CLINICAL STUDY

Pro-opiomelanocortin in human pregnancy: evolution of maternal plasma levels, concentrations in cord blood, amniotic fluid and at the feto–maternal interface

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Abstract

Objective: The human placenta normally expresses the pro-opiomelanocortin (POMC) gene. The pattern and secretory kinetics of POMC and/or POMC-derived peptides by the placenta during gestation is still debated. We recently demonstrated that full length POMC was a normal product of the human placenta. The aim of our study was to establish its normal secretory kinetics and to explore its physiological relevance.

Design: In a prospective, longitudinal study, thirty normal pregnant women had monthly measurements of plasma POMC. In a cross-sectional study of 128 healthy pregnant women, plasma POMC and human chorionic gonadotrophin (hCG) were concomitantly measured to assess their correlation. Finally, POMC levels were assessed in venous and arterial cord blood samples, in amniotic fluid and in retroplacental blood.

Methods: Plasma POMC was measured by a specific IRMA in unextracted blood or biological fluid.

Results: Plasma POMC became detectable by the 8th week of pregnancy and reached its maximum at around the 20th week, remaining stable thereafter. The relationship between POMC and gestation time (weeks) best fitted with a third degree polynomial curve. A significant negative correlation (∏=0.01) was observed between plasma levels of POMC and hCG after adjustment for gestation time to take into account the dependence of both hormones on this parameter. POMC was not secreted into the fetal circulation at term, but was present in very high levels in amniotic fluid. The highest levels of POMC were present in the retroplacental blood where the values were 35 times higher than in maternal blood; by comparison, corticotrophin releasing hormone and ACTH values in this compartment were twice or equal to those in the maternal blood.

Conclusion: Placental POMC secretion increases during the first half of pregnancy and reaches a plateau from the 20th week to delivery. The inverse correlation between POMC and hCG plasma levels, and very high POMC levels at the feto–maternal interface suggest a physiological role for this precursor during pregnancy.

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Introduction

The human placenta produces numerous pituitary hormones, including adrenocorticotropic hormone (ACTH) and β-endorphin (1–3), both generated from the same high molecular weight precursor pro-opiomelanocortin (POMC) (4, 5). The gene coding for corticotrophin releasing hormone (CRH) is also expressed at a high rate (6, 7). There is evidence that these peptides play a role in the regulation of the maternal and/or fetal hypothalamo–pituitary–adrenal axis (2, 8).

These peptides can also play a critical role in placental functions: they may influence local placental secretions in an autocrine/paracrine manner (9, 10), display vasoactive effects upon fetal-placental circulation (11), and, at least for CRH, trigger the onset of parturition (12, 13). Maternal CRH plasma levels are low in the first half of pregnancy and rise exponentially thereafter (14). They are thought to reflect the placental production of this peptide (7). Variations in the production of POMC-derived peptides throughout pregnancy are still discussed. Maternal ACTH plasma levels show a progressive rise during human gestation (2, 15) but the pituitary as well as the placenta could contribute to this change. Comparison of POMC mRNA and
immunoreactive ACTH content in the placenta at different times of gestation has given conflicting results. Some investigators reported little or no variation in placental ACTH production (3, 4, 16) while a more recent and systematic study showed significantly higher POMC mRNA in the third than in the first trimester (17). However, the precise kinetics remain unknown and their biological significance is uncertain.

Until recently, it was admitted that POMC, which is not released in significant amounts by normal pituitary corticotroph cells, could be released only by poorly differentiated ACTH secreting tumours, i.e. non pituitary tumours responsible for the ectopic ACTH syndrome or invasive corticotroph macroadenomas (18–20). We recently demonstrated that it is also a normal secretory product of human placenta (21). Thus, pregnancy is the only physiological situation in which the full-length precursor was measured in plasma, as a result of incomplete precursor processing in placental cells. Its concentration in maternal blood reflects the sole placental secretion with no possible interference from pituitary peptides. Since little hormone is stored intracellularly, it also reflects the transcriptional activity of the POMC gene.

POMC was present in maternal blood from the first trimester of gestation, displayed no nycthemeral variations, was not suppressed by glucocorticoid, and became undetectable within two days after delivery (21).

In the present study, we established the exact time course of POMC in pregnant women and addressed the question of the physiological relevance of POMC production during human pregnancy. Thus, we compared its kinetics with that of other placental hormones and examined the levels of the precursor in some compartments of particular physiological interest, i.e. cordal blood, amniotic fluid and retroplacental blood.

We propose a mathematical model for POMC variations during pregnancy. POMC and human chorionic gonadotrophin (hCG) kinetics were in opposition from the end of the first trimester, and we observed a negative correlation between hCG and POMC levels after adjustment for gestation time. We also show that POMC is not secreted into the fetal circulation at term, but is present at very high levels in amniotic fluid and retroplacental blood.

**Subjects and methods**

**Subjects**

All women gave their informed consent to participate in these studies and approval was obtained from the Institutional Review Board of Cochin Hospital.

**Longitudinal study** Thirty women with normal monofetal pregnancies were recruited at their first visit and then followed with repeated sampling (4 to 8) at routine antenatal clinical visits, at 4- to 6-week intervals.

**Cross sectional study** To examine the correlation between POMC and hCG, we analysed samples obtained from 128 healthy pregnant women sampled once between the 8th and 39th week of pregnancy, from a previously reported cross sectional study (21).

Blood (10 ml) was collected between 0800 h and 1000 h in EDTA-containing plastic tubes (Terumo Europe N.V., Leuven, Belgium) kept chilled on ice and immediately centrifuged at 4 °C.

Retroplacental blood was obtained at the time of delivery from 10 placentas obtained after elective caesarean section from term-born, normally grown infants, as previously described (22). Maternal peripheral plasma samples, arterial and venous cordal blood, as well as amniotic fluid were concomitantly collected when possible.

**Hormone measurement** POMC IRMA was performed as already described (20). Briefly, it used a C-terminal anti-β-endorphin antibody linked to a plastic bead and a 1^125^ labelled monoclonal anti-ACTH antibody. Intra- and interassay coefficients of variation were 4 and 11% respectively. Semi purified human POMC was prepared as already described (20), allowing a detection limit of 100 U/ml.

The ACTH IRMA has been described previously (ELISA ACTH, Cis Bio International, Gif-sur-Yvette, France). The assay measures intact ACTH and does not react with small ACTH fragments or POMC up to 2700 U/ml. Intra- and interassay coefficients of variation were 6 and 7% respectively.

The hCG enzymatic immunoassay has been described previously (Kit hCG Extended Range-Bayer-Immuno One: Bayer Corporation, Tarrytown, NY, USA). The assay allows direct measurement of hCG in plasma between 1000 and 1 000 000 mIU/ml. Intra- and interassay coefficients of variation were 3 and 4% respectively.

The CRH RIA performed on extracted plasma, has been described previously (23). The sensitivity of the assay was 5 pg/ml. Intra- and interassay coefficients of variation were 6 and 8% respectively.

**Statistical analysis**

Data are reported as the mean ± 1 standard deviation (s.d.). Due to non normal distribution of variables, and to the small number of subjects in certain groups of interest, non parametric statistical methods were used to examine relationships between variables: the Wilcoxon test (2-sample test and pairwise test) and the Spearman rank correlation test. Spearman partial correlation statistics were used to control for the confounding effect of gestation time when testing the non parametric correlation between two quantitative
variables (POMC and hCG). Three samples had undetectable POMC plasma levels. For these samples, a value corresponding to 50 U/ml (the middle of the interval: 0-assay detection limit), was arbitrarily chosen for data analysis. The regression curve for hCG was fitted using the Microsoft Excel analysis tools package.

Longitudinal study: the relationship between POMC plasma levels and gestation time was assessed using random-effects linear models (24). These models enable estimations of mean parameters of a polynomial curve (intercepts, slopes, etc.) and testing of the null hypothesis that these parameters are equal to zero within the whole group of women. For each model tested, residuals were checked for normality, and their squares were averaged to form the mean squared error for assessing goodness-of-fit (25). Because the distribution of POMC was skewed, a log transformation was used to yield a variable that satisfactorily met the normality assumption of random-effects linear models. Computations were performed using SAS PROC MIXED (SAS Institute Inc., Cary, NY, USA) (26).

Results

Longitudinal study: POMC kinetics (Fig. 1)

Plasma POMC became detectable by the 8th to 10th week of pregnancy and reached its maximum at around 20 weeks, remaining stable thereafter. The relationship between POMC and gestation time in weeks best fitted with third degree polynomial curve (Fig. 1, lower panel). The values estimated for the different parameters were (estimate ± standard error): intercept: 2.6181 ± 0.43266 (P = 0.0001); first-order term: 0.313517 ± 0.065453 (P = 0.0001); second-order term: 0.010687 ± 0.003028 (P = 0.0006); third-order term: 0.0001186 ± 0.00004 (P = 0.007), giving the following equation:

\[
\log \text{POMC} = 2.6181 + 0.313517 \times \text{week} + (0.010687 \times \text{week})^2 + 0.000119 \times \text{week}^3
\]

The fit was satisfactory as the mean average error was 0.336, compared with the mean log POMC equal to 5.5.
Cross sectional study: POMC/hCG relationship

hCG levels in the cross sectional study are shown on Fig. 2. From the 8th week of gestation to parturition, POMC and hCG levels displayed an opposite course. A negative relationship was observed ($P = 0.01$) (partial Spearman correlation coefficient: $r = -0.23$) after adjustment for pregnancy duration to take into account the dependence of both hormones on this parameter.

POMC in cord plasma (Fig. 3)

To test whether POMC could be secreted by the placenta into the fetal circulation, we compared values obtained in venous and arterial cord blood at delivery (Wilcoxon pairwise test): POMC levels were $113 \pm 47$ U/ml in 9 venous cord blood samples and $145 \pm 52$ in 7 arterial samples (mean ± s.d.). These values were not significantly different ($P = 0.13$). In the 11 peripheral maternal blood samples collected at the same time, POMC concentrations were $310 \pm 185$ U/ml. Maternal blood levels were significantly higher than arterial or venous cord blood levels ($P = 0.06$ and $P = 0.01$ respectively, Wilcoxon pairwise test).

POMC in amniotic fluid

Levels were also high in five amniotic fluid samples: $3400 \pm 2830$ U/ml.

Hormonal studies in retroplacental blood (Fig. 4)

POMC levels were very high in 10 retroplacental blood samples at term ($11380 \pm 8240$ U/ml) (mean ± s.d.), reaching concentrations 35-fold higher than those observed in the peripheral venous blood samples obtained from the same women ($310 \pm 185$ U/ml) ($P = 0.008$, Wilcoxon test). No correlation was found between maternal and retroplacental POMC levels in this small group ($r = 0.40$, $P = 0.29$). CRH levels were also higher in retroplacental blood than in maternal blood at term: $3170 \pm 1530$ pg/ml vs $1380 \pm 1350$ pg/ml ($P = 0.02$, Wilcoxon test). In contrast, ACTH levels in retroplacental blood and maternal blood were very similar: $29.4 \pm 19$ and $30 \pm 22$ pg/ml ($P = 0.68$, Wilcoxon test) (Fig. 4).

Discussion

Full length POMC is produced by the human placenta and released in maternal blood (21) as a result of incomplete precursor processing by placental cells. POMC levels are also high in amniotic fluid, consistent with POMC gene expression in placental membranes (17).

POMC is not released in significant amounts by normal pituitary cells, and thus its plasma levels remain
in vivo modulate CRH influence. Also, other hormones or CRH binding proteins could influence placental corticotroph production could take place at specific periods of gestation. Effective CRH control of placental corticotroph production could release ACTH in response to CRH (27), while placental trophoblast (31). At the hormonal level, gonadotrophin secretions from the 9th week of gestation (31). The cellular mechanisms that lead to the onset of hCG gene transcription and the subsequent suppression of hCG gene expression after the first trimester are still poorly understood. The onset of hCG production appears linked to differentiation of the trophoblast cells in syncytiotrophoblast (31). At the hormonal level, gonadotrophin biosynthesis (31), the trophoblast cells in syncytiotrophoblast (31). In the same way, POMC variations during gestation differ from the slight but regular increase of ACTH in maternal blood (15). This argues against a sole placental origin for excess ACTH in pregnant women.

From the 20th week of pregnancy, maternal POMC levels are similar to those observed in aggressive neuroendocrine tumours (20). That placental POMC remains, for a large part, unprocessed raises the question of the possible physiological role of this secretion. The remarkable variation in POMC levels among different subjects does not rule out this possibility: plasma hCG levels also vary widely among pregnant women at the same week of their pregnancy, whereas hCG is necessary to maintain the corpus luteum of pregnancy. Such wide inter-individual variations are also reported for CRH (12, 13, 21). The bioactivity of POMC itself has seldom been studied. There is no evidence that the precursor in itself influences the hypothalamo–pituitary–adrenal axis of the mother, as we had previously noted no correlation between plasma POMC and ACTH or cortisol in maternal blood (21).

‘ACTH precursors’ (a mixture of POMC and pro-ACTH) were proposed to exert an anti-ACTH effect at the fetal adrenals in vitro (28). Although POMC is present in cord blood at term, we found no arterial/venous blood gradient, indicating that the origin of POMC in the fetus at term would not be the placenta, but more likely secretion from immature fetal pituitary cells (29). However, this does not rule out the possibility that, earlier during gestation, POMC could be secreted from the placenta into the fetal circulation and influence fetal adrenal secretions.

The retroplacental samples consist of maternal blood collected at the materno-fetal interface. Several pieces of evidence indicate the physiological relevance and specificity of this circulation between trophoblast and maternal decidua. Lipid and prostaglandin concentrations in retroplacental blood are different from those in maternal blood (22). Bioactive molecules present there can display direct paracrine effects on fetal placental vessels and influence materno-fetal exchanges, or regulate myometrial contractility. Such properties are well established for CRH (12, 30) which, as shown here, is concentrated in retroplacental blood. More strikingly, POMC dramatically increases in retroplacental blood, reaching more than 30 times the maternal plasma levels, suggesting that POMC itself and/or some POMC-derived peptides could also be involved in a local action. It is noteworthy that not all hormones produced by the placenta are concentrated in the same way in this compartment, and that POMC levels in retroplacental blood are not correlated with maternal blood levels. This suggests a selective permeability and/or a different clearance rate of peptides and hormones in the retroplacental blood space.

Chorionic gonadotropin is a placental hormone that maintains the corpus luteum of pregnancy in higher primates including humans. hCG rises rapidly after fertilisation until the 8th week and declines thereafter to a plateau until the end of gestation. The decline in corpus luteum function is coincident with the shift in progesterone production to the placenta which is able to maintain pregnancy independently of ovarian secretions from the 9th week of gestation (31). The cellular mechanisms that lead to the onset of hCG gene transcription and the subsequent suppression of hCG gene expression after the first trimester are still poorly understood. The onset of hCG production appears linked to differentiation of the trophoblast cells in syncytiotrophoblast (31). At the hormonal level, gonadotrophin
releasing hormone (GnRH) has been proposed as a potential paracrine regulator of hCG production, as it stimulates the release of hCG in a dose-dependent manner (32, 33). However, although GnRH and hCG both increase in early gestation, GnRH remains elevated in the second and third trimesters whereas hCG concentrations fall dramatically (34). This suggests that other factors are involved in the decline of hCG production after the first trimester. Beta-endorphin, which decreases the release of hCG from placental cells in vitro (35) was a potential candidate. As POMC is the source of β-endorphin in placenta, and because POMC is not released from normal pituitary cells, we propose that the maternal POMC level is an index of placental β-endorphin production. Thus, POMC kinetics, which are symmetrically opposed to hCG variations from the end of the first trimester, and the negative relationship observed between POMC and hCG, are consistent with this hypothesis. It must be stressed that the Spearman partial correlation test was chosen to take into account the dependence of both variables on gestation time (POMC increases during pregnancy while hCG decreases), and that the correlation was assessed after correction for the gestation time at which both hormones were measured.

However, as in vitro studies were conflicting (35, 36), and because a statistical correlation does necessarily imply a causal relationship, more studies are needed to determine the possible effect of placental β-endorphin on hCG production.

In conclusion, we defined the time course of POMC variation during normal pregnancy. POMC measurement provides a new tool with which to study placental corticotroph function. Its electively high concentration at the fetomaternal interface raises the question of its physiological role. This also points out the specificity of corticotroph function. Its electively high concentration during normal pregnancy. POMC measurement provides a new tool with which to study placental corticotroph function. Its electively high concentration during normal pregnancy.

Acknowledgements

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