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Fetal Hypothalamic-Pituitary-Adrenal Stress Responses to Invasive Procedures Are Independent of Maternal Responses*

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ABSTRACT

Paired fetal and maternal samples were obtained, at fetal blood sampling and intrauterine transfusion, to study hypothalamic-pituitary-adrenal stress responses. This confirmed that the fetus mounts an hypothalamic-pituitary-adrenal stress response to transfusion via the intrahepatic vein, which involves piercing the fetal trunk, but not to transfusion via the placental cord insertion [mean cortisol response via intrahepatic vein δ = 52.6 nmol/L, 95% CI (25.3–79.9), $P = 0.001$; mean β-endorphin response δ =106 pg/mL, 95% CI (45.3–167), $P = 0.002$]. Baseline maternal fetal ratios were 13 [95% CI (10.7–15.2)] for cortisol and 0.8 [95% CI (0.5–1.0)] for β-endorphin. The novel findings were: 1) that the fetal responses were independent of those of the mother, which did not change during transfusion at either site; 2) that there was a correlation between baseline fetal and maternal cortisol levels ($r = 0.58$, $n = 51$, $P < 0.0001$) but not between baseline fetal and maternal β-endorphin levels, suggesting cortisol transfer across the placenta, rather than joint control by placental CRH; and 3) that fetal β-endorphin responses were apparent from 18 weeks gestation and independent of gestational age, whereas fetal cortisol responses were apparent from 20 weeks gestation and were dependent on gestational age ($y = -91.4 + 5.08\times$, $r = 0.51$; $n = 16$; $P = 0.04$; CI for slope, 0.16–10.0), consistent with the maturation of the fetal pituitary before the fetal adrenal. (*J Clin Endocrinol Metab* **86:** 104–109, 2001)

WE HAVE PREVIOUSLY reported that the human fetus can mount hormonal stress responses to invasive stimuli, with rises in both β-endorphin and cortisol (1), and also noradrenaline (2). However, it is possible that responses measured in fetal plasma could be secondary to maternal responses to the stress of the transabdominal invasive intrauterine procedure. We have documented a pronounced maternal sympathetic response to fetal transfusion (2), although the lack of correlation between maternal and fetal levels suggests that there was no direct transplacental passage of noradrenaline. However, in a preliminary report (3), we have found a correlation between basal maternal and fetal plasma cortisol levels, and we suggested that this was attributable to some maternal cortisol crossing the placenta. It is therefore important to study fetal and maternal responses in parallel, which has not been done previously. The aims of this study were thus to further characterize fetal hormonal stress responses and, in particular, the relationship between the maternal and fetal hypothalamic-pituitary-adrenal (HPA) axis. We used the same ethically acceptable clinical models as in our previous studies (1–4), comparing paired maternal and fetal samples after intrauterine needling at the placental cord insertion (PCI), which is not innervated and which one would not expect to be stressful for the fetus, with those at the intrahepatic vein (IHV), which has been associated with hormonal and hemodynamic fetal stress responses. One would expect both procedures to be equally stressful for the mother.

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Materials and Methods

Patients

Women with singleton pregnancies, undergoing clinically indicated fetal blood sampling or intrauterine blood/platelet transfusion at the Center for Fetal Care, Queen Charlotte's and Chelsea Hospital or at The Queen Mother's Hospital, were recruited for the study. The indications for fetal blood sampling were rapid karyotyping or suspected anemia and for intrauterine transfusion, were fetal anemia or thrombocytopenia in alloimmunized pregnancies.

Fetuses were considered suitable for inclusion when the fetus was appropriately grown for gestational age and structurally normal on preprocedural ultrasound, with no evidence of hydrops, absent enddiastolic frequencies in the umbilical artery Doppler waveform, severe anemia (hemoglobin, <5 g/dL), or hypoxia [fetal pO_2 below the reference range (5)], and had a normal karyotype, to exclude conditions that can be associated with basal elevations in the study endpoints (6, 7). Fetal sex was also determined during preprocedural ultrasound and confirmed by karyotyping. Complicated procedures, involving multiple fetal vessel punctures, or when time to access the fetal circulation exceeded 10 min [shown to cause elevations in cortisol and β-endorphin (1)], were excluded. Fifty-one fetuses satisfied the criteria for inclusion. Some of the results with cortisol have been reported previously in

correspondence form (3). Twenty-three fetuses satisfied the criteria for the transfusion group.

The site of ultrasound guided fetal blood sampling or transfusion was chosen by the operator on the basis of technical factors and ease of approach, as per usual practice. Randomization of site sampling was not considered appropriate, because anatomical factors often dictate one approach in favor of the other. Neither fetal neuromuscular blockade nor analgesia was used. The mothers did not receive sedation. Intravascular transfusion was performed using packed red blood cells or platelets, warmed to body temperature before transfusion. The purity of fetal samples was confirmed by comparison of fetal and maternal mean corpuscular volumes and subsequent Kleihauer-Betke testing. Full blood count (Coulter Counter, Coulter Electronics, Luton, UK) and blood gases (Radiometer ABL 330 blood gas analyzer, Copenhagen, Denmark) were also analyzed.

Blood samples

Two milliliters of additional venous fetal blood were drawn into a heparinized syringe, after collection of clinical samples, and placed in a chilled heparinized tube. Seven milliliters of maternal blood samples were collected by venepuncture into a heparinized Vacutainer (Becton Dickinson and Co., Meylan Cedex, France) immediately before needling or transfusion, and within 10–20 min after the procedure.

Fifty microliters (500 kIU) of Trasylol/mL blood was added, immediately after collection, to all tubes. Bloods were spun in a refrigerated centrifuge at 3,000 × *g* to separate plasma, which was stored at −80 C until assay.

Timings

Samples were collected between 1000–1800 h, the majority (90%) between 1200–1700 h. The time from first puncture of the fetal trunk or cord until accessing the fetal circulation and collection of the first blood sample was recorded as: time to access. The duration of transfusion was recorded from the time of access until the taking of the second blood sample at the end of the transfusion. Procedures were timed using a digital stopwatch and were recorded in minutes and seconds. For analysis, times were rounded to the nearest 0.1 min. Procedures with time to access that was greater than 10 min were excluded.

Written informed consent was obtained from all the mothers, for the collection of additional blood samples for research purposes, in accordance with the Institutional Ethics Committee requirements.

Assays

Cortisol levels were assayed using a standard solid-phase RIA (by DPC, Los Angeles, CA), and maternal and fetal plasma sample pairs were analyzed in the same assay run. The lower limit of sensitivity was 10 nmol/L. The intra- and interassay coefficients of variation were 5.3% and 4.3%, respectively. Plasma levels of β-endorphin were determined using a solid-phase two-site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The lower limit of sensitivity was 14 pg/mL, and cross-reactivity with lipotropin was 14%. The intra- and interassay coefficients of variation were 13.2% and 9.5%, respectively. In a few cases, there was insufficient sample volume to measure β-endorphin levels as well as cortisol.

Statistics

All ranges studied were normally distributed, except for fetal β-endorphins, time to access, and time of transfusion, which were normalized by log transformation. Data were analyzed by standard parametric statistics using SPSS, Inc. 9.0 for Windows (Chicago, IL).

Results

Patient details are given in Table 1.

Maternal and fetal basal levels

There was no significant correlation between basal concentrations of either fetal or maternal cortisol and gestational age, over the range studied (17–35 weeks), nor was there with fetal β-endorphin. However, maternal basal β-endorphin rose significantly with advancing gestational age [y = −17.7 + 2.20×; r = 0.36; n = 37; $P = 0.03$; 95% confidence interval (CI) for slope, 0.27–4.13] (Table 2). There was no correlation between maternal or fetal basal values and time of day. There was no difference in basal fetal values by sex of the fetus for either cortisol [mean (95% CI): males = 48.3 (42.2–54.4), n = 24; females = 52.3 (40.5–64.1), n = 15] or β-endorphin [geometric mean (95% CI): males = 72.9 (53.2–99.5), n = 22; females = 74.6 (51.9–104), n = 15].

Basal fetal cortisol concentrations were linearly related to their paired maternal basal cortisol levels (y = 19.5 + 0.05×; r = 0.58; n = 51; $P < 0.0001$; 95% CI for slope, 0.03–0.07) (Fig. 1a). However, fetal β-endorphin was unrelated to maternal levels [r = −0.2, n = 36, not significant (ns) (Fig. 1b)].

There was a significant linear correlation between fetal β-endorphin and time to access the fetal IHV, (y = 44.7 + 11.5×; r = 0.56; n = 29; $P = 0.001$; 95% CI for slope, 4.84–18.2) (Fig. 2b) but not cortisol (r = 0.2, n = 31, ns) (Fig. 2a). If only

TABLE 1. Patient details

Paired maternal and fetal baseline samples		
Mean ± SD	Maternal	Fetal
Number	51	51
Gestational age (weeks)	27.4 ± 4.31	27.4 ± 4.31
Cortisol (nmol/L)	605 ± 337	50.6 ± 29.9
β-Endorphin (pg/mL)	42.0 ± 27.5	67.6 (1.83 ± 0.26)[a] (n = 36)
Intrauterine transfusion		
Mean ± SD	Intrahepatic vein	Placental cord insertion
Number	16	11
Gestational age (weeks)	28.4 ± 5.14	29.5 ± 3.15
Time to access vein (minutes)[a]	2.32 (0.37 ± 0.30)	0.67 (−0.17 ± 0.53)
Cortisol (nmol/L)	52.9 ± 24.4	64.1 ± 39.0
β-Endorphin (pg/mL)[a]	85.9 (1.94 ± 0.28) (n = 14)	56.0 (1.74 ± 0.19)

[a] Geometric mean (anti-log x ± y) (x = log mean, y = log SD).

TABLE 2. Correlations with gestational age, in normal fetuses

	GA range	β-Endorphin			Cortisol		
		N	R	*P*	N	R	*P*
Maternal basal	17–35	37	0.36	0.03	46	0.23	ns
Fetal basal	17–35	46	−0.03	ns	56	0.14	ns
Maternal transfusion group delta	22–35	11	0.47	ns	11	−0.11	ns
Fetal IHV transfusion group delta	18–35	14	0.03	ns	16	0.51	0.04

GA, Gestational age range (weeks); N, number; R, Pearson correlation coefficient.

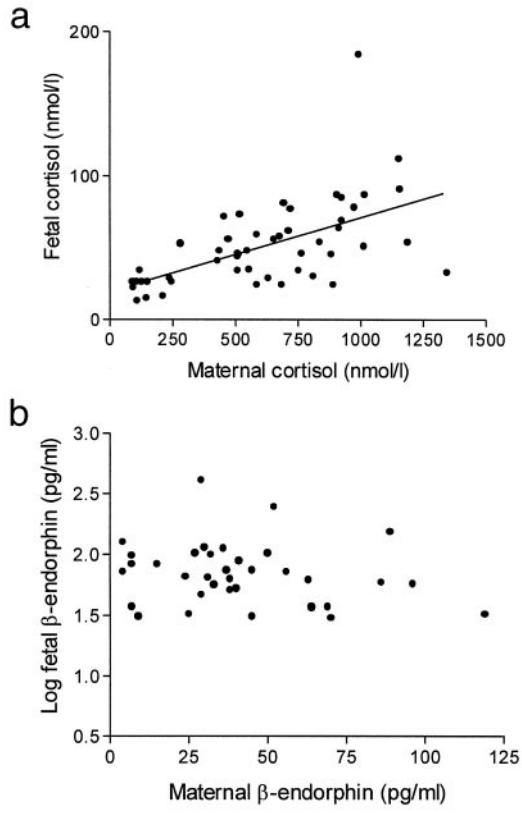

FIG. 1. a, Correlation between basal maternal and fetal plasma cortisol levels (y = 19.5 + 0.05×; r = 0.58; n = 51; $P < 0.0001$; 95% CI for slope, 0.03–0.07. b, Correlation between basal maternal and fetal plasma β-endorphin levels (r = −0.2, n = 36, ns).

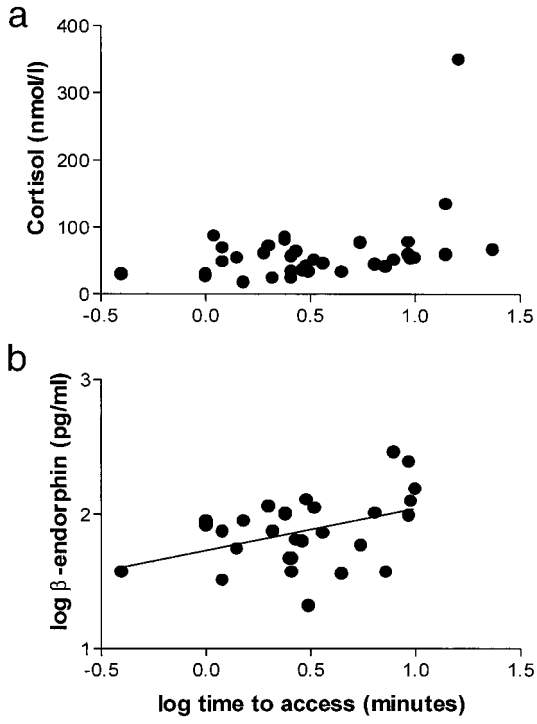

FIG. 2. a, Correlation between basal fetal plasma cortisol and time to access the fetal IHV (r = 0.2, n = 31, ns). b, Correlation between basal fetal plasma β-endorphin and time to access the fetal IHV (y = 44.7 + 11.5×; r = 0.56; n = 29; $P = 0.001$; 95% CI for slope, 4.84–18.2).

procedures lasting ≤ 6 min were considered, n = 21, the correlation with time and β-endorphin disappeared. There was no significant correlation between time to access the PCI and fetal cortisol or β-endorphin levels.

Whereas mean maternal cortisol levels were 13.0 times higher than fetal (95% CI, 10.7–15.2, n = 51), the fetal levels of β-endorphin were higher, giving a mean maternal/fetal ratio of 0.8 (95% CI, 0.5–1.03).

There was a significant correlation between maternal baseline cortisol and β-endorphin levels (r = 0.52, n = 35, $P = 0.002$). There was no such correlation between fetal baseline levels (r = 0.048, n = 46, ns).

Fetal responses

Several fetuses required multiple transfusions at the same site; in these cases, the first transfusion at each site was used.

Pretransfusion cortisol and β-endorphin levels were similar in the IHV and PCI groups (Table 1). Figure 3 shows that transfusion resulted in a significant rise in fetal plasma cortisol levels when carried out at the IHV [mean δ, 52.6 nmol/L; 95% CI (25.3–79.9); $P = 0.001$] but not when the same procedure was done via the PCI [mean δ, 3.27 nmol/L; 95% CI (−13.5 to 20.1); $P = 0.7$]. Similarly, there was a fetal β-endorphin response to IHV transfusion [mean δ, 106 pg/mL; 95% CI (45.3–167); $P = 0.002$] but not to PCI transfusion [mean δ, 12.2 pg/mL; 95% CI (−11.9 to 36.3); $P = 0.3$].

The youngest fetus transfused at the IHV was 18 weeks old. It had a large β-endorphin response (202% increase) but showed no cortisol response (−23%). The next youngest fetus, 20 weeks old, responded with a substantial increase in cortisol and β-endorphin levels (61% increase in cortisol and 27% increase in β-endorphin) to transfusion at the IHV.

The magnitude of the cortisol response at the IHV increased with gestational age (y = −91.4 + 5.08×; r = 0.51; n = 16; $P = 0.04$; 95% CI for slope, 0.16–9.99); there was no such correlation between the β-endorphin response and gestational age (r = 0.03, n = 14, ns) (Fig. 4 and Table 2).

Maternal responses

Paired pre- and posttransfusion maternal samples were available for 7 IHV and 5 PCI procedures. Maternal cortisol and β-endorphin levels did not change with transfusion [mean δs, 28.9 nmol/L (95% CI, −60.7 to 119) and 5.82 pg/mL (95% CI, −6.08 to 17.7), respectively (Fig 5)]. δ values were similar in IHV and PCI transfusions. This lack of response was equally apparent at all gestational ages studied (range, 22–35 weeks). There was no relationship between paired maternal and fetal δ cortisol or δ β-endorphin.

Discussion

Our group is the first to study human fetal stress responses *in vivo*. The present study, with a new cohort, first, confirms the findings of Giannakoulopoulos *et al.* (1), that transfusion through the IHV but not the PCI causes the fetus to mount an HPA stress response. In addition, it has examined the relationship between fetal and maternal stress hormone levels and has shown a link with baseline cortisol but not β-

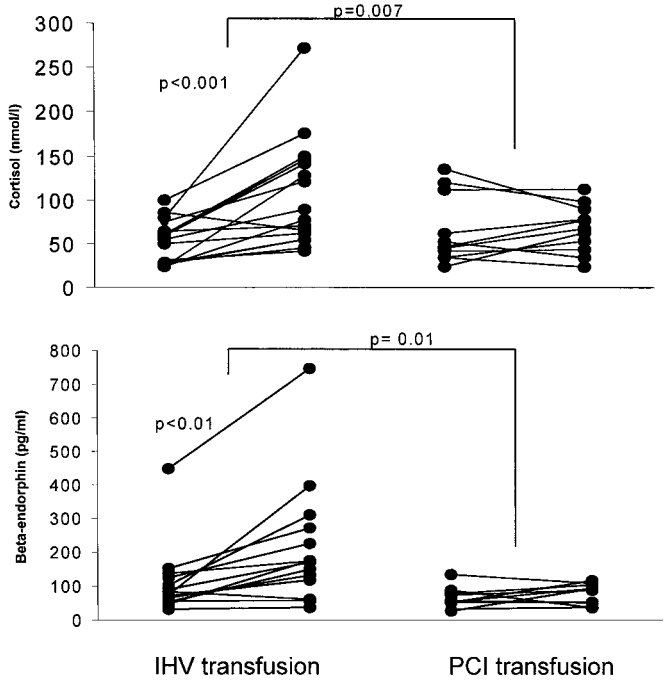

FIG. 3. Fetal plasma cortisol and β-endorphin concentrations before and after transfusion at the IHV and the PCI.

endorphin. This study has also demonstrated, for the first time, that the fetal responses, in this model, are independent of the maternal. It has also produced some evidence that the fetal β-endorphin responses are more rapid than, and develop before, the cortisol responses.

The finding of a positive correlation between basal maternal and fetal cortisol confirms and extends our pilot study (3). There was no similar correlation in β-endorphin levels. This suggests that the cortisol correlation is unlikely to be caused by joint control via placental CRH release into both maternal and fetal compartments, because then, one would expect other POMC derivatives (such as β -endorphin) also to be correlated. Indeed, Schulte *et al.* (8) have shown that CRH that is administered to the mother in the third trimester of pregnancy does not increase maternal cortisol.

It has been suggested that high levels of placental 11β-hydroxysteroid-dehydrogenase type 2 activity excludes maternal cortisol from the fetus (9, 10). A direct study of maternal-fetal cortisol transfer in the fetal-placental unit before abortion showed that 15% of ^{3}H-cortisol crossed the placenta unmetabolized (11). Our finding is compatible with substantial (80–90%) metabolism of maternal cortisol during passage through the placenta. Because fetal levels are about 13-fold lower than maternal, a rise of 10–20% in maternal levels could still double fetal concentrations. Our results indicate that 33.5% of the variance in fetal cortisol is attributable to maternal levels.

If there is direct transfer of maternal cortisol across the placenta in sufficient concentration to have a functional effect on the fetus, this provides a mechanism by which antenatal maternal stress may affect the fetus. Maternal stress in pregnancy has been shown to be associated with babies with lower birth weight and impaired brain development (12). Numerous animal studies have linked antenatal maternal stress with altered long-term hyperreactive HPA responses in the resultant offspring (13). The effects of maternal stress on the fetus can thus have long-term implications (14). The results presented here suggest that a mechanism of direct transfer occurs in humans, so that maternal stress, which results in elevated cortisol levels, may have a direct effect on development.

The correlation between maternal and fetal cortisol levels, suggesting that maternal cortisol may be transported to the fetus, raises a question about the independence of the fetal stress response to IHV transfusions. However, the fact that maternal levels of cortisol and β-endorphin did not change with transfusion to the fetus at either site (Fig. 5) suggests that maternal responses did not influence the fetal responses described here. It may seem surprising that mothers did not show any activation of the HPA axis after what one would expect to be a stressful procedure for her also (*i.e.* having a 20-gauge needle inserted under local anesthetic through her abdomen into the fetal circulation, along with a small procedure-related risk of miscarriage and fetal death). Indeed these procedures are known to elicit a noradrenaline response in the mother (2). However, others have observed a desensitization of the maternal HPA axis response during pregnancy (8), which may, in part, be caused by release of large amounts of CRH from the placenta (15). Even though maternal cortisol levels have been shown to be increased during pregnancy by stressful experiences (8, 16), it may be that a greater insult is needed to achieve the same effect as in the nonpregnant state. Alternatively, it is also possible that the mother was stressed in anticipation of the procedure and, so, was resistant to further HPA activation by the procedure itself.

The youngest fetus in the previous study was 23 weeks old (1), whereas the present study included fetuses at earlier gestational ages. A fetus at 20 weeks showed a typically large response. This is of interest because it has been suggested that the human fetal adrenal cortex cannot synthesize cortisol *de novo* before 24 weeks (17). However, the fetus at 18 weeks did not show a cortisol response, but he did show a rise in β-endorphin. The finding that the cortisol response was significantly related to gestational age in the range from 18–35 weeks but that the β-endorphin response was not, and was actually negative (Table 2, Fig. 4), suggests that the β-endorphin response from the fetal pituitary may mature before the cortisol response from the fetal adrenal. However, it must be noted that the significant cortisol correlation (Fig. 4a) depends on a relatively small number of samples and should thus be interpreted with caution.

Earlier maturation of the pituitary could explain why basal fetal β-endorphin levels are higher than those of the mother (maternal/fetal ratio, 0.8), whereas cortisol values are much lower (maternal/fetal ratio, 13). In this context, it would be of interest also to examine basal fetal and maternal ACTH levels and responses. Because of the small volumes of fetal blood available, it was not possible to do this in the present study.

Basal fetal β-endorphin levels correlated with time of needling access to the IHV (Fig. 2b), something not found in our smaller earlier study (1). A fetal cortisol link with time became apparent only in the current study, only in complicated

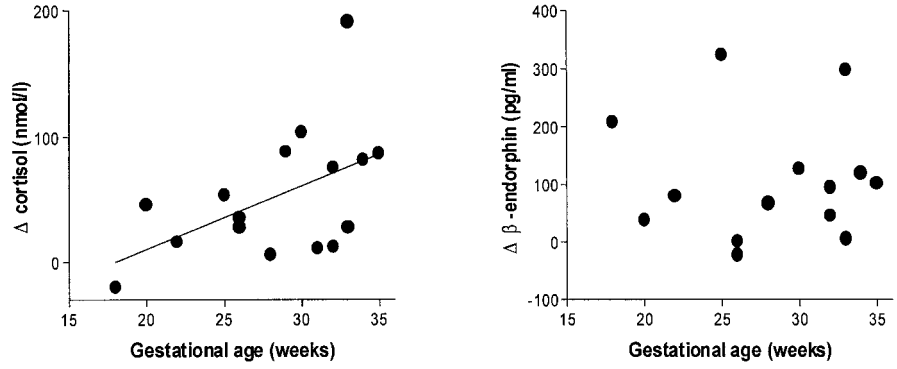

FIG. 4. Fetal cortisol and β-endorphin responses to transfusion at the IHV, as a function of gestational age. Cortisol $y = -91.4 + 5.08\times$; $r = 0.51$; $n = 16$; $P = 0.04$; 95% CI for slope, 0.16–9.99. β-endorphin, $r = 0.03$, $n = 14$, ns.

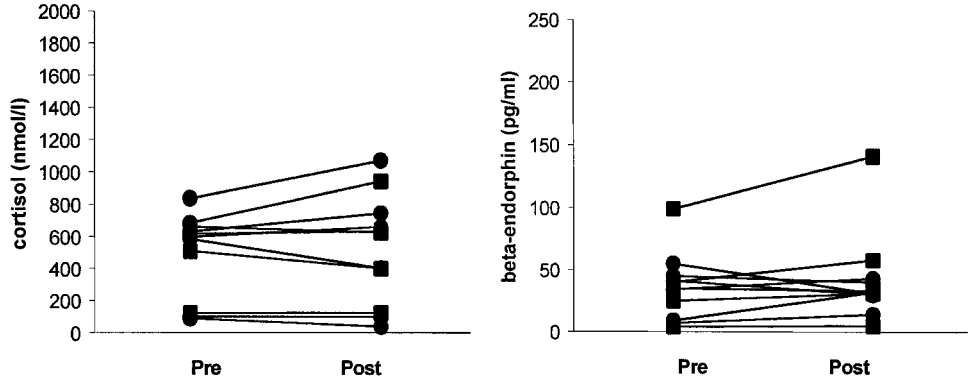

FIG. 5. Maternal plasma cortisol and β-endorphin concentrations before and after transfusion. ●, IHV; ■, PCI.

procedures in which access needling lasted more than 10 min (unpublished observations). This supports the hypothesis that the β-endorphin response is controlled by the fetal pituitary and thus is more rapid than the adrenal response; again, this should be interpreted with caution in view of the relatively small numbers of such fetal procedures.

Ours are the first studies of human fetal HPA responsiveness *in vivo*. Others have looked at basal levels of cortisol and β-endorphin, and some have examined paired maternal and fetal samples, although not in the same cohort. The finding of no relationship between basal fetal cortisol concentrations and gestational age between 17–35 weeks is similar to the results of Economides *et al.* (18) but in contrast to Donaldson *et al.* (16). However, the rise found by the latter group was predominantly in late gestation. The exact gestational period under study is important, because there could still be a rise in the last 4 weeks of pregnancy and in the first trimester (19), periods not included in the present study.

Only one previous study has compared maternal and fetal β-endorphin plasma levels during pregnancy and has found that fetal values were higher than maternal (20). Unlike our study, Rudunovic *et al.* (20 did find a correlation between maternal and fetal β-endorphin values but over a wider gestational age range, from 18–39 weeks). Our results are in agreement with those of Goland *et al.* (21), who similarly did not find any correlation in maternal and fetal β-endorphin in cord blood at delivery. Radunovic *et al.* (22) found that basal fetal β-endorphin increased with fetal blood sampling at the PCI but only when multiple punctures were required and time to access the vein exceeded 3 min. In contrast, we found no evidence for a β-endorphin response to needling at the PCI in uncomplicated procedures.

In conclusion, in the human fetus, the HPA axis seems functional from midgestation. Although there is a correlation between basal fetal and maternal cortisol levels, suggesting some placental transfer, the fetal stress responses, demonstrated in response to transfusion at the IHV, are independent of those of the mother.

Acknowledgments

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