Insulin Blunts the Natriuretic Action of Atrial Natriuretic Peptide in Hypertension

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Abstract
Hyperinsulinemia and insulin resistance are implicated in the etiology of hypertension, but the mechanisms involved have not been established. The objectives of this study were to determine whether untreated essential hypertensive patients are more sensitive to the antinatriuretic action of insulin and more resistant to the counteracting natriuretic effect of atrial natriuretic peptide in contrast to age- and sex-matched normotensive control subjects. Urinary sodium excretion was measured at baseline, during hyperinsulinemic euglycemic clamp, and during coadministration of insulin and atrial natriuretic peptide. Baseline urinary sodium excretion was not significantly different in the normotensive subjects (415±47 μmol/min, n=12) and hypertensive patients (381±18 μmol/min, n=10); with the institution of insulin infusion, there was a similar and significant decline from baseline (P<.001) to 289±35 μmol/min in normotensive subjects and 235±17 μmol/min in hypertensive patients. Atrial natriuretic peptide was able to oppose the antinatriuretic action of insulin in normotensive subjects, increasing urinary sodium excretion significantly to a mean level of 352±31 μmol/min (P<.05), which did not differ significantly from baseline. In the hypertensive group, atrial natriuretic peptide infusion had no effect on urinary sodium excretion (238±18 μmol/min), and the difference from baseline remained highly significant (P<.001). The antinatriuretic action of insulin was less effective than their normotensive counterparts, as reflected by a lower glucose utilization rate and higher mean baseline plasma insulin level (P<.05 for each). We conclude that resistance to the natriuretic action of atrial natriuretic peptide may be a pathogenetic link between insulin resistance and hypertension. (Hypertension. 1994;23[part 2]:1054-1058.)

Key Words: hypertension, essential • insulin • insulin resistance • natriuretic peptide, atrial • sodium • aldosterone • glomerular filtration rate • lithium

Hypertension and insulin resistance are implicated in the etiology of hypertension, but the mechanisms involved have not been established. Attention has focused on the sympathetic neural and vasodilator effects of insulin, but much less information is available on its effect on body fluid and sodium homeostasis, despite the importance of the kidneys in arterial pressure regulation and the pathogenesis of hypertension.

In studies we performed on healthy human subjects, the coinfusion of insulin and atrial natriuretic peptide (ANP) in a low dose completely abolished the well-described antinatriuretic action of insulin. The same dose of ANP alone, however, had no effect on urinary sodium excretion (UUN,V). We concluded that hyperinsulinemia alone is unlikely to cause hypertension because any tendency for sodium retention would ultimately stimulate ANP release and return sodium balance to normal, thus maintaining euvolemia and normal blood pressure (BP) levels. If hyperinsulinemia has an etiologic role in hypertension mediated in part through alterations in sodium balance, then there must be either exaggerated sodium retention in response to insulin or attenuation of the renal "escape" mechanisms.

The purpose of this study was to assess the relation between ANP and insulin in essential hypertension. The specific aims were to examine whether the antinatriuretic response to hyperinsulinemia is enhanced in essential hypertension and whether ANP can counteract the sodium-retaining action of insulin in hypertensive patients. We found that in contrast to normotensive control subjects, ANP in a low dose did not counteract the antinatriuretic effect of insulin in hypertensive patients and conclude that resistance to the natriuretic action of ANP may be a pathogenetic link between insulin resistance and hypertension.

Methods
Subjects
The 10 male hypertensive patients who participated in this study were recruited from hypertension clinics at university-affiliated hospitals and the 12 normotensive control subjects from local advertisement. Some control subjects were part of another report. Study subjects ranged in age from 25 to 45 years. The study protocol was approved by the Human Subjects Review Committee of the University of Toronto, and all participants gave written informed consent. Subjects were excluded if they had any significant comorbid conditions or were taking medications on a regular basis. Hypertensive patients on antihypertensive medications had them withdrawn slowly, and their BP was assessed at weekly intervals. If the diastolic BP rose to greater than 109 mm Hg, drug treatment was resumed and the patient was excluded from the study. After a drug-free period of 2 weeks, each subject had a 24-hour ambulatory BP recording. Those who were hypertensive and met the eligibility criteria were enrolled in the study. Control subjects were matched for age and sex. A clinical history was taken and physical examination performed on all study subjects. None had any significant current health problems or evidence of liver or heart disease. All had normal renal function and negative urinalysis.

Preparation and Procedures
Subjects were studied after being maintained on a constant sodium diet of 150 mmol/d for 7 days. Dietary compliance was
assessed by determining $U_{\text{Na}} V$ on the day before the study. Those whose $U_{\text{Na}} V$ deviated more than 10% from the prescribed intake were excluded from the study. Subjects were studied in the Clinical Investigation Unit, The Toronto Hospital, on an ambulatory outpatient basis. They refrained from caffeine and alcohol ingestion for 48 hours and from tobacco for at least 16 hours before the study. Subjects were given 600 mg lithium carbonate orally at midnight, and the studies were performed the following morning at 8:30 AM in the postabsorptive state after an overnight fast.

On arrival at the study center, subjects voided and then drank 10 mL/kg of water over a period of 45 minutes to induce water diuresis. Hydration was sustained by the oral administration of 200 mL of water hourly. Two 21-gauge peripheral venous catheters were inserted into veins in the left forearm and connected to infusion pumps (Harvard Apparatus) for administration of insulin, para-aminohippurate (PAH), and ANP into one catheter and insulin and glucose into the other. A third catheter (19-gauge butterfly) was placed in a retrograde fashion in a superficial vein in the contralateral hand or wrist for blood sampling. Arterialization of venous blood was achieved by placing the hand with the third sampling catheter in a warming chamber at 69°C.

A priming dose of 20% PAH (8 mg/kg) and insulin (60 mg/kg) was administered as a bolus followed by constant infusions to maintain plasma concentrations at 1.5 and 20 mg/dL, respectively. The euglycemic insulin clamp technique was used to maintain steady-state hyperinsulinenia. This entailed administration of a priming dose of insulin (840 μU/m² Humulin R, Eli Lilly) intravenously over 10 minutes followed by a continuous infusion of insulin at a rate of 40 mU/m² of body surface area per minute (Humulin). Plasma glucose infusion was started 4 minutes after the insulin infusion. Plasma glucose concentration was maintained at the fasting level by measuring arterialized venous blood for plasma glucose every 5 minutes and subsequently adjusting the 20% glucose infusion rate. Human ANP (Institut Armand Frappier) was infused at a rate of 0.5 pmol/kg per minute. Arterial BP and heart rate were measured every 15 minutes with an automatic BP measuring device (Dinamap, model 1846SX, Critikon Inc).

Protocol

The study was conducted in three sequential periods on one occasion. In period 1, the baseline period, two 30-minute accurately timed urine collections were obtained by spontaneous voiding after a 60-minute equilibration period. In period 2, the euglycemic insulin clamp was begun, and after 1 hour two additional 30-minute urine collections were obtained. In period 3, the clamp continued and an ANP infusion was begun and continued with insulin for an additional hour. Urine was collected every 30 minutes. Blood samples were drawn at the midpoint of each urine collection period and analyzed for insulin, PAH, electrolytes, osmolality, lithium, cyclic GMP (cGMP), catecholamines, and aldosterone. Urine samples were analyzed for insulin, PAH, electrolytes, osmolality, lithium, and cGMP.

Analytic Methods

Serum sodium and lithium and urinary sodium concentrations were measured by flame photometry and urine lithium by atomic absorption spectrophotometry. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments). Plasma insulin, aldosterone, and ANP were determined by radioimmunoassay and plasma catecholamines by high-performance liquid chromatography with electrochemical detection. Inulin concentrations in plasma and urine were measured by a modified method of Walser et al and PAH concentration by the spectrophotometric method of Brun.

Clearance values were obtained from standard clearance formulas. PAH clearance was taken as a measure of effective renal plasma flow (ERPF) and insulin clearance as a measure of glomerular filtration rate (GFR). Clearance results are the means of clearance determinations during each phase of the study and are corrected for body surface area. Filtration fraction was calculated as GFR/ERPF. Segmental tubular sodium handling was estimated using lithium clearance. The M value was defined as the quantity of glucose infused expressed as milligrams per kilogram per minute to maintain euglycemia during minutes 60 to 120 of the insulin infusion. The lower the M value, the greater the insulin resistance.

Statistical Analysis

The Statistical Analysis System (SAS Institute Inc) was used for statistical analysis. Results are expressed as mean±SEM. Repeated-measures ANOVA was used to assess between- and within-group differences at each study period. Student's t test for independent samples was used to test for differences in demographic and physiological characteristics between hypertensive patients and normotensive subjects. A probability value of less than .05 was considered statistically significant, and appropriate adjustments in the probability value were made to account for multiple comparisons.

Results

Study Population

By plan there was a significant difference in BP levels, with the mean pressure being 149±5/89±4 mm Hg in the hypertensive group and 123±7/73±2 mm Hg in the normotensive group ($P<.001$). The hypertensive patients were insulin resistant, having a significantly higher fasting baseline insulin level (80.2±13 pmol/L) and lower M value (4.2±0.7 mg/kg per minute) than the control subjects (43.0±5 pmol and 6.1±0.5 mg/kg per minute, respectively, $P<.05$ for each). The hypertensive patients were slightly older (34±2 versus 30±2 years) and heavier (body mass index, 28.0±1.8 versus 24.4±0.8 kg/m²) than the normotensive subjects, but the differences were not statistically significant. Baseline ANP levels were comparable and normal in the two groups (Fig 1).

Humoral Effects

The euglycemic clamp technique resulted in a 10-fold elevation in plasma insulin levels in the normotensive subjects to 434±31 pmol/L during insulin alone and 422±23 pmol/L with ANP confusion; the mean levels in the hypertensive patients were comparable, being 635±128 and 517±70 pmol/L, respectively, $P<.05$ minute, respectively, $P<.05$ for each). The hypertensive patients were slightly older (34±2 versus 30±2 years) and heavier (body mass index, 28.0±1.8 versus 24.4±0.8 kg/m²) than the normotensive subjects, but the differences were not statistically significant. Baseline ANP levels were comparable and normal in the two groups (Fig 1).

No significant difference was observed in either group in baseline plasma levels of norepinephrine (1.67±0.42 versus 1.32±0.14 nmol/L), hypertensive patients versus normotensive subjects) or epinephrine (0.30±0.11 versus 0.32±0.07 nmol/L, hypertensive patients versus non-
that observed at baseline. The reduction in mean aldosterone level between the period of insulin alone and that with ANP was significant in the hypertensive group (P=.015) and approached but did not reach conventional statistical significance (P=.056) in the control group.

Renal Hemodynamics
Baseline GFR was similar in the two groups (121±7 and 114±5 mL/min in normotensive subjects and hypertensive patients, respectively). On the other hand, ERPF was significantly higher and filtration fraction significantly lower in the normotensive than in the hypertensive group (P<.05). There was no change in these relations during the study.

Segmental Tubular Sodium Handling
Baseline U_{NaV} was not significantly different in the normotensive subjects (415±47 μmol/min) and hypertensive patients (381±18 μmol/min). With institution of insulin, there was a similar and significant decline in U_{NaV} from baseline (P<.001) to 289±35 μmol/min in the normotensive subjects and 235±17 μmol/min in the hypertensive patients (Fig 2). ANP was able to oppose the antinatriuretic action of insulin in normotensive subjects, increasing U_{NaV} significantly to a mean level of 352±31 μmol/min (P<.05), which did not differ significantly from baseline. In the hypertensive group, on the other hand, ANP infusion had virtually no effect on U_{NaV} (238±18 μmol/min), and the difference from baseline remained highly significant (P<.001). The results of fractional sodium excretion paralleled the U_{NaV} results (Table). Fractional lithium excretion, a measure of proximal tubular function, increased, and the fractional proximal sodium reabsorption rate decreased in both groups; the fractional distal sodium reabsorption rate, an indicator of distal tubular effect, increased significantly (P<.001) in both groups during insulin infusion. The effects on the fractional excretion of lithium, absolute proximal sodium reabsorption rate, and absolute distal sodium reabsorption rate were similar in the two groups.

Miscellaneous
No difference was noted between groups in baseline hematocrit, osmolality, or heart rate. BP remained significantly higher in the hypertensive group through-
In accordance with previous reports, the hypertensive patients had a lower M value, as determined by the euclidean clamp, and a higher mean baseline insulin level than their normotensive counterparts, indicating impaired insulin action on glucose metabolism. The renal sodium effect of insulin, on the other hand, was not impaired, as judged by the similar antinatriuretic response to insulin in the two groups. This finding is in keeping with the reports of Finch et al in experimental hypertension, Rocchini et al in insulin-resistant obese adolescents, and Natali et al in human hypertension. The novel finding of the present study is the lack of a natriuretic response to the infusion of ANP in the hypertensive group that was not related to a difference in plasma ANP levels. This unresponsiveness in the presence of hyperinsulinemia is another manifestation of the abnormal renal sodium handling found in hypertension.7,16

In animal models of hypertension and studies of humans with essential hypertension, the natriuretic response to ANP infusion in the absence of hyperinsulinemia is reported to be normal or even exaggerated compared with normotensive control subjects.17,18 Heightened responsiveness may be related to a higher renal perfusion pressure, because this exaggerated effect was no longer apparent in untreated essential hypertension when systemic arterial pressure was reduced to normotensive levels by concomitant nitroprusside infusion.19 The modulating role of renal perfusion pressure on the natriuresis induced by ANP has also been demonstrated in experimental studies. For example, Seymour et al20 showed that stepped reductions in renal perfusion pressure resulted in a progressive fall in the fractional excretion of sodium during ANP administration. It is suggested that a higher renal perfusion pressure in hypertensive patients is required to maintain sodium balance21,26 and may account for the failure to find elevated plasma ANP levels in uncomplicated hypertension.18 Our finding of similar ANP levels in normotensive subjects and hypertensive patients at baseline is consistent with this suggestion.

The increase in urinary and serum concentrations of cGMP, the major second messenger for ANP, in the two groups during ANP infusion suggests that ANP resistance is not related to downregulation of its receptors or to an impaired intracellular biochemical response. Instead, this finding points to systems that antagonize the effects of ANP. Resistance to the natriuretic action of ANP is well documented in many disorders21,22 and has been attributed in part to heightened renal adrenergic nerve activity.23 It has been shown that stimulation of renal sympathetic nerves or systemic administration of norepinephrine attenuates the natriuretic response to ANP.24,25 Because sympathetic nervous system overactivity has been demonstrated in essential hypertension with preferential activation of renal and cardiac sympathetic nerves,26 it is not reasonable to suspect that increased nervous system activity may account for the blunted natriuresis to ANP in our hypertensive patients. The fact that plasma catecholamine levels were not different in the two groups does not exclude this possibility in light of the well-known insensitivity of this measure to reflect overall and regional sympathetic nervous system activity.

The results of our study do not support the possibility that resistance to the natriuretic action of ANP was related to the failure of ANP to suppress aldosterone release in the hypertensive patients, which indirectly would enhance the antinatriuretic effect of insulin. Instead, there was equal or greater suppression of plasma aldosterone levels in the hypertensive group during the coadministration of insulin and ANP, and the mean levels between the two groups did not differ significantly.

We previously reported that the coinfusion of insulin and low-dose ANP completely abolished the well-described antinatriuretic action of insulin in normotensive subjects.8 In that report, as in the present study, cGMP concentration in blood and urine increased, and plasma

### Table: Segmental Tubular Sodium Handling

<table>
<thead>
<tr>
<th>Parameter Tubular Sodium Handling</th>
<th>Hypertensive</th>
<th>Baseline</th>
<th>Insulin</th>
<th>Insulin and ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td>FENa, mmol/min</td>
<td>Hypertensive</td>
<td>0.022±0.002</td>
<td>0.013±0.001*</td>
<td>0.014±0.001*</td>
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<tr>
<td></td>
<td>Normotensive</td>
<td>0.022±0.002</td>
<td>0.017±0.001*</td>
<td>0.020±0.001</td>
</tr>
<tr>
<td>UNaV, µmol/min</td>
<td>Hypertensive</td>
<td>235±17</td>
<td>238±18*</td>
<td>352±31</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>289±35</td>
<td>352±31</td>
<td>352±31</td>
</tr>
<tr>
<td>FEi, %</td>
<td>Hypertensive</td>
<td>0.20±0.01</td>
<td>0.22±0.02</td>
<td>0.24±0.02</td>
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<tr>
<td></td>
<td>Normotensive</td>
<td>0.22±0.02</td>
<td>0.25±0.02</td>
<td>0.31±0.05</td>
</tr>
<tr>
<td>APRNa, mmol/min</td>
<td>Hypertensive</td>
<td>12.3±0.6</td>
<td>12.7±0.9</td>
<td>10.9±0.6</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>12.8±0.8</td>
<td>11.1±0.7</td>
<td>11.2±0.9</td>
</tr>
<tr>
<td>FFRNa, %</td>
<td>Hypertensive</td>
<td>0.79±0.02</td>
<td>0.79±0.02</td>
<td>0.76±0.02</td>
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<tr>
<td></td>
<td>Normotensive</td>
<td>0.78±0.01</td>
<td>0.75±0.02</td>
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<tr>
<td>ADRNa, mmol/min</td>
<td>Hypertensive</td>
<td>2.9±0.4</td>
<td>3.2±0.4</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>3.3±0.3</td>
<td>3.5±0.3</td>
<td>4.3±0.6</td>
</tr>
<tr>
<td>FDRNa, %</td>
<td>Hypertensive</td>
<td>0.88±0.02</td>
<td>0.94±0.01*</td>
<td>0.94±0.01*</td>
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<tr>
<td></td>
<td>Normotensive</td>
<td>0.90±0.01</td>
<td>0.93±0.01*</td>
<td>0.92±0.01*</td>
</tr>
<tr>
<td>FLNa, mmol/min</td>
<td>Hypertensive</td>
<td>15.5±0.8</td>
<td>16.2±1.2</td>
<td>14.4±0.7</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>16.4±1.0</td>
<td>14.8±0.9</td>
<td>15.8±0.9</td>
</tr>
</tbody>
</table>

ANF indicates atrial natriuretic factor, FENa, fractional excretion of sodium; UNaV, urinary sodium excretion; FEi, fractional excretion of lithium; APRNa and FFRNa, absolute and fractional proximal sodium reabsorption rates, respectively; ADRNa and FDRNa, absolute and fractional distal sodium reabsorption rates, respectively; and FLNa, filtered load of sodium. Values are mean±SEM calculated from means of clearance determinations during each phase of the study. See text for descriptions of studies.

*P<.01 compared with baseline.
aldoosterone level fell significantly even though the dose of ANP infused did not on its own induce natriuresis. The fact that modest elevations in plasma ANP level can counteract the sodium-retaining effects of insulin in normotensive subjects strongly suggests that under normal circumstances ANP plays an important role in sodium homeostasis by blocking the actions of antinatriuretic forces that may be normally operating to increase circulating blood volume.

In experimental hypertension associated with hyperinsulinemia, alterations in renal sodium handling and the natriuretic effect of ANP have been demonstrated. In the fructose-induced model of hypertension, elevated plasma ANP and reduced plasma aldosterone levels suggest the presence of hypervolemia and indicate resistance to ANP action. Chronic infusion of insulin in rats transiently reduces $U_{NaV}$ from which the kidneys “escape”; in this model sodium balance is maintained at the expense of a higher mean arterial pressure. Although ANP levels were not measured in this study, the evidence pointing to increased sympathetic nervous system activity as the mechanism responsible for hypertension raises the possibility that the response to ANP infusion would be attenuated in this form of hypertension.

In our previous report in normotensive subjects we concluded, based on lithium clearance data, that insulin caused an antinatriuresis by stimulating reabsorption of sodium at a distal tubular site and that ANP likely inhibited this effect by acting directly on the inner medullary collecting duct. In the hypertensive group in the present study, the calculated fractional proximal sodium reabsorption rate fell, whereas the fractional distal sodium reabsorption rate increased significantly during insulin infusion, supporting the contention that the major site of the antinatriuretic action of insulin is in the distal nephron. It is tempting to speculate that the inability of ANP to overcome this effect of insulin in the hypertensive group points to resistance occurring at the level of the inner medullary collecting duct. Support for this speculation is the observation that $\beta$-adrenergic stimulation increases sodium reabsorption in the inner medullary collecting duct and the mounting evidence of the quantitative importance of this segment of the nephron in regulating renal sodium output.

Acknowledgments

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