Modulation of human chorionic gonadotrophin bioactivity during the first trimester of pregnancy*

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The objective of this study was to evaluate the bioactivity of human chorionic gonadotrophin (HCG) during first trimester pregnancy. This was done by means of a retrospective analysis of sera from patients with first trimester normal intrauterine and ectopic pregnancies. Serum samples were obtained from 38 women with amenorrhoea of <10 weeks. From these, 19 had a normal intrauterine pregnancy (IUP) and 19 an ectopic pregnancy (EP). Cases were allocated to either low serum immunoreactive HCG (HCGi), intermediate HCGi or high HCGi concentrations (HCGi <5000 mIU/ml, between 5000 and 40 000 mIU/ml and >40 000 mIU/ml respectively). HCGi and oestradiol were measured by enzyme immunoassays and bioactive HCG by the mouse Leydig cell bioassay. All results were analysed by analysis of variance and unpaired Student’s t-test. There was a significant difference between bioactive to immunoreactive HCG ratios (b/i ratio) between the subgroups of low, intermediate and high HCGi concentrations. Lower b/i ratios were found when HCGi concentrations were high (HCG b/i mean ± SEM: high subgroup, 0.33 ± 0.07 versus low subgroup: 1.50 ± 0.12; P < 0.0001). Furthermore, the b/i ratios were inversely correlated with oestradiol (P < 0.0001) and HCGi (P < 0.0001) concentrations but not with gestational age. There was no difference in the b/i ratios when comparing IUP with EP. It is concluded that, in first trimester pregnancies, there is a likely modulation of HCG bioactivity which is inversely correlated with HCGi and oestradiol concentration. The underlying mechanisms and their physiological relevance remain to be elucidated.

Keywords: bioactivity/ectopic pregnancy/HCG immunoreactivity/intrauterine pregnancy/oestradiol

Introduction

Human chorionic gonadotrophin (HCG), a member of the glycoprotein hormone family which includes the pituitary hormones: luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), is composed of two distinct non-covalently associated subunits, namely the α and β subunits. Intact HCG, i.e. combined α and β subunits and its free subunits are secreted by trophoblast cells, in particular by the syncytiotrophoblast. Recent studies demonstrated that several HCG isoforms modified in their carbohydrate moiety or even in their peptide bond (nicked HCG) could be implicated in the modulation of HCG bioactivity (Cole et al., 1993; O’Connor et al., 1994).

It is generally accepted that HCG bioactivity changes during pregnancy with the highest in-vivo biological activity during the first trimester (Wide, 1962). This observation was followed by studies demonstrating a higher molecular weight of HCG at 10 weeks than at 33 weeks of gestation (Fein et al., 1980). Furthermore, late pregnancy HCG is less negatively charged and has a lower HCG b/i ratio than the early pregnancy HCG (Wide and Hobson, 1987). A change in the nature of these HCG isoforms seems to occur at around the 13th week of gestation with the appearance of less acidic HCG molecules later in pregnancy (Wide et al., 1994). More recently, Lopata et al. (1997) demonstrated that cultured human embryos in an in-vitro fertilization (IVF) programme produce less acidic HCG isoforms with the advancement of their development. Since HCG isoforms seem to change with time during early embryo development, we decided to investigate whether these changes were measurable in the maternal circulation in terms of modification of HCG bioactivity.

Materials and methods

Only women (n = 38) with amenorrhoea of <10 weeks were included in this study. Among these, 19 patients had a normal intrauterine pregnancy (IUP) and 19 women had an ectopic pregnancy (EP) of similar gestational age (43.6 and 46.6 days respectively). EP were diagnosed by laparoscopy and all were tubal pregnancies. IUP were confirmed by first trimester transvaginal echography and all cases had a normal term delivery. All cases were allocated to three subgroups according to their immunoreactive HCG concentration (HCGi): <5000 mIU/ml (low), between 5000 and 40 000 mIU/ml (intermediate) and >40 000 mIU/ml (high).

Serum samples were obtained (one sample per patient) at the time of echographic diagnosis in normal IUP [32–66 days after last menstrual period (LMP)] or at the day of laparoscopy in the EP (35–61 days after LMP). Immunoreactivity of HCG and its free β subunit (total HCG) was measured by enzyme immunoassay (IMX, Abbott Park, Illinois, USA) at a sensitivity of 1 mIU/ml and with a between-assay coefficient of variation of 3.4%. According to the manufacturer’s data, this assay does not crossreact with α HCG. Oestradiol was measured by an automated enzyme immunoassay (Vidas, Biomérieux, © European Society for Human Reproduction and Embryology 2629
Table I. Comparison of bioactive and immunoreactive human chorionic gonadotrophin ratios (HCG b/i) in serum of first trimester pregnancies (n = 38) between the three subgroups, irrespective of the implantation site

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>n</th>
<th>b/i mean</th>
<th>SEM</th>
<th>HCGi range (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: low</td>
<td>19</td>
<td>1.50</td>
<td>0.12</td>
<td>175-3849</td>
</tr>
<tr>
<td>B: intermediate</td>
<td>10</td>
<td>1.01</td>
<td>0.24</td>
<td>8110-37332</td>
</tr>
<tr>
<td>C: high</td>
<td>9</td>
<td>0.33</td>
<td>0.07</td>
<td>45554-127820</td>
</tr>
</tbody>
</table>

Statistics on b/i: A versus B P = 0.0465, B versus C P = 0.0069, A versus C P < 0.0001.

Marcy-l’Etoile, France) at a sensitivity of 5 pg/ml and a between-assay coefficient of variation of 4.7%.

Bioactive HCG (HCGb) was measured in triplicate by a mouse Leydig cell bioassay, using WHO LH 80/552 as standard (Robertson and Binden, 1990). All samples were diluted in Dulbecco’s minimal essential medium (DMEM, 20 mM HEPES without bicarbonate or glutamine) supplemented with 1% bovine serum albumin (Sigma, St Louis, MO, USA; fraction V) and 4% calf serum. Leydig cells were obtained by mechanical dispersion of testes from Balb/c mice 6-8 weeks of age. At the end of a 4 h incubation of the cells with sera, testosterone concentrations were measured in the medium using a radioimmunoassay (DPC, Sunnyvale, CA, USA). In the first assay, similar dilutions were performed on all sera. In the second assay, sera with HCGb falling outside the reference curve were rediluted. This method allowed us to minimize the incidence of dilution on the results. In addition, this protocol was repeated in a second series of assays, confirming the observed data.

Statistical analyses were performed on a Power Macintosh computer using the Statview programme. Data were compared by analysis of variance (ANOVA) or unpaired Student’s t-test when appropriate.

Results

In Table I, the HCG b/i ratios between the three subgroups are compared for all pregnancies, irrespective of their site of implantation. The b/i ratio decreased with increasing immunoreactive HCG. There was a significant difference in HCG b/i ratios between low, intermediate and high HCGi subgroups (P = 0.0465 for low versus intermediate, P = 0.0069 for intermediate versus high and P < 0.0001 for low versus high).

A similar comparison of parameters in both IUP and EP is shown in Table II. Using an analysis of variance with Scheffe’s test, there was no difference in the gestational age between the different subgroups (A–F, Table II). Stratifying the data according to the site of implantation, we observed that in IUP and EP, the b/i ratio decreased significantly with increasing HCGi. This decrease, however, was not statistically significant when low and intermediate subgroups were compared in both IUP and EP (Table II). There was no difference in HCG b/i ratios between matched IUP and EP subgroups. Values of HCGi were not different when comparing matched IUP and EP subgroups except when the low subgroups were considered (P = 0.016, Table II). In contrast to b/i, oestradiol increased with increasing HCGi. The difference between the different subgroups for both IUP and EP was significant (P = 0.0469–0.0001), except when comparing the intermediate and high subgroups in EP. Moreover, EP had significantly (P = 0.046; 0.0469–0.0001) lower oestradiol concentrations than IUP, except when the intermediate subgroups were compared.

Considering all pregnancies, HCG bioactivity was closely correlated with HCG immunoreactivity (r = 0.869, P < 0.0001, result not shown). However, b/i ratios appeared to be negatively correlated with oestradiol (r = 0.624, P < 0.0001, Figure 1). A similar correlation was observed when considering IUP and EP separately (r = 0.586, P = 0.0020 and r = 0.567, P = 0.0115 respectively). Oestradiol and HCGi were closely correlated with gestational age in IUP only (r = 0.577, P = 0.0153). However, there was no correlation between b/i ratio and gestational age.

Discussion

Dimeric HCG and its free β subunit rise rapidly during the first trimester of pregnancy reaching a peak between 9 and 10 weeks, followed by a decline during the second trimester of pregnancy. The proliferative activity of trophoblastic cells (Kiss et al., 1997), placental progesterone (Wilson et al., 1984), placental gonadotrophin releasing hormone (GnRH) (Siler-Khodr et al., 1986) and its receptor (Lin et al., 1995) but not β2 adrenergic receptors or oxytocin receptors (Meuris et al., 1996) have been implicated in the regulation of HCG secretion. The regulation of trophoblastic GnRH expression is more complex and seems to involve factors such as oestradiol and progesterone (Dong et al., 1996) but also glucocorticoids (Chen et al., 1998). In contrast to these established regulatory processes, very little is known about the modulation of the biological activity of HCG.

The results of the present study indicate that the HCG b/i ratio decreases in the maternal circulation during early first trimester pregnancy. However, it is not clear why relatively high concentrations of bioactive HCG are associated with relatively low concentrations of immunoreactive HCG. It is clear that the changes in HCG bioactivity are not influenced by the site of implantation, as we noted a similar pattern of b/i ratio between ectopic and intrauterine gestations. To the
HCG bioactivity in pregnancy

Table II. Gestational age (GA), immunoreactive human chorionic gonadotrophin (HCGi), oestradiol and HCG b/i (bioactive/active) ratio in both normal intrauterine pregnancies (IUP) and ectopic pregnancies (EP) according to the subgroups of HCGi.

<table>
<thead>
<tr>
<th>Implantation site</th>
<th>n</th>
<th>GA in days (range)</th>
<th>HCGi (mIU/ml) ±SEM</th>
<th>HCG b/i ratio ±SEM</th>
<th>Oestradiol (pg/ml) ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. IUP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: low</td>
<td>9</td>
<td>40 (32–48)</td>
<td>2258 ± 399</td>
<td>1.3 ± 0.1</td>
<td>235.2 ± 37.5</td>
</tr>
<tr>
<td>B: intermediate</td>
<td>5</td>
<td>39 (35–42)</td>
<td>26 343 ± 3534</td>
<td>0.8 ± 0.4</td>
<td>398.8 ± 97.0</td>
</tr>
<tr>
<td>C: high</td>
<td>5</td>
<td>51 (45–66)</td>
<td>88 410 ± 5125</td>
<td>0.4 ± 0.1</td>
<td>790.4 ± 91.2</td>
</tr>
<tr>
<td>II. EP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D: low</td>
<td>10</td>
<td>46 (35–61)</td>
<td>969 ± 191</td>
<td>1.7 ± 0.2</td>
<td>96.0 ± 25.2</td>
</tr>
<tr>
<td>E: intermediate</td>
<td>5</td>
<td>44 (41–46)</td>
<td>15 584 ± 2120</td>
<td>1.3 ± 0.3</td>
<td>273.4 ± 43.8</td>
</tr>
<tr>
<td>F: high</td>
<td>4</td>
<td>50 (42–58)</td>
<td>73 104 ± 19 120</td>
<td>0.2 ± 0.1</td>
<td>406.5 ± 88.2</td>
</tr>
</tbody>
</table>

Statistics for b/i:
In IUP: A versus C, P = 0.005; A versus B, P = 0.071; B versus C, P = 0.261.
In EP: D versus F, P < 0.0001; D versus E, P = 0.220; E versus F, P = 0.002.
Statistics for HCGi: A versus D, P = 0.016.
Statistics for oestradiol:
In IUP: A versus C, P < 0.0001; A versus B, P = 0.0469; B versus C, P = 0.0001.
In EP: D versus F, P = 0.0008; D versus E, P = 0.0292; E versus F, not significant.
A versus D, P = 0.046; B versus E, not significant; C versus F, P < 0.0001.

The importance of oligosaccharides for the biological activity of HCG and other gonadotrophins is well established (Thtotakura et al., 1995). N-Linked oligosaccharides on the alpha chain (Asn 52) of HCG seem to play an important role in signal transduction, resulting in stimulation of steroidogenesis and cAMP production (Matzuck et al., 1989). Recently, Stanton et al. (1996) demonstrated for human FSH and LH, a linear correlation between their sialic acid content, radioreceptor activity and in-vitro bioactivity.

Steroids, in particular oestradiol, seem to influence the glycosylation process of gonadotrophins such as FSH and LH (Wide and Naessén, 1994), leading to a modulation of their bioactivity (Padmanabhan et al., 1988). A recent study (Dharmesh and Baenziger, 1993) suggested modulation by oestrogen of an enzyme implicated in the glycosylation of LH (β1,4 GalNAc-transferase).

In the present study, the significant negative correlation observed between the b/i ratio of HCG and plasma oestradiol concentrations could be explained by such steroid influence on HCG bioactivity. The question of whether oestradiol may directly influence the glycosylation process of HCG and its associated bioactivity will need to be addressed by future studies.

The observed lower values of oestradiol in EP as compared to IUP is not a novel finding (Milwidsky et al., 1977), and has been attributed among other reasons, to the less frequent presence of embryos in EP with a consequently lower source of oestradiol precursors (Barnea et al., 1986). It is interesting to note that despite these lower oestradiol concentrations in EP their potential effect on HCG glycosylation seems to be maintained, since we did not observe any significant difference in the HCG b/i ratio between IUP and EP. This last observation fits the data published by Buck et al. (1995), who demonstrated that there is no difference in the measurable isoforms of HCG between normal IUP and EP.

In conclusion, this study shows that changes in HCG bioactivity occur very early in pregnancy and that they might represent an adaptation to the continuously changing interactions between the embryo and the mother.

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References

P.Mock et al.


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