Relationship between insulin release, antinatriuresis and hypokalaemia after glucose ingestion in normal and hypertensive man

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1. Insulin simultaneously causes hypokalaemia and antinatriuresis, and it has been suggested that the two effects are tightly coupled. Whether these actions are preserved in patients with essential hypertension is not known.

2. Eight hypertensive patients and eight normotensive control subjects were studied before and after the ingestion of 75 g of glucose. Despite similar glycaemic profiles, the patients showed a hyperinsulinaemic response incremental area $49\pm8$ versus $27\pm6$ nmol/l^{-1} 3h, $P<0.04$) but a blunted hypokalaemic response ($-7\pm1$ versus $-16\pm1\%$, $P<0.001$). Both absolute and fractional urinary excretion of sodium and potassium were significantly decreased during glucose-induced hyperinsulinaemia in hypertensive patients as well as in normotensive subjects ($P<0.05$ for all changes).

3. To test whether hypokalaemia is required for insulin-induced antinatriuresis, each hypertensive patient received another oral glucose load during which enough potassium chloride was given to clamp the plasma potassium concentration at baseline. Under these conditions, significant insulin-induced antinatriuresis still occurred. In addition, whereas the glycaemic profile was superimposable, the response of the plasma insulin concentration was significantly greater with than without maintenance of the plasma potassium concentration (total area $79\pm14$ versus $63\pm8$ nmol/l^{-1} 3 h, $P<0.04$).

4. We conclude that (a) insulin causes antinatriuresis, antikaliuresis and hypokalaemia under physiological conditions; (b) in hyperinsulinaemic (insulin-resistant) patients with essential hypertension, the antinatriuretic action of insulin is quantitatively preserved; and (c) clamping plasma potassium levels prevents insulin-induced antikaliuresis but not antinatriuresis, and potentiates the insulin secretory response to glucose.

INTRODUCTION

Essential hypertension is often associated with hyperinsulinaemia [1] and insulin resistance [2]. Whether these metabolic abnormalities play a causal role in the genesis or maintenance of elevated blood pressure (BP) level is still largely unknown. One of the possible mechanisms through which insulin might raise BP is by reducing urinary sodium excretion. This action of insulin has been extensively documented in lean [3–5] and obese [6] normotensive subjects, but not in patients with essential hypertension. If this effect of insulin were preserved in otherwise insulin-resistant patients, then the attendant compensatory hyperinsulinaemia could chronically stimulate antinatriuresis and thereby conceivably contribute to high BP.

Insulin-induced antinatriuresis has been studied with the use of the insulin clamp technique during forced water diuresis in previously salt-loaded subjects [3, 5]. This experimental setting makes it possible to assess the action of insulin on renal sodium handling relatively independently of other hormonal or haemodynamic influences; however, it does not provide an indication of the physiological relevance of this action. Moreover, recent work has shown that insulin-induced antinatriuresis during euglycaemic hyperinsulinaemia is abolished when the hypokalaemic effect of the hormone is prevented by a simultaneous infusion of potassium chloride [7]. On these grounds it has been argued that insulin-induced antinatriuresis is not a direct action on the distal renal tubule [3, 4], but is rather the consequence of insulin action on potassium metabolism. Whether an interaction between antinatriuresis and hypokalaemia occurs under physiological conditions is not known.

The present study was undertaken (a) to measure the antinatriuretic effect of insulin under physiologi-
cal conditions in patients with essential hypertension who have an enhanced insulin secretory response to glucose, and (b) to test whether, under the same conditions, insulin-induced hypokalaemia is linked to insulin-induced antinatriuresis.

MATERIALS AND METHODS

Subjects

Eight ambulatory patients with essential hypertension were recruited from the Hypertension Clinic, and eight sex-, age- and weight-matched normotensive control subjects were enrolled (see Table 1). All hypertensive patients had undergone a recent complete clinical work-up to exclude diabetes, impaired glucose tolerance, liver disease and secondary forms of arterial hypertension. The mean known duration of the hypertensive disease was 6.6 years (range 3–15 years); no subject had ever been treated with diuretics or β-adrenoceptor blockers, and all other drugs were discontinued 1 week before the study. All subjects were instructed to maintain their dietary habits and lifestyle, and to avoid intense physical exercise for the entire duration of the study period which, on average, lasted 7 days (range 5–9 days). The nature of the investigation and the potential risks associated with it were fully explained to all subjects, who gave their consent before participating in the study. The research protocol was approved by the Institutional Review Board of the C.N.R. Institute of Clinical Physiology.

Procedures

All subjects received an oral glucose tolerance test (OGTT study). Studies began at 07.00 hours, after an overnight fast, and took place in a quiet room with constant temperature. After a Teflon cannula was inserted into an antecubital vein, subjects were invited to void, and then sat in a comfortable chair for the entire duration of the study. The intravenous line was kept patent by a slow drip delivering a volume of saline calculated to replace blood loss (see below). A period of 3 h preceded glucose ingestion, and served as baseline. To ensure an adequate urine flow, subjects were given 300 ml of water to drink at the beginning of this 3 h period. Urine was then collected at the end of the baseline period. At time zero, subjects ingested 75 g of glucose as a 50% solution in 150 ml of water. Another 150 ml of water was drunk by each subject in order to match the water load (300 ml) of the basal period. Glucose absorption was followed for 3 h, at the end of which urine was again collected. During the last hour of the baseline period, and for 3 h after glucose ingestion, blood samples were withdrawn from the antecubital vein for the determination of plasma concentrations of glucose, insulin, free fatty acids (FFA), sodium and potassium. Plasma creatinine and aldosterone concentrations were measured at hourly intervals. The sodium lost through blood sampling was exactly replaced by means of intravenously infused saline. It amounted to 4 ± 2 mmol during the 3 h of the baseline period, and 6 ± 2 mmol during the 3 h of glucose absorption. Arterial BP (mercury sphygmomanometry) and heart rate (HR) were measured at 30 min intervals throughout the study by the same physician, each reported value being the mean of three consecutive measurements.

In addition, each hypertensive patient underwent, in randomized order, two additional studies on separate days. On one occasion (termed the OGTT + K study), patients ingested 21 mmol of slow-release KCl divided into three tablets (KCl-Retard) before the glucose drink. Furthermore, after glucose ingestion the plasma potassium concentration was clamped at its baseline level by measuring the plasma concentration every 10 min and then adjusting the rate of intravenous infusion of a KCl (40 mmol/500 ml) solution. In order to minimize both the exogenous sodium load and water load, for this study protocol the KCl solution was made up as one-third saline and two-thirds water, and no additional water was given to drink. The total water load was 307 ± 40 ml (with the KCl infusion) plus 150 ml with the glucose drink. The total administered sodium was 4 ± 2 mmol during the baseline period, and 18 ± 4 mmol during the 3 h of glucose absorption. Since in the OGTT + K study blood loss was larger than with the OGTT study (due to the frequent measurements of plasma potassium concentration), sodium administration resulted in excess of sodium loss during glucose absorption by only 7 ± 4 mmol/3 h.

On a third occasion, patients underwent a time-control study, which was identical to the OGTT study except that 150 ml of water alone replaced the glucose solution at time zero.

Analytical procedures

Plasma glucose was assayed by the glucose oxidase method (Glucose Analyzer; Beckman Instruments, Fullerton, CA, U.S.A.). Plasma and urinary sodium and potassium were assayed in duplicate immediately after blood withdrawal by an ion-selective electrode method (Microlytes 6 Selective Ion Analyser; Kone Instruments, Espoo, Finland). For metabolite and hormone determinations, blood was placed into chilled tubes containing lithium–heparin, immediately spun, and the plasma was stored at −20°C until analysis. All analyses were carried out within 2 weeks. Plasma FFA were assayed by an enzymic method (Wako Chemical GmbH, Neuss, Germany). Plasma and urinary creatinine and serum cholesterol and triacylglycerol were assayed spectrophotometrically in duplicate, on an Eris Analyzer 6170 (Eppendorf Geratebau, Hamburg, Germany). Insulin and aldosterone were assayed in plasma by radioimmunoassay, (Insk 5 and Aldctk; Sorin Biomedica, Vercelli, Italy). For the latter assays, samples from
Table I. Clinical characteristics of the study groups. Values are means ± SEM. Statistical significance (unpaired t-test): *P < 0.05 compared with normotensive subjects.

<table>
<thead>
<tr>
<th></th>
<th>Normotensive subjects</th>
<th>Hypertensive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>7/1</td>
<td>7/1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 ± 2</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)†</td>
<td>25.9 ± 0.7</td>
<td>25.7 ± 1.0</td>
</tr>
<tr>
<td>Fat mass (%)‡</td>
<td>23 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115 ± 6</td>
<td>156 ± 5*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77 ± 3</td>
<td>103 ± 3*</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>40 ± 2</td>
<td>51 ± 2*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>62 ± 3</td>
<td>68 ± 2*</td>
</tr>
<tr>
<td>Serum cholesterol concn. (mmol/l)</td>
<td>5.69 ± 0.59</td>
<td>5.36 ± 0.22</td>
</tr>
<tr>
<td>Serum triacylglycerol concn. (mmol/l)</td>
<td>0.43 ± 0.06</td>
<td>0.43 ± 0.05</td>
</tr>
</tbody>
</table>

† Calculated as the ratio between weight (kg) and the squared value of height (m²).‡ Calculated by the formula of Hume [37].

all three studies of each hypertensive patient were analysed in the same run.

Data analysis and calculation

Sodium potassium and creatinine clearance rates were calculated as the ratio of the respective urinary output and the plasma concentration; the latter was the mean of three measurements during the baseline period, and the mean of six half-hourly measurements during glucose absorption. Fractional sodium and potassium excretion rates were computed as the ratio of sodium and potassium clearance, respectively, to creatinine clearance. Glucose and insulin areas under the curve were calculated by the trapezoidal rule.

All data are expressed as means ± SEM. Two-way analysis of variance with repeated measures over time was used to compare the results of time-related variables between hypertensive patients and normotensive subjects. The unpaired and paired t-test were employed to compare mean group values as appropriate. Both absolute and percentage changes were calculated and specified in the text. Two-way analysis of variance with doubly repeated measures was used to compare the results of time-related measurements between different studies in the hypertensive group. Linear regression was carried out by standard methods.

RESULTS

The characteristics of the study groups are summarized in Table I. Although hypertensive and normotensive subjects were accurately matched for gender, age, body mass index and body weight composition, the difference in resting BP level was wide. By selection, the least hypertensive patient had higher BP values (mean BP=109 mmHg) than the most ‘hypertensive’ of the control subjects (105 mmHg). Resting HR was slightly higher in the hypertensive patients than in the control subjects.

OGTT: hypertensive patients versus normotensive subjects

Fasting plasma glucose and insulin concentrations were similar in the hypertensive and normotensive groups (5.26 ± 0.09 versus 5.32 ± 0.13 mmol/l, and 62 ± 5 versus 54 ± 6 pmol/l, respectively). After glucose ingestion, in the hypertensive patients the time course of the plasma glucose level was slightly but consistently above that of the control subjects, although the difference fell short of statistical significance (P=0.12). In contrast, the insulin response to glucose (see Fig. 1) was significantly higher (P<0.04 by analysis of variance) in the hypertensive patients than in the control subjects, the incremental area under the curve being 60% greater in the former (48.5 ± 8.0 nmol l⁻¹ 3 h) than in the latter (26.9 ± 5.6 nmol l⁻¹ 3 h).

Fasting plasma potassium concentration was higher, and plasma sodium concentration was lower, in normotensive subjects than in hypertensive patients (Table 2). In both groups, glucose ingestion was followed by a decline in plasma potassium and aldosterone levels (Fig. 1). Relative to the basal (fasting) value, however, the mean decrease in plasma potassium concentration during the 3 h of glucose absorption was 7±1% in hypertensive patients as compared with 16±1% in control subjects (P<0.001). Likewise, the plasma aldosterone concentration decreased significantly (P<0.05) less in hypertensive patients than in normotensive subjects. Looking at the pooled data from both groups, the mean decrease in plasma potassium concentration during the OGTT was positively associated with the corresponding mean decrease in plasma aldosterone concentration (r=0.58, P<0.02), and negatively related to glucose-induced insulin release (expressed as the ratio of plasma insulin to plasma glucose incremental area r=0.61, P<0.02).

The basal plasma FFA concentration (0.714 ± 0.72 versus 0.385 ± 0.38 mmol/l, P<0.001) was significantly higher in hypertensive patients than in control subjects; after glucose ingestion, the plasma FFA concentration declined to a similar nadir in the two groups.

The two study groups also differed in their haemodynamic response to carbohydrate ingestion. The hypertensive group showed a small (6%) but consistent reduction in both systolic (SBP) and diastolic (DBP) blood pressure (P<0.001 and P<0.005 by analysis of variance, respectively) associated with a small (but statistically insignificant) increase in HR, so that the calculated double product index (SBP × HR) remained stable throughout the study. In the normotensive subjects, on the other hand, there was no systematic change in either BP or HR throughout the absorptive period.
Baseline urine output, creatinine clearance and urinary excretion of sodium and potassium were similar in hypertensive patients and normotensive subjects (Table 2). After glucose ingestion, both groups showed a marked reduction in urinary sodium and potassium excretion. The decrease in urinary sodium excretion tended to be greater in the hypertensive patients than in the control subjects (30 ± 13% versus 18 ± 9%), but this difference did not reach statistical significance when analysed by two-way analysis of variance (Table 2). Looking at the pooled results of both groups, glucose ingestion was found to be associated with a 23 ± 6% reduction in urinary sodium output and a 40 ± 5% drop in urinary potassium excretion.

In terms of fractional excretion, the analysis (Table 2) confirmed that glucose ingestion caused a significant decrease in fractional excretion of sodium. In addition, the fractional excretion of potassium also fell (by 26 ± 6%) with glucose feeding despite the concurrent decrease in plasma potassium concentrations. There was no significant difference between the two groups for either ion (Table 2).

### Table 2. Plasma electrolyte concentrations and urinary fluxes. Values are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Normotensive subjects</th>
<th>Hypertensive patients</th>
<th>Analysis of variance†</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Test*</td>
<td>Basal</td>
</tr>
<tr>
<td><strong>Plasma Na⁺ concn.</strong> (mmol/l)</td>
<td>137.3 ± 1.3</td>
<td>136.3 ± 1.5</td>
<td>140.6 ± 0.7</td>
</tr>
<tr>
<td><strong>Plasma K⁺ concn.</strong> (mmol/l)</td>
<td>4.54 ± 0.13</td>
<td>3.79 ± 0.12</td>
<td>3.74 ± 0.09</td>
</tr>
<tr>
<td><strong>Urinary output</strong> (ml/min)</td>
<td>1.37 ± 0.29</td>
<td>1.53 ± 0.34</td>
<td>1.72 ± 0.29</td>
</tr>
<tr>
<td><strong>Creatinine clearance</strong> (ml/min)</td>
<td>130 ± 14</td>
<td>115 ± 12</td>
<td>141 ± 9</td>
</tr>
<tr>
<td><strong>Urinary Na⁺ excretion</strong> (µmol/min)</td>
<td>115 ± 22</td>
<td>88 ± 12</td>
<td>142 ± 25</td>
</tr>
<tr>
<td><strong>Fractional Na⁺ excretion</strong> (%)</td>
<td>0.64 ± 0.11</td>
<td>0.57 ± 0.07</td>
<td>0.74 ± 0.14</td>
</tr>
<tr>
<td><strong>Urinary K⁺ excretion</strong> (µmol/min)</td>
<td>68 ± 8</td>
<td>38 ± 3</td>
<td>70 ± 11</td>
</tr>
<tr>
<td><strong>Fractional K⁺ excretion</strong> (%)</td>
<td>11.7 ± 0.9</td>
<td>8.9 ± 0.5</td>
<td>13.2 ± 1.9</td>
</tr>
</tbody>
</table>

*Mean values during 3 h after glucose ingestion.
†Two-way analysis of variance with repeated measure over the 'test' factor.
†Interaction term, P < 0.05.

Potassium replacement in hypertensive patients

No changes in plasma glucose, insulin and potassium concentrations were observed during the time-control study. After glucose ingestion, the plasma potassium concentration showed a mean decline of 0.27 ± 0.08 mmol/l during the OGTT, with a nadir (−0.40 ± 0.10 mmol/l) at 120 min. Potassium supplementation (OGTT + K) prevented this decline (Fig. 2). The amount of infused potassium necessary to maintain stable plasma levels ranged between 3 and 25 mmol/3 h, and averaged 13 ± 3 mmol/3 h. The infusion rate was highest between 90 and 120 min, when it averaged 1.4 ± 0.3 µmol min⁻¹ kg⁻¹. After glucose ingestion, the glycaemic profiles were superimposable regardless of potassium supplementation. In contrast, glucose-induced plasma insulin concentrations were higher when plasma potassium concentration was held constant (Fig. 2): the difference was more pronounced during the second hour but was consistent throughout the study, and was statistically significant both when comparing all time points (P < 0.05 by analysis of variance) and when comparing the area under the curves (63 ± 8 versus 79 ± 14 nmol l⁻¹ 3 h, P < 0.04 by paired t-test).

The plasma aldosterone concentration remained stable during the time-control study (from 330 ± 36 to 345 ± 48 pmol/l). During the OGTT, the plasma aldosterone concentration showed a slight fall (from 360 ± 42 to 289 ± 65 pmol/l), which did not reach statistical significance; in contrast, during the OGTT + K the plasma aldosterone concentration increased from 372 ± 43 to 512 ± 71 pmol/l (P < 0.05, Fig. 2).

Table 3 and Fig. 3 give the plasma concentrations and urinary fluxes of ions during this protocol. Potassium supplementation fully prevented the fall in absolute and fractional excretion of potassium after glucose ingestion. Nevertheless, urinary sodium excretion decreased during glucose absorption whether or not kalaemia was maintained. In comparison with the time-control study, both the OGTT and OGTT + K resulted in a significant decrease in both absolute and fractional natriuresis. The decrease in natriuresis was similar during the OGTT and OGTT + K study, whether expressed in...
Insulin-induced antinatriuresis in hypertension

Fig. 1. Plasma glucose, insulin, potassium and aldosterone concentrations in normotensive subjects (○) and hypertensive patients (●) in the fasting state and in response to glucose ingestion. Vertical bars are SEMs.

Fig. 2. Plasma glucose, insulin, potassium and aldosterone concentrations in eight hypertensive patients after oral glucose (OGTT, ——) oral glucose plus potassium supplementation (OGTT+K——) or saline (time-control, ———), all given at time 0. Vertical bars are SEMs.

absolute terms or normalized by the prevailing insulin concentration.

Basal plasma FFA levels were similar in the three studies. During the time-control study, the plasma FFA level increased slightly (from 0.590 ± 0.082 to 0.680 ± 0.052 mmol/l), whereas both OGTT and OGTT+K were associated with similar declines in the plasma FFA level.

DISCUSSION

Hypertensive patients versus normotensive subjects

The hypertensive patients selected for the present study were relatively young, untreated or in pharmacological washout, had normal body weight, serum lipid levels (Table 1) and glucose tolerance (Fig. 1); the known duration of their hypertensive disease was relatively short. On the other hand, the control subjects were accurately matched with the patients for age, obesity and serum lipid levels, but has low–normal BP values at rest, with no overlap with the hypertensive group. The insulin response to glucose was considerably heightened in the hypertensive patients compared with the control subjects (Fig. 1), with slightly higher plasma glucose concentrations as the likely stimulus for this hyper-response. Thus, these patients presumably were insulin-resistant. Epidemiological surveys and case-control studies have repeatedly found that high BP is associated with hyperinsulinemia [8], and that the degree of such hyperinsulinemia is directly related to the height of BP even when accounting for confounders [9–11]. In the present case-control study, the degree of hyperinsulinemia in the hypertensive group (330 pmol/l on average during 3h versus 203 pmol/l in the control subjects) is similar to that found in other case-control studies [12, 13]. The presence of fasting hypokalaemia (relative to control subjects) in the hypertensive patients may reflect the fact, consistently confirmed by large-scale investigations [14], that height of BP and plasma potassium concentrations are inversely related to one another independently of treatment. In other
Table 3. Plasma electrolyte concentrations and urinary fluxes in the hypertensive group under 3 experimental conditions. Values are means ± SEM. Statistical significance: *P < 0.05, †P < 0.01 compared with basal.

<table>
<thead>
<tr>
<th></th>
<th>Time-control study</th>
<th>OGTT study</th>
<th>OGTT + K study</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Test</td>
<td>Basal</td>
</tr>
<tr>
<td>Plasma Na⁺ concn. (mmol/l)</td>
<td>140.1 ± 0.7 140.3 ± 0.8</td>
<td>140.6 ± 0.7 140.7 ± 0.6</td>
<td>140.2 ± 0.8 139.9 ± 0.7</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>156 ± 18 149 ± 19</td>
<td>141 ± 9 135 ± 11</td>
<td>135 ± 18 144 ± 19</td>
</tr>
<tr>
<td>Urine output (ml/min)</td>
<td>1.48 ± 0.22 1.18 ± 0.18</td>
<td>1.72 ± 0.29 1.25 ± 0.30*</td>
<td>1.50 ± 0.22 1.50 ± 0.35</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion (μmol/min)</td>
<td>113 ± 26 104 ± 24</td>
<td>142 ± 25 104 ± 23*</td>
<td>143 ± 40 126 ± 39†</td>
</tr>
<tr>
<td>Urinary K⁺ excretion (μmol/min)</td>
<td>73 ± 13 57 ± 9</td>
<td>70 ± 11 39 ± 7†</td>
<td>61 ± 15 63 ± 10</td>
</tr>
</tbody>
</table>

Incidentally, fasting plasma FFA concentrations, BP and HR all were higher in the hypertensive patients than in control subjects. Those features are compatible with the possibility that in this particular group of patients the adrenergic system was overactive in the resting state, resulting in relative hypokalaemia and accelerated lipolysis [15].

The response to glucose ingestion differed between hypertensive patients and normotensive subjects in another important respect. The hypokalaemic effect of insulin was blunted in the hypertensive patients (Fig. 1) despite their relative hyperinsulinaemia. Descriptively, this is resistance to the action of endogenous insulin on potassium metabolism. The finding might reflect a true impairment of the ability of the hormone to promote potassium uptake into the liver or the peripheral tissue [16]. In the absence of direct measurements of potassium metabolism, however, it should be borne in mind that changes in the plasma concentration are the net result of uptake and release of the ion in several organs [17]. The smaller change in the plasma potassium level in the hypertensive patients may be related to their lower starting plasma concentration potassium (Fig. 1). Alternatively, the smaller hypokalaemic effect of insulin in the hypertensive group could be related to the nadir in plasma potassium concentration reached during the OGTT, which was similar in the two groups (Fig. 1). Hepatic catheterization studies [16] have shown that, with the development of hypokalaemia, the liver shifts from a net uptake to a net release of potassium despite ongoing insulin stimulation. It is therefore possible that the threshold at which the hepatic switch in potassium handling occurs was reached more quickly in the hypertensive patients than in the control subjects on account of the lower starting values. Another explanation could be down-regulation by hypokalaemia of Na⁺/K⁺-ATPase concentration in skeletal muscle tissue [18].

Finally, in response to glucose ingestion the hypertensive patients showed a significant decrease in both DBP and SBP, which was not seen in the control subjects. In normal subjects, glucose ingestion has been reported to induce a modest (5–10%) rise in SBP with no change in DBP [19, 20] or no changes in either [21]. It is possible that we failed to detect changes in BP because it was measured with a sphygmomanometer at 30 min intervals, whereas in some of the other studies continuous intra-arterial recording was employed. However, it should be noted that our subjects rested in the sitting position for 2 h before baseline BP was
measured. Furthermore, in the hypertensive group arterial BP showed a similar sustained 66% decrement during both the OGTT and the OGTT+K study (P<0.05 for both), whereas no change in SBP and a slight increase in DBP (P=0.08) were detected during the time-control study (data not shown). The mechanism underlying this phenomenon cannot be established by this study; possibly, in the hypertensive patients splanchnic [22] vasodilatation after glucose ingestion was imperfectly matched by an increase in cardiac output.

In contrast to the response of plasma insulin and potassium concentrations, hypertensive patients and control subjects were similar with regard to glucose-induced antinatriuresis and antikaliuresis. The antinatriuretic effect of insulin has been extensively documented in normal subjects [3-7, 71], in obese patients [6] and, indirectly, in diabetic patients [23], but not in patients with essential hypertension. Moreover, the experimental protocols employed to demonstrate insulin-induced antinatriuresis have all included forced water diuresis, often associated with chronic salt loading [3, 6, 7]. Under these conditions, both antidiuretic hormone and aldosterone secretion are suppressed. Consequently, the handling of both sodium and potassium by the kidneys becomes dependent mainly on tubular transport systems. Although this condition is ideal to document an independent effect of insulin on renal sodium handling, it is far from the conditions of everyday life. Our results indicate that the antinatriuretic property of insulin is operative under physiological conditions, and is preserved in essential hypertension. Although in the hypertensive patients as a group the mean decrease in fractional sodium excretion was not statistically different from that of the normotensive group, in the whole series insulin-induced antinatriuresis (i.e. the difference between basal and post-load fractional sodium excretion) was quantitatively related to the insulin area (r=0.58, P<0.02). This finding clearly suggests that insulin-induced antinatriuresis was intact in the hypertensive patients. Furthermore, insulin-induced antinatriuresis was significantly related to the basal mean BP value (r=0.56, P<0.03). Thus, the dissociation between an impaired insulin action on glucose metabolism and a normal insulin effect on sodium metabolism is consistent with the possibility that the hyperinsulinaemia of hypertension may result in inappropriate sodium retention and, in the long term, in arterial hypertension. It is possible that the small decline in arterial BP (mean BP= -4±1 mmHg) observed during the test played a role in favouring antinatriuresis. Under strictly controlled experimental conditions, the acute pressure-natriuresis curve shows a large gain and a rapid onset [24]. However, in intact animals and man, acute variations in the renal perfusion pressure are effectively counterbalanced by several reflex mechanisms (myogenic autoregulation, plasma catecholamines, angiotensin, renal nerve activity), which act in concert to maintain kidney function and haemodynamics. Moreover, in essential hypertension the slope of the acute pressure-natriuresis curve is flatter than that of normotensive subjects and borderline hypertensive subjects [25]. Therefore, it appears unlikely that the observed BP decrease in our hypertensive patients contributed to antinatriuresis significantly.

Role of potassium

In addition to hyperinsulinaemia, hyperglycaemia and hypokalaemia may also participate in the antinatriuresis induced by glucose ingestion. With regard to the role of potassium, our replacement experiments clearly demonstrate that preventing hypokalaemia does not alter insulin-induced antinatriuresis. This result is in contrast to that reported by Friedberg et al. [7], who found no effect of insulin on sodium excretion when plasma potassium was replaced during a euglycaemic insulin clamp. The discrepancy can be explained by the experimental conditions of the two studies. Under hyperglycaemic conditions, an excess of glucose enters the glomerulus and is entirely reabsorbed at the level of the proximal tubule along with sodium with a molar ratio of 1:1. During our OGTT study, the mean increase in plasma glucose concentration was 1.94 mmol/l, whereas the plasma sodium concentration remained stable at 141 mmol/l. Thus, the hyperglycaemia observed during the test was responsible for the reabsorption of 1.4% of the filtered sodium load, which, in absolute terms, is of the same magnitude as the overall fractional sodium excretion rate. However, this event takes place in the proximal tubule, in which small changes in sodium handling have little impact on the final sodium balance. In addition, sodium/glucose co-transport is determined by the gradient generated by the Na⁺/K⁺-ATPase pump at the basolateral membrane and in turn generates a gradient just like sodium transport alone. Thus, an increase in sodium/glucose co-transport simply modifies the proportions of 'alone' versus 'coupled' sodium reabsorption. Finally, in the dog the hyperglycaemic clamp induced a similar antinatriuresis when compared with the euglycaemic clamp [26]. With regard to the hormonal milieu, when insulin-induced antinatriuresis was measured under conditions of salt and water loading [7] basal aldosterone levels were suppressed by ~50% compared with our subjects. Nevertheless, hyperinsulinaemia, antinatriuresis and hypokalaemia were similar in degree in healthy subjects under clamp conditions [7] and in our hypertensive patients during oral glucose loading (Fig. 1 and Table 2). Therefore, differences in basal aldosterone concentrations (or in their changes during the tests) do not appear to play a major role in insulin-induced antinatriuresis. In support of this conclusion are studies in rats [27] showing that angiotensin-converting enzyme inhibition by capto-
potassium but also the sodium and fluid lost by
humans [3]. In our studies, therefore, much care was taken to replace not only
concentration and plasma osmolarity were clamped
specifically chosen to avoid volume-induced natriur-
when both plasma potassium concent-
the combination of high potassium
isteresis. Thus, when both plasma potassium con-
block insulin-mediated stimulation of sodium reab-
regard to this, studies in Brattleboro
vasopressin-deficient) rats have shown that insulin
insulin levels are accompanied by
results. Thus, when both plasma potassium con-
nal hypertension, the antinatriuretic action of
concentration were required to bring out
detectable changes in the plasma insulin con-
sodium infusion does not cause antinatriuresis unless anti-
levels (i.e. insulin area during OGTT + K versus OGTT) were seen in
patients with lower fasting plasma potassium
levels ($r = 0.67, P < 0.06$), higher diastolic BP ($r = 0.71, P < 0.05$), higher insulin response to glucose ($r = 0.69, P < 0.05$) and stronger insulin-induced antinatriuresis ($r = 0.72, P < 0.05$). This pattern of asso-
ciations suggests that the more insulin-resistant sub-
ject is prone to further hyperinsulinaemia when the
restraining influence of a relatively low plasma
potassium concentration on insulin secretion is
(i.e. when potassium is supplemented). It is
possible to speculate that potassium supplemen-
tation in hypertensive patients (at least, in insulin-
resistant ones), while lowering BP via multiple
mechanisms [34], has the potential to exacerbate
the hyperinsulinaemia, with the attendant conse-
quences on glucose tolerance but also on sodium
restitution. This may partly explain the variable
success of potassium supplementation in clinical
trials [35, 36].

In summary, we have found that under physiolo-
gical circumstances the insulin released in response to oral glucose causes antinatriuresis, antikaliuresis
and hypokalaemia, with no major change in BP. In
hyperinsulinaemic (insulin-resistant) patients with
essential hypertension, the antinatriuretic action of
insulin is quantitatively preserved, whereas the
hypokalaemic effect of the hormone is blunted.
Clamping plasma potassium levels under iso-
smolar conditions prevents insulin-induced antika-
liuresis but not antinatriuresis, and potentiates the
insulin secretory response to glucose. The clinical
implications of these findings are that long-term
insulin stimulation of antinatriuresis might con-
tribute to the rise in BP in some (insulin-resistant)
patients, and that, paradoxically, potassium supple-
mentation (although potentially beneficial in other
respect) might reinforce the antinatriuresis by
potentiating glucose-induced insulin release.

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