Increased Insulin Sensitivity in the High Sodium One-Kidney, One Figure-8 Hypertensive Rat

Simona Frontoni, Lynne Ohman, Joseph R. Haywood, and Luciano Rossetti

This study examines the relation between sympathetic activity and in vivo insulin-mediated glucose metabolism in a rat model of acquired hypertension. Two groups of conscious, unrestrained rats were studied in the postabsorptive state: sham-operated normotensive rats (n=10) and renal-wrapped hypertensive rats (n = 10). Mean arterial pressure was increased in the hypertensive compared with the normotensive group in the fed (184±9 versus 144±6 mm Hg; p<0.01) and in the fasting (147±8 versus 112±7 mm Hg; p<0.01) state. After a 24-hour fast, hepatic glucose production, plasma glucose, insulin, and norepinephrine concentrations were similar in the two groups. Blood pressure did not change in either group during the 3-milliunits/kg • min euglycemic insulin clamp study; however, plasma norepinephrine concentration rose significantly in hypertensive (207±24 versus 329±11 pg/ml; p<0.05) but not in normotensive rats (229±23 versus 267±27 pg/ml; p=NS). During the insulin clamp study, the hepatic glucose production was similar in the hypertensive (3.8±0.8 mg/kg • min) compared with the normotensive (4.0±0.3 mg/kg • min) rats. Insulin-mediated glucose uptake was significantly higher in hypertensive than in normotensive rats (33.0±0.7 versus 25.8±0.8; p<0.01). This increase was mostly due to a marked increase in skeletal muscle glycogen synthesis in the hypertensive versus the normotensive group (11.9±1.0 versus 6.9±0.8; p<0.01), whereas the stimulation of whole body glycolysis (production of $^3$H$_2$O) was not significantly different in the two groups (14.7±0.8 versus 15.9±0.9 mg/kg • min in normotensive and hypertensive rats, respectively; p=NS). After euglycemic insulin infusion, plasma norepinephrine concentration increased in hypertensive but not in normotensive rats; however, the blood pressure did not change in either group. Peripheral insulin sensitivity is increased in rats with acquired sodium-sensitive hypertension. These results indicate that sodium-dependent hypertension is associated with enhanced response of the sympathetic nervous system to insulin and with increased insulin sensitivity. (Hypertension 1992;20:192-198)

KEY WORDS • insulin • sodium • renovascular hypertension • sympathetic nervous system • hypertension, sodium-dependent

Recent metabolic and epidemiological observations demonstrate the association of essential hypertension and insulin resistance. Increased sympathoadrenal activity has been suggested to play a determinant role in the development and maintenance of high blood pressure in human essential hypertension and in several animal models of hypertension. These findings have prompted speculation on the role of the sympathetic nervous system in the onset of insulin resistance. Catecholamines, indeed, display potent metabolic effects. Particularly, suppression of insulin secretion by catecholamines is mediated through $\alpha$-adrenergic receptors, whereas in human subjects, the direct effects of catecholamines on peripheral and hepatic glucose metabolism appear to be mediated largely through $\beta$-adrenergic receptors. Acute $\beta$-adrenergic stimulation results in impaired insulin action, mainly through the decrease in net glycogen deposition (stimulation of glycogen phosphorylase and inhibition of glycogen synthase) and the increase in hepatic glucose output. However, the effect of the chronic elevation of the plasma catecholamine concentration on insulin action is still controversial. Some discrepancies between $\alpha$- and $\beta$-adrenergic responsiveness have been demonstrated when adrenergic sensitivity in hypertension was examined. These results suggest that a sustained sympathetic overactivity in hypertension leads to $\beta$-adrenergic downregulation, whereas $\alpha$-adrenergic response is normal or enhanced. Furthermore, biochemical and metabolic responses to an acute challenge with $\beta$-receptor-stimulating agents, such as generation of cAMP, secretion of insulin, and production of glucose and free fatty acids are blunted after chronic administration of these drugs. Mononuclear leukocyte $\beta$-adrenergic receptor densities exhibit a rapid biphasic response (an initial increase followed by subsequent decrease) to $\beta$-adrenergic agonists in vivo. It appears evident that the in vivo pathophysiology of the
sympathoadrenal system and the metabolic and hemodynamic results of its activation are not a simple function of the catecholamine release, but they are modulated by complex regulatory systems.32,33

Several experimental models of sodium-dependent hypertension have been used to investigate the relation between sodium and elevated arterial pressure.38–40 The contributions of the sympathetic nervous system to the maintenance of sodium-dependent hypertension have been firmly established.39,40 We recently found that one-kidney, one figure-8 renal-wrap hypertension is also a model of sodium-dependent hypertension and is characterized by increased nonfasting norepinephrine concentration and enhanced release.41,42

The aim of the present study was to evaluate the relation between the activity of the sympathetic nervous system and the insulin-mediated glucose metabolism in conscious, chronically hypertensive rats fed a high-sodium diet.

**Methods**

**Animal Preparation**

Two groups of 24-hour–fasted male Sprague-Dawley rats were studied. The rats were fed a sodium-supplemented rat chow containing 1.2 meq sodium per gram food, an amount equivalent to eight times the sodium content in standard rat chow. Food and tap water were given ad libitum. Two weeks after the initiation of the high-sodium diet, one group of rats (wrap, n = 18) was anesthetized with methoxyflurane and subjected to figure-8 renal wrap and contralateral nephrectomy as described by Grollman.43 The other group of rats (sham, n = 18) was sham-wrapped, which consisted of unilateral nephrectomy only. Three weeks after surgery, animals were anesthetized with methoxyflurane and subjected to pentobarbital injection. In these groups, no insulin or pentobarbital was infused at a constant rate (14 μU/min) throughout the study.

At the end of the study, rats were injected with pentobarbital (60 mg/kg body wt), the abdomen was quickly opened, and the rectus abdominal and hind limb muscles were freeze-clamped with aluminum tongs precooled in liquid nitrogen. All tissue samples were kept frozen at -70°C for subsequent analysis.

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

**Analytical Procedures**

Plasma catecholamine concentration was measured by the single-isotope radioenzymatic assay as previously described.44 The sensitivity of this assay for norepinephrine and epinephrine is 20 pg/ml, and the intra-assay and the interassay coefficients of variation are 6% and 10%, respectively.

Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Palo Alto, Calif.) and plasma insulin by radioimmunoassay using rat and porcine insulin standards.

Plasma [3H]-glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide–zinc sulphate precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate tritiated water.

Muscle glycojen synthesis was quantified by determining the increment in cold glycojen concentration above fasting levels.44–46 Fasting levels were measured in rats (n = 8) that underwent the same animal preparation (i.e., housing, catheter insertion, 24-hour fast, pentobarbital injection). In these groups, no insulin or glucose was administered, and tissue samples were obtained for determination of glycojen concentration. Muscle glycojen concentration was determined after digestion with amyloglucosidase as previously described. The intra-assay and the interassay coefficients of variation (CV) were <10% (at 0.250 g/100 g tissue wt) when a muscle homogenate was assayed as multiple aliquots.

Plasma-tritiated, water-specific activity was determined by liquid scintillation counting of Somogyi filtr-
trates before and after evaporation to dryness. Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in tritiated water or \([^{3}H-3\text{glucose}].\) Rates of whole body glycolysis were estimated from the increment per unit time in tritiated water (dpm/ml ⋅ min) ⋅ body water mass (ml)/[\text{[H]-3-glucose specific activity (disintegrations per minute per milligram). Plasma water is assumed to be 93\% of the total plasma volume, and total body mass is assumed to be 65\% of the body mass.\(^{45}\)

Calculations

Data for total body glucose uptake and suppression of hepatic glucose production represent the mean values during the last 30 minutes of the insulin clamp study. The hepatic glucose production was calculated as the difference between the tracer-derived rate of appearance and the infusion rate of glucose. Total body glucose disposal was calculated by adding the rate of residual hepatic glucose production during the last 30 minutes of each insulin clamp to the glucose infusion rate during the same 30-minute time period. The rate of net glycogen synthesis was calculated as number of \([^{3}H-3\text{glucose counts in glycogen per gram of muscle tissue divided by the time-weighted mean plasma [H]-3-glucose specific activity (dpm/mg glucose). For each rat, the mean of four determinations on rectus abdominal muscle and four on hind limb muscle were used to approximate the mean whole body muscle glycogen concentration. All values are expressed as mean±SEM. Differences between groups were determined using ANOVA or Student's \(t\) test for paired and unpaired data, as appropriate.

Results

Basal Characteristics

Resting mean arterial pressure was increased in hypertensive compared with normotensive rats in both the fed (184±9 versus 144±6 mm Hg; \(p<0.01\)) and fasting (147±8 versus 112±7 mm Hg; \(p<0.01\); Table 1) states. Table 1 also shows that the heart rate, the fasting plasma glucose and insulin, and the basal hepatic glucose production were similar in normotensive and hypertensive rats. Norepinephrine (229±23 versus 207±24 pg/ml) and epinephrine (101±14 versus 94±21 pg/ml) plasma concentrations were also similar between groups. The basal skeletal muscle glycogen concentration was similar in the normotensive (5.01±0.17 mg/g wet wt) and in the hypertensive (4.96±0.13 mg/g wet wt) groups.

Euglycemic Insulin Clamp Studies

Steady-state plasma glucose and insulin concentrations during the insulin clamp studies were similar in the hypertensive compared with the normotensive group (Table 2). The CVs for plasma glucose and insulin concentrations were <5\% and 10\%, respectively, in all groups. During the euglycemic clamp study, the mean arterial pressure failed to show any change in either group, whereas the heart rate was progressively although not significantly increased in hypertensive but not in normotensive animals (Figure 1). After euglycemic insulin infusion, plasma norepinephrine concentration rose significantly above basal in the hypertensive group (from 207±24 to 329±11 pg/ml; \(p=0.035\) by ANOVA for repeated measures) but was not significantly increased in the normotensive group (from 229±23 to 267±27 pg/ml; Figure 2). The plasma epinephrine concentration (147±25 and 134±31 pg/ml in the normotensive and hypertensive rats, respectively; Figure 2) did not change significantly during the insulin clamp studies in either group.

During the insulin clamp studies, the hepatic glucose production was similar in hypertensive and normotensive rats (Table 2), whereas the glucose infusion rate (9.3±0.21 versus 6.85±0.19 mg/min; \(p<0.01\)) and the insulin-mediated glucose uptake (33.0±0.7 versus 25.8±0.8 mg/kg  ⋅ min; \(p<0.01\); Figure 3A) were significantly increased in the hypertensive compared with the normotensive group. Muscle glycogen synthesis was also markedly increased in the hypertensive group (11.9±1.0 versus 6.9±0.8 mg/kg  ⋅ min; \(p<0.01\); Figure 3B) and accounted for most of the difference in glucose uptake observed in the two groups. Whole body glycolysis was similar in normotensive and hypertensive animals (14.7±0.8 versus 15.9±0.9 mg/kg  ⋅ min, respectively; \(p=NS\); Figure 3C).

Discussion

We have examined the effect of a physiological elevation of the plasma insulin concentration on several parameters of sympathoadrenal activity and glucose metabolism in conscious, chronically hypertensive, high-sodium–fed rats. Our results demonstrate that the one-kidney, renal-wrap hypertensive rat,\(^{46-48}\) compared with the sham-operated control rat, demonstrates increased sensitivity to the stimulatory action of insulin on periph-

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**Table 1.** General Characteristics of the Experimental Groups in 24-Hour-Fasted, Sham-Operated Control Rats and Hypertensive Renal-Wrap Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sham</th>
<th>Wrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (days)</td>
<td>94±2</td>
<td>95±2</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>320±7</td>
<td>314±7</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>108±4</td>
<td>107±3</td>
</tr>
<tr>
<td>Insulin (microunits/ml)</td>
<td>22±3</td>
<td>19±2</td>
</tr>
<tr>
<td>HGP (mg/kg ⋅ min)</td>
<td>6.8±0.2</td>
<td>6.9±0.3</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>112±7</td>
<td>147±8*</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>401±22</td>
<td>373±21</td>
</tr>
</tbody>
</table>

Sham, sham-operated control rats; Wrap, hypertensive renal-wraps; HGP, hepatic glucose production. Values are mean±SEM.

*p<0.05 vs. sham.

**Table 2.** Insulin Clamp Study

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sham</th>
<th>Wrap</th>
</tr>
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<tbody>
<tr>
<td>(n)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>SSPG (mg/dl)</td>
<td>101±2</td>
<td>100±2</td>
</tr>
<tr>
<td>SSPI (microunits/ml)</td>
<td>63±4</td>
<td>65±5</td>
</tr>
<tr>
<td>HGP (mg/kg ⋅ min)</td>
<td>4.0±0.3</td>
<td>3.8±0.6</td>
</tr>
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Sham, sham-operated control rats; Wrap, hypertensive renal-wrap rats; SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin; HGP, hepatic glucose production. Values are mean±SEM.
eral glucose uptake and glycogen synthesis despite higher activation of the sympathetic nervous system. Increased sympathetic nervous system activity may play a major role in the onset and maintenance of high blood pressure in human essential hypertension and in several animal models of hypertension. Particularly, increased sympathoadrenal function has been demonstrated in the Dahl salt-sensitive, in the deoxycorticosterone (DOC) saline, and in the high-sodium, one-kidney models of sodium-sensitive hypertension.

In the present study, the mean arterial pressure was significantly elevated in renal-wrap rats compared with sham-operated control rats both in the fasted and in the fed state. Although the fasting plasma catecholamine concentration was similar in hypertensive and normotensive animals (Figure 1), after insulin infusion, the plasma norepinephrine level increased significantly above basal in the hypertensive but not in the normotensive group. This finding is consistent with the notion that sympathetic nervous system function is increased in high-sodium, renal-wrap hypertension and in other animal models of sodium-sensitive hypertension. In hypertensive rats, despite the increment in the plasma norepinephrine concentration during the insulin infusion, the mean arterial pressure did not display any significant change throughout the study. This may suggest that an effective baroreceptor reflex response was operating in hypertensive rats and prevented any further increase in blood pressure. Additionally, because insulin is also known to produce peripheral vasodilation, the observed overall effect during the insulin infusion may represent the balance between the hormone's peripheral effect and its stimulation of the sympathetic nervous system. Alternatively, insulin may selectively stimulate sympathetic pathways specific for metabolic regulation rather than cardiovascular function. Furthermore, the sympathoexcitatory effect of insulin may contribute to hypertension, either via an enhanced sympathetic response to insulin or through an increase in its circulating levels. In this regard, our observation of a lack of a significant change in mean arterial pressure after acute physiological hyperinsulinemia does not exclude a role of the hormone in the chronic regulation of blood pressure.

The increased activation of the sympathetic nervous system has also been proposed as a possible mechanism for the association of hypertension and insulin resistance. The euglycemic insulin clamp allows us to evaluate the effect of physiological insulin concentrations on peripheral glucose disposal and hepatic glucose
production in conscious rats. The ability of insulin to promote glucose uptake was significantly increased (by 27%) in hypertensive compared with normotensive rats. Because the major fates of an infused glucose load under euglycemic hyperinsulinemic conditions are glycolysis and muscle glycogen synthesis, we examined the contribution of these pathways to glucose disposal. The rate of whole body glycolysis was not significantly increased in hypertensive compared with normotensive rats. However, the muscle glycogen concentration at the end of the insulin clamp study was higher in the hypertensive compared with the normotensive group. Because the basal muscle glycogen concentration was similar in all rats and the muscle mass in the rat represents 40% of the body weight,40 it is possible to calculate the contribution of skeletal muscle glycogen synthesis to the whole body glucose uptake from the increment in muscle glycogen synthesis.44-46 The difference in glucose incorporation into glycogen between normotensive and hypertensive rats can account for the majority of the increase in whole body glucose uptake observed in hypertensive animals. These results demonstrate an increased sensitivity to insulin of muscle glucose uptake and muscle glycogen synthesis in a sodium-sensitive model of renal hypertension that is associated with increased sympathetic nervous system function.41,42 Thus, a chronic elevation in sympathetic nervous system activity41 does not determine the onset of insulin resistance in this model of sodium-dependent hypertension.

The relation between high blood pressure and insulin resistance in human essential hypertension has stimulated interest in the role of insulin in genetic and acquired models of hypertension in animals. In fact, the presence of impaired insulin action in various animal models (and the consequent hyperinsulinemia) would support the hypothesis that high blood pressure, through the associated hemodynamic, metabolic, or both, abnormalities, may contribute to the onset of insulin resistance in human essential hypertension. Consistent with this hypothesis, Mondon and Reaven55 and Hwang et al56 found a decrease in insulin-mediated glucose metabolism in spontaneously hypertensive rats (SHR) and in fructose-induced hypertensive rats, respectively. Although glucose intolerance was shown in conscious rats, insulin-mediated glucose metabolism was assessed under β-adrenergic blockade in anesthetized, fed animals in both of these previous studies53-56; the reduction in insulin-mediated glucose metabolism may be secondary to the induction of hepatic insulin resistance by fructose diet per se as shown by Zavaroni et al57 and Tobey et al.58 By contrast, Tsutsu et al59 demonstrated an increased glucose disappearance rate (K value) in SHR compared with Wistar-Kyoto rats during an intravenous glucose tolerance test in conscious, unstressed rats. Recently, we have reported increased insulin-stimulated glucose uptake and glycogen synthesis in conscious, 24-hour-fasted SHR rats.60 It should be noted that the nutritional conditions (duration of fasting) and the experimental procedures varied greatly in the studies cited above and may largely account for the contradictory findings. However, the present study, together with previous observations, indicates that insulin resistance is not a direct consequence of hypertension.

Our findings may be interpreted as contradictory with the numerous publications that demonstrated the induction of insulin resistance at the cellular and at the whole body level after short-term exposure to catechol-
amines or β-adrenergic agonists. In fact, acute catecholamine administration determines impaired insulin action through the inhibition of net glycogen synthesis and through the stimulation of hepatic glucose production. However, it is also established that reduced sensitivity to β-receptor stimulation follows the chronic administration of β-agonists. Particularly, Scheidegger et al showed a 30% increase in insulin-stimulated glucose uptake after chronic β-receptor stimulation in healthy volunteers. This increase was completely accounted for by an enhanced insulin-stimulated nonoxidative glucose disposal ("glucose storage"). Similarly, the decrease in the number of β-receptors, which follows intensive physical training, has been suggested to contribute to the simultaneous reversal of peripheral insulin resistance. It is tempting to speculate that, in the present study, the enhancement of the ability of insulin to stimulate glucose uptake and skeletal muscle glycogen synthesis is due to a decrease in β-adrenergic sensitivity. Consistent with this hypothesis, Lupien et al have recently demonstrated a marked increase in insulin sensitivity after 10 days of norepinephrine infusion in normal rats.

Thus, the figure-S renal-wrap model of sodium-dependent hypertension is associated with an increased ability of insulin to stimulate glucose uptake and glycogen synthesis in skeletal muscle. This indicates that hypertension does not invariably result in the onset of insulin resistance and that increased sympathoadrenal function can be associated with improved insulin sensitivity.

Acknowledgments

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References


Increased insulin sensitivity in the high sodium one-kidney, one figure-8 hypertensive rat.
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