Insulin’s acute effects on glomerular filtration rate correlate with insulin sensitivity whereas insulin’s acute effects on proximal tubular sodium reabsorption correlate with salt sensitivity in normal subjects


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Abstract

Background. Insulin induces sodium retention by increasing distal tubular sodium reabsorption. Opposite effects of insulin to offset insulin-induced sodium retention are supposedly increases in glomerular filtration rate (GFR) and decreases in proximal tubular sodium reabsorption. Defects in these opposing effects could link insulin resistance to blood-pressure elevation and salt sensitivity.

Methods. We assessed the relationship between the acute effects of sequential physiological and supraphysiological insulin dosages (50 and 150 mU/kg/h) on renal sodium handling, and insulin sensitivity and salt sensitivity using the euglycaemic clamp technique and clearances of $[^{131}]$hippuran, $[^{125}]$iothalamate, sodium, and lithium in 20 normal subjects displaying a wide range of insulin sensitivity. Time-control experiments were performed in the same subjects. Salt sensitivity was determined using a diet method.

Results. During the successive insulin infusions, GFR increased by 5.9% ($P = 0.003$) and 10.9% ($P < 0.001$), while fractional sodium excretion decreased by 34 and 50% (both $P < 0.001$). Distal tubular sodium reabsorption increased and proximal tubular sodium reabsorption decreased. Insulin sensitivity correlated with changes in GFR during physiological ($r = 0.60, P = 0.005$) and supraphysiological ($r = 0.58, P = 0.007$) hyperinsulinaemia, but not with changes in proximal tubular sodium reabsorption. Salt sensitivity correlated with changes in proximal tubular sodium reabsorption ($r = 0.49, P = 0.028$), but not in GFR, during physiological hyperinsulinaemia. Neither insulin sensitivity or salt sensitivity correlated with changes in overall fractional sodium excretion.

Conclusions. Insulin sensitivity and salt sensitivity correlate with changes in different elements of renal sodium handling, but not with overall sodium excretion, during insulin infusion. The relevance for blood pressure regulation remains to be proved.

Key words: blood pressure; insulin; insulin sensitivity; lithium; salt sensitivity; sodium

Introduction

Insulin resistance has been associated with hypertension [1]. It has been suggested that the attendant hyperinsulinaemia contributes to blood pressure elevation by inducing renal sodium retention [2,3]. The renal-body fluid feedback mechanism for blood pressure control requires that compensatory mechanisms will restore sodium balance [4]. As a consequence, insulin resistance can only cause blood pressure elevation if its associated antinatriuretic stimulus is insufficiently opposed by a different natriuretic stimulus.

It has been demonstrated that insulin induces sodium retention by increasing distal tubular sodium reabsorption in insulin sensitive and insulin resistant subjects [5–12]. This antinatriuretic effect can be opposed by a concomitant decrease in proximal tubular sodium reabsorption [8,11,12] or an increase in glomerular filtration rate (GFR) [13,14]. Some previous observations support the hypothesis that insulin resistance is associated with impairments in these opposing natriuretic effects of insulin. It has been shown that insulin-mediated changes in renal plasma flow correlate with insulin-mediated changes in lithium clearance [15]. Therefore, it has been suggested that abnormal proximal sodium reabsorption due to impaired insulin-mediated renal vasodilatation may be present in insulin-resistant subjects [8,15]. Chronic hyperinsulinaemia increased GFR in normal dogs [14], but not in obese, insulin-resistant dogs [16]. These observations suggest that insulin could increase GFR, and thus the filtered sodium load, only in insulin-sensitive subjects.

Salt sensitivity is characterized by a reduction of the slope of the renal pressure natriuresis curve, suggesting
Study protocol

Informed consent was obtained from all subjects. The protocol had been approved by the local ethics committee, and the simultaneous infusion of saline was given. Urine was collected by spontaneous voiding. The week before the studies, the subjects were instructed to adhere to a diet containing 10 g NaCl daily; the extra amount and blood samples were drawn at the start and end of each study period. The subjects were given 300 ml of water orally each hour, subtracted by the amount of glucose water given during the clamp, to ensure adequate diuresis.

Blood pressure was measured using a semi-continuous blood-pressure-measuring device (Nippon Cohn BP 103 N Sphygnomanometer, Hayashi, Komaki-City, Japan). Five measurements were performed during each period of measurement.

Hyperinsulinaemic euglycaemic clamp studies

Sensitivity to insulin-mediated glucose uptake was assessed by the hyperinsulinaemic euglycaemic clamp technique, as described previously [12,27]. Insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark) diluted to 50 ml with 45 ml of 0.9% saline and 5 ml of 20% human albumin, was infused in a primed, continuous manner at a rate of 50 mU/kg/h for 90 min, succeeded by an infusion rate of 150 mU/kg/h for 180 min. Normoglycaemia was maintained by adjusting the rate of a 20% d-glucose infusion based on frequent plasma glucose measurements with an automated glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH, USA). As hepatic glucose production is completely suppressed in normal subjects at the insulin concentrations employed in the present study [28], whole-body glucose uptake (M value) was calculated from the glucose infusion rate during the last 45 min of each study period [29]. Blood samples for measurement of plasma insulin were drawn four times during last 45 min of the second, third and fourth study period. Plasma insulin concentrations were measured by radioimmunoassay (Immunoradiometric Assay, Medenix Diagnostics, Fleurus, Belgium).

Clearance studies

Renal plasma flow (RPF) and GFR were measured using the simultaneous infusion of [131I]hippuran and [125I]iothalamate, as described previously [30,31]. Briefly, a continuous infusion containing 100 μCi [131I]hippuran and 50 μCi [125I]iothalamate (Amersham, UK) in 100 ml of saline was administered at a rate of 12 ml/h, after a priming dose of 20 μCi [131I]hippuran and 10 μCi [125I]iothalamate had been given. Urine was collected by spontaneous voiding and blood samples were drawn at the start and end of each clearance period. The activities of [131I]hippuran and [125I]iothalamate in plasma, urine, and diluted infusion solution (1:100) were determined in duplicate using a well-type scintillation counter (1282 CompuGamma, LKB Wallac, Finland). Calculations of the clearance rates were made by using the formulae: I/V (plasma clearance) and U × V/P (renal clearance), where I is counts/min per millilitre of the diluted infusion solution, U is counts/min per millilitre urine, V is volume of the infusion or urine in ml/min, and P is counts/min per millilitre of plasma. RPF was calculated as the plasma clearance of [131I]hippuran (C\text{hip}). GFR was calculated as the renal clearance of [125I]iothalamate corrected for incomplete urine collection with the equation.

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Subjects and methods

Subjects

Twenty healthy Caucasian volunteers participated in the studies. They were recruited from a larger group of 47 volunteers who underwent assessment of their insulin sensitivity [26]. Both the 10 subjects with the lowest and those with the highest insulin sensitivity were requested to participate in the present study. All subjects were healthy as judged by medical history, were normotensive (blood pressure less than 140/90 mmHg) and had a normal 75-g oral glucose tolerance test. Seven subjects had a first-degree relative and seven others a second-degree relative with hypertension; one subject had a first-degree relative and seven others a second-degree relative with type II diabetes. They did not use any medication. Informed consent was obtained from all subjects. The protocol had been approved by the local ethics committee, and the study was carried out in accordance with the Declaration of Helsinki.

Study protocol

The week before the studies, the subjects were instructed to adhere to a diet containing 10 g NaCl daily; the extra amount of sodium necessary to reach this level was supplied with capsules containing 500 mg NaCl. Compliance was confirmed by measuring the 24-h urinary sodium excretion the last 2 days before the studies. The subjects received 300 mg of lithium carbonate orally at 10 p.m. the evening before the insulin infusion and the control study.

After an overnight fast, all subjects came to the clinic at 8 a.m. They were given 10 ml of sodium perchlorate before each study to block the thyroid gland. Two polytetrafluoroethylene cannulae (Venflon; Viggo, Helsinborg, Sweden) were inserted in forearm veins for intermittent blood sampling and infusions. After a 90-min stabilization period, there were four consecutive study periods of 90 min. Baseline measurements were performed during the first study period. A physiological insulin infusion was given during the second period, and a supraphysiological insulin infusion during the third and fourth period. An additional intravenous saline infusion (90 mmol NaCl/1.73 m²) was given as 0.9% NaCl during the fourth study period to test the ability of the kidney to process a sodium load under these circumstances. Measurements were performed during the last 45 min of each study period. The subjects were given 300 ml of water orally each hour, subtracted by the amount of glucose water given during the clamp, to ensure adequate diuresis.

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a disturbance in renal tubular sodium handling [14,17,18]. A possible role of insulin and insulin resistance in the development of salt sensitivity of blood pressure is not yet clear [17,19], but gains support from previous observations. Salt sensitivity has been associated with a hyperinsulinaemic response to oral glucose [20–22], and with insulin resistance in several studies [23–25]. We wanted to assess whether insulin resistance is associated with impaired natriuretic effects on insulin in the glomerulus and proximal tubule as opposed to the insulin-induced antinatriuresis in the distal tubule. Therefore we tested the hypothesis that insulin sensitivity correlates with an increase in GFR and decrease in proximal tubular sodium reabsorption during insulin infusion. Assuming an association between insulin resistance and salt sensitivity, we also hypothesized that salt sensitivity is associated with an impaired increase in GFR and impaired decrease in proximal tubular sodium reabsorption. Therefore we assessed the effects of insulin on renal haemodynamics and sodium handling in normal subjects displaying a wide range of insulin sensitivity, who also underwent a salt sensitivity test.
C_{\text{u}}(1 \times V/P)C_{\text{u}}(U \times V/P). Day-to-day coefficients of variation are 5.0 and 2.2% respectively [30].

Blood samples for measurement of sodium and lithium were drawn in the middle of the clearance periods. Urine and serum concentrations of sodium were determined by standard laboratory methods. Urine and serum concentrations of lithium were measured by atomic absorption (Atomic Absorption Spectrophotometer, Perkin–Elmer, Norwalk, CT, USA). Sodium and lithium clearances were calculated according to standard formulae. Fractional clearances were preferred to absolute clearances, because they correct for changes in glomerular filtration rate as well as for dead space or incomplete voiding. Fractional proximal sodium reabsorption was calculated as $1 - (C_{\text{u}}/C_{\text{G}}) \times 100\%$, and fractional distal tubular sodium reabsorption as $1 - (C_{\text{sodium}}/C_{\text{u}}) \times 100\%$.

**Control experiment**

On separate days, approximately 4 weeks after the insulin clamp experiment had been performed, a control experiment was carried out in an identical fashion with infusion of the same amount of solvent, including the intravenous saline load, and with blood sampling at the same time intervals. Control experiments had to be performed after the insulin clamp experiments because we could not determine beforehand the amount of 20% glucose to be infused each hour to maintain euglycaemia. To correct for any (non-specific) change in haemodynamic variables due to volume expansion as the result of 20% glucose infusion to maintain euglycaemia during the insulin infusion experiment, a corresponding amount of water was given orally each hour. Control experiments enabled us to correct for non-specific changes in the variables under evaluation, unrelated to insulin infusion.

**Salt sensitivity testing**

Salt sensitivity testing was performed using a standardized procedure [32]. During 1 week, the subjects adhered to a high-sodium diet containing 10 g NaCl/day; the extra amount of NaCl, if necessary, was supplied by capsules containing 500 mg of NaCl. The next week, the subjects were placed on a low-sodium diet containing 2 g NaCl/day. On the seventh day of both weeks, systolic and diastolic blood pressures were measured in the recumbent state at 2-min intervals for 1 h. Mean arterial blood pressure (MAP) was calculated as diastolic blood pressure plus one-third of the difference between systolic and diastolic blood pressure. Salt sensitivity was defined as the difference between the average of 30 readings on the high- and low-salt periods. Compliance with the diet was confirmed by measurements of the 24-h urinary sodium excretion during the last 2 days before each study.

**Statistical analysis**

All variables were analysed by the method of analysis of variance (ANOVA) for repeated measurements to detect differences over time and between the studies followed by paired tests. To avoid measurement of non-specific treatment effects on the variables under evaluation, the measurements during the control experiments were subtracted from the corresponding measurements obtained during each intervention before statistical analysis was performed [33,34]. Likewise, to aid interpretation of the relative magnitude of changes in different parameters in the figures, the percentage change from baseline during each study was calculated and the percentage change during the time-control study was subtracted from the percentage change after insulin.

**Results**

The characteristics of the study population are provided in Table 1. The average 24-h urinary sodium excretions did not differ before the insulin infusion study and before the control experiment. During the euglycaemic clamp, normoglycaemia (4.4 ± 0.1 mmol/l) was maintained. The whole-body glucose uptake showed an eightfold variation (range 1.6–13.1 mg/kg/min) during infusion of 50 mU insulin/kg/h. The coefficient of variation of the blood glucose level during infusion of 50 and 150 mU insulin/kg/h was 7.3 ± 0.6 and 9.0 ± 0.6% respectively.

**Effects of insulin in fusion**

The effects of insulin infusion on MAP, renal haemodynamics and sodium handling are shown in Table 2. MAP showed a slight decline during insulin infusion. Both RPF and GFR increased. Corrected for time-control studies, relative increases in GFR amounted to 5.9% ($P = 0.003$) and 10.9% ($P < 0.001$) during infusion of 50 and 150 mU insulin/kg/h respectively. Compared to the time-control studies, overall fractional sodium excretion decreased 34 and 50% (both $P < 0.001$) during infusion of 50 and 150 mU insulin/kg/h respectively. Fractional proximal tubular sodium reabsorption decreased and fractional distal
The results of the correlation analysis between insulin-mediated glucose uptake and insulin-mediated relative changes in parameters of renal sodium handling are listed in Table 3. Insulin sensitivity correlated with insulin-mediated changes in GFR during both insulin infusion rates (Figure 1). The M value did not correlate with the changes in proximal tubular sodium reabsorption and overall fractional sodium excretion.

Insulin-mediated changes in GFR correlated with insulin-mediated changes in RPF \((r = 0.64, P = 0.002)\) during infusion of 50 mU insulin/kg/h but not during infusion of 150 mU insulin/kg/h \((r = 0.28, \text{NS})\).

### Correlation analysis with salt sensitivity

The subjects showed a fairly good compliance with the high-and low-sodium diet. The average 24-h sodium excretion amounted to 190 ± 57 mmol at the end of the high-sodium diet period, and to 59 ± 35 mmol at the end of the low-sodium diet period. Individual differences in MAP between both weeks ranged from −14.9 to +13.3 mmHg. These parameters of salt sensitivity did not correlate with fasting insulin levels \((r = −0.06)\). Salt sensitivity was not inversely correlated, as hypothesized, with insulin-mediated glucose uptake during infusion of 50 mU insulin/kg/h \((r = 0.34, \text{NS})\) and 150 mU insulin/kg/h \((r = 0.44, P = 0.05)\).

During infusion of 50 mU insulin/kg/h, salt sensitivity showed a non-significant inverse correlation with the changes in overall fractional sodium excretion \((r = −0.38, P = 0.10)\). Salt sensitivity did not correlate with insulin-mediated changes in GFR \((r = 0.30, \text{NS})\) and distal tubular sodium reabsorption \((r = 0.11, \text{NS})\). In contrast, salt sensitivity correlated positively with the
changes in estimated proximal tubular sodium reabsorption ($r=0.49$, $P=0.028$; Figure 2).

**Discussion**

The hypotheses that insulin resistance is associated with impaired natriuretic effects of insulin in the glomerulus and proximal tubule as opposed to insulin’s antinatriuretic effects in the distal tubule could be partly confirmed by the results of our study. As hypothesized, insulin-mediated glucose uptake was positively correlated with insulin-mediated changes in GFR, but not with changes in proximal tubular sodium reabsorption. Besides, salt sensitivity correlated positively with insulin-mediated changes in proximal tubular sodium reabsorption.

Our observation that insulin induces sodium retention by increasing distal tubular sodium reabsorption has been amply demonstrated [5–12]. However, the finding that insulin increases GFR appears more controversial because previous human studies did not show a significant increase in GFR during insulin infusion [5–10,15,35]. This discrepancy can be explained by several factors such as a different method of measurement of GFR, a smaller study population, a shorter duration of insulin infusion, and a lack of time-control studies in almost all those previous studies. Importantly, we measured GFR by using the simultaneous infusion of $[^{131}]$hippuran and...
An increase in GFR has been demonstrated during chronic hyperinsulinaemia in normal dogs, which has been regarded as a renal 'escape' mechanism to offset insulin-induced sodium retention [14]. Considering the small absolute amount of sodium retained (84 mg on average during the final 45 min of the physiological insulin infusion, data not shown), insulin-induced sodium retention is an unlikely explanation for the immediate increases in GFR in our study. More likely, the increases in GFR can be attributed to insulin's effect on RPF, because changes in GFR correlated with changes in RPF during physiological hyperinsulinaemia. The fact that GFR increased less than RPF during insulin infusion can be explained by the presence of a filtration disequilibrium in humans and proportionally similar reductions in afferent and efferent arteriolar resistances [36].

However, the correlation between changes in RPF and changes in GFR was lost during supraphysiological hyperinsulinaemia, whereas the correlation between insulin-mediated glucose uptake and changes in GFR persisted. It is tempting to speculate that high doses of insulin may affect glomerular filtration directly or indirectly via nitric oxide. The possibility that insulin affects the ultrafiltration coefficient of the glomerular basement membrane gains support from experimental data. In vitro studies have shown that high doses of insulin attenuate the glomerular mesangial cell responses to vasoactive agents [37], and that nitric oxide stimulation antagonizes mesangial cell contraction [38]. In addition, the glomerular ultrafiltration coefficient can be reduced to approximately 50% of control with nitric oxide blockade in vivo in rats [39].

The hypothesis that insulin sensitivity correlates with the natriuretic effects of insulin to increase GFR and decrease proximal tubular reabsorption as opposed to insulin's antinatriuretic effect in the distal tubule could only be confirmed for insulin's effects on GFR. Thus, we could not confirm the hypothesis that insulin sensitivity correlates with an insulin-induced decrease in proximal tubular reabsorption. Also, the changes in proximal tubular sodium reabsorption were not related to the changes in RPF during insulin infusion, as previously suggested [11,15]. The latter indicates that glomerulotubular balance mechanisms prevail over the supposed hydrostatic effects of insulin on proximal tubular sodium reabsorption due to a stimulating effect of insulin on RPF. It also explains that we found no correlation between insulin-mediated glucose uptake and changes in overall fractional sodium excretion.

The assumption that insulin resistance and hyperinsulinaemia are linked to salt sensitivity could not be confirmed by our results. The fasting insulin levels did not correlate with salt sensitivity of blood pressure. Furthermore, salt sensitivity was not related to insulin resistance, as previously suggested [23–25]. Salt sensitivity even showed a weak positive correlation with the degree of insulin sensitivity. Notably, a positive correlation between salt sensitivity and insulin sensitivity has been reported in hypertensive subjects [40,41]. In spite of the absent association between salt sensitivity and insulin resistance, salt sensitivity still correlated with an impaired decrease in proximal tubular sodium reabsorption during insulin infusion. However, salt sensitivity only showed a weak inverse correlation with insulin-induced changes in overall fractional sodium excretion. Unfortunately, salt sensitivity showed much less variation than insulin sensitivity in our study subjects because they were selected to represent a wide range of insulin sensitivity.

The relevance of impaired natriuretic effects of insulin to oppose insulin-induced antinatriuresis in the distal tubule in insulin resistant and salt sensitive subjects is not yet clear. The impaired increase in GFR in insulin-resistant subjects, and the impaired decrease in proximal sodium reabsorption in salt sensitive subjects, during acute insulin infusion did not cause abnormal sodium retention. However, this does not exclude the possibility that these mechanisms may contribute to abnormal sodium retention and blood pressure regulation in the long-term. An alternative possibility is that insulin only causes abnormal sodium retention in insulin-resistant or salt-sensitive subjects if an additional antinatriuretic stimulus is present. Animal studies suggest that this 'third factor' could be sympathetic nervous system activation. Chronic hyperinsulinaemia increased blood pressure in rats only during concomitant sympathetic nervous system activation [42,43]. Also, chronic hyperinsulinaemia induced a blood pressure elevation and sodium retention in salt-sensitive rats, but these effects were abolished during co-administration of an α-blocker [44].

The acute intravenous saline load was handled similarly during the insulin infusion and time-control studies. It is important to consider that acute sodium loading was given superimposed on a sequential insulin infusion. Before acute sodium loading, baseline values concerning renal sodium handling differed between the insulin infusion and time-control studies, because fractional sodium excretion had already decreased during the insulin infusion studies. Thus, acute insulin infusion decreased renal sodium excretion but it did not appear to interfere with the kidney's ability to process a sodium load under these circumstances.

To summarize, insulin induces renal sodium retention by increasing distal tubular sodium reabsorption. The present study shows that an antagonistic natriuretic effect of insulin, i.e. an increase in GFR, to oppose insulin-induced sodium retention is impaired in relation to insulin resistance. Our results also indicate that insulin-induced decreases in proximal tubular sodium reabsorption are impaired in relation to salt sensitivity. It remains to be proved whether these acute effects of insulin are relevant for long-term blood pressure regulation in insulin-resistant and salt-sensitive subjects.

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