Normal pancreastatin-like and increased post-glucose insulin levels in young offspring of insulin-resistant non-obese essential hypertensive patients

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Abstract

Pancreastatin is a regulatory peptide known to inhibit insulin secretion and insulin action with a glycogenolytic effect in the liver. This peptide is present in and secreted by many endocrine and chromaffin cells. Abnormalities of glucose, insulin and lipoprotein metabolism are common in patients with hypertension, as well as their first-degree relatives. We have recently studied a group of non-obese hypertensive subjects in which pancreastatin-like levels were increased compared with controls, and correlated with norepinephrine levels. We hypothesized that pancreastatin alongside the sympathoadrenal system might have a part in the insulin resistance of these patients, and this metabolic syndrome could play a role in the pathogenesis and complications of hypertension. In this article, we studied the normotensive offspring of these non-obese hypertensive patients and looked for metabolic abnormalities as well as plasma pancreastatin, glucagon and catecholamine levels. The subjects were separated into two groups: (1) offspring from non-insulin-resistant patients and (2) offspring from insulin-resistant patients. We found that after an intravenous glucose load, offspring from insulin-resistant patients were already hyperinsulinemic, although glucose clearance was normal, suggesting an early alteration in insulin sensitivity, whereas pancreastatin and catecholamine levels were normal compared with matched controls. However, offspring from non-insulin-resistant patients had no differences with controls. These results suggest that pancreastatin and catecholamines may not play an important role in triggering insulin resistance, although they may be important once the syndrome is established.

Journal of Endocrinology (1997) 153, 313–318

Introduction

Pancreastatin (PST), a 49 amino acid peptide, was first isolated from porcine pancreas extracts and implicated in the first phase of insulin secretion (Tatemoto et al. 1986, Efendic et al. 1987). It is derived from the precursor molecule chromogranin A (CGA) by proteolytic processing, and it is present throughout the neuroendocrine system (Iancangelo et al. 1988, Schmidt et al. 1988). A role of PST as a regulatory hormone has been established, with a variety of biological effects (see Sánchez-Margalet et al. 1996ª for review). The mechanism of PST action involves calcium mobilization and activation of protein kinase C (Sánchez-Margalet & Goberna 1994a, Sánchez-Margalet et al. 1994a,b, Sánchez-Margalet et al. 1996b).

The processing of CGA is tissue specific (Winkler & Fischer-Colbrie 1992). Thus, CGA processing is more extensive in endocrine cells of the gut and pancreas (Watkinson et al. 1991). Post-secretory processing of CGA has also been reported and may play a role in the production of biologically active peptides such as PST (Simon et al. 1989, Watkinson et al. 1993). In this context, plasma CGA has been employed as a measure of exocytotic sympathoadrenal activity, since CGA correlates with norepinephrine release rate (Dimsdale et al. 1992). Besides, CGA and PST have been shown to be increased in parallel in neuroendocrine neoplasia (Syversen et al. 1994). An increase in the adrenergic activity in patients with essential hypertension has been reported (Kjeldsen et al. 1982, Eser et al. 1985). Moreover, we have recently found that plasma PST-like immunoreactivity correlates with plasma norepinephrine in essential hypertension (Sánchez-Margalet et al. 1995a). The role of PST as a counter-regulatory agent of insulin action (Sánchez et al. 1990, 1992, Sánchez-Margalet et al. 1992, 1993, Sánchez-Margalet & Goberna 1994a) and the increased levels we found in hypertensive patients (Sánchez-Margalet et al. 1995b) suggested that PST might play a role in the pathophysiology of the
hypertensive syndrome associated with metabolic abnormalities. In fact, insulin resistance and the sympathetic-adrenal system are involved in the metabolic abnormalities associated with hypertension (Reaven et al. 1996).

We have recently studied a group of non-obese hypertensive subjects in which pancreastatin-like levels were increased compared with controls (Sánchez-Margalet et al. 1995b), and correlated with noradrenaline levels (Sánchez-Margalet et al. 1995a). They had different degrees of insulin resistance and accordingly, they had different alteration in other factors for coronary artery disease, such as increased low density lipoprotein (LDL) cholesterol and triglycerides and decreased high density lipoprotein (HDL) cholesterol. In this report, we studied the young offspring of non-obese hypertensive parents. All of them were normotensive and non-obese. It is known that essential hypertension is frequently associated with insulin resistance and compensatory hyperinsulinaemia (Ferranini et al. 1987, Reaven 1988), although not all essential hypertensive patients have insulin resistance. In this line, the offsprings were separated in two groups dependent on the insulin sensitivity of the hypertensive parent (measured by somatostatin–insulin steady state glucose). They underwent an intravenous glucose tolerance test and we studied lipoprotein metabolism as well as other endocrine factors, such as insulin, glucagon, catecholamine and PST levels.

Materials and Methods

Subjects
Non-obese (body mass index <27 kg/m² and waist:hip ratio <0.95) hypertensive patients were from the Hypertension Unit of the Virgen Macarena University Hospital in Sevilla (Sánchez-Margalet et al. 1995a,b). They had different degrees of insulin resistance according to the oral and intravenous glucose-tolerance tests. To determine more accurately their insulin-resistant state, they undertook a steady-state plasma glucose (SSPG) and insulin determination (Shen et al. 1988) by a somatostatin-insulin-glucose infusion (350 mg/h, 25 mU/m²/min and 6 mg/kg/min respectively). Glucose levels were monitored every 30 min up to 120 min, and then every 10 min up to 180 min to measure glucose and insulin in the steady state (Dobner et al. 1981). This technique allowed the patients to be separated into two groups: insulin-resistant (SSPG >200 mg/dl, mean ±21) and non-insulin-resistant (SSPG <200 mg/dl, mean ±5 ±9). We then studied young non-obese, normotensive (diastolic blood pressure <95 mmHg, and systolic blood pressure <140 mmHg) subjects from the offspring of the two groups (see Table 1 for clinical characteristics). All of them had only one parent affected of essential hypertension. All the affected subjects with SSPG studies (highly motivated patients from the Hypertension Unit) that were asked to follow the study gave an affirmative answer after which we got 100% positive response with the offspring.

The offsprings of both groups of non-obese hypertensive patients and control subjects (offspring of normotensive, normoinsulinemic subjects, with basal insulin lower than 10 μU/ml) then underwent an intravenous glucose load (330 mg/kg) in the morning after an overnight fast as previously described (Sánchez-Margalet et al. 1995b). Venous blood samples were drawn before and 5, 10, 15, 30 and 60 min after glucose loading, and were collected into lithium–heparin tubes containing 0·1 ml aprotinin (Trasylol 20 000 U/ml; Bayer AG, Leverkusen, Munich, Germany). After centrifugation, plasma was made into aliquots and stored at −20°C.

### Table 1 Clinical characteristics of the subjects. Values are expressed as means ± S.D.

<table>
<thead>
<tr>
<th>Sex (Male/female)</th>
<th>Controls</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 0.15</td>
<td>21 ± 0.3</td>
<td>19 ± 1.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 0.5</td>
<td>25 ± 0.4</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>84 ± 3</td>
<td>93 ± 3</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>Diastolic</td>
<td>67 ± 4</td>
<td>69 ± 3</td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
<td>115 ± 4</td>
<td>119 ± 4</td>
</tr>
</tbody>
</table>

Homone determinations

RIA was employed to measure PST-like levels (Sánchez-Margalet et al. 1995a,b). A RIA kit from ICN Biomedicals, Inc (Costa Mesa, CA, USA) was used to determine plasma glucagon. Plasma catecholamines were determined by RIA with a kit of extraction, methylation and RIA from Immuno Biological Laboratories (Hamburg, Germany). Plasma insulin was measured by enzyme-linked immune assay with an IMX-SYSTEM autoanalyser (Abbot Cientifica, Madrid, Spain).
Biochemical analysis

Plasma glucose was determined by the glucose oxidase method. Triglycerides and total and high-density lipoprotein-cholesterol were measured by an enzymatic colorimetric test and precipitation technique (phosphotungstate-magnesium chloride; kit from Boehringer–Mannheim GmbH, Barcelona, Spain). Lipoproteins were analyzed by using an ultracentrifugation step (Havel et al. 1985). Free fatty acids (FFA) were measured using an enzymatic colorimetric kit (Boehringer–Mannheim GmbH).

Statistical analysis

Values are expressed as means ± S.E.M. Data were analysed by analysis of variance for multiple comparison, and a post test (Bonferroni) was used to test the degree of significance of the differences from control.

Results

Lipid plasma levels

Plasma values of triglycerides were very similar in all groups: 89 ± 10 mg/dl in controls; 82 ± 12 mg/dl in offspring from insulin-resistant patients or group I; and 85 ± 13 mg/dl in offspring from non-insulin-resistant patients or group II (90% confidence interval). Similarly, no differences were found in FFA: 275 ± 20, 255 ± 22, and 240 ± 23 μmol/l, controls, group I and II respectively (90% confidence interval). Total plasma cholesterol levels were normal and no significant differences were found between controls (180 ± 10 mg/dl), and group I (166 ± 11 mg/dl), or group II (172 ± 10 mg/dl), with 90% confidence interval. Cholesterol from different lipoproteins was also measured. Very low density lipoprotein cholesterol was very similar in all groups: 20 ± 3, 17 ± 2 and 17 ± 3 mg/dl, controls, group I and II respectively (90% confidence interval). No differences in LDL cholesterol levels were found between controls (106 ± 8 mg/dl) and group I (98 ± 7 mg/dl) or group II (102 ± 6 mg/dl), with 90% confidence interval. The offspring of insulin-resistant hypertensive patients (group I) had slightly lower HDL cholesterol (51 ± 4 mg/dl) than controls (64 ± 7 mg/dl), however, the difference was not statistically significant (P<0.25, 90% confidence interval). Offspring of non-hypertensive parents (group II) had HDL cholesterol levels closer to those of controls (55 ± 3 mg/dl).

Intravenous glucose-tolerance test

Values of glucose and insulin in the intravenous glucose-tolerance test are shown in Fig. 1. Offspring from insulin-resistant patients had higher insulin response to glucose, although there were significant differences only at 10 and 15 min; whereas the insulin response in the offspring from non-insulin-resistant patients was the same as the control one. Basal insulin levels were slightly higher in the offspring from insulin resistant patients (group I), but the differences were not statistically significant (P<0.06). On the other hand, there were no significant differences in glucose levels in any time point of the glucose tolerance test in the offspring from insulin-resistant patients. As expected, there were no differences in glucose levels of the offspring from non-insulin-resistant patients.

As shown in Fig. 2, plasma glucagon and FFA were decreased by the glucose loading. Plasma glucagon is expected to decrease because the secretion is inhibited by glucose; and FFA are expected to decrease because of the action of the secreted insulin. However, there were no statistically significant differences in glucagon or FFA at
any time point in the three groups studied. Figure 3 shows plasma catecholamines (epinephrine, norepinephrine) and PST levels before and after the glucose challenge. We found no significant changes in both catecholamine and PST levels after glucose loading in any group. Moreover, plasma catecholamine and PST values were similar in the three groups studied, with no significant differences.

Discussion

Essential hypertension is associated with insulin resistance and compensatory hyperinsulinemia (Ferranini et al. 1987, Reaven 1988). The most accepted hypothesis postulates that insulin resistance and compensatory hyperinsulinemia
are primary events, and enhanced sympathetic activity would be the link between the insulin action and the development of hypertension. Moreover, insulin resistance has been shown to be a characteristic feature of essential hypertension, independent of obesity (Pollare et al. 1990). Marked fasting and post-glucose hyperinsulinemia is associated with high blood pressure, in addition to an increase in total cholesterol and triglyceride levels, with lower HDL cholesterol concentrations (Ferrari et al. 1991).

Other studies in offspring of hypertensive parents and prospective studies have previously shown that hyperinsulinemia precedes the development of hypertension and other manifestations of the metabolic syndrome (Ferrari et al. 1991, Skarfors et al. 1991, Haffner et al. 1992). These data are in agreement with our results in young offspring of non-obese hypertensive patients. Thus, we have found high post-glucose insulin levels in the offspring of the insulin resistant hypertensive parents compared with matched controls. However, basal insulin levels were only slightly higher in this group and there were no significant differences with controls. It should be pointed out that there were no sex differences in the insulin resistance of the subjects (either the sex of the offspring or the affected parent). On the other hand, we have found normal levels of catecholamines and pancreastatin in these normotensive young subjects, even though their hypertensive parents had increased levels of these hormones (Sánchez-Margalet et al. 1995b). Therefore, our results suggest that catecholamines and pancreastatin might not play an important role in the triggering of the insulin resistance and hyperinsulinemia. Instead, they may have a part in the pathophysiology of the syndrome in a later step, once it is established. Alternatively, insulin may play a role in enhancing the sympathetic nervous system (SNS) as previously suggested (Daly & Landsberg 1991), and therefore mediate an increase in catecholamine and pancreastatin levels. Moreover, several lines of evidence have demonstrated that borderline hypertension is a hyperadrenergic state characterized by elevated norepinephrine (Julius 1991, Sánchez-Margalet et al. 1995b). Catecholamines and pancreastatin are potent antagonists of insulin action in vivo (Deibert & DeFronzo 1980, Lager et al. 1986, Sánchez et al. 1990, Sánchez-Margalet et al. 1992) and in vitro (Kuroda et al. 1987, Sánchez-Margalet & Goberna 1994a), and therefore they may worsen the insulin resistance in this syndrome. Follow-up studies of these subjects are needed to see whether the increase in sympathoadrenal activity precedes the appearance of hypertension.

Acknowledgements

We acknowledge the technical assistance of Isabel Gonzáles in the intravenous glucose-tolerance test. We also thank the secretarial work of Concepción Muñoz and Carmen Peña. This work was supported by grants from the Fondo de Investigacion Sanitaria (FIS 92/390 R.G., FIS 96/1411 V.S.-M.).

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Received 26 July 1996
Revised manuscript received 21 October 1996
Accepted 2 January 1997