Chronic Hyperinsulinemia Augments Deoxycorticosterone Acetate–Salt Hypertension

Shin-ichi Kitamura, Shinji Seto, Shoichi Nagao, Kiyotaka Matsuo, Masazumi Akahoshi, Katsusuke Yano

Abstract To evaluate the effect of chronic hyperinsulinemia on blood pressure in salt-dependent hypertension, we infused insulin (1.0 IU/d, n=15) or saline (n=13) for 4 weeks into deoxycorticosterone acetate–salt hypertensive rats. The insulin infusion increased plasma insulin levels to 24±2 μU/mL, which was higher than in the saline-infused rats (9±1 μU/mL) but was still within the physiological range. Blood pressure was measured by the tail-cuff method twice a week, and daily sodium intake and urinary sodium excretion were calculated for 3 weeks. At week 4, arterial pressor responsiveness to norepinephrine, angiotensin II, and hexamethonium bromide was evaluated. After 14 days of chronic infusion, the insulin group showed a higher blood pressure than the saline group (on 21st day: 178±6 versus 156±5 mm Hg, P<.05 by tail-cuff method; 171±4 versus 149±3 mm Hg, P<.05 by direct intra-arterial measurement). This blood pressure difference was eliminated after ganglionic blockade with hexamethonium bromide (86±4 mm Hg in insulin-treated and 89±4 mm Hg in saline-treated rats by direct intra-arterial measurement). Throughout the experiment, neither sodium balance nor arterial pressor responsiveness to norepinephrine or angiotensin II differed between the two groups. In conclusion, chronic hyperinsulinemia in the physiological range augments the development of hypertension in salt-dependent hypertension, and this augmentation may be mediated by sympathetic stimulation independent of salt retention. (Hypertension. 1994;24[suppl I]:I-16-I-19.)

Key Words • insulin • hypertension, mineralocorticoid • sympathetic nervous system • hexamethonium bromide

Insulin resistance and compensatory hyperinsulinemia are frequently found in cases of essential hypertension, suggesting that insulin is involved in the pathogenesis of hypertension.1,2 Heightened activity of the sympathetic nervous system (SNS) is one of several mechanisms that have been cited as potential links between blood pressure (BP) and insulin resistance.3,4 Moreover, insulin has an antinatriuretic effect, and sodium retention is a proposed mechanism for the increase of blood pressure.5,6 Deoxycorticosterone acetate (DOCA)–salt hypertension is well known to be related to increased SNS activity and sodium retention.7 Therefore, in the present study we investigated whether chronic insulin infusion augments the development of hypertension in DOCA-salt hypertensive rats and, if it does, whether or not the augmentation of this hypertension is mediated by increased SNS activity and/or sodium retention.

Methods

All surgical procedures were performed with rats under sodium pentobarbital (50 mg/kg IP) anesthesia. Seven-week-old male Sprague-Dawley rats (Charles River Japan Inc, Kanagawa, Japan) were unilaterally nephrectomized, and a silicone rubber sheet (Dow Corning 3110 RTV, Midland, Mich) containing 100 mg/kg DOCA was implanted subcutaneously. Then an osmotic minipump (model 2ML4, Alza Corp, Palo Alto, Calif) filled with either 40 IU/mL insulin (Humulin R, Eli Lilly & Co, Indianapolis, Ind) or normal saline was implanted subcutaneously. Rats were divided into two groups: insulin-treated (DOCA-ins, n=15) and saline-treated (DOCA-sal, n=13) rats. The osmotic minipump was active for 30 days, and insulin was delivered at a dose of 1.0 IU/d. Rats were placed in individual metabolic cages in a quiet, air-conditioned room with a 12-hour light/dark cycle. All rats received normal rat chow containing 0.4% NaCl and 0.9% NaCl to drink.

Measurement of Blood Pressure and Sodium Balance

BP was measured twice a week in conscious rats by the tail-cuff method, and body weight was recorded for 3 weeks. Daily 24-hour urine samples were collected, and the 24-hour amounts of food and water (0.9% saline) consumed were recorded. The 24-hour sodium excretion was calculated from the urine volume and urinary sodium content, and total 24-hour sodium intake was calculated from the amount of food and water consumed. Daily sodium balance was expressed as total sodium intake minus urinary sodium excretion.

Observation of Arterial Pressor Responsiveness

At week 4, indwelling polyethylene catheters (PE-10 fused to PE-50) filled with heparinized saline were implanted in the abdominal aorta and inferior vena cava via the left femoral artery and vein. These catheters were passed beneath the skin, exteriorized at the scapular region, and plugged with stainless steel until the day of the experiment as described previously.8 Rats were allowed to recover from surgery for 48 hours, and then the studies were conducted while rats were in a conscious and resting state. Direct mean BP was monitored continuously through the arterial catheter with a P50 Statham pressure transducer (Gould Inc, Puerto Rico) and AT-601G tachograph (Nihon Kohden, Tokyo, Japan) and was recorded continuously throughout the experiment with a WT-647G recorder (Nihon Kohden).

Pressor responsiveness to norepinephrine (graded doses of 50, 100, 200, and 400 ng/kg per minute IV for 5 minutes each), angiotensin II (Ang II) (graded doses of 5, 10, 20, and 40 ng/kg...
Blood Pressure in DOCA-salt Rats

Fig 1. Line graphs show changes in systolic blood pressure and body weight in deoxycorticosterone acetate (DOCA)-salt hypertensive insulin-treated (DOCA-ins) and saline-treated (DOCA-sal) rats. Top: Changes in blood pressure throughout the experiment and after ganglionic blockade with hexamethonium (Hx). • Indicates tail-cuff blood pressure in DOCA-ins rats; ○, tail-cuff blood pressure in DOCA-sal rats; ●, direct mean blood pressure in DOCA-ins rats; and ◦, direct mean blood pressure in DOCA-sal rats. Bottom: Changes in body weight. • Indicates DOCA-ins rats; ○, DOCA-sal rats. Data are mean±SEM.

Fig 2. Line graphs show changes in urinary sodium excretion and sodium balance (total sodium intake—sodium excretion) in deoxycorticosterone acetate (DOCA)—salt hypertensive insulin-treated (DOCA-ins) and saline-treated (DOCA-sal) rats. ● Indicates DOCA-ins rats; ○, DOCA-sal rats. Data are mean±SEM.

Results

Fig 1 depicts changes of BP and body weight in DOCA-ins and DOCA-sal rats. BP did not differ between the two groups until week 3, after which BP in DOCA-ins rats became significantly higher than that in DOCA-sal rats (at week 4, 178±6 versus 156±5 mm Hg, \( P<.05 \)); direct mean BP measured before hexamethonium administration was also significantly higher in DOCA-ins than in DOCA-sal rats (171±4 versus 149±3 mm Hg, \( P<.05 \)). In both groups, mean BP was substantially reduced by ganglionic blockade with hexamethonium to levels that were no longer significantly different (86±4 versus 89±4 mm Hg). There was no difference in body weight between the two groups.

Fig 2 shows urinary sodium excretion and cumulative sodium retention in DOCA-ins and DOCA-sal rats. There were no significant differences in either parameter between the two rat groups.

Although plasma IRI levels were higher in DOCA-ins than in DOCA-sal rats, no significant difference was observed in plasma glucose, catecholamines, or PRA between the two groups (Table). Plasma IRI levels in DOCA-ins rats were within the physiological range.

Arterial pressor responsiveness to norepinephrine and Ang II are shown in Fig 3. Neither norepinephrine nor Ang II demonstrated any augmentation in the pressor responses in DOCA-ins compared with DOCA-sal rats.

Discussion

The present study demonstrated that chronic hyperinsulinemia in the physiological range augments the devel-
Levels of Plasma Hormonal Factors and Glucose in DOCA-Ins and DOCA-Sal Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DOCA-Ins (n=7)</th>
<th>DOCA-Sal (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRI, μU/mL</td>
<td>24.4±2.1*</td>
<td>9.0±1.1</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>126.0±1.7</td>
<td>131.6±1.3</td>
</tr>
<tr>
<td>Epinephrine, ng/mL</td>
<td>0.58±0.10</td>
<td>0.62±0.03</td>
</tr>
<tr>
<td>Norepinephrine, ng/mL</td>
<td>0.32±0.08</td>
<td>0.34±0.13</td>
</tr>
<tr>
<td>PRA, ng/mL</td>
<td>0.26±0.11</td>
<td>0.08±0.03</td>
</tr>
</tbody>
</table>

DOCA indicates deoxycorticosterone acetate; DOCA-Ins, insulin-treated DOCA-salt rats; DOCA-sal, saline-treated DOCA-salt rats; IRI, plasma immunoreactive insulin; and PRA, plasma renin activity. Values are mean±SEM on the 27th experimental day.

*P<.05 between DOCA-Ins and DOCA-Sal.

These observations were indicative of sympathetic stimulation, and the effect of hexamethonium can be used to augment the BP was eliminated after ganglionic blockage. However, plasma catecholamine levels did not differ between DOCA-Ins and DOCA-Sal rats in this study. Acute insulin infusion by the euglycemic clamp technique has revealed that hyperinsulinemia increases plasma norepinephrine levels and norepinephrine turnover. But in these studies, plasma IRI levels increased to nonphysiological levels. On the other hand, it has been reported that chronic hyperinsulinemia did not increase plasma catecholamine levels when plasma IRI levels were in the physiological range. Furthermore, Supiano et al reported that there was no difference in plasma norepinephrine levels between hypertensive subjects with hyperinsulinemia and normotensive control subjects.

Because hyperinsulinemia was achieved chronically in the present study and plasma IRI levels were within the physiological range, it is possible that SNS stimulation by hyperinsulinemia was weak. Again, we did not observe any differences in plasma norepinephrine levels between DOCA-Ins and DOCA-Sal rats.

In the present study, an approximate threefold increase in plasma IRI level was observed in the DOCA-Ins group; however, this increase did not induce hypoglycemia. Tomiyama et al also reported that an increase of plasma IRI to the level of the present study by chronic insulin infusion did not elicit hypoglycemia.

Pressor responsiveness to norepinephrine and Ang II did not differ between DOCA-Ins and DOCA-Sal rats. These results are in contrast to recent acute studies demonstrating that insulin specifically increased the pressor response to norepinephrine in rats. However, it has been reported that under low-dose insulin administration, pressor responsiveness to norepinephrine was significantly high only when a high dose of norepinephrine was administered. Because hyperinsulinemia was within the normal range, the norepinephrine concentration used in the present study could not be expected to evoke an enhanced pressor response in DOCA-Ins rats. In addition, compensatory mechanisms activated by a BP increase would modify the present results. Although insulin has been reported to potentiate the pressor effect of Ang II in acute experiments, it is known that chronic infusion of insulin failed to potentiate the hypertensive action of Ang II. In the present study, the BP response to Ang II and PRA levels did not differ between DOCA-Ins and DOCA-Sal rats, a finding consistent with the results of previous chronic studies.

Many investigators have demonstrated the relation between insulin and salt-sensitive hypertension and suggested that insulin increases sodium reabsorption in the kidney. In the present study, however, we did not observe sodium retention in DOCA-Ins compared with DOCA-Sal rats. However, in another study chronic hyperinsulinemia increased BP but did not cause sodium retention. Although the exact mechanism remains unclear, it is possible that sodium retention was not observed because of pressure natriuresis.

Tomiyama et al showed that insulin infusion elevated BP in Dahl salt-sensitive but not salt-resistant rats. Their results as well as our results in DOCA-salt hypertension suggest that salt-sensitive hypertension can be a predisposing condition for the hypertensive action of insulin. However, several investigators have shown that insulin administration increases BP in non-salt-dependent hypertensive and normal rats.
We did not investigate whether the effects of insulin on the SNS were mediated centrally or peripherally. Some investigators have suggested that the sympathetic action of insulin is mediated centrally, because insulin receptors are widely distributed in the brain\textsuperscript{19} and a direct central action of insulin has already been reported.\textsuperscript{20} Further studies are needed to clarify whether the effect of chronic hyperinsulinemia on the SNS is mediated centrally or peripherally.

In summary, chronic hyperinsulinemia augments the development of hypertension in salt-dependent hypertension, and this effect may be mediated by sympathetic stimulation independent of salt retention.

References

Chronic hyperinsulinemia augments deoxycorticosterone acetate-salt hypertension.

S Kitamura, S Seto, S Nagao, K Matsuo, M Akahoshi and K Yano

*Hypertension*. 1994;23:I16
doi: 10.1161/01.HYP.23.1_Suppl.I16

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/23/1_Suppl/I16

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/