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Autonomous adrenocorticotropin reaction to stress stimuli in human fetus

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ABSTRACT

The aim of the study was to determine whether human fetuses show ACTH response to stress stimuli, to define the gestational age from which these reactions may be present and to analyze the relationship between hormone concentrations and their changes, both in fetuses and in pregnant women. The study included 81 intrauterine transfusions carried out in 19 pregnant women. 52 procedures were performed directly into the umbilical vein, which is not innervated, so neutral for the fetus (the PCI group) and 29 transfusions into the intrahepatic vein —which puncture is stressful for the fetus (the IHV group). ACTH and cortisol concentrations in fetal and maternal plasma obtained during the procedures were assayed. The initial mean plasma ACTH concentration in the PCI group equaled 18.94 pg/mL, but in the IHV group it was significantly higher and amounted 75.17 pg/mL ($p<0.001$). There was no significant change in the hormone concentration during the transfusion both in the IHV group (95.8 pg/mL, $p>0.05$) and in the PCI group (22.36 pg/mL, $p>0.05$). The observed hormonal response in the IHV group proves the existence of fetal pituitary reaction to stress. The initial fetal ACTH concentration in the IHV group correlated with the number of transfusions performed on a single fetus ($R=0.41$; $p=0.04$). No correlation with parity, gestational weeks or the volume of transfused packed red blood cells was found. There was also no correlation between fetal and maternal ACTH concentrations in any group. Presented data suggest that the human fetus shows autonomous ACTH reaction to stress stimulation.

1. Introduction

Adrenocorticotropin (ACTH) is a peptide consisting of 39 aminoacids, produced in anterior lobe β1 cells from a precursor which also forms melanotropins, lipotropins and β-endorpins — proopiomelanocortin (POMC). Adrenal gland grows in the absence of ACTH only up to 15 weeks of fetal development. Adrenocorticotropin is indispensable for its further development [1]. It is detectable in fetal plasma from 12 weeks of gestation (weeks). As from 34 weeks its concentration increases [2], but at term it is significantly lower [3–5]. It is also thought to have a crucial role in the fetal hypothalamus-pituitary-adrenal stress response. During the last three decades neonatal and fetal responses to stress and noxious stimulation have been investigated extensively. Neonatal and fetal adrenal reactions to stress were measured in many experiments on mammals, including humans. In human fetuses cortisol and noradrenaline response to stress is well documented [6–8]. Corticotropin releasing hormone and its concentration in the reaction to stress stimulation have also been an issue of a wide interest [9]. Although hypothalamic–pituitary–adrenal axis is known to function during fetal life and play a crucial role in the adaptation to stress and also in the biologic clock of pregnancy, only few studies have measured fetal adrenocorticotropin levels in stressful situations.

The aim of this study was to determine whether human fetus shows an adrenocorticotropin response to stress stimuli, define the gestational age from which these reactions may be present and to analyze the relationship between hormone concentrations and their changes, both in fetuses and in pregnant women.

2. Materials and methods

2.1. Studied groups

The studied material included 81 intrauterine transfusions due to the severe hemolytic disease carried out on 19 pregnant women hospitalized at the 1st Department of Obstetrics and Gynecology, Medical University of Warsaw, between 2005 and 2007. The fetuses were qualified for routine intrauterine treatment on the basis of the peak systolic velocity in the middle cerebral artery (equal or more than 1.5 multiples of median (MoM)). The inclusion criteria for the experiment were: singleton pregnancies, fetuses appropriate for gestational age with no severe ultrasound abnormalities or hydrops. An informed consent was signed by all 19 women qualified for the intrauterine transfusion because of fetal hemolytic disease, who decided

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to participate in the study. Those who refused had an equal access to subsequent medical procedures. The study obtained the approval of the Ethics Committee of Medical University of Warsaw and was conducted according to the Declaration of Helsinki.

The patients were divided into different subgroups, depending on the procedure technique. In the first one, with the placenta located on the anterior uterine wall the vascular access for red blood cells transfusion was obtained by puncturing the umbilical vein at placental cord insertion (the PCI group). The placental cord has no sensory innervation, so its puncturing is neutral for the fetus. In the second subgroup, with placental location other than the anterior uterine wall, red blood cells were administered directly into the intrahepatic vein, which is usually the umbilical part of the left branch of the portal vein (the IHV group). In the latter fetal peritoneal cavity was punctured prior to transfusion to administer pancuronium bromide in order to obtain fetal relaxation. It prevents the fetus from moving during the transfusion and therefore reduces the risk related to the procedure. Both punctures of the fetal trunk are thought to be stressful for it.

The site of the ultrasound-guided intrauterine transfusion was chosen basing on the location of the placenta, which is independent of any medical manipulation. No other randomization was used. Only procedures uncomplicated by multiple fetal puncturing were included in the study. Neither maternal sedation nor fetal analgesia were used. Pregnant women received only local analgesia before inserting the needle into the uterine cavity.

The PCI group consisted of 13 pregnant women. They had a total of 52 intrauterine transfusions performed. The IHV group consisted of 6 women with a total of 29 transfusions in this group. Only one fetus from the PCI group underwent a single procedure, other fetuses had several transfusions (from 2 to 8).

In the PCI group the procedures were carried out from 22 to 36 weeks and in the IHV group from 19 to 33 weeks.

2.2. Blood samples

1 to 3 ml samples of fetal venous blood were collected into polystyrene tubes and mixed with tripotassium versenate (K3-EDTA) before and after each transfusion, depending on gestational age. Simultaneously two 5 ml samples of maternal venous blood were taken Blood samples were centrifuged for 10 min at 3000 circulations per minute within 30 min from collection. The plasma was then frozen at −20 °C. The exact time from the first fetal puncture to the first and second fetal blood sample was measured using an electronic stopwatch.

To exclude the influence of circadian rhythm on the assayed hormone concentrations, only the procedures carried out at the same time of the day (between 11.00 am and 1.00 pm) were taken into account.

2.3. Assays

ACTH and cortisol were assayed in every fetal and maternal blood sample. The plasma ACTH concentration was analyzed using a standard radioimmunoassay (Lumitest ACTH, Brahms Diagnostica, Hennigsdorf, Germany). The lower limit of sensitivity was 1 pg/mL. The coefficient of variation was 10.5%. The plasma cortisol concentration was assayed using Roche Cobas Cortisol assay (Roche Diagnostics, Mannheim, Germany), with lower sensitivity limit of 0.018 μd/dL and coefficient of variation 7.5%. The fetal and maternal plasma samples were paired and analyzed in the same assay run.

2.4. Statistical analysis

Statistical analysis was performed with the use of STATISTICA (version 9.1). The characteristics of descriptive statistics were carried out using typical parameters, such as arithmetic mean, median, standard deviation and 95% confidence interval. Because of the small samples of our study groups and data not following a normal distribution, nonparametric tests were used. The procedure times were compared using Mann–Whitney *U*-test. In the analysis of ACTH and cortisol concentrations both Mann–Whitney *U*-test and the Wilcoxon signed ranks test were used. The further analysis of relations between both studied hormones within the groups was performed with intra-class correlation method. Spearman's rank correlation was applied to explore the associations between hormone concentrations and other variables. P value <0.05 was considered significant.

3. Results

3.1. Procedure times

The time of the whole procedure (from the first fetal trunk puncture to the second fetal blood sample collection) in the IHV group equaled 1238 s on average (95% confidence interval [CI]:953–1523; SD 398). In the PCI group the procedure time (from the first puncture of the umbilical vein to the second blood sample collection) was shorter and took 448 s on average (95% CI: 284–614; SD 342; $p<0.001$). 27% of the procedures in the PCI group lasted longer than 600 s. The mean time from the beginning of the procedure to the first fetal blood sample collection equaled 30 s in the PCI group (95% CI: 23–38; SD 14). In the IHV group it was much longer and lasted 774 s on average (95% CI: 524–1025; SD 350; $p<0.001$) due to the time required for obtaining fetal relaxation after pancuronium administration. The time from the first to the second fetal blood collection (the time of blood transfusion) was similar in both groups, amounting 464 s in the IHV (95% CI: 281–646; SD 255) and 418 s in the PCI group on average (95% CI: 305–637; SD 323; $p>0.05$).

3.2. Fetal and maternal ACTH

The adrenocorticotropin and cortisol results for both groups are shown in Table 1. In the PCI group, where the puncture of the umbilical vein is painless for the fetus, the mean ACTH concentration in the fetal plasma did not change during the procedure ($p>0.05$) (Fig. 1). The mean hormone concentration before the procedure in the IHV group, where the fetal abdominal puncture is required, was significantly higher than the initial concentration in the PCI group ($p<0.001$). In the IHV group there was no significant change in hormone concentrations over the treatment, either ($p>0.05$) (Fig. 2).

The youngest fetus in the IHV group underwent the transfusion at 20 weeks. Its plasma ACTH concentration before the procedure amounted 62.11 pg/mL and after the transfusion 78.3 pg/mL. Both levels were about four times higher than the average ACTH concentration in the PCI group.

In the PCI group the average time of the transfusion was over 7 min, but almost 30% of the procedures lasted more than 10 min. The mean initial concentration of ACTH in those fetuses from the PCI group in whom the treatment lasted over 10 min equaled 16.62 pg/mL and did not change significantly during the procedure — 20.55 pg/mL ($p>0.05$). Both the initial and final hormone concentrations were significantly lower than in the IHV group ($p<0.001$). The shortest procedure in the IHV group lasted 700 s. ACTH levels in this case did not differ from the mean hormone concentration in the whole group (initial concentration 68.13 pg/mL, final 73.99 pg/mL).

The initial fetal ACTH concentration in the IHV group showed an average correlation with the number of transfusions performed on a single fetus ($R=0.41$; $p=0.04$). No correlation with parity, gestational weeks or the volume of transfused red blood cells concentrate was found (Table 2). Insignificant change in fetal plasma hormone concentration during the procedure in the IHV group and initial