusate sample was counted and the isotope infusion rate was substituted for urine isotope excretion rate to calculate clearance.12

Values are presented as mean±SEM and were analyzed by repeated measures ANOVA. Supplemental within-group comparisons were made with Dunnett’s t test, and between-group comparisons were made with unequal t tests.14 A value of P<.05 was considered statistically significant.

Results

MAP and urinary sodium excretion for vehicle and AC rats are presented in Figs 1 and 2. Baseline MAP was not different between vehicle and AC rats (100±3 versus 101±2 mmHg) and was decreased 18±2 mmHg by ACEI. Addition of Ang II to the infusate restored blood pressure in AC rats to 99±1 mmHg, a value not different from baseline (ie, before ACEI). During insulin infusion, MAP increased in both groups; however, after 7 days of insulin infusion the change in MAP (Fig 3) from control was approximately twofold greater in the AC rats (12±1 versus 5±1 mmHg).

Baseline urinary sodium excretions were not different between groups and were not changed significantly by continued vehicle administration or by ACEI and restoration of baseline Ang II levels. During the control period, urinary sodium excretion averaged 2.7±0.1 and 2.8±0.1 mmol/d in vehicle and AC rats, respectively, and the sodium excretory response to insulin infusion (Figs 1 and 2) was similar in both groups.
On day 1 of insulin infusion, urinary sodium excretion decreased to \(1.2 \pm 0.6\) and \(1.5 \pm 0.2\) mmol/d in vehicle and AC rats, respectively. Thereafter, both groups maintained sodium balance at control levels of sodium excretion for the remainder of the insulin infusion. In addition, urinary sodium excretion was increased transiently, although not significantly, in both groups on the first day after stopping the insulin and glucose infusion.

GFR and renal plasma flow were not different between groups during the baseline period and were not changed significantly by continued vehicle administration or by ACEI and restoration of baseline Ang II levels. On day 1 of insulin infusion (Fig 4), GFR and renal plasma flow increased to \(113 \pm 3\%\) and \(116 \pm 4\%\) control, respectively, and renal vascular resistance decreased to \(91 \pm 3\%\) control in vehicle rats. These variables were not changed significantly on day 1 in AC rats. By day 3 of insulin infusion, GFR, decreased significantly and to similar levels \((85 \pm 3\%\) and \(87 \pm 4\%\) of control\) in vehicle and AC rats. In contrast, the reductions in renal plasma flow to \(73 \pm 5\%\) and \(89 \pm 4\%\) of control and the increases in renal vascular resistance to \(153 \pm 10\%\) and \(119 \pm 6\%\) of control, in AC and vehicle rats, respectively, were significantly greater in AC rats. The values on day 7 were not significantly different from those on day 3 in either group.

Urinary thromboxane B\(_2\) excretion averaged \(15 \pm 2\) ng/d in vehicle and AC rats during the baseline period and was unchanged by continued vehicle administration; however, it was decreased, although not significantly, to \(10 \pm 2\) ng/d by ACEI. Addition of Ang II to the infusate increased urinary thromboxane excretion in AC rats to \(16 \pm 2\) ng/d, a value not different from baseline. Urinary thromboxane excretion did not change significantly during insulin infusion in vehicle rats, but there was a modest and significant increase to \(149 \pm 11\%\) control in AC rats (Fig 5). Urinary excretions of 6-keto-PGF\(_{1\alpha}\) averaged \(11 \pm 1\) and \(10 \pm 1\) ng/d in vehicle and AC rats, respectively, during the baseline period and were not changed significantly by continued vehicle administration or by ACEI and restoration of baseline Ang II levels. There was, however, an approximately twofold increase in 6-keto-PGF\(_{1\alpha}\) excretion during insulin infusion in both groups, which returned toward control during the recovery period (Fig 5).

PRA and fasting plasma concentrations for insulin and glucose are presented in the Table. Fasting plasma insulin was not different between vehicle and AC rats and increased approximately twofold during insulin infusion in both groups.
There was no significant difference in fasting plasma glucose between groups, and no significant change in plasma glucose occurred during any experimental period in either group. PRA tended to be higher in the AC rats during the control period, which suggests that the Ang II replacement may have been too slight. However, the difference was not statistically significant, and the agreement in MAP and thromboxane B2 excretion between the two groups during this period strengthens the likelihood that baseline Ang II levels were not markedly different during the control period. PRA decreased by 25% by day 3 of insulin infusion in vehicle rats and remained suppressed on day 7. There was no significant change in PRA during the insulin infusion in AC rats.

Discussion

The main finding from this study is that maintenance of baseline Ang II potentiates both the renal vasoconstriction and the hypertension associated with chronic insulin infusion in rats. In addition, preventing the insulin-induced fall in Ang II revealed a significant stimulatory effect of insulin on renal thromboxane production. These results suggest that the decrease in PRA during insulin infusion in normal rats limits the hypertensive action of insulin, possibly because of a decrease in Ang II-mediated thromboxane production.

In this study, as in previous studies from our laboratory,1–6 physiological increases in plasma insulin by continuous intravenous infusion increased blood pressure in rats over a 7-day infusion period. The mechanisms for this increase are not understood completely, but because the insulin-infused rats maintained sodium balance in the face of significantly elevated blood pressure, it is clear that the infusion induced an antinatriuretic shift in the pressure-natriuresis relationship.7 Without this shift, the increase in blood pressure could not have been sustained because pressure-induced natriuresis would have ensued and continued until normal blood pressure was restored.7

Increased sodium reabsorption is one potential mechanism for the shift in pressure natriuresis, and a consistent observation from this study and our previous insulin-infusion studies1–6 is reduced urinary sodium excretion on the first day of insulin infusion. Moreover, the new finding in this study that GFR increased on the first day of the insulin infusion provides evidence that the transient decrease in sodium excretion was caused by an insulin-induced increase in sodium reabsorption. This action is consistent with reports from acute insulin infusion studies;14 however, the decrease in sodium excretion subsided after 1 day, and there was no increase in cumulative sodium balance1–6 or cardiac output1 for the 7-day infusion period. The finding of decreased GFR during the later phases of insulin hypertension provides evidence that the increase in tubular reabsorption was not sustained and, moreover, suggests that renal vasoconstriction most likely underlies the shift in pressure natriuresis with sustained insulin infusion.

Further support for a link between decreased GFR and insulin hypertension is our recent observation that chronic α,-

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<th>Recovery</th>
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*P<.05 compared with control period; †P<.05 compared with vehicle rats.
and β-adrenergic receptor blockade failed to blunt the GFR-lowering actions of insulin and likewise did not alter the hypertensive response to insulin infusion. In contrast, thromboxane synthase inhibition and ACE inhibition attenuated both the insulin-induced renal vasoconstriction and the hypertensive response to insulin. In addition, clamping Ang II at baseline in the present study markedly potentiated both the renal vasoconstriction and the hypertension associated with insulin infusion. Thus, chronic insulin infusion in rats shifts pressure natriuresis to a higher pressure level, most likely because of a decrease in GFR.

The site of renal vasoconstriction during insulin infusion is not known, but similar reductions in GFR and renal plasma flow are suggestive of afferent arteriolar constriction. This is consistent with a role of thromboxane as a mediator of the decrease in GFR because several studies have reported that the afferent arteriole is the predominant renal vascular site of action of thromboxane. However, thromboxane also has been reported to increase efferent arteriolar resistance, and previous results suggest that thromboxane potentiates the hypertensive and efferent arteriolar constrictor actions of Ang II. Such an interaction between thromboxane and Ang II on the efferent arteriole may explain why renal plasma flow decreased more in the Ang II-clamped rats whereas GFR was not different between groups.

Interaction between thromboxane and Ang II also may explain the findings that insulin-induced hypertension and renal vasoconstriction are dependent on these hormones, yet no increase in their production occurs during insulin infusion. The increase in urinary thromboxane B2 excretion in the Ang II-clamped rats during insulin infusion suggests that insulin stimulates thromboxane synthesis, and Ang II also has been reported to increase thromboxane production. One explanation for the finding of no change in thromboxane B2 excretion in the vehicle rats during insulin infusion therefore may have been the decrease in Ang II and thus Ang II-mediated thromboxane synthesis.

The decrease in Ang II (as evidenced by the decrease in PRA) in the vehicle rats has been measured in our previous studies in rats during insulin infusion and may be a consequence of enhanced pressor actions of thromboxane in the presence of hyperinsulinemia. In support of this possibility, Yanagisawa-Miwa et al reported recently that insulin increased the vasoconstrictor response to thromboxane. Thus, insulin not only stimulates thromboxane synthesis but also appears to enhance its vasoconstrictor actions. In normal rats (the vehicle group in this study), the increase in blood pressure during insulin infusion therefore may be caused primarily by enhancement of thromboxane’s vasoconstrictor actions by insulin. The results from the Ang II-clamped rats suggest further that the decrease in Ang II that normally occurs masks the effect of insulin to stimulate thromboxane synthesis and thereby limits the rise in blood pressure.

The stimulatory effect of insulin on thromboxane production in rats may explain, in part, the opposite hemodynamic responses to chronic insulin infusion that we have measured in dogs. We have reported that a similar insulin/glucose infusion protocol either does not change or decreases arterial pressure in normal dogs, dogs with reduced kidney mass and a high salt intake, dogs with norepinephrine or Ang II hypertension, and obese insulin-resistant dogs. Because there also is no experimental evidence that hyperinsulinemia by itself causes hypertension in humans, these observations suggest that insulin requires the presence of a secondary factor to initiate the vasoconstrictor responses measured in rats. We have not measured prostaglandin or thromboxane excretion in dogs, but reports that arachidonic acid tends to be metabolized preferentially to vasodilatory prostaglandins in dogs, rather than to thromboxane as observed in rats, suggest that differences in the thromboxane/prostaglandin ratio may be an important factor underlying the apparent species-dependent blood pressure responses to insulin. If insulin does not increase thromboxane production markedly in dogs, this also could explain the apparent lack of Ang II dependence of the blood pressure response to insulin in dogs. The role of thromboxane remains to be tested in dogs or humans, but it offers intriguing possibilities about the potential hemodynamic responses to hyperinsulinemia in disease states associated with enhanced thromboxane production.

The mechanism through which insulin stimulates thromboxane production is not clear. Platelet thromboxane production was reported to decrease on incubation with insulin, and insulin treatment decreases urinary thromboxane excretion in diabetes mellitus. Moreover, perfusion of mesenteric vascular beds with insulin at physiological concentrations had no effect on thromboxane production, which suggests that hyperinsulinemia per se does not have a significant effect to stimulate thromboxane production. Another possibility, however, is that alterations in glucose metabolism secondary to elevated insulin levels may be partly responsible for the increased thromboxane formation. The insulin-inducible glucose transporter (GLUT4) has been demonstrated in renal arteriolar vascular smooth muscle cells and in glomerular mesangial cells, and glucose uptake in these cell types should be increased during insulin infusion because of insulin-induced upregulation of the GLUT 4 transporter. Moreover, the finding that glucose per se stimulates vascular and mesangial cell thromboxane production suggests the possibility that increased renal thromboxane production during insulin infusion might be attributable to an insulin-induced increase in glucose uptake, and that possible sources of this increased production are vascular tissue and mesangial cells.

Thus, Ang II is a potent modulator of the hypertensive response to insulin. Clamping Ang II at baseline potentiated both the renal vasoconstriction and the hypertension associated with chronic insulin infusion in rats. In addition, the finding that thromboxane production, a requirement for development of insulin hypertension, is enhanced during insulin infusion when Ang II levels are prevented from falling suggests that at least part of the potenitation of insulin hypertension in these rats may have been the result of increased thromboxane-mediated renal vasoconstriction. These results provide additional evidence linking the chronic hypertensive action of insulin with the ability of insulin to cause renal vasoconstriction, and they also suggest that the fall in PRA that normally occurs during insulin infusion is an important compensatory mechanism that limits the hypertensive response by attenuating renal thromboxane production.
Acknowledgments
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References


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