Hyperinsulinemic rats are normotensive but sensitized to angiotensin II

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1Department of Molecular and Clinical Medicine/Clinical Physiology, Institute of Medicine and 2Department of Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, Göteborg University, Göteborg, Sweden; and 3Department of Physiology, University of Southern Denmark, Odense, Denmark

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Johansson ME, Andersson IJ, Alexanderson C, Skøtt O, Holmång A, Bergström G. Hyperinsulinemic rats are normotensive but sensitized to angiotensin II. Am J Physiol Regul Integr Comp Physiol 294: R1240–R1247, 2008.—The effect of insulin on blood pressure (BP) is debated, and an involvement of an activated renin-angiotensin aldosterone system (RAAS) has been suggested. We studied the effect of chronic insulin infusion on telemetry BP and assessed sympathetic activity and dependence of the RAAS. Female Sprague-Dawley rats received insulin (2 units/day, INS group, n = 12) or insulin combined with losartan (30 mg·kg−1·day−1, INS+LOS group, n = 10), the angiotensin II receptor antagonist, for 6 wk. Losartan-treated (LOS group, n = 10) and untreated rats served as controls (n = 11). We used telemetry to measure BP and heart rate (HR), and acute ganglion blockade and air-jet stress to investigate possible control of BP by the sympathetic nervous system. In addition, we used myograph technique to study vascular function ex vivo. The INS and INS+LOS groups developed euglycemic hyperinsulinemia. Insulin did not affect BP but increased HR (27 beats/min on average). Ganglion blockade reduced mean arterial pressure (MAP) similarly in all groups. Air-jet stress did not increase sympathetic reactivity but rather revealed possible blunting of the stress response in hyperinsulinemia. Chronic losartan markedly reduced 24-h-MAP in the INS+LOS group (−38 ± 1 mmHg P < 0.001) compared with the LOS group (−18 ± 1 mmHg, P ≤ 0.05). While insulin did not affect vascular function per se, losartan improved endothelial function in the aorta of insulin-treated rats. Our results raise doubt regarding the role of hyperinsulinemia in hypertension. Moreover, we found no evidence that insulin affects sympathetic nervous system activity. However, chronic losartan treatment revealed an important interaction between insulin and RAAS in BP control.

insulin/hyperinsulinemia; hypertension; air-jet stress

HYPERINSULINEMIA (or insulin resistance) often associates with hypertension, and epidemiological studies have suggested a positive correlation between hyperinsulinemia and hypertension, especially in obese subjects (28). An activated renin-angiotensin aldosterone system (RAAS) might provide a mechanistic link between obesity, insulin, and hypertension. Indeed, several studies have reported intricate cross talk between insulin and RAAS; such cross talk is fairly well established regarding deranged glucose metabolism. RAAS interventions enhance insulin signaling, lower the risk of new onset of diabetes in hypertensive subjects (23), and improve insulin sensitivity in obese hypertensive subjects (9). However, other studies suggest that cross talk occurs between insulin and angiotensin II signaling in nonglucose-related actions of insulin, e.g., mitogenic and growth-promoting effects (19) and also in vasoregulation mediated by nitric oxide (NO) production (36). Furthermore, activity in the sympathetic nervous system is reportedly stimulated by RAAS activity, and both angiotensin II and insulin stimulate and/or facilitate sympathetic nervous system (SNS) activity, thus forming another level of interaction between these hormonal systems (26, 29, 31).

The literature on the hemodynamic effects of hyperinsulinemia in rats is vast. Most studies report increased blood pressure (BP) following insulin infusion (5, 7, 26, 35); however, there are also reports of no change in BP levels (22). Most of these studies are well controlled with respect to insulin’s metabolic actions but relatively short term (7–10 days). The more long-term studies (>10 days) are less well controlled regarding metabolic effects of insulin and, importantly, have not used state-of-the-art technique to measure BP (7, 35). Thus, there is inconsistency regarding the effect of insulin on BP in rats, and there is a lack of more long-term studies using appropriate techniques to measure BP.

We reported earlier that chronic insulin infusion increases cardiac weight, an effect possibly mediated by angiotensin II (32). Other reports suggest that an activated RAAS is involved in the effect of insulin on BP showing that RAAS interventions ameliorate insulin-mediated hypertension (5, 7, 30). We are aware of no long-term studies that have addressed the issue of insulin-RAAS interactions in hemodynamic control using state-of-the-art techniques to measure BP.

Therefore, the aim of the current study was to use telemetry to measure carefully the true effect of RAAS intervention and chronic insulin infusion on BP in freely moving rats. We used a previously described model (13, 14) in which insulin is infused by minipump, and glucose is supplemented orally to counteract hypoglycemia. The model enables us to study the long-term effects of hyperinsulinemia per se. We further examined whether insulin infusion increases RAAS and SNS activity as well as cardiovascular reactivity.

METHODS

Animals

Female Sprague-Dawley rats (B&K Universal, Sollentuna, Sweden) weighing ∼180 g were fed standard rat chow and tap water ad libitum. Animals were housed at 21–25°C in a room with a 12:12-h light-dark cycle. We acclimated animals to their surroundings for 1 wk before initiating the experiment. All animal procedures were approved by the Animal Ethics Committee of Göteborg University.

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Experimental Protocol

Study 1. Chronic interactions between insulin and RAAS. We treated the rats with insulin [2 units/day, Insam Infusat (Apoteksbolaget), INS group, n = 12] or insulin combined with losartan [30 mg·kg⁻¹·day⁻¹, the ANG II receptor antagonist (Merck, Sharp and Dohme, Sweden), INS+LOS group, n = 11] for 6 wk. Losartan-treated (LOS group, n = 10) and untreated rats (control group, n = 11) served as controls. To counteract hypoglycemia, rats were supplemented with 10% glucose in drinking water throughout the study. With this regimen, plasma glucose at 2 and 6 wk were not different between groups (see data from 6 wk in Table 4). Blood samples were drawn from conscious animals after 2 and 6 wk by cutting the tip of the tail. To measure mean arterial pressure (MAP) and heart rate (HR), we surgically implanted telemetry probes after 4 wk. Measurements were performed at week 5. To determine whether SNS participates in MAP control, we used acute ganglion blockade (20 mg/kg ip hexamethonium chloride; Sigma, St. Louis, MO) and air-jet stress. We killed all animals after 6 wk of treatment by overdosing them with pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget). After collecting blood from the right ventricle, we carefully removed the aorta and pentobarbital (0.015 ml/g body wt ip; Apoteksbolaget).

Study 2. Acute interactions between insulin and RAAS. Study 2 explored the time course of BP and HR alterations. In addition, we used acute selective antagonism to examine RAAS and SNS activity during insulin treatment and used air-jet stress to activate SNS. After fitting the rats with telemetry probes, we allowed 1-wk of recovery before initiating insulin treatment. Insulin-treated rats were supplemented with 10% glucose in drinking water throughout the study. We measured 24-h MAP and HR during 48 h, starting 3 days after insulin treatment and then every second week throughout the entire treatment period (6 wk). At weeks 2, 4, and 6, we administered acute dosing of losartan (30 mg/kg ip) and two different ganglionic blockers (20 mg/kg hexamethonium and 10 mg/kg ip pentolinium trtarate; Acros Organics, Geel, Belgium) as well as air-jet stress.

Drug Administration

Insulin was given as a continuous subcutaneous infusion using osmotic pumps (Alzet, Scanbur, Sollentuna, Sweden). The groups receiving insulin also received 10% glucose in their drinking water to maintain euglycemia. Losartan was also administered in osmotic pumps and implanted subcutaneously.

24-h BP Monitoring

To measure conscious 24-h BP, we used a telemetry technique described previously (3). Briefly, we anesthetized rats with isoflurane, implanted a radio telemetric transducer catheter (Data Science International, St. Paul, MN) into the lower aorta ~1 cm below the renal arteries, and glued the catheter into position (3M Vetbond; 3M Animal Care Products, St. Paul, MN). Following a recovery period (1 wk), we placed the animals on a receiver plate and collected the signal using Dataquest ART Version 3.1 (Data Science International). We corrected the pressure signal for electronic offset, i.e., the average of one measurement outside the animal before implantation and after explantation. Offset values greater than ~10 or +10 were excluded. To analyze 24-h MAP, we averaged samples gathered on two subsequent days.

Stress Test and Acute Dosing

During stress exposure and drug testing, we sampled telemetry BP using a short sampling duration and a high rate of repetition (5 s, averaged each 10 s). We obtained stable and undisturbed baseline values (10 min) at ~8:00 AM, before entering the animal room. We transferred the animals to a specially designed cage wherein a stream of compressed air was blown for 10 min. MAP was then followed for another 10-min recovery. To evaluate the involvement of SNS in BP control, we administered acute intraperitoneal doses of ganglion blockers hexamethonium or pentolinium, generating prompt and stable effects. We assessed the MAP response during a 5-min period starting 4-min after dosing. To evaluate RAAS dependency on BP, we administered a single dose of losartan (30 mg/kg ip). During week 2, we noted a stable BP response 1 h after dosing; therefore, we used the average BP value between 60–120 min and ceased sampling at 120 min. However, at weeks 4 and 6, BP response did not stabilize until after 2 h, resulting in prolonged recording time. Therefore, we calculated average BP 120–180 min after acute dosing.

The tests lasted 1 wk and were performed according to the following schedule. Day 1, air-jet stress before noon and acute losartan in the afternoon. Two days later, hexamethonium was administered before noon followed by pentolinium in the afternoon, which allowed the animals to recover for a minimum of 24 h between drug administration.

Ex Vivo Vascular Function

Using myograph technique described previously (4, 10, 11, 16, 17), we examined ex vivo vascular function in the aortae and mesenteric arteries. Briefly, we dissected aortic segments from the fifth intercostal branch (midthoracic aorta). After equilibration, we preactivated the vessel strips by adding KCl (100 mmol/l) and NE (5–10 mol/l norepinephrine, Arterenol, Sigma). After subsequent equilibration, we studied the NE concentration response relationship (10⁻⁶–10⁻⁵ mol/l) to verify NE sensitivity. We examined endothelium-dependent vasodilatory responses with ACh-induced vasodilation (10⁻⁴–10⁻³ mol/l) following NE preconstriction (~75% of maximal constriction). Using sodium nitroprusside (10⁻⁵–10⁻⁴ mol/l, SNP; Sigma), we evaluated endothelium-independent relaxation. Finally, we performed ACh (10⁻⁹–10⁻⁵ mol/l) concentration response in the presence of NO synthase inhibitor Nω-nitro-L-arginine (3 × 10⁻⁴ mol/l; L-NNa; Sigma).

After separating the second or third branch of the mesenteric artery from adjacent connective tissue, we recorded isometric wall tension in a MultiMyograph (model 610M; Danish Myo Technology, Aarhus, Denmark). Following equilibration, the vessel segments were normalized by stepwise extension according to the manufacturer’s protocol. We activated the vessels with KCl and NE before initiating the experiment. Following NE concentration response relationship (5.6 × 10⁻³–3.2 × 10⁻⁵), we performed the endothelium-dependent relaxation response (10⁻¹–10⁻² mol/l ACh), endothelium-independent relaxation responses (10⁻¹–10⁻⁵ mol/l SNP), and NO-dependent response (10⁻¹–10⁻⁵ mol/l ACh) in the presence of the NO synthase inhibitor L-NNa (3 × 10⁻⁴ mol/l) as described above.

Quantification of Gene Expression

The protocol has been described previously (16). Briefly, we extracted total mRNA with Trizol reagent (Life Technologies, Invitrogen, Paisley, Scotland) followed by reverse transcription with Thermoscript RT-PCR system (Invitrogen) according to the manufacturer’s protocol. We performed relative quantification of mRNA expression on a LightCycler (Roche Diagnostics, Mannheim, Germany) using SYBR Green I. We designed PCR primers for α₁β-receptor using LightCycler Probe Design Software version 1.0 (Roche Diagnostics). GAPDH served as an internal control (11).

Immunoblotting

We performed protein extraction and Western blot analysis as previously described (27, 33) with some minor modifications. Briefly, mesenteric adipose tissues were homogenized on dry ice by using a
mortar and pestle and then homogenization in PE buffer (10 mM potassium phosphate buffer, pH 6.8 and 1 mM EDTA). After sonication and centrifugation, we stored the supernatants at −70°C until analysis. Protein concentrations were determined using the Bradford assay. Protein (40 μg) was loaded on 4–12% NuPAGE Bis-Tris gels (Novex, Invitrogen) and electroblotted to a PVDF membrane (GE Healthcare, Buckinghamshire, UK). We then incubated the membranes with primary antibodies against AT1 receptor (sc-1173, diluted 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), ACE (sc-20791, diluted 1:1,000; Santa Cruz), and β-actin (A5441, diluted 1:1,000; Sigma). Immunolabeling was visualized by chemiluminescence using an alkaline-phosphatase-conjugated secondary antibody and CDP-Star (Tropix; Applied Biosystems, Foster City, CA) as substrate. Relative density of the bands was evaluated by densitometry using Image Gauge software (version 3.45; Fuji Film). To correct for loading differences, the density of each band was normalized to its corresponding β-actin band. Each gel/blot contained a positive control (rat kidney). Mean densities of β-actin-normalized bands are expressed as percentage of positive control for the respective gel.

**Biochemical Analysis**

Plasma renin concentrations (PRC) were measured by RIA of angiotensin I, using the antibody-trapping technique (24). Only results with linearity in serial dilutions (between 50- and 1,000-fold) were accepted. Renin values, expressed in standard milliGoldblatt units per milliliter (mGU/ml), were standardized with renin standards obtained from the National Institutes for Biological Standards and Control (Potters Bar, Herts. UK). We determined plasma glucose concentration with an autoanalyzer using the glucose oxidase method (YSI).

**Table 1. Effects of ganglion antagonist hexamethonium (20 mg/kg; Hexa) and air-jet stress on mean arterial pressure (MAP) and heart rate (HR) in study 1, after 5 wk of treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Hexa MAP/HR Baseline</th>
<th>Hexa</th>
<th>Air-Jet Stress MAP/HR Baseline</th>
<th>Stress</th>
<th>Poststress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + losartan</td>
<td>10</td>
<td>93 ± 7/418 ± 12</td>
<td>74 ± 7/417 ± 17</td>
<td>83 ± 5/424 ± 17</td>
<td>112 ± 5/391 ± 19</td>
<td>98 ± 6/443 ± 7</td>
</tr>
<tr>
<td>Insulin + losartan</td>
<td>11</td>
<td>68 ± 5/432 ± 16</td>
<td>45 ± 6/386 ± 11</td>
<td>64 ± 4/461 ± 14</td>
<td>83 ± 4/436 ± 30</td>
<td>73 ± 5/481 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of rats per group.
Scientific, Yellow Springs, OH). Commercial assay kits determined the plasma levels of insulin (Linco Research, St. Charles, MO) according to the manufacturer’s protocol.

**Statistics**

All data that fulfilled the criteria for parametric testing were analyzed using one-way ANOVA (version 12.0.1; SPSS, Chicago, IL) followed by Tukey’s honestly significant difference test. We evaluated 24-h MAP and HR using repeated-measurements ANOVA (STATISTICA 6 for Windows, version 7; StatSoft, Tulsa, OK) followed by one-way ANOVA using group as factor, followed by Bonferroni post hoc test. Because PRC data did not fulfill the criteria, we examined such data using nonparametric statistics. The Kruskal-Wallis test verified differences among means, and the Mann-Whitney U-test assessed differences in individual groups. To evaluate time as a factor, we also analyzed PRC data with repeated-measurements ANOVA after logarithmic transformation. We used the Student’s t-test to compare control and INS groups in study 2. All data are expressed as means ± SE. A value of \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Study 1**

Similar MAP but marked hypotensive effect of losartan. Insulin did not affect MAP per se (Fig. 1, repeated-measurements ANOVA; time, \( P < 0.0001 \), group, \( P < 0.0001 \) time*group, \( P = 0.0095 \)). Losartan decreased BP significantly compared with control (\(-18 \pm 1 \) mmHg, \( P < 0.05 \)). MAP reduction was more marked in the INS+LOS group (\(-38 \pm 1 \) mmHg, \( P < 0.01 \)). Although there were numerical differences, we observed no significant HR differences (repeated-measurements ANOVA; time, \( P < 0.0001 \), group, \( P = 0.02 \), time*group, \( P = 0.721 \)).

**Sympathetic activity and air-jet stress.** Hexamethonium reduced MAP similarly in all groups (control, \(-29 \pm 4 \); INS, \(-32 \pm 3 \); control-LOS, \(-26 \pm 5 \); and INS+LOS, \(-34 \pm 6 \) mmHg).
compared with baseline, Table 1). Hexamethionum did not affect HR (control, -3 ± 6; INS, -9 ± 2; control-LOS, +1 ± 5; and INS+LOS, -10 ± 3% of baseline, Table 1). Air-jet stress caused no significant differences in absolute BP changes. However, the INS group showed a tendency toward lower MAP response compared with control, (MAP: control, +32 ± 3; INS, +19 ± 4; control-LOS, +36 ± 7; INS+LOS, +29 ± 7% of baseline). HR response to air-jet stress was similar in all groups (HR: control, -7 ± 8; INS, -7 ± 8; control-LOS, -6 ± 6; INS+LOS, -5 ± 11% of baseline, Table 1).

Losartan improves ACh-induced vasodilation in hyperinsulinemic rat aortae. Insulin did not affect ACh-dependent vasodilation significantly (control, 90 ± 5 vs. INS, 81 ± 4%, Fig. 2A). However, losartan significantly improved NO-mediated vasodilation in insulin-treated rats (INS, 81 ± 4 vs. INS+LOS, 97 ± 2%, P ≤ 0.01, Fig. 2C). We observed no difference in SNP-induced vasodilation (~95% in all groups). In addition, normalized internal diameter, potassium response, NE response, or ACh-mediated vasodilation did not differ in the mesenteric arteries (data not shown).

**PRC increased after 2 wk of insulin treatment.** While insulin treatment increased PRC significantly in the INS group at 2 wk (control, 37 ± 3 vs. INS, 76 ± 13*10⁻² mGU/ml, P < 0.05), we observed no significant difference after 6 wk (control, 51 ± 7 vs. INS, 68 ± 15*10⁻² mGU/ml). Losartan treatment increased PRC in both groups at 2 and 6 wk (2 wk: control-LOS, 1,402 ± 160 and INS+LOS, 1,567 ± 143*10⁻² mGU/ml, P < 0.001, 6 wk: control-LOS, 807 ± 181 and INS+LOS, 2,005 ± 385*10⁻² mGU/ml, P < 0.001). Whereas PRC remained more or less constant in all groups between weeks 2 and 6, PRC decreased significantly in the control-LOS group during the same time period (P < 0.05).

**Gene and protein expression.** We observed no difference in mRNA expression of the α₁a-receptor in the mesenteric arteries (control, 3.4 ± 1; control-LOS, 2.5 ± 0.4; INS, 5.2 ± 1.2; and INS+LOS, 3.0 ± 0.8 arbitrary units). Western blot analysis of mesenteric adipose tissue showed no differences in AT1 receptor expression (Fig. 3A) or ACE expression (Fig. 3B).

**Study 2**

**Similar MAP, increased HR in hyperinsulinemic rats.** MAP did not differ in INS and control animals in the second study (repeated-measurements ANOVA; time, P = 0.018; group, P = 0.552; time*group, P = 0.987). However, HR increased significantly 3 days after commencing insulin treatment, and this difference remained throughout the study (repeated-measurements ANOVA; time, P < 0.0001; group, P = 0.016; time*group, P < 0.0001, Fig. 4).

**No differences in acute effects of losartan.** To study whether hyperinsulinemic rats develop sensitization to RAAS, we administered a single dose of losartan 2, 4, and 6 wk after initiating insulin treatment. Acute dosing showed a similar MAP response in the control and INS groups at 2, 4, and 6 wk of insulin treatment (2 wk: control, -13 ± 2 vs. INS, -16 ± 3% of baseline; 4 wk: control, -12 ± 3 vs. INS, -19 ± 3% of baseline; 6 wk: control, -10 ± 3 vs. INS, -17 ± 2% of baseline, Table 2). HR did not change significantly following losartan treatment (2 wk: control, +12 ± 3 vs. INS, +5 ± 4% of baseline; 4 wk: control, +17 ± 3 vs. INS, +4 ± 5% of baseline; 6 wk: control, +16 ± 6 vs. INS, +97 ± 4% of baseline, Table 2).

**BP and HR respond similarly to ganglionic blockade.** Study 2 used pentolinium to block ganglionic traffic. Compared with hexamethionum (study 1), pentolinium has a slightly different pharmacological profile, and the effect of pentolinium lasted

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**Table 2. Effects of angiotensin II receptor antagonist losartan (30 mg/kg) on MAP and HR after 2, 4, and 6 wk of insulin treatment in study 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 2 MAP/HR</th>
<th>Week 4 MAP/HR</th>
<th>Week 6 MAP/HR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline/Post</td>
<td>Baseline/Post</td>
<td>Baseline/Post</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of rats per group.
longer. Pentolium did not affect MAP or HR response (MAP week 2: control, $-14 \pm 6$; INS, $-14 \pm 8\%$ of baseline; week 4: control, $-30 \pm 2$; INS, $-35 \pm 3\%$ of baseline; week 6: control, $-27 \pm 2$; INS, $-24 \pm 7\%$ of baseline, Table 3).

Blunted MAP response during air-jet stress? MAP response to air-jet stress did not change after 2 wk of insulin treatment (control, $+30 \pm 2$ vs. INS, $+24 \pm 3\%$ of baseline, Table 3). Although MAP response in the insulin-treated animals decreased significantly during week 4 (control, $+36 \pm 6$ vs. INS, $+28 \pm 2\%$, $P<0.05$, Table 3), we observed no difference during week 6 (control, $+30 \pm 3$ vs. INS, $+27 \pm 2\%$, Table 3). To increase the statistical power of our analyses, we pooled data from week 5 of study 1 and week 6 of study 2. Subsequent analysis of the pooled data showed a significantly smaller increase in BP in response to air-jet stress in the INS group (control, $+30 \pm 2$ vs. INS, $+23 \pm 2\%$, $P=0.03$).

Rats are hyperinsulinemic and euglycemic. Since none of the following parameters differed significantly between the control and INS groups in studies 1 and 2, we combined them (Table 4).

Hyperinsulinemic INS and INS+LOS rats had normal blood glucose levels (Table 4). Body weight increased in INS rats compared with control animals ($P<0.001$, Table 4). INS+LOS treatment prevented an insulin-mediated increase in body weight ($P<0.001$, Table 4). Losartan did not influence body weight in control animals (Table 4).

Left ventricular weight. Hyperinsulinemia resulted in increased left ventricular weight (LVW; $P<0.01$ vs. C, Table 4), and losartan reduced LVW ($P<0.001$). However, after normalizing LVW to body weight, we observed no differences between control and INS. Losartan treatment reduced LVW significantly in the control animals ($P<0.001$, Table 4), a difference that persisted after normalization for body weight ($P<0.001$ for both, Table 4).

**DISCUSSION**

We show here that chronic insulin treatment in rats sensitizes BP control to RAAS. Hyperinsulinemia per se does not affect BP, but chronic treatment with the angiotensin receptor blocker losartan lowers BP nearly 40 mmHg, suggesting that an intact RAAS participates importantly in BP control. However, we found no convincing evidence of increased baseline sympathetic activity in hyperinsulinemic rats and no increased vascular reactivity to stressful stimuli.

**Insulin Infusion Does Not Affect BP Chronically in Unrestrained Rats**

The literature is widely inconsistent regarding the effects of hyperinsulinemia on BP. Some studies show increased BP (5, 8, 26, 35), while others show no effect (12, 22) or even acutely reduced BP (1, 34). Some of these apparent inconsistencies most likely depend on the level of hyperinsulinemia achieved and resultant glucose levels, the length of the observation period, and differences between species. The current study was designed to look at both the immediate and more long-term effects of insulin on BP. We used a model in which glucose is supplemented orally, achieving normal plasma glucose at 2 wk. Despite oral supplementation, glucose kinetics are altered, evidenced by an increased insulin sensitivity (13, 14). Our study, which used telemetry and allowed freely moving unrestrained BP measurements in home cages, shows that insulin does not affect BP in rats rendered hyperinsulinemic for 6 wk. The data is in disagreement with a previous study using telemetry and infusing insulin for 10 days (26). In the study by Meehan et al. (26) oral glucose was administered to both control and insulin group, and the achieved plasma insulin was comparable to the current study (8 and 12 times increase, respectively). The administration of glucose to the control groups makes it hard to compare the two studies since glucose per se resulted in a marked increase in systolic BP. The data is also in disagreement with the study by Fang and Huang (7) who reported increased BP after insulin in a 42-days-long study. However, in this study BP was measured using tail cuff technique, and glucose was not supplemented in drinking water. We believe that our data are solid since we have used state-of-the-art BP technique and reproduced identical findings in two separate studies. It is interesting to note that all previous studies reporting an increase in BP after insulin is performed in male rats (8, 26, 35). This would suggest a possible sex difference in the susceptibility toward insulin. Another possibility is that the data by Fang and Huang (7) might result from interactions between insulin’s sympathofacilitation and the stressful stimuli of tail cuff BP measurement.

**No Differences in Sympathetic Activity or Reactivity**

Therefore, we sought to assess in hyperinsulinemic rats the degree of baseline sympathetic activity as well as sympathetic reactivity. Earlier reports suggested that hyperinsulinemia contributes to hypertension by a sympathostimulatory effect in humans (28) and animals (38). In the current study, HR consistently increased after insulin treatment and also throughout the study, possibly indicating altered autonomic tone. However, ganglion blockade reduced BP and HR similarly in all groups and at all time points in both studies. Furthermore, mesenteric resistance vessels showed similar mRNA expression of the $\alpha_{1a}$-receptor. These findings do not support increased resting sympathetic tone in hyperinsulinemic rats as an explanation to the increased HR. However, ganglion blockade does not test for sympathetic reactivity. Indeed, a recent report suggested that rats rendered hyperinsulinemic by fructose feeding develop

**Table 3. Effects of ganglion antagonist pentolium (10 mg/kg) and air-jet stress on mean arterial pressure (MAP) and heart rate (HR) after 2, 4, and 6 weeks of insulin treatment in study 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Week 2 MAP/HR</th>
<th>Week 4 MAP/HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pentolium Baseline/Post</td>
<td>Pentolium Baseline/Post</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>$100 \pm 3/393 \pm 13$</td>
<td>$86 \pm 7/413 \pm 13$</td>
</tr>
<tr>
<td>Insulin</td>
<td>8</td>
<td>$102 \pm 2/390 \pm 9$</td>
<td>$86 \pm 8/389 \pm 16$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n$ = number of rats per group.
stress-sensitive hypertension (6). Therefore, we submitted our hyperinsulinemic rats to air-jet stress. Such stress did not augment MAP or HR response; on the contrary, hyperinsulinemic rats showed blunted stress response compared with control rats. This finding was not totally consistent and will require further study. However, we show here that hyperinsulinemic rats are neither hyperreactive nor more stress sensitive during air-jet stress. The explanation to the observed increase in HR could be vagal withdrawal or regional alterations in sympathetic tone.

**Chronic Interaction of Angiotensin II and Insulin**

Interestingly, chronic treatment of hyperinsulinemic rats with the AT1 receptor antagonist losartan resulted in markedly reduced BP (40 mmHg) compared with a more modest reduction in the losartan-treated control group with (18 mmHg), suggesting that RAAS may contribute importantly to BP control in hyperinsulinaemia. Since administration of angiotensin II during hyperinsulinaemia results in greater BP response, others have suggested that insulin increases angiotensin II sensitivity (30). Increased RAAS sensitivity might result from differences in receptor quantity or increased RAAS activity. Although we observed transiently increased PRC after 2 wk of insulin treatment, thus corroborating earlier reports (15, 19, 20) and also on renin release in diabetic animals (18), such action does not explain satisfactorily the effect of chronic losartan treatment. Earlier reports suggest that angiotensin II can impair insulin-induced phosphorylation of endothelial NO synthase, thereby reducing NO availability (36). This is supported by our data showing that losartan treatment significantly enhances ACh-induced vasodilatation in the aorta of insulin-treated rats. However, since we found no similar changes in the mesenteric resistance vessels, this finding likely does not affect BP control. Small vessel myograph revealed no differences in contractile response to potassium, suggesting that no functionally important vascular remodeling occurs in resistance vessels; thus, the increased RAAS dependency is not due to vascular changes induced by trophic actions of insulin. Local RAAS in adipose tissue (21) likely contributes to angiotensin II production. Insulin increased adipose tissue (2) and thus increased locally-derived angiotensin II and RAAS dependency. Therefore, we performed immunoblotting against the AT1 receptor and ACE in mesenteric adipose tissue. We observed no differences in the expression of these RAAS components.

Our data shows that single-dose losartan does not increase RAAS dependency during acute antagonism of the angiotensin II receptor. Furthermore, BP reduction following acute losartan treatment was less than the chronic effect of losartan in hyperinsulinemic rats, suggesting that cross talk between angiotensin II and hyperinsulinaemia, revealed in our chronic treatment protocol, results from long-term changes in insulin/angiotensin signaling not affected by acute receptor antagonism.

**Losartan Reverses LVW**

LVW increased following insulin treatment and decreased following losartan treatment. Moreover, losartan decreased LVW in control animals in relation to achieved BP reduction (17% BP reduction, 17% reduction in LVW). However, decreased LVW in the INS+LOS group was less than expected in relation to achieved BP (39% BP reduction compared with 25% reduction in LVW), suggesting that insulin per se acts as a cardiac growth factor. However, such data requires cautious interpretation since insulin affects body weight, fat mass, and tibia length (37), making it harder to normalize LVW between groups. Using body weight as normalization factor diminishes differences in LVW. In a parallel study (Johansson ME, Alexanderson C, Holmäng A, Bergström G, unpublished data), we estimated lean body mass using dual-energy X-ray absorptiometry. This normalization procedure also diminished LVW differences between the insulin-treated and control group.

**Perspectives and Significance**

The worldwide epidemic of diabetes has stimulated research on the hemodynamic importance of insulin. Our results raise doubt regarding the role of insulin per se in hypertension, and further-

### Table 4. Effect of chronic insulin and/or angiotensin receptor antagonist (losartan) treatment on body weight (BW), insulin, and glucose levels, left and right ventricle weight (LVW, RVW) normalized to body weight (LVW/BW, RVW/BW)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>Insulin, mU/ml</th>
<th>Glucose, mmol/l</th>
<th>LVW, g</th>
<th>LVW/BW, g/kg</th>
<th>RVW, g</th>
<th>RVW/BW, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>303±5</td>
<td>33±3</td>
<td>7.7±0.2</td>
<td>0.714±0.013</td>
<td>2.36±0.03</td>
<td>0.193±0.004</td>
<td>0.640±0.013</td>
</tr>
<tr>
<td>Control losartan</td>
<td>10</td>
<td>295±6</td>
<td>37±5</td>
<td>8.9±0.3</td>
<td>0.593±0.021</td>
<td>2.01±0.05</td>
<td>0.160±0.011</td>
<td>0.541±0.031</td>
</tr>
<tr>
<td>Insulin</td>
<td>21</td>
<td>350±6e</td>
<td>395±103e</td>
<td>6.9±0.3</td>
<td>0.788±0.018</td>
<td>2.25±0.04</td>
<td>0.215±0.007</td>
<td>0.619±0.024</td>
</tr>
<tr>
<td>Insulin-losartan</td>
<td>11</td>
<td>304±7</td>
<td>429±170e</td>
<td>7.1±0.9</td>
<td>0.590±0.012</td>
<td>1.94±0.04</td>
<td>0.178±0.012</td>
<td>0.581±0.035</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of rats per group. *P < 0.001 vs. control, **P < 0.001 vs. insulin, †P < 0.05 vs. control, ‡P < 0.01 vs. insulin, §P < 0.01 vs. control.
more, we found no evidence for insulin to activate SNS or to increase cardiovascular reactivity toward stressful stimuli. Importantly, however, we show that insulin combined with an angiotensin receptor blocker results in marked reductions in BP. These increase cardiovascular reactivity toward stressful stimuli. Importantly, we found no evidence for insulin to activate SNS or to activate angiotensin II that could be of importance in understanding the beneficial effects of angiotensin receptor blockers in diabetics.

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