Alteration of Plasma Ghrelin Levels Associated With the Blood Pressure in Pregnancy

Yasuo Makino, Hiroshi Hosoda, Kazuhiro Shibata, Ikuko Makino, Masayasu Kojima, Kenji Kangawa, Tatsuhiko Kawarabayashi

Abstract—Ghrelin, an endogenous ligand for the growth hormone secretagogues, was originally isolated from rat stomach. It stimulates the release of growth hormone from primary pituitary cell cultures. We investigated the plasma concentration of ghrelin peptide in 16 nonpregnant women, 18 normal pregnant women, 20 patients with pregnancy-induced hypertension, and 10 postpartum women. The plasma concentration of ghrelin in nonpregnant women was 239.5 ± 16.9 fmol/mL. The plasma concentration of ghrelin in normal pregnant women at the third trimester was 127.1 ± 5.6 fmol/mL. There was a negative correlation between plasma ghrelin concentration and systemic blood pressure in normal pregnant women (systolic: \( r = -0.564, P < 0.05 \); diastolic: \( r = -0.610, P < 0.01 \)). Pregnant women with pregnancy-induced hypertension (177.9 ± 14.6 fmol/mL, \( P < 0.05 \)) also had significantly higher levels of ghrelin compared with those of normal pregnant women. In addition, there was a significant correlation between plasma ghrelin levels and systemic blood pressure (systolic: \( r = -0.482, P < 0.05 \); diastolic: \( r = -0.466, P < 0.05 \)). These results suggest for the first time that ghrelin might have some role in cardiovascular control during normal pregnancy and in pathophysiological conditions in pregnancy, such as pregnancy-induced hypertension. (Hypertension. 2002;39:781-784.)

Key Words: blood pressure ■ growth substances ■ preeclampsia ■ pregnancy

G

ghrelin, an endogenous ligand for the growth hormone (GH) secretagogues, was identified from rat stomach and subsequently cloned in rats and humans.\(^1\) Ghrelin stimulates GH release in rat pituitary cell cultures, and intravenous ghrelin administration resulted in an increase in serum GH in rat and human.\(^1,2\) Rat and human ghrelin differ by only 2 amino acids, suggesting an important physiological role. In addition, ghrelin immunoreactivity has been located in the hypothalamic arcuate nucleus.\(^3\) On the other hand, ghrelin receptors are expressed in various tissues such as the brain\(^3–5\) and blood vessels.\(^6\)

It has been recently demonstrated that intracerebroventricular injection of ghrelin strongly stimulates feeding in rats and increased body weight gain.\(^7,8\) These findings suggest that ghrelin may function not only as GH secretagogues but also as a regulator of energy balance.

It has been recently reported that ghrelin is present in other tissue, such as human placenta, both at the first trimester and after delivery,\(^9\) which suggests that this peptide may play an important role in the reproductive physiology. However, there have been no reports on the amount of ghrelin peptide in human fetal-maternal circulation in pregnancy.

Pregnancy-induced hypertension (PIH) is a common cause of maternal or fetal mortality. Shallow endovascular cytotrophoblast invasion in the spiral arteries and endothelial cell dysfunction are 2 key features in the pathogenesis of PIH; however, the etiology of preeclampsia is still unknown.\(^10\)

Therefore, in the present study, to elucidate whether ghrelin has any implications as a physiological or pathophysiological substance in pregnancy, we examined the plasma concentration of ghrelin in maternal venous blood and umbilical artery blood in pregnant women.

Methods

Subjects

The study population consisted of 16 nonpregnant women, 18 normal pregnant women, 20 patients with PIH, and 10 postpartum women, who were admitted to the Obstetrical Clinic of the School of Medicine of Fukuoka University between March 1, 1998, and April 30, 2001. The normotensive group had no abnormal alterations in blood pressure, proteinuria, or edema and did not undergo any drug administration during pregnancy. The diagnosis of PIH was made on the basis of systolic blood pressure of ≥140 mm Hg or diastolic pressure of ≥90 mm Hg, with or without proteinuria and edema. Only singleton pregnancies delivered from 28 to 41 weeks of gestation with no evidence of fetal anomalies were included in this study. There were no remarkable past histories except for previous cesarean section. This protocol was approved by the Human Investigational Review Board for the School of Medicine of Fukuoka University.
Statistical Analysis

Data for ghrelin peptide levels are expressed as the mean±SEM. Data of clinical characteristics are expressed as the mean±SD. The data were compared using Newman-Keul’s multiple comparison test (maternal blood) or Student’s t test (clinical data and umbilical cord blood). Correlations between peptide level and blood pressure was examined by linear regression analysis. A value of P<0.05 was considered significant.

Results

There were no significant differences in maternal age and gestational age at sampling of the umbilical artery blood (Table). The systolic and diastolic blood pressures were significantly higher in the patients with PIH than in nonpregnant and normal pregnant women (P<0.01) (Table).

Immunoreactive Ghrelin

The plasma concentration of ghrelin was 239.5±16.9 and 221.5±16.0 fmol/mL in the nonpregnant and postpartum women, respectively, and there was no significant difference between the 2 groups (Figure 1). On the other hand, the

<table>
<thead>
<tr>
<th>Variables</th>
<th>Nonpregnant Women</th>
<th>Normal Pregnant Women</th>
<th>Pregnant Women With PIH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>30.8±4.0</td>
<td>30.4±4.7</td>
<td>32.0±3.0</td>
</tr>
<tr>
<td>Systemic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic, mm Hg</td>
<td>109.1±8.5</td>
<td>110.9±13.0</td>
<td>157.5±16.7*</td>
</tr>
<tr>
<td>Diastolic, mm Hg</td>
<td>66.0±7.0</td>
<td>67.1±8.6</td>
<td>99.6±9.0*</td>
</tr>
<tr>
<td>Gestational age at delivery, week</td>
<td>—</td>
<td>38.1±1.4</td>
<td>35.2±3.6*</td>
</tr>
<tr>
<td>Gestational age at venipuncture, week</td>
<td>—</td>
<td>34.3±3.6</td>
<td>35.0±3.8</td>
</tr>
<tr>
<td>Infant birth weight, g</td>
<td></td>
<td>2,955.8±323.6</td>
<td>1,951.2±828.6*</td>
</tr>
<tr>
<td>Gestational age at sampling of the umbilical artery, week</td>
<td>—</td>
<td>37.6±1.6</td>
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Values are mean±S.D. *P<0.01 vs normal pregnant women.

University. Written agreement from all participants was obtained before this study.

Quantification of Immunoreactive Ghrelin in Plasma

Blood samples were taken from the antecubital vein in the morning between 7:00 AM and 8:00 AM after an overnight fast, because plasma ghrelin level has been shown to be altered by food intake. 11,12 Also, 36-hour incubation at 4°C, 100°/H9262 at a dilution of 1:10 000. After 12-hour incubation at 4°C, 100°/H9262, ghrelin level has been shown to be altered by food intake. 11,12 Also, the RIA incubation mixture consisted of 100°/H9262 against the C-terminal fragment 13

60% acetonitrile containing 0.1% TFA. The eluate was then eluted with 3 mL saline and then loaded into a Sep-Pak C18 cartridge. After the column was washed with 5 mL each of saline and 5% acetonitrile containing 0.1% TFA, the absorbed materials were eluted with 3 mL 60% acetonitrile containing 0.1% TFA. The eluate was then lyophilized.

Radioimmunoassay (RIA) for plasma ghrelin was performed as described previously. 13 Briefly, a polyclonal antibody was raised against the C-terminal fragment 13–18 of rat ghrelin in a rabbit. A maleimide-activated maiculture keyhole limpet hemocyanin (mKHL)-[Cys 0]-ghrelin 13–18 conjugate was used for immunization. The RIA incubation mixture consisted of 100 μL of standard ghrelin or unknown sample, normal rabbit serum, and 200 μL of antiserum at a dilution of 1:10 000. After 12-hour incubation at 4°C, 100 μL of 125I-labeled ligand (15 000 cpm) was added to the mixture. After a 36-hour incubation at 4°C, 100 μL of goat-rabbit IgG antiserum was added. Free and bound tracers were separated by centrifugation at 3000 rpm for 30 minutes after incubation for 24 hours at 4°C. Pellet radioactivity was quantified using a γ-counter. The minimum detectable dose of ghrelin was <6 fmol/tube. The antiserum exhibited 100% cross-reactivity with rat or human ghrelin. 13–18 No significant cross-reactivity with other peptides was observed. Intraobserver variability of ghrelin measurement was <6%, and its interobserver variability was <9%. Day-to-day variation was <9%. The recovery of human ghrelin (1 ng) added to the plasma sample was 95%.

Figure 1. Concentration of plasma ghrelin in maternal (A) and umbilical cord (B) blood. Values are mean±SEM. *P<0.05 vs normal pregnant women. Non-preg indicates non-pregnant women; pregnant, normal pregnant women.

Comparison of Pregnancy Outcomes for Normotensive and PIH Women

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Values are mean±S.D. *P<0.01 vs normal pregnant women.

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plasma concentration of ghrelin in normal pregnant women at the third trimester was 127.1±5.6 fmol/mL. Moreover, the plasma concentration of ghrelin was 177.9±14.6 fmol/mL in the pregnant women with PIH, and the levels were significantly higher than those in the normal pregnant women (P<0.05). There was no significant difference between 8 normal pregnant women and 12 patients with PIH in regard to concentration in the umbilical artery blood (183.9±22.0 and 213.2±19.1 fmol/mL, respectively). However, in the normal pregnant women, the plasma ghrelin concentrations in umbilical artery blood were significantly higher than those in maternal blood (P<0.05).

The Relationship Between Plasma Ghrelin Level and Systemic Blood Pressure

Figure 2 shows the relationship between plasma ghrelin concentration and systemic blood pressure in 3 groups. In nonpregnant women, there was no correlation between plasma ghrelin concentration and systemic blood pressure. However, in normal pregnant women (Figure 2B), plasma ghrelin concentrations were negatively correlated with the systemic blood pressure women (systolic: r = 0.564, P<0.05; diastolic: r = -0.610, P<0.01). Furthermore, plasma ghrelin concentrations were also negatively correlated with the systolic and diastolic blood pressure in the patients with PIH pressure (systolic: r = -0.482, P<0.05; diastolic: r = -0.466, P<0.05).

Discussion

Ghrelin, an endogenous ligand for the GH secretagogues receptor, was originally isolated from rat stomach. Ghrelin stimulates GH release in rat pituitary cell cultures, and intravenous ghrelin administration resulted in an increase in serum GH in rat and human.1,2 It is well documented that GH plays an important role in metabolic changes occurring in late pregnancy in the rat.14 Also, it has been demonstrated that plasma GH levels increased during pregnancy in human.15 However, in the present study, the maternal plasma concentration of ghrelin was markedly decreased in normal pregnant women in the third trimester. The present results thus suggest that plasma ghrelin levels do not play an important role in maintaining circulating GH levels during pregnancy.

We also demonstrated that plasma ghrelin concentrations were negatively correlated with systemic blood pressure in normal pregnant women but not in nonpregnant women.

During pregnancy, a number of physiological changes occur in the maternal circulation to accommodate the growing fetus. These changes usually include an increase in cardiac output and a decrease in arterial blood pressure and total peripheral resistance. However, the mechanisms of this physiological adaptation remain to be elucidated. On the other hand, the cardiovascular effects of ghrelin are still unknown at present. However, recent reports have demonstrated that ghrelin peptide and its specific binding sites are detected in cardiovascular systems, including the heart, kidney, and blood vessels.6,13 Therefore, it is interesting to speculate that ghrelin may have some role in the cardiovascular system. In fact, very recently, Nagaya et al reported that ghrelin decreased mean arterial pressure but did not increase heart rate in healthy volunteers.13 They have also mentioned that ghrelin induced hypotension in hypophysectomized rats and speculated that ghrelin has a direct vasorelaxant effect. From these findings, it is thus possible that ghrelin may be an important mediator in cardiovascular control during pregnancy.

Furthermore, plasma ghrelin concentration was significantly higher in patients with PIH than in normal pregnant women, and there was significant correlation between plasma ghrelin concentration and systemic blood pressure in patients with PIH. Therefore, we speculate that a compensatory increase of ghrelin occurred in patients with PIH.

Previously, we developed 2 RIAs specific for the C- and N-terminal regions of ghrelin.16 Using these 2 RIAs combined with high-performance liquid chromatography, we found 2 major molecular forms of ghrelin, an n-octanoylated and des-acyle ghrelin, in rat stomach and intestine.16 RIA for C-terminal region of ghrelin detected both n-octanoylated and des-acyle ghrelin. RIA for N-terminal region of ghrelin
detected only n-octanoylated ghrelin. Des-acyl ghrelin has no biological activity to increase the intracellular calcium concentration. In the present study, we also measured the plasma ghrelin level by the RIA for N-terminal region of ghrelin in nonpregnant women. The plasma n-octanoylated ghrelin (active form) concentration in nonpregnant women was 26.7 ± 8.3 fmol/mL. Therefore, we think that the percentage of active form in plasma ghrelin is ≈10%. However, further studies are needed to determine whether des-acyl ghrelin is a premodified form or a n-octanoyl–deleted form.

Recently, it has been demonstrated that administration of ghrelin, in addition to its role in regulating GH secretion, caused weight gain by reducing fat utilization in mice and rats. It is well documented that energy requirements are increased during pregnancy. These increased demands can be met through metabolic adaptations and increased dietary intakes. The significant decrease in circulating maternal ghrelin during the third trimester of normal pregnancy is hypothesized to be a response to the marked changes in maternal weight and energy expenditure. Therefore, it is hypothesized that the decreased plasma ghrelin concentrations during normal pregnancy may represent a physiological adaptation to the positive energy balance during pregnancy.

It has been recently demonstrated that plasma ghrelin concentration was decreased in obesity and that plasma ghrelin concentration was negatively correlated with body mass index. However, in the present study, it is unclear whether the decreased plasma ghrelin concentration is involved in energy balance during pregnancy. Further investigations are needed to clarify the possible involvement of ghrelin in energy homeostasis during pregnancy.

Another interesting finding in the present study was that ghrelin concentrations in umbilical artery blood were higher than those in maternal blood normal pregnant women, suggesting that the fetal-placental unit may contribute to its production. Furthermore, Gualillo et al. recently demonstrated that ghrelin mRNA and ghrelin peptides are present in the human and in rat placentae. These findings thus also suggest that ghrelin may have some role not only in maternal circulation but also in fetal-placental circulation.

In conclusion, although it still remains to be elucidated whether ghrelin has biological actions other than growth hormone secretion and food intake, our results suggest for the first time that ghrelin may have some important role in cardiovascular control during normal pregnancy and in pathophysiological conditions in pregnancy, such as PIH.

Acknowledgments
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