Hyperinsulinemia and the Aldosterone and Pressor Responses to Angiotensin II

Albert P. Rocchini, Catherine Moorehead, Susan DeRemer, Theodore L. Goodfriend, and Dennis L. Ball

To determine whether hyperinsulinemia alters angiotensin II-mediated aldosterone secretion, the increase in plasma aldosterone after intravenous angiotensin II (5, 10, and 20 ng/kg/min for 15 minutes each) was measured before and after euglycemic hyperinsulinemia in seven chronically instrumented dogs. In a random sequence on 4 successive days, dogs received either 0, 2, 4, or 8 milliunits/kg/min insulin. Euglycemic hyperinsulinemia, at all insulin doses, resulted in a significantly greater (p<0.01) change in the angiotensin II-stimulated increments of plasma aldosterone than was observed when angiotensin II was administered alone. However, there was no dose-dependence of insulin's effect on angiotensin II-stimulated aldosterone. The effect of weight gain on the angiotensin II response was also evaluated in five dogs. After weight gain, euglycemic hyperinsulinemia augmented angiotensin II-stimulated aldosterone to the same magnitude that was observed before weight gain. Possible mechanisms whereby insulin could increase angiotensin II-stimulated aldosterone production include: increased intracellular potassium, reduced plasma free fatty acids, and a direct action of insulin to induce increased adrenal steroidogenesis. In addition to altering the angiotensin II-aldosterone dose-response curve, hyperinsulinemia also increased the pressor action of angiotensin II. In contrast to the angiotensin II-aldosterone response, progressive hyperinsulinemia resulted in a progressive increase in the pressor response to angiotensin II. The increased pressor response is probably due to an increased activation of the sympathetic nervous system by insulin. (Hypertension 1990;15:861-866)

One of the physiological abnormalities that may be partially responsible for the hypertension and sodium retention observed in obese individuals is excess mineralocorticoid activity. We have demonstrated in the dog that 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 norepinephrine, insulin, and aldosterone concentrations. Tuck et al demonstrated in obese adults that weight loss lowered both plasma renin activity and aldosterone concentrations. Tuck et al demonstrated in obese adults that weight loss lowered both plasma renin activity and aldosterone concentration. Hiramatsu et al documented in obese hypertensives that with increasing body weight there is a progressive increase in the ratio of plasma aldosterone to plasma renin activity. Scavo and colleagues reported that, although obese adults have a normal plasma renin activity, they have an increased plasma aldosterone concentration and an increased aldosterone secretion rate. Spark et al have reported that in obese patients during the early stages of fasting there is a dissociation between plasma renin activity and aldosterone. Finally, we have shown that obese adolescents have higher aldosterone concentrations than nonobese adolescents. Upright posture in obese adolescents produced a greater increment in aldosterone for a given rise in renin. Our speculation that increased plasma aldosterone concentration in some obese subjects is caused by increased adrenal sensitivity to angiotensin II is based on these studies. Insulin has been shown to influence the renin-angiotensin-aldosterone system in normal subjects and patients with diabetes. Since hyperinsulinemia is a characteristic feature of obesity, we hypothesize that increased aldosterone levels observed in obese individuals are caused by hyperinsulinemia and its ability to augment angiotensin II-mediated aldosterone production. The purpose of this report is to evaluate whether euglycemic hyperinsulinemia can alter the angiotensin II-aldosterone dose-response curve in nonobese and obese dogs.
Methods

Seven adult mongrel dogs (five females and two males) were trained to lie quietly on a padded table. All dogs were surgically instrumented with an ascending aortic catheter and a right atrial catheter. After surgery, the dogs were allowed to recover for 2 weeks before the experiments were started. In a random sequence on 4 successive days, dogs received either 0, 2, 4 or 8 milliunits/kg/min insulin in conjunction with an intravenous infusion of angiotensin II. Baseline measurements were made after a 10-minute control period, which was followed either by the beginning of the euglycemic insulin clamp studies or a 30-minute infusion of 0.9% saline at 0.5 ml/min (Figure 1). Angiotensin II (Sigma Chemical Co., St. Louis, Missouri) diluted in 0.9% saline was given by constant infusion (1 ml/min) at rates of 5, 10, and 20 ng/kg/min for 15 minutes each. The euglycemic insulin clamp studies were performed in the following fashion. Insulin (Eli Lilly and Company, Indianapolis, Indiana) was administered intravenously as an initial bolus injection (50 milliunits/kg), which was followed by a continuous infusion at rates of either 2, 4, or 8 milliunits/kg/min.12 Insulin was diluted in normal saline, and its concentration was adjusted so that it could be delivered at a rate of 0.5 ml/min. Concomitantly with the insulin, an intravenous infusion of 20% glucose was administered by a variable infusion syringe pump (Harvard Apparatus, South Natick, Massachusetts). Blood samples were obtained at 5-minute intervals for determination of blood glucose concentration. The plasma glucose concentration was held constant at baseline by varying the glucose infusion rate every 5 minutes. Blood pressure and heart rate were monitored every 5 minutes throughout the infusion studies. Blood was drawn for measurement of sodium, potassium, insulin, norepinephrine, and aldosterone during the control period and every 15 minutes throughout the study. Blood was drawn for measurement of plasma oleic acid during the control period, after 30 minutes of euglycemic hyperinsulinemia, and after the angiotensin II infusion. In addition to the four angiotensin II studies, each dog had a 75-minute euglycemic insulin clamp study performed at each insulin dose but without any angiotensin II. To evaluate the role of the sympathetic nervous system on the pressor response to angiotensin II, two dogs received an intravenous infusion of angiotensin II in conjunction with insulin (0 or 4 milliunits/kg/min) and α-adrenergic blockade by use of a continuous intravenous infusion of phentolamine hydrochloride (8 μg/kg/min at a rate of 0.1 ml/min). The α-adrenergic blockade was started for 15 minutes before starting either the 30-minute saline infusion, when angiotensin II alone was administered, or the euglycemic clamp study. To ensure that adequate α-adrenergic blockade was achieved, each dog received two bolus doses of phenylephrine, one after 10 minutes of the phentolamine infusion and the other at the end of the experiment. The phenylephrine dose was chosen so that it would cause a 10-mm Hg increase in mean arterial pressure before the start of the phentolamine infusion.

To assess the effect of weight gain on the angiotensin II responses, five of the dogs were fed a high fat diet consisting of 2 lb cooked beef fat or lard in addition to their regular diet of one can of dog food.1 After 5 weeks of the high fat diet, the previously described angiotensin II and insulin infusion studies were repeated.

Laboratory Measurements

Arterial pressure was measured with P23Db Statham pressure transducers (Statham, Oxnard, California) and recorded on an AR6 optical recorder (PPG Biomedical Systems, Hershey, Pennsylvania). Plasma glucose was assayed by the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, California). Plasma insulin and aldosterone were measured by radioimmunoassay,14 plasma and urinary electrolytes were measured by flame photometry, and plasma norepinephrine was measured by high-pressure liquid chromatography with electrochemical detection.15 Free fatty acids in plasma were extracted by the method of Parmelee et al16; this extraction was followed by high-pressure liquid chromatography of the p-nitrobenzyl esters.17 A Waters Novapak (Rochester, Minnesota) C18 cartridge column was used, and the fatty acid esters were eluted with a convex gradient of water and acetonitrile, which contained 50 ppm trifluoroacetic acid. Results are reported in terms of free oleic acid only, for reasons discussed below.

Statistical Analysis

All values are mean±SEM. Differences in the following variables (arterial pressure, electrolyte concentration, norepinephrine, and aldosterone) between the regular diet and after 5 weeks of the high fat diet were assessed by paired t test. Differences between angiotensin II alone and angiotensin II plus insulin were evaluated for statistical significance by two-way analysis of variance. Differences between angiotensin II infusion experiments before weight gain and after 5 weeks of the high fat diet were also assessed by two-way analysis of variance. The change in mean blood pressure and the change

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**Figure 1. Schematic representation of experimental protocol. C, control; AH, angiotensin II.**
in aldosterone that occurred after angiotensin II infusion, with or without insulin, were made by subtracting the mean arterial pressure or aldosterone concentration just before the start of the angiotensin II infusion from the values measured after 15 minutes of each of the three angiotensin II doses (5, 10, and 20 ng/kg/min).

**Results**

**Effects of Insulin on Aldosterone Production in the Dog**

**Before weight gain.** The control plasma aldosterone before either angiotensin II or insulin administration was 42±5 pg/ml. Within the first 30 minutes of euglycemic hyperinsulinemia, a small but not statistically significant decrease in aldosterone concentration (42±5 to 38±6 pg/ml) occurred, and no further change was observed over an additional 45 minutes of hyperinsulinemia. As demonstrated in Figure 2, euglycemic hyperinsulinemia, at all insulin doses (2, 4, and 8 milliunits/kg/min), resulted in a significantly greater change in the angiotensin II-stimulated increments of plasma aldosterone than was observed when angiotensin II was administered alone (p<0.01). However, there was no dose-dependence of insulin's effect on angiotensin II-stimulated aldosterone.

Basal concentrations of insulin, glucose, sodium, and potassium did not change during infusion of angiotensin II. Euglycemic hyperinsulinemia resulted in a rapid decrease in serum potassium concentration that was unaffected by the subsequent angiotensin II infusion (3.84±0.15 meq/l [control], 3.06±0.21 meq/l [after 45 minutes of insulin alone], and 2.96±0.25 meq/l [after angiotensin II and insulin]). The magnitude of the insulin-induced decrease in plasma potassium was not dose dependent, since all three insulin doses decreased serum potassium to the same extent. Plasma renin activity and glucose and sodium concentrations did not significantly change during the intravenous infusion of insulin. Infusion of insulin lowered the levels of free oleic acid in plasma by 68–83% (Table 1). Other fatty acids fell by comparable percentages (data not shown).

**After 5 weeks of a high fat diet.** The five dogs that received the high fat diet for 5 weeks significantly increased their body weight from 22.7±2.1 to 25.9±1.9 kg. Weight gain was associated with a significant increase in mean arterial pressure, fasting insulin concentration, plasma norepinephrine concentration, and aldosterone concentration (Table 2). After weight gain, euglycemic hyperinsulinemia augmented the angiotensin II-induced change in aldosterone to the same magnitude that was observed before weight gain. Similarly, weight gain did not alter the ability of the insulin infusion to lower the plasma levels of free oleic acid (from 179±32 to 38±19 nM/ml after insulin).

**Effect of Insulin on Pressor Action of Angiotensin II**

Mean arterial pressure and plasma norepinephrine increased during euglycemic hyperinsulinemia in a

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**TABLE 1. Change in Plasma Free Oleic Acid Levels After Euglycemic Hyperinsulinemia**

<table>
<thead>
<tr>
<th>Insulin dose (milliunits/kg/min)</th>
<th>0 (n=6)</th>
<th>2 (n=4)</th>
<th>4 (n=6)</th>
<th>8 (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma insulin (microunits/ml)</td>
<td>7.4±2.1</td>
<td>136±13</td>
<td>232±24</td>
<td>671±36</td>
</tr>
<tr>
<td>Oleic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (nM/ml)</td>
<td>202±23</td>
<td>190±42</td>
<td>185±33</td>
<td>207±39</td>
</tr>
<tr>
<td>After 30 minutes of insulin</td>
<td>...</td>
<td>43±4*</td>
<td>59±25*</td>
<td>36±5*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of dogs. *p<0.01 vs. control.
TABLE 3. Change in Norepinephrine and Mean Arterial Pressure During Euglycemic Hyperinsulinemia in Seven Dogs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>BP* (mm Hg)</th>
<th>Norepi* (pg/ml)</th>
<th>BP+* (mm Hg)</th>
<th>Norepi+* (pg/ml)</th>
<th>BP++ (mm Hg)</th>
<th>Norepi++ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86±5</td>
<td>145±32</td>
<td>87±8</td>
<td>149±38</td>
<td>83±9</td>
<td>140±40</td>
</tr>
<tr>
<td>30</td>
<td>91±4</td>
<td>195±30</td>
<td>95±6</td>
<td>330±49</td>
<td>101±8</td>
<td>480±54</td>
</tr>
<tr>
<td>45</td>
<td>92±5</td>
<td>210±32</td>
<td>96±7</td>
<td>325±44</td>
<td>102±9</td>
<td>474±62</td>
</tr>
<tr>
<td>60</td>
<td>93±4</td>
<td>204±35</td>
<td>95±6</td>
<td>342±35</td>
<td>100±8</td>
<td>491±52</td>
</tr>
<tr>
<td>75</td>
<td>92±6</td>
<td>212±36</td>
<td>98±6</td>
<td>326±48</td>
<td>103±7</td>
<td>485±48</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BP, blood pressure; Norepi, norepinephrine.

*P<0.05 increase over time.
**P<0.05 vs. 2 milliunits/kg/min insulin.
***P<0.05 vs. 2 and 4 milliunits/kg/min insulin.

Dose-dependent fashion (Table 3). Plasma norepinephrine and arterial pressure both reached a stable level after 30–45 minutes and did not change further over an additional 45 minutes of euglycemic hyperinsulinemia. Because euglycemic hyperinsulinemia altered mean arterial pressure, to compare the change in pressure induced by angiotensin II with and without insulin we used the mean arterial pressure after 30 minutes of euglycemic hyperinsulinemia as our reference blood pressure. As can be seen in Figure 3, when compared with angiotensin II alone euglycemic hyperinsulinemia plus angiotensin II resulted in a larger pressor response. In contrast to the angiotensin II–aldosterone response, progressive hyperinsulinemia resulted in a progressive increase in the pressor response to angiotensin II. Because activation of the sympathetic nervous system could be in part responsible for insulin's augmentation of the angiotensin II pressor response, two of the dogs were studied during α-blockade with phentolamine. The phentolamine infusion prevented both the increase in pressure caused by hyperinsulinemia alone and the increased angiotensin II pressor response (Figure 4).

Discussion

In the present study we have demonstrated that hyperinsulinemia increases both the pressor response to angiotensin II and the angiotensin II–mediated secretion of aldosterone. Insulin has previously been reported to affect aldosterone secretion. Pratt et al demonstrated that insulin is necessary for normal renin and aldosterone secretion in the diabetic rat. They also concluded that, although aldosterone secretion may be affected by insulin secondarily through renin release, a local adrenal action of aldosterone could not be ruled out. Farfel et al suggested that insulin's influence on aldosterone regulation in humans is mediated through renin and not through changes in serum potassium. Both of these studies evaluated the effect of hyperinsulinemia on basal aldosterone secretion; however, they did not evaluate the effect of hyperinsulinemia on the angiotensin II–aldosterone dose-response relation, as we did in this study.

Vierhapper et al demonstrated in six healthy men that, during euglycemic hyperinsulinemia, graded doses of angiotensin II augment aldosterone secretion. However, unlike the present study, in which

![Figure 3](https://hyper.ahajournals.org/figure3.png)

**Figure 3.** Bar graphs showing angiotensin II (All) pressor response with and without euglycemic hyperinsulinemia. INS, insulin. The change in mean arterial pressure (ΔBP) was calculated as the mean arterial pressure after 15 minutes of each angiotensin II infusion minus the pressure immediately before starting the angiotensin II infusion (time 0 in Figure 1). Statistical significance was performed by two-way analysis of variance.

![Figure 4](https://hyper.ahajournals.org/figure4.png)

**Figure 4.** Bar graph showing effect of phentolamine hydrochloride (PHEN) on the angiotensin II (All) pressor response with and without euglycemic hyperinsulinemia, in one dog. The change in mean arterial pressure (ΔBP) was calculated as the mean arterial pressure after 15 minutes of each angiotensin II infusion minus the pressure immediately before starting the angiotensin II infusion (time 0 in Figure 1). INS, insulin.
hyperinsulinemia resulted in a greater than fourfold increase in angiotensin II–augmented aldosterone secretion. Vierhapper et al observed that hyperinsulinemia produces less than a 25% increase in plasma aldosterone. Although we do not know the exact explanation for the differences between the two studies, one obvious difference between the studies is that our study was performed in dogs and the study of Vierhapper et al study was performed in humans.

In the present study we have also demonstrated that the effect of insulin on the angiotensin II–aldosterone relation is independent of insulin dose at plasma insulin concentrations above 100 microunits/ml. Since we have not studied lower doses of insulin, we do not know the threshold for induction of insulin’s effect on aldosterone production. However, the plasma insulin levels (136±13 microunits/ml) achieved by 2 microunits/kg/min are comparable to levels observed in obese humans after an oral glucose meal. Thus, it is possible that the regulation of aldosterone may be affected by physiological increases in insulin. This hypothesis is consistent with the increased aldosterone production that has been observed by us and others in obese subjects. In addition, since selective insulin-resistance and resultant hyperinsulinemia are present in some patients with essential hypertension, it is possible that insulin may in part explain the previously reported enhanced adrenal responsiveness to angiotensin II, which is observed in patients with low renin essential hypertension. 

The mechanism whereby insulin can increase aldosterone production is unknown. The secretion of aldosterone in humans and animals is mainly regulated through changes in concentration of adrenocorticotrophic hormone, angiotensin II, and potassium. Insulin infusion resulted in an acute reduction in serum potassium. That effect alone would have been expected to reduce adrenal responsiveness to angiotensin II. A reciprocal change in intracellular potassium could be invoked as a cause of the sensitization to angiotensin by insulin, but this effect has not been demonstrated in the adrenal glomerulosa. Furthermore, the role of intracellular potassium on aldosterone production is unclear.

Another possible explanation of insulin’s effect on aldosterone production is through its effects on plasma free fatty acids. Goodfriend and Ball showed that certain unsaturated fatty acids inhibit the binding of angiotensin II to its receptors in bovine adrenal glomerulosa cells and inhibit the cells’ production of aldosterone. The most potent fatty acid tested was oleic acid, which is also the predominant free fatty acid in dogs and humans. For that reason, we reported only the effects of insulin on oleic acid in the current experiments.

Goodfriend and Ball also found that removal of fatty acids from glomerulosa cells by fatty acid–free albumin sensitized the cells to angiotensin. In the present study, we demonstrated that euglycemic hyperinsulinemia resulted in a profound fall of fatty acids in plasma. Thus, it is possible that insulin increases angiotensin II–mediated aldosterone secretion by reducing plasma fatty acid levels; this reduction facilitates removal of fatty acids from the adrenal into the circulation and thereby relieves inhibition of glomerulosa responsiveness. Further studies are planned to more clearly define the potential role that changes in plasma fatty acids may have on the regulation of plasma aldosterone.

Finally, insulin could act by directly affecting metabolic conditions within the adrenal gland or by an adrenotrophic action. The addition of insulin to ovarian cells is associated with increased steroidogenesis. Receptors for insulin have been identified in the adrenal gland, including in the zone glomerulosa. Therefore, it is possible that insulin could directly affect steroidogenesis.

In addition to demonstrating that insulin increases angiotensin II–mediated aldosterone production, we have shown that insulin also increases the pressor response to angiotensin II. We demonstrated that unlike the aldosterone response, the pressor response increases with increasing insulin dose. Euglycemic hyperinsulinemia also resulted in a dose-dependent increase in both plasma norepinephrine and arterial pressure. We speculate that the increased pressor response of angiotensin II observed in the presence of hyperinsulinemia was due in large part to insulin’s activation of the sympathetic nervous system and to the resultant increase in arterial pressure and plasma norepinephrine. This hypothesis is supported by the fact that angiotensin II is known to increase responsiveness to norepinephrine and that, in two dogs, a-blockade with phentolamine obliterated the insulin-associated increased pressor response to angiotensin II. It is important to note that the results of our study contradict the report of Vierhapper et al, who demonstrated that, in six healthy men, hyperinsulinemia did not modify the pressor action of angiotensin II. A possible explanation for the difference between these two studies may be that, in the study of Vierhapper et al, euglycemic hyperinsulinemia may not have resulted in an activation of the sympathetic nervous system. Although euglycemic hyperinsulinemia has been shown to increase plasma norepinephrine in dogs and humans, there has been at least one study in which hyperinsulinemia failed to result in an increase in plasma norepinephrine. The mechanism by which insulin stimulates the sympathetic nervous system is unknown, but it has been speculated that this stimulation occurs through a central action of insulin, perhaps in insulin-sensitive areas in the hypothalamus.

References


**Key Words** • sympathetic nervous system • fatty acids • potassium
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