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THE BINDING OF SEX STEROIDS IN HUMAN MATERNAL AND FETAL BLOOD AT DIFFERENT STAGES OF GESTATION

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SUMMARY

The concentrations of the steroid binding proteins albumin, α_1-acid glycoprotein (AAG) and steroid-binding β-globulin (SBβG) have been measured in human fetal and maternal serum at mid pregnancy and term using highly specific immunochemical methods. AAG appears to be relatively unimportant as a sex steroid binding protein in fetal serum, while albumin probably serves as a low affinity, high capacity carrier of such steroids throughout much of fetal life and also in the maternal serum. SBβG levels in maternal serum are approximately twenty times greater than the levels of testosterone plus oestradiol-17β. In the fetus, SBβG levels are much nearer to the combined concentration of the ligands, testosterone and oestradiol-17β, and at term the levels of ligand and SBβG are almost the same. Steady-state electrophoretic studies of fetal and maternal serum have failed to demonstrate any additional oestrogen or androgen binding proteins.

INTRODUCTION

A large proportion of serum oestrogens and androgens, both conjugated and unconjugated, appear to circulate bound to proteins. For convenience, two types of binding may be distinguished: first, high affinity, limited capacity binding and second, low affinity, high capacity binding. In the human, steroid binding β-globulin (SBβG[1], SHBG[2], SBP[3] or SP_2[4]), present at a relatively high concentration in the blood of pregnant women and at lower concentrations in normal males and females, is known to bind oestradiol with high affinity and testosterone and 5α-dihydrotestosterone with still higher affinities while albumin and α_1-acid glycoprotein (AAG) appear to be low affinity binders of several steroid hormones. It is not clear, however, whether a similar pattern of steroid binding occurs in the fetus at different stages of its development or whether additional binding proteins are present. In the present study, we have measured, using specific immunoprecipitation methods, the concentrations of albumin, AAG and SBβG in maternal and fetal blood at mid preganancy and term and have carried out preliminary studies to look for evidence of additional androgen- or oestrogen-specific binding proteins in fetal blood.

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MATERIALS AND METHODS

[1, 2, 6, 7 (n)-^{3}H]-testosterone (approximately 90 Ci/mmol), [1, 2, 4, 5, 6, 7 (n)-^{3}H]-5α-dihydrotestosterone (130 Ci/mmol), [2, 4, 6, 7 (n)-^{3}H]-oestrone (90 Ci/mmol) and [2, 4, 6, 7 (n)-^{3}H]-oestradiol-17β (90 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, England) and unlabelled testosterone and oestradiol-17β from Steraloids (Croydon, England). Other reagents were of the highest purity available and were obtained from BDH (Poole, England) or Sigma (London) Ltd. (Kingston-upon-Thames, England).

Serum was prepared from maternal venous blood samples and umbilical cord blood (mainly venous) immediately after delivery and from fetuses aborted therapeutically at 17–22 weeks gestation. Separated serum was stored at −20°C until required.

The serum concentrations of α_1-acid glycoprotein (AAG) and albumin were measured using the appropriate LC- or M-Partigen immunodiffusion plates and standard solutions (Behringwerke AG, W. Germany). The coefficients of variation of replicate measurements of both AAG and albumin were between 2% and 3%.

Steroid-binding β-globulin (SBβG) was measured by electroimmunoassay as described by Bohn[5], using a specific rabbit antiserum raised

against purified SBβG (6) at an antiserum in agarose concentration of 1.5% (v/v) (maternal serum) or 0.5% (v/v) (fetal serum). When used in the immunoelectrophoresis of whole pregnancy plasma this antiserum produced only one precipitin arc, thus confirming the specificity of the assay. Standard solutions used in this assay were calibrated against purified protein. The coefficient of variation of replicate measurements was about 5%.

The binding of oestradiol-17β, oestrone, testosterone or 5α-dihydrotestosterone to components of maternal and fetal serum was studied by a steady-state polyacrylamide gel electrophoresis technique as described by Cowan *et al.*[7]. Plasma was diluted 5-fold for oestrogen binding experiments and 10 or 20-fold when studying androgen binding. Radiolabelled steroid (10^{-9} mol/l) was incubated with diluted plasma for 15 min at 30°C before applying 150 μl samples to the polyacrylamide gels.

Unconjugated oestradiol-17β was measured as described by Hotchkiss *et al.*[8] using a specific antiserum raised in sheep against oestradiol-6-0 (carboxymethyl) oxime linked to bovine serum albumin and testosterone by the method of Rowe *et al.*[9]. The latter assay measures approximately 28% dihydrotestosterone in addition to testosterone.

RESULTS AND DISCUSSION

Testosterone and oestradiol levels in mother and fetus

Table 1 shows the serum concentrations of testosterone and oestradiol-17β in mother and fetus at term and mid-pregnancy (16–22 weeks). The term oestradiol-17β levels in the present study are of a similar magnitude to those reported by Shutt, Smith and Shearman[10] although, in contrast to our findings, they found that the maternal levels were approximately three-fold higher than the levels in cord venous blood. The term testosterone levels reported in the present study are similar to those reported by Abramovich[11]. Since no significant sex difference in fetal serum concentrations of testosterone is observed at term[11], the appropriate value in Table 1 is calculated from values obtained for both male and female fetuses.

Albumin and α_1-acid glycoprotein

From the data in Table 2 it is clear that α_1-acid

Table 1. Maternal and fetal serum concentrations of testosterone and oestradiol-17β at mid-pregnancy and term: values are shown as mean ± 1 S.D. of the number of observations shown in parentheses

Samples		Testosterone (nmol/l)	Ref.	Oestradiol-17β (nmol/l)	Ref.
Mid pregnancy	Maternal	1.90 ± 0.92 (n = 13)	[10]	21.6 (n = 9)	[9]
	Fetal	♂7.67 ± 4.07 (n = 8) ♀0.93 ± 0.42 (n = 6)	[10]	3.6 (n = 2)	[9]
Term	Maternal	4.18 ± 1.45 (n = 6)	P.S.	41.14 ± 18.30 (n = 11)	P.S.
	Fetal	2.83 ± 1.40 (n = 6)	P.S.	78.84 ± 45.61 (n = 11)	P.S.

P.S. = Present study.

Table 2. Concentrations of albumin, AAG and SBβG in maternal and fetal serum at mid-preganancy and term (Fetal values in parentheses)

Subject	Albumin (mg/dl)		AAG (mg/dl)		SBβG (mg/dl)	
J (20 weeks)	—	(1500)	—	(3.05)	—	(0.50)
K (19 weeks)	—	(1785)	—	(4.7)	—	(0.15)
De (22 weeks)	—	(1470)	—	(4.3)	—	(0.56)
Y (19 weeks)	—	(1260)	—	(5.9)	—	(0.66)
Du (18 weeks)	—	(1755)	—	(4.7)	—	(0.47)
McN (17 weeks)	—	(2055)	—	(4.5)	—	(0.47)
Mean	—	(1637.5)	—	(4.5)	—	(0.47)
Cr (term)	2990	(3190)	63.0	(12.0)	7.64	(0.44)
Cu (term)	1750	(3190)	60.0	(33.0)	9.58	(1.10)
F (term)	2640	(2750)	43.0	(21.0)	7.60	(0.55)
L (term)	2120	(2830)	43.0	(26.0)	6.19	(0.77)
T (term)	2830	(3520)	54.0	(29.0)	6.41	(0.94)
W (term)	1960	(2695)	56.0	(29.0)	5.38	(0.64)
Mean	2381	(3029)	53.2	(25.0)	6.97	(0.74)

glycoprotein (AAG) concentration in fetal blood increases some 5–6 fold over the second half of pregnancy but at term the fetal levels are still only about 50% of the maternal serum concentration. The low levels of AAG in fetal serum at mid-pregnancy together with the low affinity of AAG for oestradiol and testosterone[12] suggests that AAG is relatively unimportant as an oestradiol or testosterone binding protein.

Although fetal serum albumin concentrations increase only some 1.8 fold over the second half of pregnancy, the mid-pregnancy levels (about 1640 mg/dl) are nearly 70% of the term maternal levels. Thus albumin must be considered to be a potentially important low affinity steroid binding protein throughout much of fetal life.

Steroid-binding β-globulin

In this study, we have used a highly specific assay to measure SBβG levels in terms of the amount of immunologically reactive protein present (Table 2) rather than as the number of available testosterone or dihydrotestosterone binding sites[13, 14]. By electroimmunoassay, the mean mid-pregnancy fetal serum concentration of immunoreactive SBβG is found to be 4.7 mg/l. Since the molecular weight of SBβG is approximately 65,000 daltons[4, 6] this is equivalent to a molar concentration of 7.2×10^{-8} mol/l. Assuming one steroid binding site per molecule of SBβG this concentration is hence somewhat greater than the total circulating levels of testosterone and oestradiol-17β, plus of course, dihydrotestosterone.

In term fetal serum, the mean molar concentration of SBβG (1.1×10^{-7} mol/l) is very close to that of testosterone plus oestradiol-17β (approximately 0.8×10^{-7} mol/l).

In maternal serum, the SBβG levels at term (and mid pregnancy[15]) are in excess of the circulating testosterone and oestradiol levels by a factor of about 20. In the light of this finding it is suggested that these sex steroids remain bound with high affinity in the maternal serum and that little transfer from mother to fetus occurs. However, *in vitro* assays using purified SBβG would be required to confirm the above suggestion and to investigate the influence of other competing steroids.

Other high affinity binding proteins

Using steady state polyacrylamide gel electrophoresis, the androgens, testosterone and 5α-dihydrotestosterone showed only one major peak of bound radioactivity in both maternal and fetal serum at mid-pregnancy and term. It is probable that this binding was due to SBβG, since the binding of radioactive steroid was abolished in the presence of a hundred-fold excess of unlabelled dihydrotestosterone.

Oestradiol-17β appeared to be bound to at least 2 separate components of maternal serum at term and mid-pregnancy. One of these is believed to be SBβG since the oestradiol binding was completely abolished in the presence of a hundred-fold excess of dihydrotestosterone while the other, an anodal protein migrating just behind the front of the bromophenol blue marker, was not saturable and was probably albumin. In fetal serum, both at term and mid-pregnancy, only binding of oestradiol-17β to the anodal protein could be demonstrated by this technique. This binding was not reduced in the presence of excess unlabelled oestradiol. Oestrone showed a similar pattern of binding as did oestradiol-17β in all plasma samples examined. It appears, therefore, that fetal plasma does not contain a high affinity oestrogen-binding protein other than SBβG, since AAG has only a low affinity for such ligands[12].

Although fetal serum appears to contain sufficient SBβG to bind most if not all the oestradiol present, our inability to demonstrate such binding using steady-state electrophoresis requires further comment. First, we cannot be certain that all the SBβG measured immunologically is in fact biologically active as a steroid binder. The alternative conclusion is that in the presence of a greater than 3000-fold excess of albumin, the oestrogens of fetal blood are not significantly bound to the high affinity sites on SBβG but are more loosely-bound to albumin from which they may readily dissociate to become biologically active.

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