Effects of acute hyperinsulinemia on plasma atrial and brain natriuretic peptide concentrations

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Evidence has accumulated to show that hyperinsulinemia and insulin resistance play important roles in the development of essential hypertension (1-4). The mechanisms suggested for the increased blood pressure include reduction of renal sodium excretion followed by plasma volume expansion (5, 6), activation of the sympathetic nervous system (7), vascular hypertrophy with vascular smooth-muscle cell proliferation (8) and increased intracellular sodium and calcium concentrations (9). Although insulin was reported previously to be antinatriuretic via the direct action on the renal distal tubules (5, 6), possibly by activating Na+/K+ ATPase (10), other reports suggest that insulin could be natriuretic through its vasodilatory action (11, 12). Therefore, the pathophysiological role of insulin in the impaired natriuresis in patients with hypertension remains unclear.

The plasma level of atrial natriuretic peptide (ANP), one of the cardiac hormones involved in the regulation of blood pressure and body fluid volume (13), is elevated in patients with diabetes mellitus with insulin resistance (14-16). The increase in plasma ANP level in a hyperinsulinemic condition may be attributed to an increase in atrial pressure associated with sodium retention. However, the ubiquitous distribution of insulin receptors in various tissues, including cardiac myocytes, and insulin’s diverse biological actions (17) suggest a possible direct action on ANP secretion. This hypothesis is supported by the fact that the secretion of natriuretic peptides is under the regulation of various humoral factors (i.e., vasopressin, angiotensin II, endothelin) (18). In the present study, therefore, we have investigated the relationship between insulin and natriuretic peptides by determining plasma ANP and brain natriuretic peptide (BNP) levels during physiological and non-physiological acute hyperinsulinemia in man.

Subjects and methods
Physiological and non-physiological hyperinsulinemias were achieved by three different procedures: euglycemic-hyperinsulinemic glucose clamp, oral glucose challenge test and insulin challenge test. Informed consent was obtained from each patient and volunteer normal subjects after explaining the purpose of the studies.
Euglycemic–hyperinsulinemic glucose clamp

Twenty patients with non-insulin-dependent diabetes mellitus, aged 54 ± 2 years (mean ± SEM, range 32–64 years: 15 males and five females), were studied. None of the patients had advanced diabetic complications such as proliferative retinopathy and/or impaired renal function. Three patients were receiving insulin. Twelve patients were receiving oral hypoglycemic agents and five patients were treated by diet alone.

The euglycemic–hyperinsulinemic glucose clamp was performed according to the method described initially by De Fronzo et al. (19) in the morning after an overnight fasting. Catheters were inserted into a brachial vein for blood sampling to measure continuously the blood glucose level and to determine plasma hormones and another peripheral vein for constant infusion of insulin and 10% glucose solution, respectively. Insulin was infused at a rate of 1.12 mU·kg⁻¹·min⁻¹ (40 mU·m⁻²·min⁻¹), while 10% glucose solution was infused to maintain the blood glucose level at 0.8 g/l over a period of 60–180 min. Blood samples were collected before and every 60 min after the start of the clamp to determine plasma immuno-reactive insulin (IRI), ANP, BNP, plasma osmolarity and serum total protein concentration.

Glucose challenge test

Twenty-two healthy subjects aged 35 ± 3 years (range 19–53 years; nine males and 13 females) were studied. Seventy-five grams of glucose in 225 ml of water (Toleran G, Otuka Pharmaceutical Co., Osaka, Japan) was administered orally in the morning after an overnight fasting. Blood samples were collected before and every 30 min after the administration of glucose for determination of blood glucose, plasma IRI, ANP, BNP, plasma osmolarity and serum total protein concentration.

To ascertain the effects of volume loading by water contained in the glucose solution, six normal subjects (aged 30 ± 3 years, range 26–43 years; four males and two females), who took the above-mentioned glucose challenge test were given 225 ml of water orally in the morning after an overnight fasting. Blood samples were collected before and every 30 min after the administration of water for measurement of plasma ANP, BNP and serum total protein concentration.

Insulin challenge test

The insulin challenge test was performed in six patients aged 37 ± 4 years (range 38–50 years; three males and three females) as part of the diagnostic procedures to evaluate the hypothalamic–pituitary function. Insulin (0.05–0.1 U/kg) was administered as a bolus in the morning after an overnight fasting. Blood samples were collected before and every 30 min after insulin administration to determine blood glucose, plasma IRI, ANP, BNP, growth hormone (GH), ACTH, cortisol, plasma osmolarity and serum total protein concentration.

Assay methods

Plasma ANP and BNP were determined by their respective immunoradiometric assays as described previously (20). Plasma IRI, GH, ACTH and cortisol levels were determined by commercially available RIA kits. Plasma osmolarity was measured by freezing-point depression methods. The plasma total protein concentration was measured by using an autoanalyzer.

Estimation of plasma volume

In the glucose clamp and the glucose challenge tests, the percentage changes in plasma volume were calculated based on the changes in serum protein concentration determined before and after the tests using the following equation:

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\text{Percentage change of plasma volume} = \frac{\text{Serum protein concentration (after)} - \text{Serum protein concentration (before)}}{\text{Serum protein concentration (before)}} \times 100(\%)
\]

Statistical analysis

Values were expressed as means ± SEM. The statistical significance was analyzed by Student’s t-test or
Mann–Whitney’s U test, as appropriate; p < 0.05 was considered statistically significant.

Results

The average steady-state plasma IRI level during the euglycemic–hyperinsulinemic glucose clamp was 74.9 ± 30.1 mU/l. Basal plasma ANP and BNP levels were 9.6 ± 1.7 and 8.1 ± 1.6 ng/l, respectively. The plasma ANP level increased by about 39% 60min after the clamp, while the plasma BNP level did not change (Fig. 1). There was no correlation between plasma IRI and ANP levels of the peak and the area under the curve of the responses (data not shown). While plasma osmolarity did not show a significant change (before, 280 ± 2 mOsm/kg·H₂O; after, 281 ± 2 mOsm/kg·H₂O), plasma volume estimated from the changes in serum total protein concentration showed a significant increase (9.0 ± 2.0%, p < 0.05).

Both blood glucose and plasma IRI levels showed a significant increase after glucose administration. The plasma IRI reached a peak value of 59.0 ± 11.2 mU/l 90min after glucose administration (Fig. 2a). Basal plasma ANP and BNP levels were 17.7 ± 2.6 and 6.8 ± 1.3 ng/l, respectively. The plasma ANP but not BNP level showed a significant increase, with a peak value 90min after administration (Fig. 2b). However, there was no significant correlation between the changes in the plasma IRI and ANP levels in terms of their peak value and the area under the curve. Although plasma osmolarity did not change (before, 280 ± 2 mOsm/kg·H₂O; after, 275 ± 1 mOsm/kg·H₂O), plasma volume increased by an average of 7.3 ± 2.3% (p < 0.01).

Plasma ANP and BNP levels, plasma osmolarity and plasma volume showed no significant change during the oral water loading, which was done as a control study for the glucose challenge test (data not shown).

After intravenous bolus administration of insulin, plasma IRI showed a significant increase, with the highest level of 997.7 ± 628.4 mU/l after 30 min, while the blood glucose level became as low as 0.39 ± 0.09 g/l after 30 min (Fig. 3a). Basal plasma ANP and BNP levels were 13.9 ± 4.3 and 8.9 ± 2.0 ng/l, respectively. By contrast to the changes in IRI and blood glucose, plasma ANP and BNP levels did not show any significant change throughout the course (Fig. 3b). The changes in plasma osmolarity (before, 279 ± 3 mOsm/kg·H₂O; after, 281 ± 3 mOsm/kg·H₂O) and plasma volume (7.3 ± 1.9%) were not significant. In addition, plasma GH, ACTH and cortisol showed normal responses to the

Fig. 2. Levels in blood glucose (open column in a), plasma immunoreactive insulin (IRI: hatched column in a), plasma atrial natriuretic peptide (ANP: hatched column in b) and plasma brain natriuretic peptide (BNP: open column in b) levels during the glucose challenge test. Number of patients is shown in parentheses. Values are means ± SEM; * p < 0.0001 and ** p < 0.05 vs before glucose administration.
insulin-induced hypoglycemia in all the patients (data not shown).

Discussion

The relationship between plasma insulin and natriuretic peptides was examined under physiological and non-physiological acute hyperinsulinemic conditions. Although the plasma ANP level increased significantly during the euglycemic–hyperinsulinemic glucose clamp and the glucose challenge test, there was no significant correlation between changes in plasma ANP and IRI. In addition, plasma ANP did not show any significant change despite the marked increase in plasma IRI during the insulin challenge test. Plasma BNP did not change in any of the hyperinsulinemic conditions. All these findings provide in vivo evidence that insulin does not affect, at least acutely, the secretion of ANP and BNP. These findings suggest that the increased plasma ANP reported in patients with essential hypertension associated with hyperinsulinemia (14–16) may not be attributed to the direct action of insulin on the heart but may be an indirect result that reflects the impaired sodium retention and plasma volume expansion (21–23).

The mechanism responsible for the increased plasma ANP during the glucose clamp and glucose challenge tests remains unclear. However, there was a significant increase in plasma volume in patients subjected to the glucose clamp, because approximately 500 ml of 10% glucose and saline was administered intravenously over a period of 3–5 h. It has been reported that intravenous infusion of isotonic glucose, hypertonic saline or mannitol causes elevation of plasma ANP and that there is a positive correlation between plasma volume and plasma ANP level (24). Therefore, the increase in plasma volume may have stimulated the ANP secretion in the glucose clamp procedure.

Because oral water load also increases plasma volume (24), the increase in plasma volume associated with glucose challenge may be the result of water load contained in the glucose solution. However, this may not be the case in the present study, because the same volume (225 ml) of water intake did not produce any significant change in plasma volume or plasma ANP level. The increase in plasma volume is therefore attributed to the administration of glucose itself. Although plasma osmolarity did not show a significant change, its transient increase associated with hyperglycemia after glucose administration may lead to a significant increase in plasma volume.

In the chronic hyperinsulinemic state (21), insulin has been shown to retain sodium, to cause plasma volume expansion and then to stimulate ANP secretion through an increase in atrial pressure. However, Anderson et al. (21) have demonstrated that sodium retention and volume expansion caused by acute administration of insulin can be counterbalanced by its vasodilative effect in the kidney. In addition, the plasma ANP level did not show a significant increase.

Fig. 3. Levels in blood glucose (open column in a), plasma immunoreactive insulin (IRI; hatched column in a), plasma atrial natriuretic peptide (ANP; hatched column in b) and plasma brain natriuretic peptide (BNP; open column in b) levels during the insulin challenge test. Number of patients is shown in parentheses. Values are means ± SEM; *p < 0.01 and **p < 0.05 vs before insulin administration.
The increase of the plasma volume seen in the glucose clamp and glucose challenge test in the present study, therefore, appears not to be the result of acute hyperinsulinemia but the result of volume overload related to the test procedures.

Plasma osmolarity has been shown to be one of the factors affecting the secretion of natriuretic peptides (25). However, plasma osmolarity did not show any significant change in the glucose clamp and the glucose challenge test, despite the increased blood glucose level. In addition, there was no significant correlation between changes in plasma osmolarity and plasma ANP level. Therefore, plasma osmolarity was not a major factor affecting the plasma ANP level.

It has been reported that some diabetic states have been associated with increases in circulating levels of ANP (26, 27). The diabetic patients, however, had lower basal plasma ANP levels than the control subjects in the present study, although the difference did not reach the level of statistical significance. The exact reason for this is not known. However, it is expected that the venous return and the atrial pressure are reduced in poorly controlled, slightly dehydrated non-ketotic diabetic patients, which may lead to the decrease in ANP secretion. In addition, the role of the autonomic nervous system in the regulation of ANP secretion has been well described (28). Therefore, an impaired function of the autonomic nervous system due to diabetic neuropathy possibly present in the patients may affect the plasma ANP levels, although precise evaluation of the autonomic nerve function was not performed in the present study.

In contrast to the changes in plasma ANP, plasma BNP did not change during the glucose clamp and the glucose challenge test. This clearly indicates that acute hyperinsulinemia does not stimulate BNP secretion. Although both ANP and BNP reflect cardiac overload and share similar vasodilative and natriuretic actions (13), various differences have been reported in their biological aspects. Atrial natriuretic peptide is stored in the secretory granules and can be secreted rapidly by acute stimulation (18), while the rate of BNP secretion is regulated at the step of gene transcription (29). The plasma BNP level was shown not to change in response to acute cardiac pacing (20).

In addition, BNP reflects more the hemodynamic overload to the ventricle, while ANP reflects more the hemodynamic overload to the atrium. The increase in the plasma volume seen by the glucose clamp and the glucose challenge test is likely to be not large enough to produce the ventricular stress to stimulate BNP secretion. Whatever the mechanism, the difference seen in the changes in plasma ANP and BNP support the concept that the two natriuretic peptides are secreted by different regulatory mechanisms (13).

In conclusion, the present study clearly indicates that acute hyperinsulinemia does not have a direct stimulatory effect on ANP and BNP secretion. Further studies are required, however, to elucidate the effects of chronic hyperinsulinemia on the natriuretic peptide system.

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References


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