

Presence of pro-opiomelanocortin peptides and corticotropin-releasing factor in human placenta

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Abstract

Immunoreactive adrenocorticotropin (ACTH), beta-endorphin (BEP) and corticotropin-releasing factor (CRF) were detected in human term placenta obtained from elective Caesarian surgery. The concentrations of ACTH, BEP and CRF in placenta detected by radioimmunoassay (RIA) were 2.83 ± 0.36 , 0.52 ± 0.05 and 0.56 ± 0.15 ng/g wet weight of tissue respectively. Pro-opiomelanocortin (POMC) peptides were also detected in the amnion and chorion membranes and in the decidua. The concentrations of ACTH were 1.72 ± 0.20 , 4.43 ± 0.39 and 5.80 ± 0.17 ng/g and the levels of BEP were 0.42 ± 0.18 , 0.65 ± 0.20 and 3.66 ± 1.10 ng/g in the amnion, chorion and decidua respectively. In contrast to placenta, immunoreactive CRF was not detected in the amnion, chorion and decidua. Immunoreactive N-acetylated BEP was also not detected in all the placental subfractions. Comparison of the amounts of both ACTH and BEP in the various placental components indicated the following distribution: decidua > chorion > placenta > amnion. In decidua, POMC peptides were present in an equi-molar ratio but in the other three placental fractions, ACTH levels were three to five-fold higher than BEP. In immunohistochemical studies, only a positive staining for ACTH was obtained for decidua. Our results confirm the presence of POMC peptides and CRF in placenta and their physiological roles in pregnancy and parturition.

Key words: Pro-opiomelanocortin, corticotropin-releasing factor, placenta, immunohistochemistry

INTRODUCTION

In the pituitary, the peptide hormones adrenocorticotropin (ACTH), beta-lipotropin (LPH) and beta-endorphin (BEP) are post-translationally derived from the common glycoprotein precursor pro-opiomelanocortin (POMC).¹ The hypothalamus regulates the pituitary-adrenal axis through the secretion of corticotropin-releasing factor (CRF).² It has been suggested that the POMC-derived peptides, ACTH and BEP play an important role in human reproductive physiology and in parturition. This proposal is supported by the detection of POMC peptides in both male and female reproductive systems by radioimmunoassays, bioassays and immunohistochemistry.³⁻⁷ Human placental tissues?-" cultured placental cells^{12,13} and secretory endometrium¹⁴ are also known to contain POMC peptides. There is also a wide distribution of CRF in human tissues as reported by Suda *et al*¹⁵ in the adrenal, liver, pancreas, duodenum and stomach albeit in lower concentrations as compared to the central nervous system. Since immunoreactive CRF is also present in human placenta¹⁶⁻¹⁸ and

maternal plasma of third trimester pregnancy,¹⁹ the release of placental POMC peptides may be regulated in a paracrine manner.

The validity of the detection of immunoreactive POMC peptides in human placenta has been challenged.²⁰⁻²² A report by Julliard *et al*²⁰ claimed that the immunoreactive BEP in human placental extracts is a fragment of immunoglobulin G. In the other two studies, the authors concluded based on their negative immunohistochemical findings, that the POMC peptides found in maternal blood are not derived from the placenta. In view of these discrepancies, we further examined in this study the presence and distribution of POMC peptides, ACTH, BEP and its inactive congener N-acetylated BEP, and CRF in the various components of human placenta.

MATERIALS AND METHODS

Samples

Human placental tissues (n=6) were obtained from elective Caesarian surgery of normal term pregnancies from Queen Victoria Hospital,

Melbourne. The placenta was separated into its components, placenta, amnion, chorion and decidua and then further dissected into smaller pieces. These samples were rapidly frozen in liquid nitrogen and stored in a -80°C freezer. Duplicate samples were fixed in Bouin's fixative for immunohistochemistry.

Radioimmunoassay

For RIA of POMC peptides in placental tissues, samples were homogenised in 5ml/g 0.1M HCl at 4°C using a polytron. The homogenate was centrifuged at 10 000g for 30 min at 4°C and the supernatant was aliquoted and lyophilised in the Savant Evaporator. Lyophilised samples were stored in a -20°C freezer and then later reconstituted in buffer for RIA. Placental samples for RIA of CRF were homogenised in 50% (v/v) glacial acetic acid, centrifuged and the lyophilised and stored as above. Radioimmunoassays of ACTH, BEP, N-acetylated BEP and CRF were performed following the methods and using highly specific antisera as previously described.^{4,7,23} The sensitivities of the-assays were 1.95, 3.90, 1.95 and 3.90 pg/tube for ACTH, BEP, N-acetylated BEP and CRF respectively. Intra- and inter-assay coefficient of variations (CVs) were <10% and <15%.

Immunohistochemistry

After fixation of tissues in Bouin's fixative for 4 h, tissues were rinsed in 70% (v/v) ethanol and then further dehydrated and embedded in paraffin wax. Paraffin sections (5 um thick) were cut, mounted onto glass slides and then cleared by sequential incubation in two changes of xylene, absolute ethanol, 80% (v/v) ethanol and water. Sections were finally incubated in phosphate buffered saline (PBS) pH 7.4 for 15 min before proceeding on to immunohistochemical staining

by the peroxidase anti-peroxidase (PAP) method.²² The sections were immersed in 3% (v/v) H₂O₂ in absolute methanol for 5 min to block endogenous peroxidase activity. After rinsing twice in PBS, sections were incubated with primary antisera (R56 and R43 for BEP; R72 for ACTH) diluted 1:50, 1:100 and 1:200 in PBS containing 0.25% Triton X-100, 0.1 mol/l lysine-HCl, 1 mg/ml bovine serum albumin and 0.1% sodium azide. The sections were sealed with coverslips and then left to incubate at 4°C for 18-24 h. After washing in three changes of PBS, 1:20 swine anti-rabbit immunoglobulin (Dakopatts) was applied onto the sections. After 30 min incubation at room temperature and three rinses in PBS, 1:50 PAP solution (Dakopatts) was applied and left for a further 30 min incubation. Peroxidase activity was detected in the sections using 0.05% (w/v) diaminobenzidine.HCl (Sigma) in PBS containing 0.03% (v/v) H₂O₂. After counterstaining in haematoxylin, sections were dehydrated and mounted in Depex. Slides were viewed in a light microscope (Zeiss).

RESULTS

Table 1 shows the concentrations of immunoreactive ACTH and BEP as detected by RIA in the various placental components. All the four placental components:- placenta, chorion, amnion and decidua were found to contain both ACTH and BEP but the presence of N-acetylated BEP could not be detected (Table 2). Comparison of the amounts of both ACTH and BEP in the various placental components gave the following distribution:- decidua > chorion > placenta > amnion. The decidua and chorion membranes contain the highest amounts of ACTH while placenta and amnion membranes contain significantly lower (p<0.02) ACTH levels (Table 1). The lowest ACTH level was detected in amnion

TABLE 1: Immunoreactive ACTH and BEP concentrations in human placenta

Placenta fraction	n	ACTH (ng/g)	BEP (ng/g)	ACTH:BEP
Placenta	6	2.83±0.36 ^{ac}	0.52±0.05 ^b	3.6:1
Amnion	6	1.72±0.20 ^{acd}	0.42±0.18 ^b	2.7:1
Chorion	6	4.43±0.39 ^a	0.65±0.20 ^b	4.5:1
Decidua	6	5.80±0.17	3.66±1.10	1.1:1

^ap<0.01, ^bp<0.001 compared to decidua

^cp<0.02 compared to chorion

^dp<0.05 compared to placenta

TABLE 2: Immunoreactive CRF and N-acetylated BEP concentrations in human placenta

Placenta fraction	n	CRF (ng/g)	N-acetyl BEP (ng/g)
Placenta	6	0.56±0.15	ND
Amnion	6	ND	ND
Chorion	6	ND	ND
Decidua	6	ND	ND

ND = Not detectable (<0.3 ng/g)

membrane. These results are in agreement to those reported earlier.¹¹⁻¹³ In contrast, BEP levels were similar in placenta, amnion and chorion, all being significantly lower ($p < 0.001$) than decidua. Comparison on a molar basis showed that the POMC peptides were present in an equi-molar ratio only in decidua. In the other three placental fractions, ACTH was the predominant peptide, being three to five fold higher than BEP (Table I). Table 2 shows the detection of CRF in placental fractions. Of the four fractions, only placenta was found to contain CRF 0.56 ± 0.15 ng/g wet weight, while immunoreactive CRF was not detectable (sensitivity <0.3 ng/g) in chorion, amnion and decidua (Table 2).

In the immunohistochemical studies, only a positive staining for ACTH was obtained for decidua (Fig. 1). The number of samples used in the staining tests was n=6 for placenta, amnion and chorion and n=3 for decidua. Parallel

immunoperoxidase staining for BEP was unsuccessful with two different primary antisera (R56 and R43), both of which appropriately stain rat anterior pituitary corticotropes and melanotropes. This difference may reflect the lower content of immunoreactive BEP in placental tissues as compared to immunoreactive ACTH.

DISCUSSION

In this present study, we have used radioimmunoassays and immunohistochemistry to determine the presence and distribution of POMC peptides and CRF in human placental fractions. Using radioimmunoassays we have been able to detect ACTH and BEP in all placental components. The highest amounts of both these POMC peptides were present in decidua, followed by chorion, placenta and amnion. In the decidua, POMC peptides were present in an equi-molar

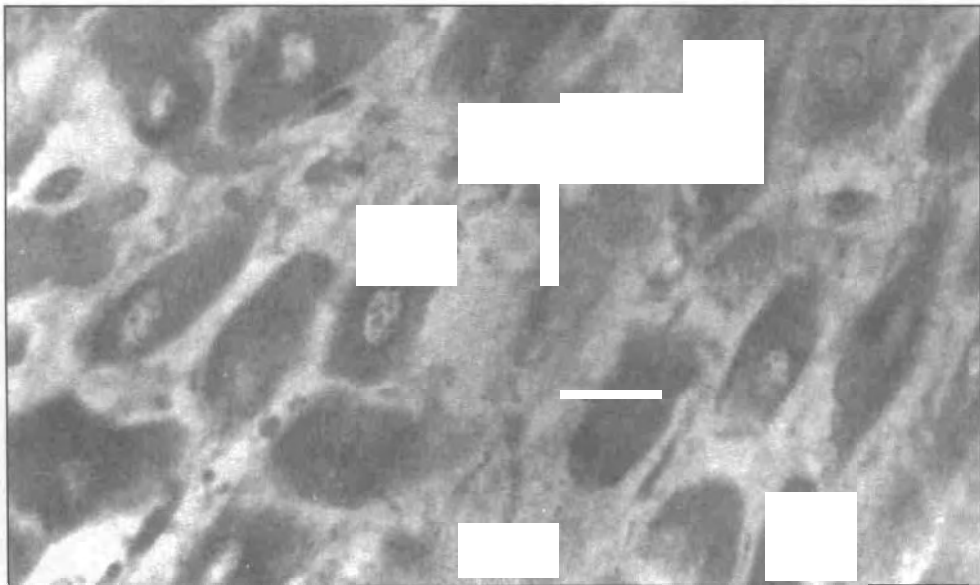


FIG. 1: Light photomicrograph of human decidua showing positive staining of ACTH-like immunoreactive cells. Magnification $\times 400$.

ratio while in the other three placental fractions, ACTH predominates, being three to five fold more than BEP. Our POMC peptide results are comparable with those of earlier reports⁹⁻¹³ and the higher concentration of these peptides in the chorion as compared to amnion membranes has also been previously documented.⁹

In the placenta, the presence of POMC peptides have been localised predominantly in syncytiotrophoblasts.^{14,18} This paper reports for the first time the immunohistochemical localisation of ACTH in decidua. In contrast to our findings, Demura *et al*²⁵ observed that trophoblast contain more POMC peptides than decidua as measured by radioimmunoassays. However, we believe that our observations are correct based on our immunohistochemical results as well as our measurements of POMC peptides by RIA. The detection of BEP in secretory but not proliferative endometrium has been reported by Wahlstrom *et al*.¹⁴ It has been suggested that the human decidua is probably a specialized tissue²⁶ since it is known to contain a variety of hormones including prolactin.²⁵ We conclude that POMC peptides are present in human placenta and the negative immunohistochemical findings of previous studies^{21,22} are probably due to problems of tissue fixation and/or the peculiar characteristics of the primary antibodies used. More recently, the POMC gene has been identified in placenta and ovary²¹ which further supports the earlier suggestion^{13,28} that placental ACTH and BEP, like the pituitary-derived hormones are contained within a larger POMC peptide precursor. Chen *et al*²⁷ reported that the expression of the POMC-like gene in the ovary but not in the placenta is regulated by gonadotropins. The synthesis of placental and endometrial POMC peptides may be induced by steroid hormones such as progesterone.¹⁴ Since we could not detect any presence of N-acetylated BEP in human placenta, we conclude that placental BEP unlike the testis⁶ is probably not inactivated within the placental cells.

We have shown in accordance with earlier reports¹⁶⁻¹⁸ that CRF is found in placenta. We further demonstrated that CRF is only found in placenta and not in decidua, chorion and amnion. In contrast to POMC peptides, the cellular distribution of immunoreactive CRF has been identified in cytotrophoblasts.^{18,29} Placental CRF has biological activity since it is able to stimulate the release of POMC peptides from both anterior pituitary cells^{16,18,30} and placental cells.^{18,31} Unlike the anterior pituitary, glucocorticoids and vasopressin were shown to have no effect on the

secretion of ACTH by the placenta, while oxytocin and prostaglandins stimulated the secretion of both placental ACTH and CRF.³¹ The authors concluded that placental CRF may have paracrine actions on the synthesis of placental POMC peptides and steroidogenesis, in addition to its endocrine role at the maternal pituitary level. Placental CRF may be an important stimulator of the maternal pituitary-adrenal axis during late pregnancy and parturition^{30,32} since maternal plasma CRF levels are well correlated with ACTH and cortisol levels in blood samples collected at early labour, delivery and postpartum.³⁰ The high concentrations of CRF during late pregnancy are thought to be regulated by CRF-binding proteins which are also produced by the placenta.³³

It has been suggested that maternal CRF levels are important in the initiation of parturition since its levels are increased in hypertension, pre-eclampsia and intrauterine growth retardation.³² During parturition, circulating ACTH and BEP are also significantly raised and are thus actively involved in stress and analgesia management during labour and delivery.^{34,35} In addition to its stimulatory effects on maternal and fetal steroidogenesis, production of progesterone and oestradiol by the placenta is controlled by paracrine actions of placental ACTH.³⁶ In conclusion, the human placenta is thus a complex endocrine organ involved in synthesis and secretion of bioactive hypothalamic- and pituitary-like peptide hormones in addition to its major role as producer of maternal steroids for gestation.

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