Simultaneous Determinations of Total Cortisol and Progesterone by Radioimmunoassay during Late Pregnancy

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Total cortisol and progesterone in the maternal peripheral sera were determined by radioimmunoassay. Total cortisol was measured in 196 sera, and progesterone in 203 sera obtained randomly throughout pregnancy. And serial estimations of both steroid hormone levels were also made simultaneously in 6 normal pregnant women during late pregnancy, labour and puerperium. Total cortisol and progesterone increased with the advance of gestation. During the last few weeks preceding labour total cortisol increased further, whereas progesterone decreased. After the onset of labour a rising tendency in total cortisol levels and a falling tendency in those of progesterone were also observed, but at puerperium both hormones decreased rapidly to non-pregnant level in the 6 pregnant women.

Key Words: cortisol, progesterone, onset of labour, radioimmunoassay

1. Introduction

The causes of term labour are still unknown. Changes in hormonal environment prior to labour have recently been investigated as a possible predisposing factor. In several mammalian species, the onset of parturition was found to be associated with a marked increase in fetal adrenal corticoid production. More information, however, is needed, for the confirmation of the role of fetal adrenal cortex in the initiation of parturition in human.

It has been shown that progesterone is a prerequisite for maintenance of pregnancy in many species of animals. Indeed, progesterone withdrawal, as evidenced by its decrease in the maternal peripheral blood, precedes the onset of parturition in many species. Most studies, however, have failed to demonstrate any significant correlation between maternal peripheral progesterone levels and the onset of human labour. Only 2 studies with a competitive protein-binding technique and a specific radioimmunoassay method have demonstrated a fall in plasma progesterone levels before the onset of labour. Thus the question remains unresolved and further studies are required.

The aims of the present study are: to identify more accurately the patterns of the changes in serum cortisol and progesterone during normal pregnancy, and to determine whether or not these steroid levels undergo a noticeable variation before the onset of labour.

2. Experimental

Women attending the antenatal clinic and subsequently admitted to the labour room of Shizuoka Saiseikai Hospital were the subjects of the present study. All of the women were 30 years of age or less, were sure of the date of the last menstrual period, and had a history of regular menstrual cycles of 28−30 days before pregnancy. They were apparently healthy pregnant patients; all cases went into labour spontaneously and were delivered vaginally of a single normal healthy infant, weighing 2.5 kg or more, within ±14 days of the expected date of delivery.
A total of 196 and 203 blood specimens were collected randomly from the patients, and subjected to cortisol and progesterone measurements, respectively. Both steroid levels were examined by grouping the values at weekly intervals on the gestational timetable based on the last menstrual period. Blood samples were also collected sequentially from 6 normal patients during late pregnancy, labour and pureperium. These samples were subjected to the same measurements.

The samples were drawn in the morning (between 9.00-11.00 a.m.) from the maternal antecubital vein except when the spontaneous onset of labour occurred in 6 cases of serial measurement. The samples were immediately centrifuged, and the serum was stored at -20°C until assay.

Serum total cortisol was measured by the highly specific nonchromatographic radioimmunoassay of Yoshimi and Tachibana\textsuperscript{12}, using the kit supplied by Daiichi Radioisotope Laboratories (Tokyo, Japan). The antiserum used in this assay, which was generated in rabbit against cortisol-21-hemisuccinate-bovine serum albumin (BSA), was not specific for cortisol, and thus the specificity of the antibody was determined by checking cross reactivities with 15 steroids. The steroids that displayed considerable cross-reactions were only 11-deoxycortisol (25.9 %) and 17-α-OH-progesterone (23.6%)\textsuperscript{13}. But the levels of these steroids in pregnancy sera are too low\textsuperscript{8,14} to interfere with our radioimmunoassay for cortisol.

The plasma sample was treated with the solvent methanol/ethanol 1:1 to precipitate the plasma protein\textsuperscript{15}. No other extraction procedures were needed in this assay method. Free and bound steroids were separated using polyethylene glycol. The coefficients of variation were 12% (intraassay) and 16% (interassay). Recoveries ranged from 95% to 105%.

Serum progesterone was measured by a direct, simple radioimmunoassay method of Kutas, et al.\textsuperscript{16} In this method, ether extraction is the only purification step. Assay reagents and the antibody produced by the CEA-IRESORIN were supplied from Midorijuji Co. Ltd. (Osaka, Japan).

All serum samples were measured in duplicate. Paired t-test was used for statistical analysis.

3. Results

Figure 1 illustrates the serum levels of cortisol. Mean value of serum cortisol in non-pregnant cases was 8.2 µg/dl (n=12). During pregnancy, the level of cortisol increased progressively with advancing gestation, reaching a high value (26.7 µg/dl, n=5) at the 36th week of pregnancy, although considerable fluctuations were observed. A further increase occurred in the last few weeks of pregnancy, reaching a maximum at the 40th week (41.0 µg/dl, n=9).

Figure 2 illustrates the serum levels of progesterone during pregnancy. The mean levels of serum progesterone showed a 19-fold increase between the 7th and the 38th week of gestation and then fell significantly (p<0.01) from a peak mean of 222.5 ng/ml (n=5) at 38th week to 143.5 ng/ml (n=5) at 40th week.

Figure 3 illustrates the serial changes in total cortisol and progesterone levels in 6 pregnant women. Even though marked fluctuations of both steroid hormone levels were observed in...
the successive measurements in each patient, there was an overall tendency to rise in total cortisol level and a fall in that of progesterone before the onset of labour (Fig. 3). It is clear that the total cortisol level increased in 3 patients (Cases 3, 5 and 6), while the progesterone level decreased in 4 patients (Cases 1, 4, 5 and 6) prior to the onset of labour. During delivery the same tendency was observed, but after parturition both hormones decreased rapidly to non-pregnant levels.

4. Discussion

Turnbull, et al. have suggested that cross-sectional studies on the levels of steroids may be of little value to estimate the changes of steroid hormones before the onset of labour, owing to the marked variation in individual patients. The present study showed a rising tendency in total cortisol level and a falling tendency in that of progesterone in the maternal peripheral serum before the onset of labour in both the randomly collected samples and the serial samples of 6 pregnant women.

Many workers have assayed these plasma steroids by a competitive protein-binding technique, by which a proportion of the steroid metabolites in the plasma can also be measured. The availability of highly specific antibodies against cortisol and progesterone in the radioimmunoassay allowed us to determine these hormones more precisely.

This is the first report to demonstrate that the total cortisol concentration in maternal peripheral serum increases in the prepartum period. These results are consistent with those for amniotic fluid by Murphy, et al., but contrast with others. Campbell and Murphy proposed that the total cortisol levels give an adequate indication of the level of cortisol unbound to plasma protein, which is generally regarded as the physiologically active form of the circulating hormone. It is of interest to speculate that the increase in the total cortisol in the maternal peripheral serum in part derives from a progressive production of cortisol by fetal adrenal during pregnancy.

Our results demonstrate a steady rise in serum progesterone levels during pregnancy although there was a late fall, which is in accord with the observations of Csapó, et al. and Turnbull, et al. The late fall of this steroid was statistically significant.

Anderson, et al. reported that in sheep the late fall in serum progesterone levels is the result of a enzyme induction by fetal cortisol; cortisol induce placental 17-α-hydroxylase which metabolize progesterone. Until near term, sheep placental biosynthesis of steroids is restricted mainly to those containing carbon-21 atoms because of inactivity of the 17-α-hydroxylase. When the sheep placenta is exposed to elevated levels of glucocorticoids induced either by a enhanced production of cortisol in the fetus or by the administration of dexamethasone to the fetus, the placental 17-α-hydroxylase is rapidly induced. Taking these into consideration it is possible that the decrease in plasma progesterone levels of human pregnancy observed in the last few weeks (Fig. 2) is due to similar mechanism reported in the sheep.
Fig. 3 Serial measurements of total cortisol and progesterone levels in 6 normal pregnant women during late pregnancy, labour and puerperium. Case 3 and 5 were of forceps delivery.

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References

2) T. B. Pokoly: ibid., 117, 549 (1973)
3) S. Sybulski and G. B. Maughan: ibid., 125, 239 (1976)
13) Directions for Use of Cortisol Kit "DAI-ICHI"
要 旨

ラジオイムノアッセイによる妊娠血中コルチゾールとプロゲステロンの測定

——とくに陣痛発来との関連について——

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妊娠後期, とくに陣痛発来前における妊娠血中コルチゾールとプロゲステロンの動態は陣痛発来機序との関連から興味が持たれるが, 未だそれらの動態は諸家の報告の一致をみず, 不明確な点が多い。そこで, ラジオイムノアッセイ法により, まず正常妊娠妊娠各期のコルチゾールとプロゲステロンを測定し, これらの正常妊娠血中動態を検討した。コルチゾールは妊娠週数の進行とともに増量し, 妊娠満期の40週に最高値を示した。一方, プロゲステロンも同様に妊娠の進行とともに増加するが, 妊娠38週が最高値であり, 以後, 妊娠満期の40週には有意の減少がみられた。つぎに6例の妊娠末期の連続測定例から, 陣痛発来前に同様なコルチゾールとプロゲステロンの変動を観察した。これらステロイドホルモンの動態を胎盤のステロイドホルモン代謝酵素との関連のもとに考察を加えた。