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Corticosteroid-binding Globulin (CBG) in Fetal Development

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In fetal sheep the prepartum increase in plasma cortisol concentration is associated with an increase in high affinity corticosteroid binding activity in plasma. This appears to reflect an increase in corticosteroid-binding globulin (CBG) biosynthesis from the fetal liver, and evidence is presented that hepatic CBG gene expression is increased by exposure to glucocorticoids in the fetus. Immunoreactive CBG is found in other fetal tissues, and CBG mRNA is present in fetal pituitary. CBG reduces the ability of cortisol to exert negative feedback on basal or CRH-stimulated ACTH output by fetal sheep pituitary cells in culture. We suggest that CBG interacts with cortisol in a manner that maintains a low negative feedback on the pituitary, and perhaps hypothalamus. This constitutes a component of the cascade of events that is associated with hypothalamic–pituitary–adrenal activation in the late gestation fetus, and with the onset of parturition.

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INTRODUCTION

In most, if not all, animal species studied there is an increase in the activity of the hypothalamic–pituitary–adrenal (HPA) axis in the fetus during late gestation [1]. This results in increased output of corticosteroid, either cortisol or corticosterone, from the fetal adrenal gland. The increase in corticosteroid in the fetal circulation contributes to the stimulus to maturation of the organ systems necessary for extrauterine life. In species such as sheep, fetal adrenal cortisol provides the signal that triggers the onset of parturition. More recent studies, however, have also recognized potential adverse effects of glucocorticoids, since these may decrease insulin-like growth factor gene expression in fetal tissues [2], and thus indirectly impair fetal growth through this anti-mitogenic activity.

The fetal sheep has been used extensively for *in vivo* and *in vitro* studies of fetal HPA maturation [1]. In this species, evidence has accumulated to show that corticotrophin releasing hormone (CRH), and arginine vasopressin (AVP) from the paraventricular nucleus of the fetal hypothalamus act on the corticotrophs of the fetal pars distalis to stimulate proopiomelanocortin (POMC) gene expression, translation, and ACTH secretion. Increased concentrations of ACTH appear in the fetal circulation, and act on the adrenal gland to induce expression of steroidogenic enzymes in the fetal adrenal cortex, and to provoke increased cortisol output. A critical question is why the increase in fetal cortisol concentrations in plasma during late gestation does not feed back to inhibit ACTH secretion; instead both ACTH and cortisol continue to rise concomitantly. In this review we suggest that one mechanism to diminish the negative feedback effect of glucocorticoids is through stimulation of corticosteroid-binding globulin (CBG) production by the fetal liver. Hepatic CBG is secreted into the systemic circulation, binds cortisol, and maintains a relatively low free-cortisol concentration in plasma. In this way the negative feedback effects of glucocorticoids at the hypothalamus and pituitary may be attenuated.

Critical to this supposition is evidence that hypothalamic CRH and pituitary POMC gene expression increase through late gestation rather than being suppressed. Measurements of CRH mRNA in the parvocellular region of the paraventricular nucleus by *in situ* hybridization has shown that there is a progressive increase in its levels between day 120 of gestation to the highest values at term [3]. The presence of immuno-

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reactive CRH in this region shows that the mRNA is translated. At the pituitary level, early studies using Northern blotting suggested that POMC mRNA levels increased progressively from day 120 to term [4]. More recently, using *in situ* hybridization, we have shown unequivocally that there is an increase in levels of POMC mRNA between day 60 and day 120 of gestation, and a further increase between day 120 of gestation and term [5]. This latter increase is associated with a redistribution of POMC expressing cells towards the basal region of the pars distalis. Since POMC levels increase further after birth as plasma cortisol falls, it is possible that during fetal life there is a degree of negative feedback suppression on the hypothalamus and pituitary. However, the progressive increase in CRH and POMC mRNAs indicate that this effect is minimal or partially offset by positive inputs from higher centers.

MECHANISMS FOR REDUCING NEGATIVE FEEDBACK

There are several potential mechanisms by which the negative feedback effects of glucocorticoids might be regulated at both hypothalamic and pituitary level. First, it is possible that there may be downregulation of glucocorticoid receptor (GR) number. However, early studies showed that there was an increase in triamcinolone binding to fetal anterior pituitary cells, and hypothalamic cells from late gestation fetuses, which would not be consistent with this possibility [6]. Recently we have also shown that GR mRNA levels increase in pituitary tissue from fetuses near term [7].

A second possibility is that there is a change in expression or activity of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) which interconverts cortisol and its biologically inactive metabolite cortisone. The levels of 11β-HSD mRNA in the fetal pituitary increase during the latter part of gestation, and rise particularly between day 140 and term [8]. The increase in mRNA is associated with an increase in both dehydrogenase and reductase enzymes, but under the conditions of *in vitro* assay employed, in which Type-1 11β-HSD activity predominates, the enzyme favours the dehydrogenase rather than reductase pathway. Thus, conversion of cortisol to cortisone at the level of the pituitary may lead to diminished negative feedback potential of biologically active glucorticoids [1].

A third possibility is that CBG, by binding active glucocorticoid in the circulation, diminishes the availability of free cortisol for negative feedback at the level of the pituitary. Measurements of total cortisol, percent free cortisol and absolute free cortisol concentrations are consistent with this suggestion [9–11]. In the plasma of chronically catheterized fetal sheep, the total cortisol concentration increases from approx. 2 ng/ml at 20 days before parturition to about 85 ng/ml at term. Percent free cortisol falls slightly, although not significantly, from about 6.5 to 4.7% between -20 and -5 days prior to parturition [9]. The effect of this is that the free cortisol concentration does not change significantly between -20 and -15 days prepartum, with values of around 0.2 ng/ml. Nor does it change between -10 and -5 days prepartum when the mean value has risen to $\sim$0.5 ng/ml, despite a doubling of total cortisol over these 5 days. Thus the rise in free cortisol at parturition is much more abrupt that the change in total cortisol concentration that is generally reported [see 1].

CHANGES IN FETAL CBG

The difference between changes in total and free cortisol concentration are accomplished in large part by an increase in the concentration of CBG, measured as the cortisol binding capacity (CBC) in the circulation of the fetal lamb during late pregnancy [9–11]. Using the assay of Ballard *et al.* [10] as modified by us [12] we found that the CBC rose from values of around 20 ng/ml at 15 days prepartum to levels in excess of 80 ng/ml at the day of parturition. This profile agrees closely with that reported by Fairclough and Liggins [9], calculated from results of equilibrium dialysis studies, and is in general agreement with the observations of Ballard *et al.* [10]. The CBC fell rapidly postnatally. By 10 days of life the plasma CBC has already fallen to the low values that are characteristic of the adult sheep.

Using Northern blot analysis we showed that CBG mRNA was present as a single 1.8 kb transcript in the fetal sheep liver [13]. The levels of CBG mRNA increased significantly between day 120 and day 140 of gestation but had begun to decrease at term, although not significantly. There was a further significant decrease in CBG mRNA levels in the newborn period.

Purified ovine CBG [13] was used to generate polyclonal antibodies in rabbits. We used this antiserum for immunohistochemical localization of immunoreactive (IR)-CBG to fetal sheep tissues. In the liver, IR-CBG was present in the hepatocytes as early as day 63 of gestation. This is consistent with observations showing high concentrations of CBG in fetal serum at this stage of pregnancy [14], a time when fetal adrenal responsiveness to ACTH stimulation is also high [1]. In the liver, immunoreactive CBG was not associated with haematopoeitic tissue. It continued to be associated with hepatocytes through the course of gestation, although subjectively the intensity of staining of tissue from term animals was less than that at the earlier times of pregnancy.

It is of interest to compare the spatial and temporal pattern of CBG expression in the fetal sheep with that described in other species. In the rabbit, (term $\simeq$ 31 days), the highest levels of CBG mRNA in the liver were achieved by about day 17 of gestation [15]. There was a progressive decrease thereafter until term. This

profile corresponded with the finding that the highest corticosteroid binding activity in fetal plasma was at about day 17–18 of gestation, with a subsequent decline. In the rat, Smith and Hammond [16] demonstrated that the highest levels of CBG mRNA in the liver were present on day 15 of pregnancy (term $\simeq$ 21 days). Hepatic CBG mRNA levels, and plasma corticosteroid binding activity decreased from this time towards term. In the mouse, CBG mRNA was localized to the liver by day 13, increased to high values by day 15 of gestation (term 19 days), and then decreased by day 18 [17]. The changes in CBG mRNA, determined by *in situ* hybridization, were mirrored by changes in IR-CBG. Thus in each of these species, as in the sheep, high levels of CBG mRNA are found in the liver during fetal life. The timing of this peak varies, as does the subsequent decrease in CBC mRNA levels towards term. In the sheep, the peak of CBG mRNA is a relatively late event, whereas in the rodent species, peak levels of expression occur at an earlier stage of gestation.

In the fetal mouse and in other species, IR-CBG and CBG mRNA was localized to other fetal tissues. During fetal life in the mouse there was intense staining for IR-CBG in the proximal and distal tubules of the kidney, but this was not associated with detectable CBG mRNA [17]. Similarly, IR-CBG was present in the pancreas and in the adrenal gland. CBG mRNA was transiently expressed in the fetal mouse pancreas at day 15 of gestation. Postnatally there was an increase in CBG mRNA in the mouse kidney to peak values at day 21 [18]. At this time the abundance of CBG mRNA in the kidney exceeded that in the liver. After day 21 there was a rapid fall in CBG mRNA levels in the kidney, with a concomitant increase in levels in the liver.

We used reverse transcription-polymerase chain reaction (RT-PCR) to confirm the presence of CBG mRNA in tissue obtained from late gestation (day 140) sheep fetuses [11]. A 416 bp RT-PCR product was identified by Southern blotting in fetal liver, as control, in some samples of kidney, and abundantly in fetal pituitary tissue. There was no reaction product from fetal hypothalamus, adrenal or lung at this stage of gestation [11]. Previous studies in the rat and guinea pig had localized IR-CBG to pituitary tissue of adult animals [19, 20]. In particular, in the guinea pig, intense CBG immunostaining was present in the pars intermedia and corticotrophs of the pars distalis, but not in the pars nervosa [19]. Studies are now required to determine the distribution and localization of CBG mRNA and IR-CBG in pituitary tissue from fetal sheep at different times during the course of pregnancy.

By immunostaining, IR-CBG was present in the ovine fetal kidney as early as day 63 of gestation (ETM Berdusco, JRG Challis, unpublished observations). Intense immunostaining was present in the proximal/distal convoluted tubules and extended into the loops of Henle; the glomeruli were immunonegative. This pattern of staining persisted throughout fetal life. We found that IR-CBG was also present in the villi and circular muscle of the fetal small intestine, in the bronchiolar epithelium, and in the epithelium of the chorion, an important observation to which we return below.

EFFECTS OF CBG ON NEGATIVE FEEDBACK

A crucial aspect of our thesis was that CBG should be able to attenuate negative feedback effects of cortisol on the pituitary. To examine this we maintained pituitary cells from term fetal lambs in monolayer tissue culture for 96 h, and measured the output of IR-ACTH into the culture medium after a 6 h period in the basal state, and after a subsequent 2 h challenge with 10 nM oCRH. Studies were performed in the presence of increasing concentrations of cortisol or dexamethasone, and in the presence or absence of purified ovine CBG [11].

In the basal state there was a dose-dependent inhibition of IR-ACTH output by dexamethasone which was relatively unaffected by the further addition of CBG. Cortisol also exerted negative feedback, although was less potent than dexamethasone. The effect of cortisol was completely attenuated by concurrent addition of CBG. We found similar results with stimulated ACTH output. In these experiments, CBG + CRH produced a modest increase in IR-ACTH output over CRH alone. It is possible that exogenous CBG bound endogenous cortisol in the culture medium, and decreased its feedback effects, although these would have to be exerted at very low levels since cortisol was undetectable ($<$ 50 pg/ml) in the medium by radioimmunoassay. Alternatively, CBG can itself, under some circumstances, bind to pituitary cell membranes and stimulate protein kinase A [21]. Dexamethasone and cortisol both inhibited CRH-stimulated ACTH output. The effect of dexamethasone was not altered in the presence of CBG whereas the dose–response to cortisol was decreased by at least one order of magnitude.

CBG may have similar effects on other tissues. De Los Rios and Hill (1994, unpublished) have shown that basic fibroblast growth factor (bFGF) stimulates proliferation of chondrocytes from fetal growth plates. This effect of bFGF is inhibited by increasing concentrations of cortisol. In turn, the effect of cortisol can be inhibited by addition of CBG.

REGULATION OF CBG PRODUCTION

In 1985 we obtained evidence to suggest that activation of pituitary–adrenal function might lead to the prepartum increase in fetal CBG biosynthesis in the sheep [12]. Subsequently Berdusco *et al.* [13] infused dexamethasone (2 μg/min for 15 min every 2 h) into

chronically catheterized fetal sheep at about day 125 of gestation, and showed that there was a significant increase in plasma CBC by 48 h. The CBC continued to rise throughout 96 h of dexamethasone infusion. The rise in plasma CBC was associated with a significant increase in the levels of CBG mRNA in the fetal liver. Interestingly when dexamethasone was administered to adult sheep, there was a fall in plasma CBC and a decrease in hepatic CBG mRNA levels. Thus, the transcriptional regulation of the CBG gene in the sheep by glucocorticoids may differ between the fetus and the adult.

Infusion of dexamethasone to the fetal sheep also increased the proportion of CBG that was retained on a Concanavalin A-Sepharose column [13]. Before dexamethasone administration, and in fetuses treated with saline, the proportion of CBG that was Concanavalin A (Con A) bound was about 6%. This increased to approx. 15% after dexamethasone administration. These values are of interest in relation to ontogenic changes in CBG glycoforms in the plasma of normal fetuses and newborn lambs. During late fetal life the proportion of CBG with bi-antennary carbohydrate side chains (Con A bound) is about 10%. This begins to increase during the last week of intrauterine life to about 20% at the time of birth, but then rises further to adult values of 70–80% by about 30 days of postnatal age [11]. Thus, most of the CBG present in the fetus lacks biantennary side chains. Moreover, because its molecular size, as assessed by denaturing polyacrylamide gel electrophoresis, is greater than CBG from neonatal or maternal blood, it probably contains more highly processed carbohydrate chains. This change in the glycosylation status of CBG during fetal life may be associated with a glucocorticoid-induced change in hepatic glycosyl transferase activity, and may continue in postnatal life.

These experiments could be criticized in that they used dexamethasone, a synthetic glucocorticoid that does not bind to CBG. To overcome this objection, Berdusco *et al.* [22] infused very small amounts of cortisol into fetal sheep at day 100 of gestation, a time prior to activation of HPA function. They found that amounts of cortisol which elevated the plasma cortisol concentration by only approx. 5 ng/ml, produced a significant increase in plasma CBC, and in hepatic CBG mRNA levels. There was, however, no change in the Con A binding pattern of CBG, suggesting that the glycosyl transferase could not be upregulated by this level of glucocorticoid at this time.

To mimic the prepartum rise in endogenous glucocorticoids, we infused cortisol in an incremental fashion to fetal sheep beginning at about day 130 of gestation and continued the infusion for 10 days [23]. There was a progressive increase in plasma cortisol, and a progressive rise in plasma CBC, similar to that seen in normal animals with the approach of term. In related studies we have found that continuous infusion of cortisol to fetal sheep at this gestational age produced a significant increase in hepatic CBG mRNA within 24 h. There was also a significant rise in the mean concentration of ACTH in the fetal circulation, and in the maximum increment of plasma ACTH achieved in multiple daily samples compared to the pre-infusion control values.

Finally, to provide evidence for the importance of endogenous cortisol, studies were conducted using pairs of fetal lambs in which one of the fetuses had been bilaterally adrenalectomized, with the second fetus remaining intact [23]. These procedures were performed by Dr Abigail Fowden, University of Cambridge, England. Bilateral fetal adrenalectomy was conducted at about day 116–120 of gestation. When the animals were delivered at day 140–144 of gestation, there was a significant increase in the plasma ACTH concentration of the adrenalectomized fetus, as reported previously [24]. The adrenalectomized fetuses did not have the prepartum increase in cortisol, although small amounts of cortisol presumably derived from transplacental transfer from the mother [25] were still present in their circulation. Importantly, the adrenalectomized fetuses, lacking the prepartum rise in cortisol, did not have the elevations of plasma CBC that were found in their intact twins. Thus, these studies provide compelling evidence that abolition of the endogenous prepartum rise in cortisol, also abolishes prepartum changes in plasma CBC, and presumably in hepatic CBG gene expression.

CONCLUSIONS

These studies have provided evidence for a rise in CBG levels in fetal sheep plasma during late gestation. They have shown that CBG is derived primarily from the fetal liver, under the influence of increasing fetal plasma glucocorticoid concentration. In *in vitro* studies we found that CBG could attenuate the negative feedback effect of cortisol on both basal and CRH stimulated ACTH output from fetal pituitary cells. However, the finding that the fetal pituitary itself produces CBG raises the additional exciting possibility that the pituitary may regulate glucocorticoid concentrations in a local manner. We suggest that this effect provides at least one facet of the diminished negative feedback control that allows hypothalamic and pituitary function to continue, and gives rise to the progressive increase in IR-ACTH concentrations of the late gestation fetal lamb.

A crucial question remains, if CBG blocks the effects of cortisol on the fetal pituitary, then why does it not do so at other tissue sites, particularly those that are glucocorticoid-dependent. This objection could be overcome if local pituitary production of CBG were more important than systemic concentrations. Alternatively, many tissues, including decidua, syncytiotrophoblast and spleen, contain CBG receptors [26–28],

that could interact with CBG derived from the circulation in a manner that facilitates glucocorticoid delivery.

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REFERENCES

1. Challis J. R. G. and Brooks A. N.: Maturation and activation of hypothalamic–pituitary–adrenal function in fetal sheep. *Endocrine Rev.* 10 (1989) 182–204.
2. Lü F., Han V. K. M., Milne W. K., Fraser M., Carter A. M., Berdusco E. T. M. and Challis J. R. G.: Regulation of IGF-II gene expression in the ovine fetal adrenal gland by adrenocorticotropic hormone and cortisol. *Endocrinology* **134** (1994) 2628–2635.
3. Matthews S. G. and Challis J. R. G.: Developmental regulation of corticotropin-releasing hormone (CRH) and vasopressin (AVP) messenger ribonucleic acid (mRNA) expression in the developing ovine hypothalamus: effects of stress and glucocorticoids. *Am. J. Physiol.* (1995) In press.
4. Yang K., Challis J. R. G., Han V. K. M. and Hammond G. L.: Proopiomelanocortin messenger RNA levels increase in the fetal sheep pituitary during late gestation. *J. Endocr.* **131** (1991) 483–489.
5. Matthews S. G., Han X., Lü F. and Challis J. R. G.: Developmental changes in the distribution of proopiomelanocortin (POMC) and prolactin (PRL) messenger ribonucleic acid (MRNA) in the pituitary of the ovine fetus and lamb. *J. Molec. Endocr.* **13** (1994) 175–185.
6. Yang K., Jones S. A. and Challis J. R. G.: Changes in glucocorticoid receptor number in the hypothalamus and pituitary of the sheep fetus with gestational age and after ACTH treatment. *Endocrinology* **126** (1990) 11–17.
7. Matthews S. G., Yang K. and Challis J. R. G.: Developmental changes in glucocorticoid receptor (GR) gene expression and immunoreactivity in the ovine fetal pituitary and the effects of exogenous cortisol. *J. Endocr.* **144** (1995) 483–490.
8. Yang K., Matthews S. G. and Challis J. R. G.: Developmental and glucocorticoid regulation of 11β-hydroxysteroid dehydrogenase gene expression in the ovine pituitary. *J. Molec. Endocr.* **14** (1995) 109–1160.
9. Fairclough R. J. and Liggins G. C.: Protein binding of plasma cortisol in the foetal lamb near term. *J. Endocr.* **67** (1975) 333–341.
10. Ballard P. L., Kitterman J. A., Bland R. D., Clyman R. I., Gluckman P. D., Platzker A. C. T., Kaplan S. L. and Grumbach M. M.: Ontogeny and regulation of corticosteroid binding globulin capacity in plasma of fetal and newborn lambs. *Endocrinology* **110** (1982) 359–366.
11. Berdusco E. T. M., Yang K., Hammond G. L. and Challis J. R. G.: Corticosteroid-binding globulin in the late gestation ovine fetus and newborn: biosynthesis and function. *J. Endocr.* (1995) In press.
12. Challis J. R. G., Nancekievill E. A. and Lye S. J.: Possible role of cortisol in the stimulation of cortisol binding capacity (CBC) in the plasma of fetal sheep. *Endocrinology* **116** (1985) 1139–1144.
13. Berdusco E. T. M., Hammond G. L., Jacobs R. A., Grolla A., Akagi K., Langlois D. and Challis J. R. G.: Glucocorticoid-induced increase in plasma corticosteroid-binding globulin levels in fetal sheep in associated with increased biosynthesis and alterations in glycosylation. *Endocrinology* **132** (1993) 2001–2008.
14. Ali S., Bassett J. R., Jones M. R. and Wynn P. C.: The development of corticosteroid binding globulin-like activity in fetal sheep blood. *J. Devl. Physiol.* **18** (1992) 13–18.
15. Seralini G-E., Smith C. L. and Hammond G. L.: Rabbit corticosteroid-binding globulin: primary structure and biosynthesis during pregnancy. *Molec. Endocr.* **4** (1990) 1166–1172.
16. Smith C. L. and Hammond G. L.: Ontogeny of corticosteroid-binding globulin biosynthesis in the rat. *Endocrinology* **128** (1991) 983–988.
17. Scrocchi L. A., Orava M., Smith C. L., Han V. K. M. and Hammond G. L.: Spatial and temporal distribution of corticosteroid-binding globulin and its messenger ribonucleic acid in embryonic and fetal mice. *Endocrinology* **132** (1993) 903–909.
18. Scrocchi L. A., Hearn S. A., Han V. K. M. and Hammond G. L.: Corticosteroid-binding globulin biosynthesis in the mouse liver and kidney during postnatal development. *Endocrinology* **132** (1993) 910–916.
19. Perrot-Applanat O. R. and Milgrom E.: Specific localization of plasma corticosteroid-binding globulin immunoreactivity in pituitary corticotrophs. *Endocrinology* **132** (1984) 559–569.
20. Kuhn R. W., Green A. L., Raymoure W. J. and Siiteri P. D.: Immunocytochemical localization of corticosteroid-binding globulin in rat tissues. *J. Endocr.* **108** (1986) 31–36.
21. Koch B., Lutz-Bucher B., Briaud B. and Mialhe C.: Specific interaction of corticosteroids with binding sites in the plasma membranes of the rat anterior pituitary gland. *J. Endocr.* (1978) 215–222.
22. Berdusco E. T. M., Milne J. K. and Challis J. R. G.: Low-dose cortisol infusion increases plasma corticosteroid-binding globulin (CBG) and hepatic CBG mRNA abundance in fetal sheep at day 100 of gestation. *J. Endocr.* **140** (1994) 425–430.
23. Jeffray T., Berdusco E. T. M., Wallace M., Fowden A. and Challis J. R. G.: Effects of incremental cortisol and adrenalectomy (ADX) on plasma corticosteroid binding capacity (CBC) in fetal sheep. *Soc. Gynec. Invest.* (1995) p. 256. Abstract.
24. Wintour E. M., Coghlan J. P., Hardy K. G., Henessey D. P., Lingwood B. E. and Scoggins B. A.: Adrenal corticosteroids and immunoreactive ACTH in chronically cannulated ovine fetuses with bilateral adrenalectomy. *Acta endocr. (Copenh.)* **95** (1980) 546–552.
25. Hennessy D. P., Coghlan J. P., Hardy K. J., Scoggins B. A. and Wintour E. M.: The origin of cortisol in the blood of fetal sheep. *J. Endocr.* **95** (1982) 71–79.
26. Hryb D. J., Khan M. S., Romas N. A. and Rosner W.: Specific binding of human corticosteroid-binding globulin to cell membranes. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 3253–3256.
27. Avvakumov G. V. and Strel'chyonok O. A.: Evidence for the involvement of the transcortin carbohydrate moiety in the glycoprotein interaction with the plasma membrane of human placental syncytiotrophoblast. *Biochim. Biophys. Acta* **938** (1988) 1–6.
28. Krupenko S. A., Avvakumov G. M. and Strel'chyonok.: A transcortin-binding protein in the plasma membrane of human syncytiotrophoblast. *Biochim. Biophys. Res. Commun.* **177** (1991) 834–839.