Circulating levels of GH-releasing hormone and GH during human pregnancy


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ABSTRACT

To study the potential role of GH-releasing hormone (GHRH) in maintaining circulating levels of GH during pregnancy, 302 maternal plasma samples were collected from non-fasted subjects at various stages of pregnancy and assayed for GHRH using a ‘two-site’ immunoradiometric assay. The GH and placental lactogen levels were also determined. In addition, maternal plasma samples taken during labour, amniotic fluid and cord blood were also assayed for these hormones.

Maternal plasma GHRH levels were similar to non-pregnant levels throughout gestation despite fluctuations in GH values which were always higher than non-pregnant levels. There was no significant difference between GHRH levels in maternal plasma and cord blood although high GH levels were observed in the latter. These findings suggest that peripheral GHRH levels do not play an important role in maintaining circulating GH levels during pregnancy. Journal of Endocrinology (1990) 125, 161–167

INTRODUCTION

Several hypothalamic hormones—thyrotrophin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH), corticotrophin-releasing hormone (CRH) and somatostatin—have been identified in the human placenta (Siler-Khodr & Khodr, 1978; Fitzpatrick & Patel, 1979; Shambaugh, Kubeck & Wilber, 1979; Shibasaki, Odagiri, Shizume & Ling, 1982) and elevated levels of LHRH and CRH have been reported in maternal plasma (Sasaki, Liotta, Luckey et al. 1984; Siler-Khodr, Khodr & Valenzuela, 1984; Campbell, Linton, Wolfe et al. 1987; Economides, Linton, Nicolaides et al. 1987). However, little is known about growth hormone-releasing hormone (GHRH) levels during pregnancy. Information on growth hormone (GH) itself is also confusing: maternal plasma GH levels have variously been reported as increased (Kletzky, Rossman, Bertolli et al. 1985), decreased (Frankenne, Closet, Gomez et al. 1988) or unchanged (Kaplan & Grumbach, 1965). These conflicting findings may be due to the fact that most radioimmunoassays (RIA) for GH cross-react with human placental lactogen (hPL) (Cornblath, Parker, Reisvier et al. 1965; Kaplan & Grumbach, 1965; Spellacy, Buhi, Bradley & Holsinger, 1973; Kletzky et al. 1985).

In the present study, specific and sensitive ‘two-site’ immunoradiometric assays (IRMA) were used to measure GHRH and GH in unextracted maternal plasma during pregnancy and in cord blood, amniotic fluid and maternal plasma at parturition. Preliminary reports of this work have been published elsewhere (Mazlan & McLean, 1988; Mazlan, Spence-Jones, Kye-Mensah et al. 1989).

MATERIALS AND METHODS

Materials

Bacitracin, bovine thyroglobulin, 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC), glutaraldehyde, Iodogen, N-ethyl-maleimide, phenylmethylsulphonyl fluoride (PMSF), p-hydroxymercuribenzoate, Poly-p-pep and thimerosal were purchased from Sigma Chemicals, Poole, Dorset, U.K. Sephadex G-50 and cyanogen-bromide activated Sepharose 4-B were obtained from Pharmacia Fine Chemicals, Hounslow, Middx, U.K. GHRH(1–44)NH₂ and the hypothalamic and gut peptides used for cross-reaction studies...
were from Peninsula Laboratories Europe Ltd, St Helens, Merseyside, U.K. GHRH fragments were gifts from Dr E. Penny, Department of Chemical Endocrinology, St Bartholomew’s Hospital, London. Human GH for immunization was an in-house preparation (Jones, Benker, Salacinski et al. 1979) and the human GH used as standard was the International Reference Preparation from the National Institute of Biological Standards and Control, South Mimms, Herts, U.K. Human serum albumin was obtained from Blood Products Laboratories, Elstree, Herts, U.K. I25Iodine was purchased from Amersham International plc, Amersham, Bucks, U.K., keyhole limpet haemocyanin (KLH) was from Calbiochem Behring Diagnostics, La Jolla, CA, U.S.A., and normal rabbit serum (NRS), normal sheep serum (NSS) and sheep anti-rabbit Fc (SARFp) serum from Polyclonal Antibodies Ltd, Llandysul, S. Glam., U.K. All other salts and solvents were AnalR grade from BDH Chemicals, Poole, Dorset, U.K.

Subjects

This study was approved by the Ethical Committee of the City and Hackney Health Authority, London and informed consent was obtained from all subjects. Venous blood (10 ml) was collected from non-fasting pregnant (n = 302) and non-pregnant (n = 30) women aged 15–45 years. The period of gestation was determined from the date of the last menstrual period and, in most cases, by ultrasound. Umbilical venous cord blood was obtained from term infants (n = 17) delivered vaginally after spontaneous onset of labour. Amniotic fluid (n = 17) and maternal venous blood samples at parturition (n = 30) were also collected.

Specimens were collected into lithium-heparinized tubes, centrifuged (3000 g, 15 min, 4 °C) and the supernatants separated and stored at −20 °C until assayed for GHRH, GH and hPL.

Tissue extractions

Previously frozen human hypothalami from two patients (A and B) who had died of non-neurological diseases were homogenized at 4 °C in HCl (0-1 mol/l) containing PMSF, p-hydroxymercuibenzoate, N-ethylmaleimide and EDTA, each to a final concentration of 0-001 mol/l and bacitracin and thimerosal (both 0-01%, w/v), using a Silver homogenizer. The homogenate was centrifuged (10 000 g, 90 min, 4 °C) and the supernatants freeze-dried in the presence of mannitol (100 mg/ml) in silanized glassware to prevent physical losses. They were reconstituted in dilute HCl (0-1 mol/l) and 1 ml was neutralized with NaOH (5 mol/l), double-diluted in treated human plasma and assayed for GHRH using the GHRH IRMA. The remainder was chromatographed on Sephadex G-50 equilibrated and eluted with formic acid (1%, v/v) containing Polypep (0-01%, w/v). The fractions were assayed for GHRH-like immunoreactivity using the GHRH IRMA.

Plasma extractions

Previously frozen third trimester maternal plasma (n = 30) was centrifuged (3000 g, 15 min, 4 °C) to remove fibrin clots, pooled (50 ml), PMSF (5 mmol/l) added at 4 °C, and 1 ml taken for GHRH measurement. Concentrated HCl (1 ml) was added to the pooled plasma, dropwise, with stirring at 4 °C to give a final HCl concentration of 0-2 mol/l. Cold methanol (100%, 50 ml) was added slowly with stirring. The mixture was stirred for a further 16 h at 4 °C and centrifuged (10 000 g, 90 min, 4 °C) to give a clear and colourless supernatant (50 ml). The volume of the supernatant was reduced (25 ml) under a stream of nitrogen, freeze-dried with mannitol, reconstituted in 1 mol HCl/l (5 ml), centrifuged, and chromatographed on a Sephadex G-50 column (1.5 x 88 cm), eluted with formic acid:Polypep buffer at 6 ml/h. Fractions (3 ml) were collected and assayed for GHRH-like immunoreactivity.

GHRH IRMA

Sheep anti-GHRH sera were raised against GHRH(1-44)NH2 (1 mg) linked to KLH (4 mg) using EDC (100 μg). Rabbit anti-GHRH(1-40)OH serum was a gift from Dr E. Penny.

Labelled specific IgG was prepared by affinity purification of the sheep antiserum (40 ml) according to the method described by Hodgkinson & Lowry (1982) and iodinated using the iodogen method of Salacinski, McLean, Sykes et al. (1981).

GHRH(1-44)NH2 standards were diluted in human plasma previously treated as described by Linton, McLean, Kruseman et al. (1987). Duplicates of standards (range 0–5000 pg/ml) and samples (200 μl each) were incubated with a reagent mixture (200 μl) comprising phosphate buffer (0-05 mol/l; pH 7-4), human serum albumin (HSA; 0-5%, v/v), polyethylene glycol (PEG; 2%, w/v), NRS (0-1%, v/v), NSS (0-2%, w/v), thimerosal (0-001 mol/l), rabbit anti-GHRH serum (1:1000), labelled specific IgG (100 000 c.p.m./200 μl) and PMSF (0-001 mol/l) overnight at 4 °C. Separation was achieved by the addition of SARFp (1:20 in phosphate buffer 0-1 mol/l; pH 7-4; 200 μl), incubation for 30 min at room temperature and centrifugation (3000 g, 30 min, 4 °C). The supernatants were aspirated and the precipitates counted for radioactivity.

Blanks for each sample and standard were set up by incubating the reagent mixture with the link antibody (rabbit anti-GHRH serum) replaced by NRS.
This assay is specific for GHRH(1-44) (Fig. 1a) and human hypothalamic extracts gave a parallel response (Fig. 1b). No cross-reactions were observed with CRH, rat GHRH, human oxytoxin, angiotensin, somatostatin-14, neurophysin, secretin, vasoactive intestinal peptide, peptide-histidine-methionine, gastrin-inhibiting polypeptide, Polypep, purified bovine serum albumin and human globin, gelatin, insulin, C-peptide, glucagon, glucagon-like peptide I, glucagon-like peptide II, oxyntomodulin, human chorionic gonadotrophin (hCG) and pancreatic polypeptide up to a concentration of 2 nmol/l.

The sensitivity of the assay expressed as 2.5 S.D. from the mean of repeated measurements (n = 8) of the zero standard was 12.5 ng/l. The between-assay precision expressed as a coefficient of variation was 6.8%, 3.2% and 4% for mean values (n = 6) of GHRH (50, 200 and 2500 ng/l). The precision profile
shows a coefficient of variation of less than 2.5% over the range of 40–2500 ng/l and less than 5% between 10–40 ng/l.

**GH IRMA**

Labelled specific antibodies were prepared from sheep anti-GH serum (20 ml) as described above. The link antiserum was raised in rabbits against GH (10 mg) conjugated to muramyl-alanyl-D-glutamine (0.5 g) using EDC (100 μg).

GH standards (0–80 mU/l) were diluted in NSS. Duplicates of standards/samples (100 μl) were incubated with GH-reagent mixture (200 μl) overnight at room temperature. The GH-reagent mixture was phosphate buffer (0-05 mol/l; pH 7-4) containing HSA (0.5%), PEG (2%), NRS (1%), NSS (2%) and mannitol (1%). The precipitating reagent (200 μl) was a phosphate buffer (0.1 mol/l; pH 7.4) containing SARFe (1:20), NRS (1%) and PEG (2%) which was mixed and incubated overnight at 4°C prior to addition. Tubes were vortexed, left at room temperature for 30 min and NaCl (0-15 mol/l; 1 ml) added before centrifugation (3000 g, 30 min, 4°C); the supernatants were aspirated and the precipitates counted. The sensitivity of the assay (n=8) was 0.2 mU/l and the intra-assay precision (n=8) determined on pooled human samples with mean values of 4.1 mU/l, 16.2 mU/l and 26.0 mU/l were 2.0%, 4.6% and 3.2% respectively. The interassay precision determined from the means of eight assays using the same samples were 3.2%, 6.5% and 7.1% respectively. Prolactin and hCG cross-reacted less than 0.01%, the cross-reaction of hPL was maximal (0.18%) at 0.04 mg/l falling to 0.005% at 6.7 mg/l hPL.

**hPL RIA**

Samples were measured for hPL using the RIA described by Houghton, Shackleton, Obieke & Chard (1984).

**Measurement of GH in the presence of hPL**

The cross-reactions of hPL at concentrations found in the first, second and third trimesters and in amniotic fluid and cord blood in the GH IRMA were determined. GH standard curves were set up with each tube containing the average hPL concentration for each of these situations and the GH level read from the standard curve containing the appropriate hPL level for the sample.

**Statistical evaluation**

Results are expressed as means ± s.e.m. and the significance of differences between the means were analysed using both the Student's t-test and the Mann–Whitney U test.

**RESULTS**

Table 1 summarizes the mean levels of GHRH, GH and hPL in maternal plasma throughout gestation and in cord blood, amniotic fluid and maternal plasma at parturition. The maternal plasma levels of GHRH did not alter significantly during the course of pregnancy and were no different from the levels found in non-fasting, non-pregnant women. At parturition, the mean maternal plasma GHRH was not significantly different from the third trimester maternal level or the GHRH level in cord blood or in amniotic fluid. The level of GHRH in cord blood was not significantly different from that of amniotic fluid.

<table>
<thead>
<tr>
<th>Samples</th>
<th>GHRH (ng/l)</th>
<th>GH (mU/l)</th>
<th>hPL (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td></td>
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<tr>
<td>First trimester</td>
<td>52.6 ± 5.8 (67)</td>
<td>9.8 ± 1.2 (30)</td>
<td>0.5 ± 0.1 (36)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>57.2 ± 5.2 (95)</td>
<td>6.7 ± 0.8 (30)</td>
<td>2.0 ± 0.2* (31)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>57.6 ± 3.4 (140)</td>
<td>4.9 ± 0.6 (30)</td>
<td>6.4 ± 0.2* (53)</td>
</tr>
<tr>
<td>At parturition</td>
<td></td>
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</tr>
<tr>
<td>Maternal plasma</td>
<td>38.7 ± 5.4 (30)</td>
<td>4.0 ± 0.4 (30)</td>
<td>6.2 ± 0.3 (30)</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>44.7 ± 8.0 (17)</td>
<td>3.7 ± 0.4 (17)</td>
<td>0.6 ± 0.4* (17)</td>
</tr>
<tr>
<td>Cord blood</td>
<td>60.3 ± 11.8 (17)</td>
<td>37.2 ± 7.3 (17)</td>
<td>0.05 ± 0.01* (17)</td>
</tr>
<tr>
<td>Non-pregnant women</td>
<td>60.6 ± 6.4 (30)</td>
<td>1.2 ± 0.3* (30)</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.001 compared with preceeding group; †P < 0.04 compared with first trimester; ‡P < 0.0001 compared with all pregnancy samples (Student's t-test).
The mean GH level in the first trimester was not significantly different from that in the second trimester, and the GH level in the second trimester was not significantly different than that of the third trimester. However, the first trimester level was significantly higher than that in the third trimester and GH levels were higher throughout pregnancy than in non-pregnant women. The mean GH level in cord blood was significantly higher than that of term maternal plasma and amniotic fluid. This trend was also observed when the GH values were read off standard curves with the appropriate hPL levels added. Added hPL had no significant effect on the GH standard curve in this assay.

The maternal plasma hPL level increased as pregnancy progressed with the mean level in the third trimester being significantly higher than in the second trimester which, in turn, was significantly higher than during the first trimester. The mean hPL level in maternal plasma during labour was some ten times higher than that in amniotic fluid and more than a hundred times the level in cord blood. The hPL level in amniotic fluid was significantly higher than the level in cord blood. These data agree with previous reports (Spellacy & Buhi, 1969; Spellacy, Buhi & Birk, 1970; Braunstein, Rasor & Wade, 1980; Houghton, Shackleton, Obiekwe & Chard, 1984).

Figure 2 shows the results of gel permeation chromatography of the pooled third trimester maternal plasma. The GHRH IRMA revealed a single peak corresponding to the expected position of hypothalamic and synthetic GHRH(1-44)NH₂.
In this study the levels of plasma GHRH in pregnant women were similar to non-pregnant levels. Saito, Saito, Yamazaki & Hosoi (1984), using an extraction RIA, made a similar observation on a smaller group of women. In contrast, plasma GH levels were found to be significantly higher than in non-pregnant, non-fasting normals from the earliest weeks of pregnancy. This agrees with reports by Kletzky et al. (1985). The high GH level in early pregnancy is unlikely to be due to placental GH or hPL because both of these molecules circulate in large amounts only in the later stage of pregnancy (Frankenne et al. 1988). Similarly prolactin is present at low concentrations during early pregnancy (Knuth & Friesen, 1983).

These results suggest that circulating maternal GHRH plays no direct role in the control of GH levels during pregnancy, a conclusion similar to that reported for somatostatin (Saito, Saito, Sano & Hosoi, 1983; Webb, Wass, Penman et al. 1985). A possible explanation for the elevated maternal GH in early pregnancy could be increased pituitary sensitivity to stimulators of GH release such as GHRH. This has been demonstrated in the rat in vitro (Sheppard & Bala, 1987).

Maternal plasma GH levels decreased from the second trimester onwards. This agrees with the observations of Frankenne et al. (1988). A possible explanation for the reduction in GH levels may be a decrease in responsiveness of the maternal anterior pituitary to GHRH. Diminished serum GH responses to stimulation by insulin or arginine from the second trimester onwards have been reported (Spellacy & Buhi, 1969; Spellacy et al. 1970; Tyson & Friesen, 1973; Artenisio, Volpe, Rayonese et al. 1980). Hence, the high GH levels of the first trimester may be the result of hypersensitivity of the maternal anterior pituitary but as pregnancy progresses the pituitary becomes less responsive. The resulting decrease in pituitary GH may be functionally replaced by placental GH.

The GHRH levels in amniotic fluid and maternal plasma during labour have not previously been reported. GHRH levels in cord blood were similar to those reported earlier (Saito et al. 1984; Nagashima, Yagi, Suzuki et al. 1986; Argente, Acquafredda, Cavallo et al. 1987). There was no significant difference between the levels of GHRH in cord blood and that of the maternal plasma; this contrasts with the findings of both Saito et al. (1984) and Nagashima et al. (1986). However, these investigators compared GHRH levels in cord blood with those of fasting adults, while in the present study the subjects were not fasted. Furthermore Nagashima et al. (1986) also reported that there was no correlation between the GHRH and GH levels in the cord blood and adult samples. This agrees with the present conclusion that increased release of GHRH is unlikely to be the cause of the high GH level in cord blood.

Increased sensitivity of the fetal pituitary to fetal GHRH may account for the increase in GH in cord blood. Rat fetal pituitary cells have been shown to be more responsive to GHRH in vitro compared with adults (Khorram, De Palatis & McCann, 1983).

It is also possible that TRH might be responsible for the high GH levels in cord blood. TRH can preferentially stimulate GH in immature compared with adult animals including man both in vivo (Gill-Ad, Cocchi, Pannauti et al. 1976; Roti, Gnudi, Robuschi et al. 1982; MacDonald, Spencer & Hallet, 1985) and in vitro (Rietort, 1981; Khorram et al. 1983). High levels of TRH have also been demonstrated in cord blood (Perelman, Klein & Fischer, 1981).

In conclusion, GHRH levels did not correlate with the variations in GH levels seen during pregnancy and in cord blood. It is possible that changes in sensitivity of the anterior pituitary to GHRH, somatostatin and TRH (in the fetus and neonates) may play a more important role in maintaining the GH levels during this period.

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REFERENCES


