CLINICAL STUDY

Corticotrophin-releasing hormone and ACTH levels in maternal and fetal blood during spontaneous and oxytocin-induced labour

Tomasz Ochędalski¹, Krystyna Żylińska², Tadeusz Laudański¹ and Agnieszka Lachowicz²

Institutes of ¹Gynaecology and Obstetrics and ²Endocrinology, Medical University of Łódź, 37 Wileńska Str., 94-029 Łódź, Poland

(Correspondence should be addressed to T Ochędalski, Institute of Gynaecology and Obstetrics, 37 Wileńska Str., 94-029 Łódź, Poland; Email: tom@psk2.am.lodz.pl)

Abstract

The changes in corticotrophin-releasing hormone (CRH), ACTH and dehydroepiandrosterone (DHEA) in maternal and fetal plasma were estimated in women undergoing spontaneous and oxytocin-induced labour to correlate hormone changes with the mode of parturition. Blood was sampled from a maternal peripheral vein 2 days before labour, during the second stage of labour and on the second postnatal day, and also from umbilical vessels just after delivery. Hormone concentrations were measured by RIA and ELSA methods. The maternal plasma CRH concentration before labour was significantly higher in the group of women delivered spontaneously and declined during the labour through to the second postnatal day. Measured in umbilical vessels, CRH as well as ACTH concentrations were higher in the umbilical vein than artery. The mean maternal plasma ACTH was similar in both groups before delivery, then increased significantly in both groups during the labour, decreasing on the second day after delivery. There were no changes in DHEA concentrations among the groups and at all time points of collection. No correlations between CRH and ACTH or DHEA were observed. Our results suggest that the maternal pituitary can respond to stress factors during delivery but peripheral CRH, probably mainly of placental origin, is not a major modulator of pituitary action.

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Introduction

During pregnancy and in the peripartum period, specific functional adaptation of the hypothalamo–pituitary–adrenal (HPA) axis is a well-known phenomenon. The placenta is able to produce corticotrophin-releasing hormone (CRH) and adrenocorticotrophin (ACTH), hormones crucial for functioning of the HPA axis.

CRH, first found to be synthesized in the paraventricular nucleus of the hypothalamus and named for its ability to secrete ACTH from the pituitary, plays a major role in regulating the pituitary–adrenal axis in the physiological response to stress. During late pregnancy, the placenta, which has been shown to contain both the CRH peptide and its mRNA, is likely to be a major source of circulating CRH. Little is known about the regulation of the hormonal response to stress during pregnancy, despite its obvious implications for fetal and maternal well-being (1). Early studies on CRH within pregnant women focused on whether the peptide would influence the maternal HPA. Placental CRH, due to its similarity in structure to that of the maternal hypothalamus, has been postulated to stimulate

maternal as well as fetal pituitary ACTH release (2, 3). On the other hand, there is one crucial difference between hypothalamic and placental CRH. In contrast to the negative control on hypothalamic CRH, glucocorticoids stimulate the expression of human CRH mRNA in cultured cytotrophoblast, amnion, chorion and decidual cells (4). Experiments conducted by Schulte *et al.* among others showed a disturbance of regulation of the maternal HPA axis during pregnancy, probably due to pituitary desensitization to CRH (5, 6). Still there is discussion not only about the origin but also about the mechanism of action of CRH during labour.

Another potential role for CRH is regulation of parturition; CRH has been shown to potentiate significantly oxytocin-induced myometrial contractility *in vitro* (7). It is also well known that oxytocin, widely used in obstetrics, has the ability to influence neuroendocrine functions, including the pituitary response to CRH. In order to examine the function of the maternal HPA axis during the stress of childbirth, we have investigated changes in concentration in plasma CRH, ACTH and dehydroepiandrosterone (DHEA) in women undergoing spontaneous labour or induction of labour.

The aim of this study was to investigate whether there is a correlation between CRH and maternal and fetal ACTH and DHEA, and possible association with obstetric variables.

Materials and methods

Subjects and protocol

The study was carried out at the Department of Perinatology, Institute of Gynaecology and Obstetrics, Medical University of Łódź, Poland. The protocol of the experiment was accepted by the Ethical Committee of the Medical University of Łódź. Blood samples were obtained from 36 healthy women at term without uterine contractions 2–4 days before delivery (at 0800 h), then at the beginning of the third stage of labour, and finally on the second day after delivery (again at 0800 h). Blood samples were also taken from the umbilical vein and artery immediately after the placenta was expelled. Following centrifugation, plasma was apportioned for different assays. After birth, the weight, length and biochemical conditions of the newborn were checked.

Participants were divided into two groups. The first group of women (18 subjects) gave birth in a spontaneous labour at term; the first stage of labour was no longer than 8 h. None of the women from this group received any oxytocin supplementation or any other pharmacological treatment during the course of labour. The second group underwent elective induction of labour at term. The method of induction involved oxytocin infusion due to the lack of spontaneous contractions. The indication of induction was post-maturity. The gestational age of all the women was 39–42 weeks. The mean gestational ages of the group of spontaneously delivered women and women with oxytocin induction were similar (39 ± 1 and 39 ± 0.6 weeks respectively). The subjects' age ranged from 21 to 30 years; 16 women were primigravida. None of the women had any pregnancy-related diseases (e.g. hypertensive disorders, intrauterine growth retardation, infections), nonpregnancy-related diseases, pharmacological treatment during pregnancy or anaesthesia during labour. All the pregnancies were single.

The protocol of the induction of labour involved an i.v. oxytocin infusion followed by amniotomy if regular contractions had begun. The oxytocin infusion was commenced at a rate of 1 mU/min and increased at 15 min intervals until effective labour was established, to a maximum rate of 40 mU/min. The first stage of labour in this group lasted an average of 5 h.

Hormone assays

The plasma concentrations of CRH and DHEA were measured with RIA kits according to the procedure

provided by the companies (CRH, Peninsula Laboratories Inc., San Carlos, CA, USA; DHEA, Immunotech, Marseille, France). Assays were performed in duplicate. The intra- and interassay coefficients of variation for DHEA were 4.5 and 5.8% respectively, and for CRH 3.8 and 8.2% respectively.

The concentrations of ACTH were estimated in duplicate by ELSA-ACTH-IRMA assay kits obtained from CIS Bio International, ORIS Group, Gif sur Yvette, France. The intra- and interassay coefficients of variation were 2.9 and 5.3% respectively.

Analysis

All results are reported as means $\pm$ S.E.M. Comparison of means (by *t*-test) were performed in the analysis of CRH and ACTH levels because they fit a Gaussian distribution. Comparison of means within the groups at three time points were tested using repeated measures ANOVA, from the Statistica package (Stat Soft Inc., Tulsa, OK, USA). Association between variables was assessed by correlation.

Results

Plasma CRH levels measured before labour in women with spontaneous contractions 48 h later were significantly higher than in women without spontaneous uterus activity at 1604 ± 313 pg/ml and 780 ± 136 pg/ml respectively. During labour, in both groups CRH concentration declined, to 799 ± 143 pg/ml in spontaneously delivered and 618 ± 108 pg/ml in the second group of subjects. Forty-eight hours after labour there was no difference between the groups. The mean final plasma level was 326 ± 62 pg/ml (Fig. 1).

The mean plasma ACTH prior to the delivery was at the same level in all subjects, 68 ± 10.2 pg/ml, and 65 ± 10.4 pg/ml. Measured during the third stage of

Figure 1 The changes in CRH concentration in maternal plasma before labour, during the third stage of labour and on the second day after labour. $\blacklozenge$ Spontaneous labour; $\square$ oxytocin induction. * $P < 0.02$. Results are means $\pm$ S.E.M.

Figure 2 The changes in ACTH concentration in maternal plasma before labour, during the third stage of labour and on the second day after labour. ◆ Spontaneous labour; □ oxytocin induction. * $P < 0.02$. Results are means $\pm$ S.E.M.

labour ACTH rose, but in the group of women after induction the rise was much higher than in the women with spontaneous contractions at 330 ± 9.4 pg/ml and 123 ± 19.6 pg/ml respectively. After the delivery, the hormone concentration dropped to the level observed before labour (25 ± 3.4 pg/ml) (Fig. 2). There was no correlation between CRH and ACTH concentration.

The DHEA concentration was similar in both groups, did not change during labour, and there was no difference between its level in the umbilical vein and artery.

The CRH concentrations, but not those of ACTH were higher in the umbilical vein than in the artery in both groups. In the group with spontaneous labour, levels of CRH and ACTH were higher than in the group of women with induction (Fig. 3).

None of the examined obstetric variables (duration of labour, weight, length and biochemical parameters of the newborn) correlated with changes in hormone concentration.

Discussion

In agreement with previous reports (6–8), we have observed higher levels of CRH before labour in women

delivered spontaneously, versus women without spontaneous contractions. Although CRH alone does not stimulate contractions, it has been demonstrated that the contractile response of myometrium to oxytocin (which is endogenously secreted during the late stages of pregnancy), is greatly enhanced by the presence of CRH or by previous exposure to CRH (7). Thus, our data confirm the existence of a correlation between maternal CRH levels and the occurrence of spontaneous contractions. CRH concentrations were the same in both groups and much lower than before delivery. Some investigators found rising CRH levels during term labour (9, 10); others reported no change in plasma CRH as labour progressed (11, 12). In all of these studies CRH concentration was measured at the second stage of delivery, whereas we measured its level after delivery of the placenta. These results may suggest that labour can progress without an increase in placental CRH secretion.

Views on the role of CRH in the modulation of pituitary–adrenal function during pregnancy and parturition have been conflicting. A well-known hypothesis suggests that activity of the maternal HPA axis during pregnancy is under efficient inhibitory control before parturition. Schulte *et al.* (5) found that injection of exogenous CRH in pregnant women does not induce an increase in circulating ACTH. This is probably because the high cortisol levels that occur during pregnancy may desensitize maternal pituitary corticotrophs, which is supported by data obtained by Thomson *et al.* (13). Additionally, placental CRH could be ineffective because the CRH-binding protein counteracts the secretory action of CRH on maternal and placental ACTH as well (14). It has been shown also that plasma CRH and ACTH levels both rise during the latter half of pregnancy, supporting the hypothesis that placental CRH stimulates maternal corticotrophs (15, 16). On the other hand discrepancies occur between CRH and ACTH levels during pregnancy, because plasma ACTH remains within the range of nonpregnant subjects, despite extremely elevated maternal CRH concentrations (17). Our report confirms this observation (Figs 1 and 2). Additionally, we did not observe the

Figure 3 The changes in CRH (light bars) and ACTH (darker bars) in umbilical vein and artery; blood was sampled immediately after the placenta was expelled. Sp. lab. = spontaneous labour; oxytoc. = oxytocin-induced labour. * $P < 0.05$ between vein and artery. Results are means $\pm$ S.E.M.

correlations between CRH and ACTH levels reported by Stalla *et al.* (18). High levels of CRH measured before labour in spontaneously delivered women did not result in a higher ACTH concentration. Goland *et al.* (15), suggested that placental CRH stimulates maternal pituitary ACTH secretion, and hypothesized that the rise of placental CRH is accompanied by only a minimal rise of ACTH, which may be difficult to measure. Interestingly, in our study, ACTH concentrations in the maternal blood of women receiving oxytocin treatment were much higher than in the second group. The extremely high elevation of ACTH in this group might be correlated with oxytocin influence on the modulation of anterior pituitary secretion.

It is known that oxytocin stimulates luteinizing hormone, prolactin and ACTH release from the anterior pituitary (19, 20). However, this peptide directly only weakly stimulates ACTH release, but potentiates the rat CRH-stimulated ACTH release both *in vivo* and *in vitro* (21, 22). In sheep, simultaneous exposure to oxytocin stimulated an increase in ACTH output that was significantly greater than that induced by oxytocin and CRH alone (23). This means that the signal transmitted by CRH might be amplified by oxytocin, even if CRH levels are relatively low, as we have observed in this group, before labour. This fact raises the question of how deeply involved the high concentration of CRH observed in prepartum is in the modulation of maternal pituitary function. The differences between CRH levels in the umbilical vein and artery as well as the rapid decrease of hormone concentrations after delivery observed in our study indicate that the main source for CRH in our study is the placenta. On the other hand, after the second stage of delivery, in both groups CRH concentration decreased, whereas ACTH levels were significantly elevated (Figs 1 and 2). It is possible that despite the similarities in structure, placental and hypothalamic CRH have different roles. The rapidly lowering CRH level observed involves probably only CRH from the placenta, not from the hypothalamus. Maternal stress during the last weeks of pregnancy may influence the birth outcome (24), but, due to the elevated concentration of placental CRH, it is obviously difficult to estimate the changes in the functioning of the maternal HPA axis and the mother's hypothalamus secretion. In the group of women delivered spontaneously, we hypothesize that the elevation of ACTH levels, moderate but statistically significant, was due to the central regulation of the maternal hypothalamo-pituitary axis by the hypothalamic response to stress. The extremely high concentration of ACTH observed in the group receiving oxytocin treatment might possibly have a similar mechanism, with the primary stress response being amplified by oxytocin. Nevertheless, elevation of ACTH in both groups, despite a decline of CRH concentration, indicates that the maternal hypothalamo-pituitary axis is not completely suppressed, but is

able to answer to the stimuli regardless of the level of placental CRH. Hypothalamic CRH, like many hormones produced and released by the hypothalamus (e.g. thyrotrophin-releasing hormone and somatostatin), is difficult to measure peripherally; in most cases we can detect only the same peptides synthesized and released by peripheral organs like the placenta, gut or heart. It is also possible that at the end of pregnancy, when the pituitary is desensitized to the action of CRH, another stimulus can still serve as regulator of the pituitary stress response. This stimulus might be vasopressin, a peptide modulating ACTH release from the pituitary.

Goland *et al.* (25) suggested that the chronic placental CRH stimulation of the pituitary-adrenal axis during pregnancy leads to an enhanced response to vasopressin and a down-regulation of the response to exogenous CRH. We hypothesize that the main function of placental CRH is the preparation of the uterus for the delivery and maturation of the fetus. It is well known that placental CRH can reach the myometrium by paracrine diffusion and potentiate the effects of prostaglandin and oxytocin (26). It is also possible that the spontaneous contractions observed in a group of women with high prepartum CRH could be due to the placental CRH preparation of the uterus as well as to other factors released due to maternal response to stress. Another consequence of elevated levels of CRH might be the partial desensitization of the pituitary corticotrophs and further specific 'protection' of women from stress.

In our results we did not find changes in DHEA levels between the groups at all time points of collection, as well as in umbilical vessels. It is very likely that DHEA, which is known to be a prohormone, is rapidly converted to oestrogens in the placenta.

In summary, during parturition the maternal hypothalamo-pituitary axis seems to answer to the normal stimuli, although its function is difficult to estimate due to the high levels of placental CRH. The high concentration of this hormone may play an important role not only in the timing of parturition but also in the protection of pregnant women from stress. The physiological significance of this adaptation to the mother's well-being needs to be elucidated.

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