Human fetal and maternal corticotrophin releasing hormone responses to acute stress

R Gitau, N M Fisk, V Glover

Objectives: To study the effect of acute stress, caused by intrauterine needling at the intrahepatic vein (IHV), on fetal plasma concentrations of corticotrophin releasing hormone (CRH), and to compare paired fetal and maternal samples for CRH concentration to determine the extent of their joint control.

Design: Venous blood samples were obtained from fetuses (gestational age 17–38 weeks) undergoing fetal blood sampling (n = 29) or intrauterine transfusion (n = 17) through either the IHV or the placental cord insertion (PCI).

Setting: The Centre for Fetal Care, Queen Charlotte’s and Chelsea Hospital, London, UK.

Patients: Pregnant women undergoing clinically indicated fetal blood sampling or intrauterine blood/platelet transfusion.

Results: Fetal plasma cortisol increased with intrahepatic vein transfusion (mean (SD) cortisol response Δ64.7 (54.5) nmol/l; \( p < 0.0001 \), \( n = 11 \)), and fetal corticotrophin concentrations were higher after IHV (\( n = 7 \)) than PCI needling (\( n = 6 \)). Neither fetal nor maternal plasma CRH increased after IHV transfusion. Fetal CRH levels did not rise with gestation, whereas maternal CRH levels did (\( r = 0.58; n = 36; p < 0.0001 \)). There was a modest correlation between paired maternal and fetal values (\( r = 0.36; n = 36; p = 0.03 \)).

Conclusions: Acute fetal stress, caused by IHV needling of the fetal abdomen, resulted in hypothalamic-pituitary-adrenal axis activation, as shown by a rise in fetal cortisol and corticotrophin. However, it did not result in measurable CRH release into fetal plasma. This suggests that fetal plasma CRH is not derived from the hypophyseal-portal circulation, but from another source, presumably the placenta.

Although corticotrophin releasing hormone (CRH) is normally very low in adult plasma, maternal concentrations increase substantially during pregnancy, with the largest rise in the two weeks before delivery.1-7 High maternal levels have been associated with preterm labour.4 Although CRH is present in fetal plasma, few studies have measured fetal CRH,2,3 and none have examined levels in response to stress.

We report CRH concentrations in human fetal venous samples obtained by needling at the placental cord insertion (PCI) or the intrahepatic vein (IHV), either before and after transfusion or from a single fetal blood sampling procedure. The PCI is not innervated, and we have shown in previous studies that needling at this site is not stressful for the fetus, whereas needling through the IHV, which involves piercing the fetal abdomen, is stressful.7-9 Such procedures using the IHV cause a rise in fetal venous concentrations of the stress hormones cortisol, \( \beta \) endorphin, and noradrenaline (norepinephrine), and also Doppler changes suggestive of blood flow redistribution to the brain.2,9-10

Our hypothesis was that, unlike other stress hormones, fetal peripheral blood levels of CRH would not rise after needling at the IHV, as stress related release of CRH should be confined to the hypophyseal-portal circulation, and, as in the adult, not reach the plasma in any measurable quantity.3 This would be in contrast with the suggestion of Lockwood et al11 that some fetal plasma CRH may derive from the fetal hypothalamus.

Maternal samples were obtained at the same time as the fetal samples, to determine the relation between maternal and fetal CRH, because of the possibility that they are jointly influenced by placental as opposed to hypothalamic CRH, or that there is transplacental passage from mother to fetus, as we have previously shown for cortisol.11 Finally, we confirmed fetal hypothalamic-pituitary-adrenal axis responsiveness to acute stress by measuring cortisol and corticotrophin concentrations.

Although corticotrophin has been measured in fetal blood,7-11 the fetal corticotrophin response to a stressful procedure has not.

MATERIALS AND METHODS

Patients

Women undergoing clinically indicated fetal blood sampling or intrauterine blood/platelet transfusion at the Centre for Fetal Care, Queen Charlotte’s and Chelsea Hospital, London, UK were recruited. Ethical approval for the study was granted by the Hammersmith Hospitals ethics committee. Cortisol and \( \beta \) endorphin results from some of these fetuses have been reported previously.9

Inclusion criteria were singleton fetuses appropriately grown for gestational age and structurally normal on ultrasound. Exclusion criteria were similar to those used in our previous studies.7-10 The indications for fetal blood sampling and for intrauterine transfusion were also similar to those used previously.7-10

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. Neither fetal neuromuscular blockade nor analgesia was used. The mothers did not receive sedation. Intravascular transfusion was performed as previously described.7-10 Thirty two fetuses underwent procedures at the IHV, 11 of these receiving a blood transfusion. Fetal blood was obtained from 14 PCI procedures, six receiving blood transfusions. Mean (SD) duration of transfusion was 17.5 (7.2) minutes.

Abbreviations: CRH, corticotrophin releasing hormone; IHV, intrahepatic vein; PCI, placental cord insertion
fusion was 16.5 (9.8) minutes, and the geometric mean (anti-ln 95% confidence interval) of needle insertion to access the fetal circulation was 4.26 (3.26 to 5.58) minutes.

**Blood samples**

After collection of clinical samples, up to 1–2 ml venous fetal blood was placed in a chilled heparinised tube. If there was sufficient, an additional 0.5–1 ml was placed into a chilled EDTA tube for corticotrophin determination. Maternal blood (7 ml) was collected by venepuncture into a heparinised Vacutainer (Becton Dickinson, Meylan, France) immediately before transcervical needle insertion and again 10–20 minutes after the procedure. Trasylol (50 µl/ml (500 KIU); Bayer AG) was added to all blood samples immediately after collection. Bloods were prepared as described previously.9

**Assays**

Plasma CRH was assayed using a direct plasma radioimmunoassay (Peninsula, San Carlos, California, USA). The lower limit of sensitivity was 10 pg/ml, and the assay coefficient of variation was 5.3%. Maternal and fetal plasma sample pairs were analysed in the same assay run. In general, cortisol was measured in parallel, but in a few cases, there was insufficient sample volume to measure cortisol as well as CRH. Limited sample volume also meant it was only possible to determine corticotrophin in a small number of blood samples taken before transabdominal procedures. EDTA plasma corticotrophin was assayed using a standard solid phase two site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, California, USA). The lower limit of sensitivity was 1.0 pg/ml, and the assay coefficient of variation was 8.6%.

**Statistical analysis**

Normally distributed data were analysed by standard parametric statistics using SPSS 10.0 for Windows (Chicago, Illinois, USA). Non-parametric initial values (fetal cortisol and maternal CRH levels, time to access) and maternal and fetal transfusion data were first normalised by natural log transformation. Geometric means and anti-ln 95% confidence intervals (95%CI) are displayed. Responses to transfusions were analysed using Δ values (hormone concentration after transfusion stress – hormone concentration before transfusion stress).

**RESULTS**

**Fetal samples**

Table 1 gives details of the pre-transfusion and needling samples. Fetal plasma cortisol concentrations increased with transfusion at the IHV (fig 1A); mean (SD) Δ64.7 (54.5) nmol/l (n = 11, p < 0.0001). The few transfusions at the PCI with sufficient sample (n = 3) showed no significant change (fig 1B). Corticotrophin levels were significantly higher in the needling only—that is, non-transfusion—samples from the IHV than from the PCI (88.1 (73.6) pg/ml (n = 7) v 18.7 (5.7) pg/ml (n = 6); p = 0.04) (fig 2). There was insufficient plasma volume available to assay corticotrophin in the transfusion group samples.

Figures 3A and 3B show that there was no change in fetal CRH level with transfusion at either the IHV or PCI. Similarly, fetal CRH concentrations were similar after needling at the two sites (fig 3C).

There was no significant difference in Δ fetal CRH levels in procedures that involved transplacental insertion of the needle (median Δ 15 pg/ml; range (−40 to 163); n = 11) and those that did not (median Δ −4 pg/ml; (−51 to 362); n = 6). Similarly, there was no significant difference in initial fetal CRH levels in procedures that involved transplacental insertion of the needle (mean (SD) 185 (49.4) pg/ml; n = 20) and those that did not (216 (48.0) pg/ml; n = 26).

**Table 1** Characteristics of blood samples

<table>
<thead>
<tr>
<th></th>
<th>Before IHV transfusion</th>
<th>Before PCI transfusion</th>
<th>Needling</th>
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<tbody>
<tr>
<td>Number</td>
<td>16</td>
<td>6</td>
<td>66</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>29.1 (5.10)</td>
<td>30.5 (4.59)</td>
<td>28.4 (4.88)</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>31.6 (7.11)</td>
<td>29.8 (5.46)</td>
<td>34.0 (7.20)</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>60.7 (41.8 to 88.2)</td>
<td>50.6 (9.63 to 266)</td>
<td>57 (50.0 to 65.4)</td>
</tr>
<tr>
<td>CRH (pg/ml)</td>
<td>219 (191 to 250)</td>
<td>210 (163 to 271)</td>
<td>210 (147.6)</td>
</tr>
<tr>
<td>Maternal cortisol (nmol/l)</td>
<td>719 (613 to 843)</td>
<td>769 (598 to 989)</td>
<td>736 (240)</td>
</tr>
<tr>
<td>Maternal CRH (pg/ml)</td>
<td>368 (293 to 463)</td>
<td>377 (313 to 454)</td>
<td>334 (305 to 366)</td>
</tr>
<tr>
<td>Time to access (min)</td>
<td>3.02 (1.84 to 4.96)</td>
<td>2.74 (0.91 to 8.23)</td>
<td>3.40 (2.63 to 4.41)</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>17.9 (10.1)</td>
<td>11.9 (5.49)</td>
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Values are mean (SD) or geometric mean (anti-ln 95%CI). IHV, Intrahepatic vein; PCI, placental cord insertion; CRH, corticotrophin releasing hormone.

**Figure 1** Fetal cortisol levels before and after transfusion at the intrahepatic vein (A) (paired t test p < 0.001; n = 11) and at the placental cord insertion (B) (paired t test p = 0.3; n = 3).

**Figure 2** Fetal corticotrophin levels after blood samplings from the intrahepatic vein (IHV) and placental cord insertion (PCI) sites (t test p = 0.04; IHV, n = 7; PCI, n = 6).
Maternal CRH levels did not change with transfusion at either site (fig 4).

Figure 5 shows the relation between paired fetal and maternal CRH levels and gestational age between 20 and 38 weeks. Whereas maternal levels increased substantially with advancing gestation, fetal levels did not (maternal \( r = 0.58, n = 36, p \leq 0.0001; y = 98.4 + 1.04 \text{anti-ln } x, \text{anti-ln } 95\%CI \text{ for slope (1.02 to 1.07)}; \) fetal CRH, \( r = 0.27 \)). Figure 6 shows a modest correlation between paired fetal and maternal CRH levels (\( r = 0.36, n = 36, p = 0.03; y = 170 + 0.11x, (0.01 \text{ to } 0.22) \)).

There was no significant relation between initial maternal or fetal CRH and cortisol levels, except for a correlation between fetal CRH and fetal cortisol levels (\( r = 0.32, n = 38, p = 0.048 \); table 2).

**DISCUSSION**

This study confirmed our hypothesis, in that needling the IHV was not associated with an increase in fetal plasma CRH levels, either after transfusions, which we confirmed are associated with an increase in cortisol (fig 1), or after fetal blood sampling only procedures, which we showed were associated with an increase in corticotrophin (fig 2). This shows that the fetal hypothalamus-pituitary-adrenal axis,

<table>
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<th>Table 2</th>
<th>Correlations between maternal and fetal corticotrophin releasing hormone (CRH) and cortisol levels (after needling or before transfusion)</th>
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<tr>
<td>Maternal CRH</td>
<td>Maternal cortisol</td>
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<tr>
<td>( r = -0.002; n = 33, ns )</td>
<td>( r = 0.04; n = 33, ns )</td>
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although functional from mid-gestation,6 does not involve any measurable release of CRH into fetal plasma. This is similar to the situation in adults.

The finding that transfusion at the IHV leads to a rise in fetal cortisol supports our previous findings,7,8 and the lack of effect on fetal CRH levels indicates that placental CRH does not mediate the acute response to the stress of percutaneous needling in the human fetus.

The corticotrophin response to stress has not been previously studied in the human fetus. The increase in levels at the IHV but not the PCI, after needling, is consistent with a more rapid release than that of cortisol. This is compatible with release from the pituitary rather than the adrenal, and is also similar to findings in adults and animal models.19 In a previous study we have shown that noradrenaline, which is also released more quickly than cortisol, was increased in initial values after IHV fetal blood sampling compared with PCI.18

Our results suggest that fetal plasma CRH is derived from another source, presumably the placenta. It is well established that the placenta produces large amounts of CRH10-14 and that both maternal and fetal aspects of the syncytiotrophoblast contain binding proteins for CRH, with significantly more on both maternal and fetal aspects of the syncytiotrophoblast.17-19 This study shows that, whereas maternal plasma CRH levels did not change with increasing gestation, fetal cortisol was increased in initial values after IHV fetal blood sampling compared with PCI.8

The lack of correlation between maternal CRH and cortisol levels did not (fig 5); indeed, although there was some joint control of fetal and maternal CRH levels, it was not substantial (r = 0.36). The results suggest that the placenta secretes CRH preferentially into the maternal rather than the fetal circulation,13 although functional from mid-gestation,6 does not involve any measurable release of CRH into fetal plasma. This is similar to the situation in adults.

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