Increased Plasma Pancreastatin-Like Levels in Gestational Diabetes

Correlation with catecholamine levels

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OBJECTIVE — To investigate plasma pancreastatin (a chromogranin A–derived peptide) and catecholamine levels (counterregulatory hormones) in subjects with gestational diabetes compared with normal pregnant subjects.

RESEARCH DESIGN AND METHODS — Fasting blood samples were obtained from 11 normal pregnant and 12 nonobese gestational diabetic subjects at late pregnancy (30 ± 1 weeks). Selection criteria were those recommended by the National Diabetes Data Group (modified from O'Sullivan original criteria). Plasma glucose, insulin, glucagon, pancreastatin-like, epinephrine, and norepinephrine were measured.

RESULTS — Gestational diabetic subjects had significantly higher insulin levels than control pregnant subjects (18 ± 1 vs. 15 ± 1 μU/ml), whereas glucose and glucagon levels where comparable in the two groups. However, increased catecholamine levels (epinephrine and norepinephrine) were found in the gestational diabetic group. We also found increased pancreastatin-like levels in these patients compared with the pregnant control group (46 ± 2 vs. 30 ± 2 pmol/l). Actually, pancreastatin levels positively correlated with both epinephrine (r = 0.34) and norepinephrine (r = 0.80) levels.

CONCLUSIONS — Catecholamine and pancreastatin-like levels were found elevated in gestational diabetic subjects. These counterregulatory hormones may play a role in the insulin resistance syndrome of gestational diabetes.

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Pancreastatin (PST), a 49–amino acid peptide that was first isolated from porcine pancreas (1), arises by proteolysis of the precursor molecule chromogranin A (CGA) (2) and is present throughout the neuroendocrine system (3). It was first described as an inhibitor of insulin secretion (1), but many different effects were then reported (4). In summary, the effects of PST on endocrine and exocrine secretion in different tissues raised the hypothesis that this peptide was a general autocrine, paracrine, and endocrine inhibitor of secretion. However, the best-characterized effect of PST has been studied in the rat liver (5), where we found a glycogenolytic effect (6,7), as well as a counterregulatory effect on insulin action (8). The mechanism of PST action is mediated by a specific receptor (9,10) and involves calcium mobilization (11) and activation of protein kinase C (12) by activating a $G\alpha_{q/11}$ protein (13), which in turn activates phospholipase C-β (13,14).

The processing of the precursor of PST, CGA, is tissue-specific (15,16), and post-secretory processing of CGA may also produce biologically active peptides such as PST (17,18); the primary structure of human CGA and PST has been described (19). Serum levels of CGA and PST-like immunoreactivity (PST-LI), measured by immunoassay methods, are raised in patients with neuroendocrine tumors (20–22), and plasma CGA has been used as a measure of exocytotic sympathoadrenal activity, since CGA correlates with norepinephrine release rate (23). In addition, CGA and PST have been shown to be increased in parallel in neuroendocrine neoplasia (24), and we have found that PST-LI correlates with norepinephrine in essential hypertension (25,26).

Recently, the presence of CGA and PST-LI in placenta and amniotic fluid (27) has been shown. Moreover, plasma PST-LI levels seem to be significantly higher at term of gestation (3rd trimester) than at 6–11 weeks and in nonpregnant women (27). Pregnancy is characterized by insulin resistance and increased insulin secretion as a compensatory mechanism to maintain normal glucose tolerance (28,29). Besides, ~3% of pregnant women develop glucose intolerance in the 3rd trimester of gestation, i.e., they suffer gestational diabetes (30).

Because we had previously demonstrated the counterregulatory effect of PST on insulin action (31) and because PST levels seem to increase in the 3rd trimester of pregnancy (27), we raised the question of whether PST may be involved in the pathophysiology of gestational diabetes. Therefore, these considerations prompted us to look at the PST-LI levels, as well as related hormones, catecholamines, insulin, and glucagon, in gestational diabetic and normal pregnant subjects.

The objective of the present study was to evaluate plasma levels of glucose and hormones involved in glucose metabolism in lean subjects with gestational diabetes compared with those in lean subjects with normal pregnancy.

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Abbreviations: CGA, chromogranin A; PST, pancreastatin; PST-LI, pancreastatin-like immunoreactivity; RIA, radioimmunoassay.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of the pregnant subjects*

	Control subjects	Diabetic subjects
n	11	12
Age (years)	28 ± 1	30 ± 1
Height (cm)	160 ± 2	161 ± 3
Weight (kg)	64 ± 5	67 ± 6
BMI (kg/m²)	25.0 ± 0.5	25.9 ± 0.5
Fasting glucose (mg/dl)	74 ± 1	76 ± 1

Data are means ± SEM. There were no significant differences between the two groups.

Table 2—*Hormone plasma levels of the pregnant subjects*

	Control subjects	Diabetic subjects
Insulin (μU/ml)	15 ± 1	18 ± 1*
Glucagon (pg/ml)	145 ± 9	140 ± 8
Epinephrine (pg/ml)	124 ± 20	173 ± 21*
Norepinephrine (pg/ml)	172 ± 16	271 ± 17†
PST (pmol/l)	30 ± 2	46 ± 2†

Data are means ± SEM. *$P < 0.05$; †$P < 0.001$.

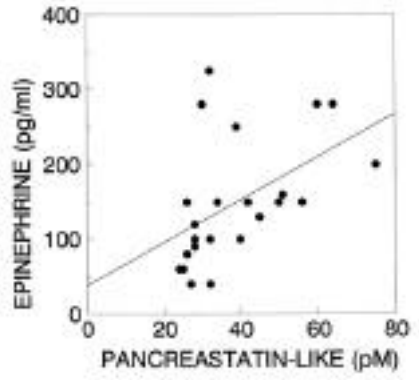

Figure 1—*Correlation between plasma values of PST-LI and epinephrine in pregnant subjects (r = 0.34, P < 0.05).*

RESEARCH DESIGN AND METHODS

Subjects

There were 23 nonobese (BMI <0.27 kg/m²) pregnant subjects used for the study. Of these women, 12 had previously been diagnosed with gestational diabetes and 11 were control subjects. Selection criteria for gestational diabetes were those recommended by the National Diabetes Data Group (modified from O'Sullivan's original criteria) (30). None of the subjects was treated with insulin. A fasting blood sample was taken by venipuncture at week 30 ± 1 under standard conditions of supine rest for 30 min. Blood samples were collected into lithium-heparin tubes containing 0.1 ml aprotinin. After centrifugation, plasma was aliquoted and stored at −20° C. The clinical characteristics of pregnant women with or without gestational diabetes are shown in Table 1. Both groups had similar clinical features with no significant differences, including fasting plasma glucose levels.

Glucose and hormone determinations

Glucose was determined by the glucose oxidase method. A radioimmunoassay (RIA) was used to measure PST-LI levels (kit from Peninsula Laboratories Europe, St. Helens, Merseyside, U.K.). The antiserum does not recognize CGA and detects a wide range of concentration of PST-LI (2–250 pmol/l). An RIA kit from ICN Biochemicals (Costa Mesa, CA) was used to determine plasma glucagon. The glucagon antiserum is very specific and does not cross-react with gut glucagon, insulin, or ACTH. The detection range was from 25 to 1,000 pg/ml. 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 immunoassay with an IMX-SYSTEM autoanalyzer (Abbot Cientifica, Madrid, Spain).

Statistical analysis

Values are means ± SEM. Student's *t* test was used for comparisons, with differences considered significant at $P < 0.05$.

RESULTS

Table 2 shows the fasting plasma insulin and counterregulatory hormone levels of pregnant subjects with or without gestational diabetes. Diabetic subjects had slightly, but significantly, higher insulin levels than the control group (18 ± 1 vs. 15 ± 1 μU/ml, $P < 0.05$). However, plasma glucagon levels were comparable in both groups. On the other hand, plasma catecholamine levels were found increased in the gestational diabetes group. Epinephrine levels were only slightly significantly increased in the gestational diabetic group (173 ± 21 vs. 124 ± 20 pg/ml, $P < 0.05$), whereas norepinephrine levels were very significantly increased (271 ± 17 vs. 172 ± 16 pg/ml, $P < 0.001$). Finally, plasma PST-LI levels were found to be very significantly increased in the gestational diabetic group (46 ± 2 vs. 30 ± 2 pmol/l, $P < 0.001$), whereas PST-LI levels in control subjects were similar to those previously described in normal pregnant women in their 3rd trimester (27).

Because we found plasma catecholamines and PST-LI levels highly increased in the gestational diabetes group, we studied the correlation between PST-LI levels with epinephrine and norepinephrine as we had previously done in hypertensive patients (22). As shown in Fig. 1, correlation of plasma PST-LI with epinephrine was only modest ($r = 0.34$, $P < 0.05$). However, plasma PST-LI correlated very positively and significantly with norepinephrine ($r = 0.80$, $P < 0.001$) (Fig. 2).

CONCLUSIONS

In the present study, we have found increased counterregulatory hormone levels (catecholamines and PST-LI) in subjects with gestational diabetes compared with matched pregnant control subjects.

Pregnant women have raised endocrine activity with increased production of several hormones, regulatory peptides, and growth factors by the neuroendocrine organ placenta (31). Thus, increased CGA and PST-LI have been previously found in sera from pregnant subjects in their last trimester (27), with a progressive increase from 1st trimester to term. This has been related to the stress in connection with delivery, with increased release of catecholamines and corelease of CGA. Along these lines, studies in rats have shown a strong increase in CGA biosynthesis at the end of the gestational period that parallels the rise in epinephrine (32).

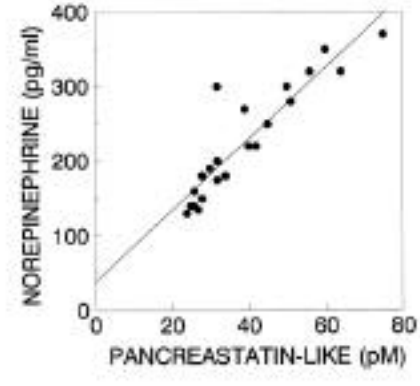

Figure 2—*Correlation between plasma values of PST-LI and norepinephrine in pregnant subjects (r = 0.80, P < 0.001).*

In this context, the endocrine changes in pregnancy may be responsible for the insulin resistance state in pregnant subjects (28,29). Moreover, the glucose intolerance observed in gestational diabetes may be caused by an exaggerated increase in counterregulatory hormones. This assumption prompted us to undertake the present study, to measure counterregulatory hormones in gestational diabetes and to compare them with those in normal pregnant subjects. Because our current hypothesis is that PST, along with catecholamines (33,34), has a counterregulatory effect on insulin action, we checked plasma PST-LI concentration and studied the possible correlation with catecholamines.

We found plasma PST-LI concentrations (30 pmol/ml) at 30 weeks of gestation in a range similar to those previously described in sera from normal pregnant subjects (27), where others have found 38 pmol/l at 37–42 weeks and 9 pmol/l at 6–11 weeks (27). In the group with gestational diabetes, we found significantly higher plasma PST-LI concentrations (46 pmol/l). Moreover, as we have previously described for hypertensive patients (26), plasma catecholamine levels (especially norepinephrine) positively and significantly correlated with PST-LI, and therefore, we found higher catecholamine levels in the gestational diabetic group, especially norepinephrine. We cannot conclude that these increased counterregulatory hormones cause the insulin resistance in women with gestational diabetes (27); however, we are positive that they may play a role in the glucose intolerance of these subjects. Moreover, they may also play a role in the insulin resistance and type 2 diabetes that these subjects experience after delivery. This hypothesis remains speculative but warrants further investigation. Therefore, it will be worthwhile to study these counterregulatory hormones in gestational diabetic subjects after delivery and see whether these hormone abnormalities correlate with the development of type 2 diabetes.

In summary, pregnancy is characterized by insulin resistance, probably due to hormone changes caused by the endocrine role of the placenta. We have found that these changes are exaggerated in gestational diabetic subjects, and these subjects have higher plasma PST-LI and catecholamine levels. These counterregulatory hormones may play a role in deteriorating insulin sensitivity and, therefore, in the appearance of gestational diabetes.

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