Inhibition of Thromboxane Synthesis Attenuates Insulin Hypertension in Rats

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Chronic insulin infusion in rats increases mean arterial pressure (MAP) and reduces glomerular filtration rate (GFR), but the mechanisms for these actions are not known. This study tested whether thromboxane synthesis inhibition (TSI) would attenuate the renal and blood pressure responses to sustained hyperinsulinemia. Male Sprague-Dawley rats were instrumented with arterial and venous catheters, and MAP was measured 24 h/day. After 4 days of baseline measurements, endogenous synthesis of thromboxane was suppressed in 7 rats by infusing the thromboxane synthetase inhibitor, U63557A, intravenously (30 µg/kg/min) for the remainder of the experiment; 7 other rats received vehicle. Baseline MAP was not significantly different between vehicle and TSI rats (96 ± 1 vs 99 ± 1 mm Hg). After 3 days of U63557A or vehicle, a 5-day control period was started, followed by a 7-day infusion of insulin (1.5 mU/kg/min, intravenously). Glucose (22 mg/kg/min, intravenously) was infused along with insulin to prevent hypoglycemia. In the control period, MAP was not different between vehicle and TSI rats (99 ± 2 vs 100 ± 1 mm Hg), but MAP increased throughout the 7-day infusion period only in the vehicle rats with an average increase in blood pressure of 7 ± 2 mm Hg. In the control period, GFR was lower in vehicle rats compared with TSI rats (2.5 ± 0.1 vs 3.1 ± 0.2 mL/min, P = .06), and the decrease to 81% ± 4% and 91% ± 6% of control, respectively, during insulin was significant only in the vehicle rats. All variables returned toward control during a 6-day recovery period. These results suggest that full expression of hypertension and renal vasoconstriction during hyperinsulinemia in rats is dependent on a normal ability to synthesize thromboxane. Am J Hypertens 1997;10:1125–1131 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Thromboxane, insulin, blood pressure, hypertension.

Chronic hyperinsulinemia in rats increases blood pressure significantly, but the mechanism is not known. Our consistent finding that cumulative sodium balance is not increased during the insulin infusion suggests that the rise in blood pressure is not mediated by increased extracellular fluid volume. A decrease in glomerular filtration rate (GFR) during insulin infusion, however, suggests that renal vasoconstriction could play a role in the pressor response.

The sympathetic nervous system has been proposed as a potential mediator of a hypertensive action of insulin. However, we have demonstrated that chronic blockade of both α- and β-adrenergic recep-
tors does not attenuate either the blood pressure raising or the GFR lowering actions of insulin.

This suggests that increased blood pressure in this experimental model of insulin hypertension is not dependent on the sympathetic nervous system and, in addition, confirms the link between the renal vasoconstriction and the rise in blood pressure.

There is considerable evidence that thromboxane is produced in the kidney and is an important mediator of renal vasoconstriction and hypertension in several experimental models. Moreover, Yanagisawa-Miwa et al recently reported that insulin potentiated thromboxane induced vasoconstriction in the coronary circulation. A similar interaction between insulin and thromboxane in the kidney might explain the renal vasoconstriction and increased blood pressure during chronic insulin infusion. Therefore, the goal of this study was to test the hypothesis that the normal ability to produce thromboxane is required for chronic insulin infusion to increase blood pressure and reduce GFR in rats.

**METHODS**

Male Sprague-Dawley rats weighing 325 to 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 Sensorcaine, and both catheters were routed subcutaneously to the scapular region and exteriorized through a stainless steel button that was implanted subcutaneously.

After recovery from surgery, the rats were placed in individual metabolic cages in a quiet, air conditioned room with a 12 h light cycle. The catheters were connected to a dual-channel infusion swivel (Instech, Plymouth Meeting, PA) mounted above the cage and protected by a stainless steel spring. The arterial catheter was filled with heparin solution (1000 USP U/mL) and connected, via the swivel, to a pressure transducer (Cobe, Lakewood, CO) 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 sec each minute, 24 h/day.

The rats received food and water ad libitum throughout the study. An intravenous infusion of 18 mL sterile 0.9% saline/day containing 18.6 mg potassium chloride/mL, combined with sodium and potassium deficient rat chow, allowed sodium and potassium intakes to be clamped at approximately 2.8 and 4.5 mEq/day, respectively, independently 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/day. This infusion was started immediately after placement of the rats in the metabolic cages, and 5 to 7 days were allowed for recovery and acclimation before control measurements were made. All solutions contained antibiotic (30,000 U/day penicillin G potassium and 27 mg/day mezlocillin), and were infused intravenously with a syringe pump (Harvard Apparatus, Millis, MA) through a Millipore filter (22 μm, Cathivex, Millipore, Bedford, MA).

**Experimental Protocol**

**Insulin Infusion in Vehicle Rats (n = 7)** After 5 days of control measurements, an intravenous infusion of insulin was started at a rate of 1.5 mU/kg/min and continued for 7 days. The water vehicle was substituted for a 50% dextrose solution that delivered glucose at 22 mg/kg/min along with the insulin to prevent hypoglycemia. Six days of recovery measurements with the control infusate were made after stopping the insulin/glucose infusion. On the second day of the control period, the third day of insulin, and the fourth day of the recovery period, 1.8 mL of arterial blood was collected following a 4 h fast via the arterial catheter and placed in chilled sodium EDTA tubes for measurement of: 125I-iodothalamate; 131I-iodohippuran; plasma renin activity (PRA); hematocrit and plasma insulin; glucose; and protein concentrations. The sample was replaced with an equal volume of 0.9% saline.

**Thromboxane Synthesis Inhibition and Insulin Infusion (n = 7)** In a separate group of rats, a thromboxane synthetase inhibitor, U63557A (Upjohn Co., Kalamazoo MI), was added to the infusate to deliver 30 μg/kg/min throughout the experiment. This dose has been reported to effectively inhibit thromboxane synthetase activity.

Control measurements were started 3 days after beginning receptor blockade and the remainder of the experiment followed the same protocol as that for the vehicle rats.

**Analytic Methods** To measure the urinary excretion of thromboxane B2 and 6-keto-PGF1α, urine was collected into glycine buffer (pH = 2.0) on the first day of the control period, the sixth day of insulin, and the third day of the recovery period. Samples were ex-
tracted on the same day and were stored at −30°C. Urinary thromboxane B₂ and 6-keto-PGF₁α concentration were measured by radioimmunoassay. Plasma insulin concentration and PRA also were measured by radioimmunoassay; plasma glucose was determined by an automatic analyzer using the glucose oxidase method (YSI Scientific, Yellow Springs, OH); plasma protein concentration was measured by refractometry (American Optical, Buffalo, NY); and urinary sodium and potassium concentrations were determined using flame photometry (Instrumentation Laboratories).

GFR and effective renal plasma flow (ERPF) were measured using a 4-h fasted plasma sample following a 24-h intravenous infusion of 125I-iothalamate (Glofil, Iso-Tex Diagnostics Inc., Friendswood, TX) and 131I-iodohippuran. Steady-state is achieved following 24 h of intravenous isotope infusion in this protocol; therefore, a sample of the infusate was counted and the infusion rate of isotope was substituted for urinary excretion rate of isotope to calculate clearance.¹³

Data were analyzed with a two factor analysis of variance, with repeated measures on one factor (time). Supplemental within group comparisons were made with Dunnett’s test and between group comparisons were made with unpaired t tests.¹⁴ P < .05 were considered statistically significant.

RESULTS

Baseline mean arterial pressure (MAP) was not significantly different between rats used for vehicle and TSI infusions (96 ± 1 vs 99 ± 1 mm Hg). MAP did not change with TSI or continued vehicle infusion, and MAP during the control period averaged 99 ± 2 and 100 ± 1 mm Hg in vehicle and TSI rats, respectively. Insulin infusion (Figure 1) for 7 days in vehicle rats raised MAP by an average of 7 ± 2 mm Hg with a maximal increase of 9 ± 1 mm Hg occurring on day 7 of the infusion. This response was markedly attenuated in TSI rats, with an average MAP elevation of only 1 ± 1 mm Hg and a maximum increase of 3 ± 1 on day 7 of insulin.

Baseline urinary sodium and potassium excretions were not different between the two groups, and were not changed by either TSI or continued vehicle administration. During the control period, urinary sodium excretion averaged 2.6 ± 0.1 and 2.7 ± 0.2 mEq/day in vehicle and TSI rats, respectively. The sodium excretory response to insulin (Figure 2) was similar in both groups. On day one of the insulin infusion period there was a consistent decrease in urinary sodium excretion to 2.0 ± 0.3 and 2.2 ± 0.2 mEq/day in vehicle and TSI rats, respectively, that was not statistically significant by ANOVA. Thereafter, both groups were in sodium balance at control levels of sodium excretion for the remainder of the insulin infusion period. Urinary potassium excretion during the control period was not different between vehicle and TSI rats (3.3 ± 0.1 vs 3.3 ± 0.2 mEq/day); it increased significantly during the insulin infusion period to 4.0 ± 0.2 and 3.8 ± 0.2 mEq/day in vehicle and TSI rats, respectively, and returned to control levels during the recovery period.

Glomerular filtration rate (GFR) during the control period was approximately 20% higher in TSI compared with vehicle rats (3.1 ± 0.2 vs 2.5 ± 0.1 mL/min, P = .06). Renal plasma flow (RPF) also tended to be higher (7.8 ± 1.0 vs 6.0 ± 0.5 mL/min) in the TSI rats. As shown in Figure 3, GFR decreased significantly to 81% ± 4% of control in the vehicle infused rats. This response was attenuated markedly in TSI rats, and GFR remained significantly higher during the insulin infusion period in the TSI rats (2.8 ± 0.1 vs 2.1 ± 0.2 mL/min). RPF decreased during the insulin infusion period in both groups. The percent decrease in RPF was similar in vehicle and TSI rats (18% ± 6% vs 16% ± 4%).

Pretreatment with the thromboxane synthetase inhibitor, U63557A, reduced urinary thromboxane B₂ excretion by >60% while having no effect on urinary 6-keto-PGF₁α excretion (Figure 4). Insulin infusion resulted in a twofold increase in 6-keto-PGF₁α excretion.
in both groups, but thromboxane B₂ excretion was not changed by the insulin infusion.

Fasting plasma concentrations for insulin, glucose, and plasma renin activity (PRA) are presented in Table 1. Insulin infusion caused an approximate fivefold increase in plasma insulin concentration in both groups. Fasting plasma glucose was lower prior to the insulin infusion in the TSI rats (125 ± 2 vs 134 ± 3 mg/dL), and did not decrease in either group during the insulin infusion. In fact, plasma glucose increased slightly in both groups although this increase was significant only in the vehicle rats. Plasma renin activity was higher in TSI rats compared to vehicle rats (3.5 ± 0.3 vs 3.0 ± 0.3 ng angiotensin I/mL/h), but was suppressed similarly in both groups during the insulin infusion period.

DISCUSSION

The main finding from this study is that an intact ability to synthesize thromboxane is required for insulin-induced hypertension in rats. In addition, TSI attenuated the decrease in GFR associated with the insulin infusion, consistent with our previous reports suggesting that renal vasoconstriction may underlie the shift in pressure natriuresis.

In this study and previous studies, chronic insulin infusion was not associated with a significant increase in cumulative sodium balance, and the tendency for cardiac output to decrease during the infusion is further evidence that insulin hypertension is not mediated by extracellular fluid volume expansion. However, pressure natriuresis is shifted to a higher pressure in normal rats during insulin infusion, because the rats are in sodium balance in the face of significantly elevated mean arterial pressure. Thus, the natriuretic effect of increased arterial pressure and an antinatriuretic shift in renal excretory function offset each other to maintain sodium balance. Two mechanisms that could account for the antinatriuretic shift are increased renal tubular sodium reabsorption or decreased GFR, and the measurement of decreased GFR on day 3 of insulin infusion suggests that renal vasoconstriction rather than increased tubular reabsorption is a principal determinant of the hypertensive shift in pressure natriuresis.

In a previous attempt to isolate the mechanism for the hypertensive response to insulin infusion and the decrease in GFR, we tested the role of the sympathetic nervous system by infusing insulin in rats with chronic α- and β-adrenergic receptor blockade. Despite nearly complete adrenergic receptor blockade, however, there was no attenuation of the hypertensive
response to the 7-day insulin infusion. Moreover, the changes in GFR and renal plasma flow were nearly identical to those in rats without receptor blockade. Those results not only suggested that the sympathetic nervous system was not required for insulin induced hypertension in rats, but also confirmed the link between renal vasoconstriction during chronic insulin infusion and the increase in blood pressure.

Yanagisawa-Miwa et al\textsuperscript{11} recently reported that insulin enhanced thromboxane induced vasoconstriction in the coronary vascular bed, and there is evidence that thromboxane contributes to the reduction in GFR and to the increase in arterial pressure in several experimental models of hypertension. Chronic administration of a thromboxane synthesis inhibitor almost completely prevents the decrease in GFR and the hypertension in the SHR.\textsuperscript{6} In addition, thromboxane receptor blockade attenuates the reduction in GFR caused by high salt diet in Dahl salt sensitive rats,\textsuperscript{16} and a similar beneficial effect on renal function is observed in diabetes mellitus\textsuperscript{17} and after acute cyclosporine administration in rats.\textsuperscript{18} Moreover, acute infusion of a thromboxane mimetic was reported to decrease GFR through an afferent arteriolar constriction action.\textsuperscript{19} These findings raised the possibility that an interaction between insulin and thromboxane at the level of the afferent arteriole could explain the changes in renal function and blood pressure in insulin hypertensive rats.

We tested this in the present study by using a specific inhibitor of thromboxane synthetase to inhibit the thromboxane system, and the decrease in urinary thromboxane B\textsubscript{2} levels suggests that there was a significant reduction in renal thromboxane production.\textsuperscript{20} However, inhibition of this enzymatic step also has been suggested to redirect prostaglandin endoperoxides into pathways leading to increased production of vasodilatory prostaglandins.\textsuperscript{21} In support of this idea, furosemide stimulated renal prostaglandin production is enhanced by pretreatment with a thromboxane synthetase inhibitor.\textsuperscript{21} In the present study, there was a significant increase in urinary excretion of 6-keto-PGF\textsubscript{1a}, the stable hydrolysis product of PGI\textsubscript{2}, during the insulin infusion period. However, there was no difference in urinary excretion of 6-keto-PGF\textsubscript{2a} between the two groups of rats during any experimental period. This suggests, therefore, that the effects of TSI were due to a reduction in thromboxane rather than an elevation in vasodilatory prostaglandins.

Inhibition of thromboxane synthesis under baseline conditions had no effect on blood pressure or sodium excretion in this study. Although numerous studies have reported a possible role for thromboxane in various pathological conditions and in several experimental models of hypertension,\textsuperscript{6–10} there is little evidence that thromboxane is an important determinant of blood pressure under normal conditions.\textsuperscript{6} There were, however, modest changes in baseline renal hemodynamics in response to TSI. GFR and renal plasma flow were 15% to 20% higher in TSI rats as compared with vehicle rats. The lack of natriuresis and a reduc-

![Figure 4](https://academic.oup.com/ajh/article-abstract/10/10/1125/156201/1129)

**TABLE 1. FASTING PLASMA CONCENTRATIONS DURING CONTROL PERIOD, INSULIN INFUSION PERIOD, AND RECOVERY PERIOD IN VEHICLE (N = 7) AND TSI (N = 7) RATS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Insulin</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (ng AI/ml/h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.0 ± 0.3</td>
<td>1.6 ± 0.2*</td>
<td>2.0 ± 0.2*</td>
</tr>
<tr>
<td>TSI</td>
<td>3.5 ± 0.3</td>
<td>1.7 ± 0.2*</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>113 ± 20</td>
<td>580 ± 144*</td>
<td>118 ± 16</td>
</tr>
<tr>
<td>TSI</td>
<td>90 ± 10</td>
<td>511 ± 156*</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>134 ± 3</td>
<td>150 ± 5*</td>
<td>127 ± 2</td>
</tr>
<tr>
<td>TSI</td>
<td>125 ± 2†</td>
<td>138 ± 7</td>
<td>127 ± 1</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity; TSI, thromboxane synthesis inhibition.

\* P < .05 compared with control period.

† P < .05 compared with normal rats.

n = 6 during recovery for normal and TSI rats.
tion in blood pressure in response to the increase in GFR may be due to the modest increase in PRA. PRA was increased by approximately 15% in TSI as compared with vehicle rats.

In this study, insulin infusion in vehicle rats raised blood pressure by approximately 10 mm Hg and decreased GFR by 19% by day 3 of the infusion. In the rats with chronic blockade of thromboxane synthetase, however, the increase in blood pressure and decrease in GFR were almost completely attenuated. This suggests that thromboxane is an important mediator of the renal vascular and hypertensive response to insulin infusion in rats.

However, it is important to note that this significant effect of TSI occurred despite the fact that insulin infusion did not raise urinary thromboxane B2 excretion. What is the mechanism, then, for the effect of TSI if insulin infusion did not stimulate increased renal thromboxane production? One possibility is that we were unable to detect any potential insulin induced increases in thromboxane production by measuring urinary thromboxane B2 excretion. However, we have reported that chronic angiotensin II (ANG II) infusions of 5 and 20 ng/kg/min in rats yielded respective 30% and 100% increases in urinary thromboxane B2 excretion above a baseline similar to that in the present study. In addition, TSI in that study completely prevented the increase in urinary thromboxane B2 excretion and attenuated the ANG II induced 40 mm Hg blood pressure rise by approximately 50%. Thus, our methods are capable of detecting increases in urinary thromboxane B2 excretion, and those increases are consistent with increased renal thromboxane production.

Another explanation for the effectiveness of TSI, despite no increase in thromboxane production during insulin infusion, is that vascular sensitivity to thromboxane was enhanced by hyperinsulinemia. Alterations in glucose metabolism secondary to the increased insulin levels have been proposed to increase sensitivity of vascular smooth muscle to vasoconstrictor stimuli. In support of this idea, Yanagisawa-Miwa et al have shown that insulin enhances thromboxane induced vasoconstriction in the coronary vascular bed through a process dependent on extracellular glucose, suggesting that this enhancement might be due to insulin stimulated glucose uptake into the cells. A similar action on the afferent arteriole, therefore, could explain the decrease in GFR and the link between renal vasoconstriction and the hypertensive shift in pressure natriuresis.

Constriction of the afferent arteriole by thromboxane has been linked both to a direct action and to an action dependent on tubuloglomerular feedback (TGF). Insulin increases chloride transport in the thick ascending limb of the loop of Henle, and a similar action at the macula densa would enhance the TGF signal for afferent arteriolar constriction. This would be a mechanism for direct interaction of insulin on the sensor limb of TGF, and also could explain the decrease in PRA measured during insulin infusion. However, there are no published reports to date on the action of insulin at the macula densa. It also is possible that a normally functioning TGF system simply is required for expression of insulin’s actions, which was suggested by the effect of chronic angiotensin converting enzyme inhibition to blunt the renal and pressor responses to insulin. Thromboxane could be involved at the effector limb of any potential TGF mediated action of insulin or, again, simply may be required for a direct afferent arteriolar action of insulin.

In summary, this study suggests that thromboxane is important in mediating the increased mean arterial pressure and decreased GFR responses associated with chronic insulin infusion in rats. Evidence that thromboxane is an important factor in mediating ANG II dependent hypertension provides a potential explanation for the similar effects of TSI and ACEI to attenuate the hypertensive and renal constrictor responses to insulin infusion, but it remains unclear how insulin, thromboxane, and ANG II interact in this model. There is suggestive evidence for a TGF related mechanism underlying the responses to insulin infusion, but further studies will be required to confirm this and to determine how these variables act on the sensor or effector limbs of TGF.

ACKNOWLEDGMENTS

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REFERENCES


