We investigated the role of insulin in salt-sensitive hypertension in Dahl salt-sensitive and salt-resistant rats. The rats were kept in metabolic cages, and sodium intake and urinary sodium excretion were measured. In salt-sensitive rats receiving a 0.3% NaCl diet, sodium retention was significantly greater at weeks 1 and 2 in rats that received an insulin infusion than in those receiving a saline infusion. Mean arterial blood pressure and plasma norepinephrine levels were significantly higher at week 3 in insulin-treated rats than in saline-treated rats (mean arterial pressure, 137±3 mm Hg versus 119±3 mm Hg, p<0.05; plasma norepinephrine, 0.40±0.02 ng/ml versus 0.27±0.01 ng/ml, p<0.05). Insulin did not influence sodium retention, mean arterial pressure, or plasma norepinephrine in salt-resistant rats. Coadministration of an α-blocker (bunazosin, 10 mg/kg per day for 3 weeks) in salt-sensitive rats abolished the insulin-induced elevations in mean arterial pressure and sodium retention. When salt-sensitive rats were fed a low salt diet (0.03% NaCl), insulin did not raise mean arterial pressure. Thus, insulin elevated blood pressure only in the salt-sensitive model. The sympathetic nervous system and sodium retention in the early phase of insulin overload may contribute to elevation of mean arterial pressure in this model. (Hypertension 1992;20:596–600)

**Key Words** • insulin • rats, Dahl salt-sensitive • sympathetic nervous system

Obesity and non–insulin-dependent diabetes mellitus are frequently accompanied by hypertension.1,2 In obese hypertensive patients, a loss of body weight is often associated with parallel decreases in blood pressure and insulin levels.3 An inverse relation between whole body glucose uptake and blood pressure in nonobese subjects has been reported.4,5 These observations have focused attention on the relation between hyperinsulinemia and hypertension,6 but since hyperinsulinemia is not always accompanied by hypertension, it is not clear whether hyperinsulinemia increases the blood pressure.

It is possible that insulin may increase the blood pressure of some subjects but not others. Data from several studies have suggested that the activation of the sympathetic nervous system and sodium retention may play roles in insulin-induced increases in blood pressure.7,8 These two mechanisms have also been reported to be involved in salt-overload hypertension (salt-sensitive hypertension).9 These observations suggest that hyperinsulinemia may affect salt sensitivity.

We investigated the effects of chronic hyperinsulinemia on blood pressure and its mechanisms in Dahl salt-sensitive (DS) and salt-resistant (DR) rats.

**Methods**

Four-week-old male DS and DR rats were obtained from Brookhaven National Laboratories, Upton, N.Y. DS rats were divided into two groups: one group received normal chow containing 0.3% NaCl (FM Chow, Funabashi Nojyo Co. Ltd., Chiba, Japan) and the other group received low salt chow containing 0.03% NaCl (Modified FM Chow, Funabashi Nojyo Co.). All DR rats were fed a normal chow. Rats were maintained at a constant room temperature (24°C) on a 12-hour light/dark cycle, and food and water were available ad libitum throughout the study. After 1 week, the animals were housed individually in metabolic cages equipped with a drinking bottle and food cup on the outside of the cage so that urine could be collected without contamination from food and water. After a control period of 3 days, an osmotic minipump (2002 Minipump, Alza Corp., Palo Alto, Calif.), filled with insulin (Actrapid MC, 40 IU/ml, Novo Corp., Denmark) or normal saline was implanted subcutaneously between the scapulas while the rat was under ether anesthesia, then insulin or saline was infused subcutaneously.

Rats were divided into the following groups: DS rats infused with insulin and fed normal chow (DS-ins, n=9); DS rats infused with normal saline and fed normal chow (DS-sal, n=8); DS rats infused with insulin and fed low salt chow (DS-L-ins, n=7); DS rats infused with normal saline and fed low salt chow (DS-L-sal, n=7); DR rats infused with insulin and fed normal chow (DR-ins, n=10); and DR rats infused with normal saline and fed normal chow (DR-sal, n=10). In addition, some of the DS rats infused with insulin received normal...
chow that contained an α-blocker (bunazosin, Eisai Corp., Tokyo, bunazosin/chow, 0.1 mg/g) (Dahl-S-A-ins, n=7). After 1 week, the first minipump was removed and replaced with two new minipumps, each filled with the same concentration of insulin or saline, while the rats were under ether anesthesia. Thus, insulin was infused at a dose of 0.4 IU/day during the first week and at a dose of 0.8 IU/day during the second and third weeks. A 0.1-ml blood sample was taken from a tail vein for measurement of blood glucose level with the glucose-oxidase-impregnated test strip method (Dextrometer II, Miles Laboratories, Inc., Elkhart, Ind.).

Nineteen days after the minipumps were implanted, the rats were anesthetized with ether, and a polyethylene catheter (PE-50, Clay Adams, Parsippany, N.J.) was inserted into the descending aorta via the left carotid artery and tunneled subcutaneously to the back of the neck. The next day, mean arterial pressures were recorded on a polygraph (RM-6100, Nihon-Koden Corp., Tokyo) with the rats in a conscious state. Pressures were allowed to stabilize for 20 minutes, and the mean of blood pressures determined over a 10-minute period was calculated. Subsequently, a 1.5-ml blood sample was drawn from the catheter to determine plasma catecholamines; another 2.0-ml blood sample was drawn to determine plasma aldosterone, plasma insulin, and glucose levels.

The systolic blood pressure was measured once a week in conscious, prewarmed restrained rats by tail-cuff plethysmography (KN 210 Series, Natsume Corp., Tokyo). Body weight was measured on the same day. On the third day of the control period and the third through sixth, ninth through 12th, and 15th through 18th days of the experimental period (after initial implantation of the minipump), 24-hour urine samples were collected into flasks containing 6N HCl to determine catecholamines and sodium content. The total 24-hour sodium intake was calculated from the amount of food consumed. The 24-hour sodium excretion was calculated from the urine volume, and the urinary sodium concentration was measured by flame photometry. Daily sodium balance was expressed as total sodium intake minus urinary sodium excretion. Plasma sodium and potassium levels were also determined by flame photometry. Plasma and urinary catecholamine and plasma aldosterone levels were estimated by high-performance liquid chromatography with electrochemical detection. Plasma insulin level was measured by radioimmunoassay (Amersham International plc, Buckinghamshire, England), and plasma glucose concentration was determined by the glucose-oxidase H₂O₂ electrode method (Mel-Auto Glucose, Kanto-Kagaku Corp., Tokyo). All procedures were in accordance with "The Guideline of the Nihon University School of Medicine on Research Animal Use." Data are expressed as the mean±SEM. Analysis of variance was used to compare values from repeated measurements in the same group of rats (Scheffe's test) and in rats from different groups (Tukey's test). A value of p<0.05 was considered statistically significant.

**Results**

The weekly tail-cuff data and changes in body weight for each group are depicted in Figure 1. Insulin and simultaneous administration of bunazosin did not affect tail-cuff data or body weight. The mean arterial pressures at the third experimental week are shown in Figure 2. The mean arterial pressure in the DS-ins group (137±3 mm Hg) was significantly higher than in the DS-sal (119±3 mm Hg) and DS-A-ins (115±7 mm Hg) groups. There were no significant differences in mean arterial pressure between the DR-ins (119±3 mm Hg) and DR-sal (117±3 mm Hg) groups or between the DS-L-ins (119±2 mm Hg) and DS-L-sal (112±5 mm Hg) groups.

Figure 3 shows the urinary sodium excretion and the 24-hour sodium balance in the DS and DR groups. The urinary sodium excretion at week 1 was significantly lower in the DS-ins group than that in the DS-sal group. In DR rats, insulin did not influence the urinary sodium excretion throughout the experiment. During the first week, sodium retention was significantly higher in the DS-ins than in the DS-sal group, but there was no significant difference in sodium retention between the DS-sal and DS-A-ins groups. Insulin did not affect urinary catecholamine excretion in any group (Figure 4).
Hypertension

Vol 20, No 5 November 1992

Dahl-R Dahl-S with Low Salt Diet

FIGURE 2. Bar graphs show mean blood pressure at third experimental week in Dahl salt-sensitive (Dahl-S) rats, Dahl salt-resistant (Dahl-R) rats, and Dahl-S rats in the low salt diet groups. Results are expressed as mean±SEM. Closed bars, groups infused with insulin (ins); open bars, groups infused with saline (sal); hatched bar, Dahl-S group infused with insulin and simultaneous administration of bunazosin (α-blocker). p<0.05 between Dahl-S-ins and Dahl-S-sal groups; p<0.01 between Dahl-S-ins and Dahl-S-ins-α-blocker groups.

Table 1 shows plasma levels of catecholamines, aldosterone, insulin, glucose, sodium, and potassium. Although the plasma norepinephrine level was higher in the DS-ins than in the DS-sal group, and plasma insulin levels were significantly higher in the DS-ins and DR-ins groups than in the DS-sal and DR-sal groups, insulin did not affect plasma aldosterone, glucose, sodium, or potassium levels in DS and DR rats.

Table 2 shows blood glucose levels at the end of week 1. Insulin did not affect blood glucose levels in DS or DR rats.

Discussion

Our results showed that hyperinsulinemia, even in the physiological range (about twice the normal plasma insulin level), elevated the blood pressure in DS but not in DR rats. This elevation in blood pressure was associated with an elevation in plasma norepinephrine and transient sodium retention. The simultaneous administration of an α-blocker prevented the elevation in blood pressure and sodium retention. These observations suggest that insulin elevates blood pressure, although different blood pressure responses to insulin may exist, and that the sympathetic nervous system may affect this elevation.

We selected DS rats for the present study because sodium retention and the sympathetic nervous system, which have been identified as important factors in the pathogenesis of hypertension in DS rats, also have been suggested as mechanisms of the insulin-induced elevation of blood pressure.

Chronic insulin infusion elevated the blood pressure and plasma norepinephrine level only in DS rats, suggesting that insulin overload may evoke different responses in the sympathetic nervous system in DS and DR rats. Acute insulin overload has been found to activate the sympathetic nervous system and enhance sodium reabsorption at distal renal tubules. Salt overload affects the sympathetic nervous system directly in DS rats. Thus, insulin's direct action, insulin-induced sodium retention, or both, may be involved in elevation of plasma norepinephrine levels. McCarty et al have demonstrated that salt overload alone does not affect plasma catecholamine levels in DS rats; thus, the elevation of plasma norepinephrine levels in our present study may have been related to the direct effect of insulin rather than to sodium retention induced by insulin. Sustained hyperinsulinemia may elevate insulin levels in the central nervous system. Minano et al reported that insulin may affect the activity of monoamine neurotransmitters in the brain. This is the first report indicating that sustained hyperinsulinemia affects the sympathetic nervous system, but we were unable to determine whether insulin affects the peripheral adrenergic system, the central nervous system, or both. Further study is needed to examine the effect of insulin on the sympathetic nervous system.
Investigations into the relation between insulin and salt-sensitive hypertension\(^{15,16}\) have suggested that insulin affects salt sensitivity and that hyperinsulinemia is a salt-sensitive state that influences the response of blood pressure to a salt overload. However, we found that hyperinsulinemia increased the blood pressure in the absence of salt overload. It is not clear whether hyperinsulinemia always increases the blood pressure in salt-sensitive hypertension. Numerous investigators have demonstrated that salt sensitivity is modulated by a variety of factors, including genetics, the sympathetic nervous system, the renin-angiotensin system, vascular reactivity to sodium, and renal function.\(^{17,18}\) Hall et al\(^9\) failed to produce hypertension with chronic insulin infusion in partially nephrectomized dogs, which is considered to be a model of salt-sensitive hypertension.\(^{19}\) We believe that insulin may elevate blood pressure in some salt-sensitive states, especially those modulated by the sympathetic nervous system.

We observed a transient sodium retention in the DS-ins rats but not in the DR-ins group. Previous studies have demonstrated that insulin enhanced sodium reabsorption at distal renal tubules\(^{22}\) and that the sympathetic nervous system\(^7\) also induced sodium retention. Although we were unable to explain why insulin induced the retention of sodium only in DS rats, changes in sodium metabolism during insulin infusion are crucial factors in blood pressure elevation in DS rats. Rocchini et al\(^{20}\) reported that in dogs, the increase in blood pressure associated with weight gain is directly related to sodium retention and that this sodium retention is in part accompanied by an increase in plasma insulin and aldosterone concentrations,\(^{20}\) but chronic insulin infusion did not affect the plasma aldosterone level in our study. Although numerous studies have demonstrated that sodium retention increases the blood pressure,\(^{17}\) it is not clear if sodium retention directly contributes to the insulin-induced elevation of blood pressure. Hall et al\(^{21}\) reported that chronic intrarenal insulin infusion induced transient sodium retention without an accompanying elevation of blood pressure in dogs. Insulin has been shown to stimulate the Na\(^+\)-H\(^+\) exchange through cell membranes.\(^{22}\) The increased intracellular

![Figure 4](figure.png)

**Figure 4.** Line graphs show changes of urinary catecholamine excretion in Dahl salt-sensitive (Dahl-S) rats and Dahl salt-resistant (Dahl-R) rats. Results are expressed as mean±SEM. Solid lines, groups infused with insulin; dotted lines, groups infused with saline.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DS-ins</th>
<th>DS-sal</th>
<th>DR-ins</th>
<th>DR-sal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>120±7</td>
<td>126±7</td>
<td>112±8</td>
<td>110±5</td>
</tr>
</tbody>
</table>

Blood glucose levels at the end of the first experimental week, using glucose-oxidase-impregnated test strip method, are expressed as mean±SEM. DS-ins, Dahl salt-sensitive rats infused with insulin; DS-sal, Dahl salt-sensitive rats infused with saline; DS-A-ins, Dahl salt-sensitive rats infused with insulin and simultaneous administration of bunazosin; DR-ins, Dahl salt-resistant rats infused with insulin; and DR-sal, Dahl salt-resistant rats infused with saline.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DS-ins</th>
<th>DS-sal</th>
<th>DR-ins</th>
<th>DR-sal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine (ng/ml)</td>
<td>0.17±0.04</td>
<td>0.20±0.02</td>
<td>0.31±0.03</td>
<td>0.36±0.05</td>
</tr>
<tr>
<td>Epinephrine (ng/ml)</td>
<td>0.40±0.02*</td>
<td>0.27±0.01</td>
<td>0.25±0.04</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>131.7±57.3</td>
<td>156.2±65.1</td>
<td>176.1±68.8</td>
<td>155.9±29.2</td>
</tr>
<tr>
<td>Sodium (meq/l)</td>
<td>139.1±0.7</td>
<td>140.4±1.2</td>
<td>140.4±1.8</td>
<td>137.9±1.9</td>
</tr>
<tr>
<td>Potassium (meq/l)</td>
<td>4.3±0.2</td>
<td>4.2±0.2</td>
<td>4.7±0.3</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>97.0±4.6</td>
<td>109.4±4.3</td>
<td>119.4±2.7</td>
<td>120.6±5.2</td>
</tr>
<tr>
<td>Insulin (microunits/ml)</td>
<td>25.9±1.5t</td>
<td>11.5±0.7</td>
<td>29.2±2.3t</td>
<td>15.2±1.1</td>
</tr>
</tbody>
</table>

Table 1. Levels of Plasma Hormonal Factors, Electrolytes, and Glucose in Dahl Salt-Sensitive and Salt-Resistant Rats

Levels of catecholamines (norepinephrine and epinephrine), aldosterone, sodium, potassium, glucose, and insulin in plasma on the 20th experimental day, in Dahl salt-sensitive (DS) rats and Dahl salt-resistant (DR) rats, are expressed as the mean±SEM.

* *p<0.05 between DS rats infused with insulin (DS-ins group) and DS rats infused with saline (DS-sal group).

† *pp<0.01 between DS or DR rats infused with insulin (DS-ins or DR-ins group) and saline (DS-sal or DR-sal group).
Na\(^+\) induced by this mechanism could elevate intracellular Ca\(^{2+}\), contributing to a pressor response to insulin. However, insulin also stimulates Na\(^+\),K\(^+-\)ATPase, which may decrease intracellular Na\(^+\) and Ca\(^{2+}\). In fact, insulin has been shown to elicit vasodilation in humans at least during the acute phase of insulin overload. These observations suggest that the effect of insulin on blood pressure is not uniform. It is not clear whether the blood pressure responses to hyperinsulinemia are related to a species difference, to the duration of hyperinsulinemia, or to some other factors. Our data suggest that differences in salt sensitivity modulate the effect of insulin on blood pressure. Insulin did not elevate the blood pressure in the low salt diet group, suggesting that a small amount of salt is needed for insulin to cause an increase in blood pressure. Thus, insulin may enhance the blood pressure response in salt-sensitive animals. Different responses of the sympathetic nervous system to hyperinsulinemia, salt, or both appear to be important in the different blood pressure responses in the salt-sensitive and salt-resistant animals.

In the present study, insulin was infused without a simultaneous infusion of glucose. Although hypoglycemia induced by insulin activates the sympathetic nervous system, this protocol of insulin infusion has not been found to induce hypoglycemia, even on the fifth or 14th days of insulin infusion, as determined by the glucose-oxidase H\(_2\)O\(_2\) electrode method (unpublished data from our laboratory). In addition, there was no significant difference on the seventh day of this experiment between the insulin or saline groups in blood pressure. The absence of hypoglycemia suggests that the chronic infusion of insulin induces insulin resistance to glucose metabolism. A similar finding was reported by Brands et al. in Sprague-Dawley rats. The mechanism whereby sustained hyperinsulinemia induces insulin resistance to glucose metabolism remains to be elucidated.

Previous studies of the effect of insulin on blood pressure have been inconclusive. Although obesity and non-insulin-dependent diabetes mellitus represent hyperinsulinemic states, they are not always accompanied by hypertension. Our findings suggest that insulin may be a factor in elevating blood pressure and that different blood pressure responses to insulin may exist. The sympathetic nervous system and salt may influence insulin's effect on blood pressure.

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References


2. Fuller JH: Epidemiology of hypertension associated with diabetes mellitus. Hypertension 1985;7(suppl II):II-3-II-7


Blood pressure response to hyperinsulinemia in salt-sensitive and salt-resistant rats.

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