Effect of Insulin on Renal Sodium Handling in Hypertensive Rats

David Finch, Gary Davis, John Bower, and Kent Kirchner

Spontaneously hypertensive rats have reduced peripheral insulin sensitivity. To determine whether hypertensive rats demonstrate reduced response to the antinatriuretic effect of insulin, urinary sodium excretion was determined in hypertensive and normotensive rats (n=7 per group) before and during euglycemic insulin administration at two infusion rates (21 milliunits/kg load and 4 milliunits/kg/min or 85 milliunits/kg load and 8 milliunits/kg/min). Hypertensive and normotensive time controls received the vehicle for insulin administration. Mean arterial pressure was greater ($p<0.05$) and inulin clearance was less ($p<0.05$) in hypertensive than normotensive rats before insulin infusion. Baseline fractional sodium excretion was not different between groups. Low dose insulin infusion reduced ($p<0.05$) fractional sodium excretion from 0.81±0.43% to 0.31±0.07% in hypertensive rats and from 1.05±0.37% to 0.47±0.18% in normotensive rats. High dose insulin infusion reduced ($p<0.05$) fractional sodium excretion from 0.67±0.22% to 0.21±0.08% in hypertensive rats and from 0.81±0.15% to 0.30±0.09% in normotensive rats. Sodium excretion was unchanged in time controls. The reduction in sodium excretion was similar in both rat groups during low dose and high dose insulin infusions. Mean arterial pressure and inulin clearance were unchanged from baseline values during insulin infusion in all rat groups. Glucose requirement to maintain euglycemia was greater ($p<0.05$) in normotensive than hypertensive rats at both insulin infusion rates. Thus, while hypertensive rats have reduced sensitivity to the hypoglycemic effects of insulin, the antinatriuretic response to insulin is not different from that of normotensive rats. Furthermore, the presence of hypertension does not modulate the antinatriuretic activity of insulin. (Hypertension 1990;15:514–518)

Elevated plasma insulin levels and resistance to the hypoglycemic effect of insulin have been associated with hypertension in human and animal models of hypertension.1–4 This observation has led to speculation that insulin may play a role in the development of increased blood pressure.5 Such a hypothesis is strengthened by studies demonstrating that normotensive rats fed a fructose-enriched diet developed insulin resistance, hyperinsulinemia, and hypertension.5 Inasmuch as insulin reduces urinary sodium excretion,6–8 an attractive hypothesis is that sodium-dependent hypertension develops as a result of persistent insulin antinatriuresis. However, no data are available that examine the effect of insulin on renal sodium reabsorption under conditions of elevated blood pressure. This information is fundamental for evaluation of any hypothesis implicating a role for insulin in development or maintenance of hypertension. The current study was performed to examine the effect of insulin on urinary sodium excretion in glucose-clamped, volume-expanded spontaneously hypertensive rats (SHR). Effect of insulin on urinary sodium excretion in normotensive Wistar-Kyoto (WKY) rats was also performed for comparison.

Methods

SHR and WKY rats were obtained from Taconic Laboratories, Germantown, New York, and maintained on a 20 mmol sodium intake/g diet for 14–21 days before study. On the day of study, rats were anesthetized with intraperitoneal injections of 5 secbutyl-5-ethyl-2-thiobarbituric acid (Inactin, Promont, Hamburg, FRG) in a dose of 80 mg/kg body wt and placed on a heated animal table. Rectal temperatures were maintained between 37° and 38° C with a servo-activated controller (Yellow Springs Instr. Co., Yellow Springs, Ohio). After tracheostomy, PE-50 catheters

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Portions of the data were presented at the annual meeting of the Southern Section of the American Federation for Clinical Research in New Orleans, Louisiana, February 1989, and was published in abstract form in Clinical Research 1989;37:16A.

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Received July 17, 1989; accepted in revised form December 19, 1989.
were introduced into the right jugular vein for inulin and insulin infusions and into the left jugular vein for infusion of glucose and saline. A PE-50 catheter was placed in the femoral artery for blood sampling and continuous blood pressure monitoring. This catheter was connected to a transducer (model 4-327-I, Trans-America Delvel, Pasadena, California), and arterial pressure was continuously recorded on a polygraph (Lafayette Instrs., Lafayette, Indiana). A flanged PE-50 catheter was placed into the bladder through a suprapubic incision for urine collection from the right kidney. From the start of surgery, isotonic Ringer's solution composed of the following (meq/l): Na+ 140, Cl− 115, HCO3, 30, K+ 5, and containing 5% polyfructose (Inunest, Laevosan Gesellschaft, Linz, Austria) was infused at a rate of 3 ml/hr for 20 minutes and at 1.5 ml/hr thereafter. The left kidney was exposed through a subcostal incision and gently separated from the adrenal gland and perirenal fat. The kidney was placed in a Lucite cup, and the upper ureteral segment was cannulated with PE-50 tubing. The kidney was bathed continuously with mineral oil. After the surgical procedure, a volume of 0.15 M NaCl, equivalent to 3% of the body weight, was infused over a 60-minute interval. The rate of the 0.15 M NaCl infusion was then adjusted to match urinary output. Urine was collected under oil in preweighed vials from the left kidney over a 45-minute interval for determination of baseline flow rate, inulin, and sodium concentrations. Blood was obtained at 15-minute intervals for glucose determination. A larger plasma sample was determined at the beginning and end of this period for measurement of inulin and sodium concentrations. After the baseline interval, SHR or WKY rats were subdivided into three groups. The first group (n=7) received an insulin load of 21 milliunits/kg and a continuous insulin infusion of 4 milliunits/kg/min. This insulin infusion rate produced plasma insulin concentrations determined by radioimmunoassay of 46±8 microunits/ml in SHR and 43±8 microunits/ml in WKY rats. The second group (n=7) received an insulin load of 85 milliunits/kg and a continuous insulin infusion of 8 milliunits/kg/min. This insulin infusion rate produced plasma insulin concentrations determined by radioimmunoassay of 198±79 microunits/ml in SHR and 165±85 microunits/ml in WKY rats. One minute after initiation of the insulin infusion a 25% glucose infusion was begun at a rate of 30 µl/min. A 40-minute stabilization period was then begun during which time blood glucose levels were determined at 5-minute intervals, and the rate of glucose infusion was adjusted to maintain the blood glucose concentration constant at the mean value measured during that animal’s baseline period. After this interval, a second 45-minute experimental period was begun. Urine and blood samples were obtained as described above. The glucose infusion was adjusted to maintain blood sugars constant. A second group of SHR and WKY rats (n=8 per group) served as time controls. These rats received the vehicle for insulin infusion and 0.15 M NaCl in place of the 25% glucose infusion. Urine and blood were collected as described above.

Analytical Techniques

Sodium concentrations in serum and urine were measured by flame photometry (model 143, Instrumentation Labs., Lexington, Massachusetts). Inulin concentration in urine and plasma was determined by the diphenylamine method of Walser et al.9 Blood glucose concentration was determined by the glucose oxidase method (Accu-Check II, Boehringer Mannheim, Indianapolis, Indiana). This technique has been demonstrated to correlate well (r=0.953) with measurements of serum glucose.10

Analysis of Data

Determination of the concentration of inulin, sodium, and chloride in blood and urine and urinary flow rate permitted calculation of whole-kidney glomerular filtration rate and urinary excretion rate of sodium according to standard expressions. Glucose concentrations for each period were the mean value of the measurements determined for that period. In insulin-infused rats, the rate of glucose use by the body was determined by weighing the glucose infusion syringe before and after the euglycemic insulin clamp and determining the rate of glucose administration. The Student's t test for paired data was used to determine statistical significance within each group. Analysis of variance was used to determine statistical significance among the groups. If analysis of variance indicated that a statistical significance existed, then Bonferroni's modification of the t test was used to determine statistical significance among the groups. Statistical significance was set at the p<0.05 level.

Results

Table 1 and Figures 1 and 2 summarize the results of blood values and urine electrolyte excretion data during baseline and experimental periods in SHR and WKY rats. Baseline blood sugars and plasma sodium concentrations did not differ between any rat group. Baseline mean arterial pressures were higher (p<0.05) and inulin clearances lower (p<0.05) in SHR compared with WKY rats. Baseline mean arterial pressures and inulin clearances, however, were not different between subsequent time control and insulin-infused subgroups of either SHR or WKY rats. Baseline urine flow rates and absolute urinary sodium excretion rates were slightly but not statistically lower in SHR than WKY rats. Baseline urine flow rates and absolute urinary sodium excretion rates, however, were not different between SHR or WKY rat time controls and their respective insulin-treated counterparts.

Values in SHR or WKY time control rat groups were not different between baseline and second collection periods for any parameter measured. Blood glucose concentrations were not different between baseline and experimental periods in any insulin-treated SHR or insulin-treated WKY rat.
TABLE 1. Effect of Insulin on Blood Glucose, Blood Pressure and Renal Function

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Blood glucose (mg/dl)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>CIN (μl/min)</th>
<th>V (μl/min)</th>
<th>UNaV V (neq/min)</th>
<th>PNa (meq/l)</th>
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<td></td>
<td>B EXP</td>
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<td>WKY time control</td>
<td>126±72</td>
<td>119±74</td>
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<td>WKY low insulin</td>
<td>120±7</td>
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<td>116±7</td>
<td>117±2</td>
<td>1,282±109</td>
<td>1,284±84</td>
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<tr>
<td>WKY high insulin</td>
<td>110±8</td>
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<td>112±5</td>
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<tr>
<td>SHR time control</td>
<td>121±7</td>
<td>121±7</td>
<td>161±5</td>
<td>165±8</td>
<td>914±64</td>
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<tr>
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<td>116±7</td>
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<td>173±4</td>
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<tr>
<td>SHR high insulin</td>
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<td>155±8</td>
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<tr>
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</tbody>
</table>

Values are mean±SEM. B, baseline period; EXP, experimental period; CIN, inulin clearance; V, urine flow rate; UNaV, absolute urinary sodium excretion rate; PNa, plasma sodium concentration; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*p<0.05 vs. WKY time control.

groups. Mean arterial pressure was unchanged between baseline and experimental periods in SHR or WKY insulin-treated rats. Absolute and fractional urinary sodium excretion rates were markedly reduced during both high dose and low dose insulin administration in both SHR and WKY rat groups (Table 1, Figure 2). The percent reduction in absolute urinary sodium excretion produced by either high or low dose insulin infusion was not different between SHR and WKY rats (Figure 1). Furthermore, the percent reduction in sodium excretion was not different between rats receiving high dose and low dose insulin infusion. The glucose infusion rate required to maintain euglycemia during low dose insulin administration was 7.5±1.2 mg/kg/min in WKY rats and 2.3±1.1 mg/kg/min in SHR (p<0.05). The glucose infusion rate required to maintain euglycemia during high dose insulin administration was 16.5±2.6 mg/kg/min in WKY rats and 6.0±2.6 mg/kg/min in SHR (p<0.05).

Discussion

The current study demonstrates that administration of insulin at rates that produce plasma insulin levels in the pathophysiological or pharmacological range significantly reduce absolute and fractional urinary sodium excretion rates in both hypertensive SHR and in normotensive WKY rats. In both rat groups these effects occur without alterations in mean arterial pressure or glomerular filtration rate. Furthermore, when the reduction in urinary sodium excretion rate produced by insulin administration was expressed as a percent of baseline values, the magnitude of the antinatriuretic response of insulin was similar in both SHR and WKY rats. This occurred despite the fact that the amount of glucose required to maintain euglycemia in SHR was less than half that required to maintain euglycemia in WKY rats. Thus, while SHR and WKY rats demonstrate differences in insulin-stimulated glucose disposal, the antinatriuretic effect of insulin is qualitatively and quantitatively similar in both strains.

Whether the antinatriuretic effect of insulin is preserved during the insulin resistance associated with hypertension has been uncertain. Based on studies in isolated perfused kidneys, Restand and associates suggested that variations in circulating
insulin concentrations reduce the availability of insulin receptor sites in the kidney and significantly attenuate the antinatriuretic effect of subsequent insulin administration. Consequently, insulin may be less antinatriuretic in the setting of the insulin resistance associated with hypertension. On the other hand, Ferrannini and associates suggested that insulin resistance associated with essential hypertension in humans is limited to the nonoxidative pathways of intracellular glucose disposal. In that study the ability of insulin to promote cellular potassium uptake and to inhibit lipolysis remained unimpaired. In our current study, the observation that insulin produces similar reductions in urinary sodium excretion in both SHR and WKY rats demonstrates that the antinatriuretic response to insulin is also unimpaired in the resistance that accompanies hypertension. The observation that doubling the insulin infusion rate produced insignificant additional reductions in urinary sodium excretion further suggests that insulin antinatriuresis is maximal in both hypertensive and normotensive rats at plasma insulin concentrations within the pathophysiological range. Thus, resistance to the action of insulin may involve some biological effects but not others. A similar situation has recently been demonstrated in obesity. In that condition, equivalent decreases in urinary sodium excretion have been reported in insulin-resistant obese adolescents and normal subjects during euglycemic insulin administration at rates producing plasma insulin concentrations similar to those observed in our high dose insulin rat groups.

Most investigators have suggested that insulin reduces urinary sodium excretion through a direct tubular effect. Although the mechanism responsible for the antinatriuretic response to insulin is not discernible from the present study, the observation that insulin reduced urinary sodium excretion without altering glomerular filtration rate, filtered sodium load, or mean arterial pressure in either SHR or WKY rats is consistent with a primary tubular rather than a hemodynamic mechanism. The observation that the antinatriuretic response was not different between SHR and WKY rat groups despite the large difference in mean arterial pressure and, presumably, renal perfusion pressure would also be consistent with a tubular mechanism for the effect of insulin.

The factors responsible for the differences in carbohydrate metabolism observed between hypertensive and normotensive conditions in humans and between SHR and WKY rats are incompletely elucidated. Reduced rates of insulin removal, higher plasma glucagon concentrations, and reduced intracellular glucose disposal have all been reported. Abnormalities in the suppression of hepatic glucose production, although not a significant component of insulin resistance associated with hypertension in humans, have yet to be excluded in the SHR. Although the mechanisms responsible for the lower amount of glucose required to maintain euglycemia in the hypertensive rats in the current study are uncertain, the observation that insulin-induced sodium retention is not prevented by the increases in mean arterial pressure suggests an attractive link between mechanisms that lead to increases in plasma insulin concentration and the development or maintenance of hypertension. It should be recognized, however, that mean arterial pressure was not altered by the short duration of insulin administration in any rat group in the current study. Thus, if insulin does increase mean arterial pressure, a more chronic exposure to the hormone is required. However, development of volume-dependent hypertension through persistent insulin-induced antinatriuresis is possible only if escape from insulin-mediated sodium retention does not occur with time. In this regard, Hall and associates noted no increase in mean arterial pressure after 28 days of insulin administration to two-thirds nephrectomized dogs. Clearly, further studies will need to be performed before the role of insulin in the development and maintenance of hypertension can be fully evaluated.

Finally, although the current study demonstrates that the antinatriuretic response is equivalent and maximal at pathophysiological insulin levels in both normotensive and hypertensive rats, whether the threshold for induction of insulin antinatriuresis and dose–response curve for this effect are truly identical remains to be determined.

In summary, the current study demonstrates that insulin produces equivalent antinatriuresis in hypertensive and normotensive animals. This antinatriuretic response appears to be mediated through tubular rather than hemodynamic mechanisms.


**KEY WORDS** • insulin • sodium excretion • essential hypertension • spontaneously hypertensive rats
Effect of insulin on renal sodium handling in hypertensive rats.
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Hypertension. 1990;15:514-518
doi: 10.1161/01.HYP.15.5.514

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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