J. Steroid Biochem. Molec. Biol. Vol. 42, No. 5, pp. 527–532, 1992
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0960-0760/92 $5.00 + 0.00
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THE TIME-COURSE OF ACTH STIMULATION OF CORTISOL SYNTHESIS BY THE IMMATURE OVINE FOETAL ADRENAL GLAND

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(Received 12 November 1991)

Summary—The aim of this study was to establish the time-course of foetal adrenal gland activation by ACTH at a period of intra-uterine development during which adrenal function is minimal (100–120 days of gestation). Blood samples for cortisol analysis were collected at 6-h intervals during the 24 h ACTH (0.05, 0.5 and 5.0 μg/h) infusion and during the subsequent 24-h period following cessation of the infusion. Plasma cortisol concentrations were measured using a newly developed radioimmunoassay, whose sensitivity was found to be comparable to that of the validated double-isotope dilution derivative method. There was a significant increase in foetal plasma cortisol concentration, from 3.9 ± 1 to 17.8 ± 1.9 nmol/l, within 12 h of commencement of the 2 higher doses of ACTH. Values are mean $\pm$ SEM; $n = 5$. Following termination of the infusion, cortisol levels fell significantly by the first 6 h, returning to basal levels thereafter. An increase in plasma ACTH from 4.6 ± 0.6 to 8.4 ± 1.0 pmol/l was sufficient to initiate a significant increase in cortisol production. The results suggest that the normal low values of cortisol at this period of gestation result from inadequate endogenous ACTH production at this stage.

INTRODUCTION

During one period of intra-uterine development, approx. 90–120 days of gestation (term = 147 ± 5), the concentration of cortisol in ovine foetal blood is < 5 nmol/l [1–3]. This value is indistinguishable from that in bilaterally adrenalectomized foetuses [4] and can be accounted for by the small transplacental passage of maternal cortisol [5]. The capacity of the foetal adrenal, *in vitro*, to synthesize cortisol in response to exogenous ACTH, over a 2–4 h period, is also low at this time [1, 6]. During this period there is very little, if any, expression of the genes encoding for two important enzymes in the biosynthetic pathway of cortisol–cholesterol side-chain cleavage ($P450_{scc}$) and 17α-hydroxylase ($P450_{17\alpha}$) [7]. The infusion of ACTH for 24 h, *in vivo*, can stimulate increased expression of the genes for $P450_{scc}$ and $P450_{17\alpha}$ [8]. If the ACTH infusion is stopped for 24 h prior to the removal of the adrenals, expression of these genes is switched off. However, the time-course of activation and inactivation of adrenal function was not determined. In the present study, we aimed to establish the time-course of this action of exogenous ACTH, by analysis of the changes in plasma cortisol, at 6-h intervals, during the 24 h ACTH infusion and during the subsequent 24-h period when the ACTH infusion was terminated. A second set of experiments were conducted to try to establish the minimum change in plasma ACTH that was necessary to activate the foetal adrenal at this stage. This required the use of fewer animals than would have been required had foetuses been killed for adrenal collection at various times. In order to perform these experiments, a radioimmunoassay for ovine foetal plasma cortisol was developed and validated by comparison with cortisol measurements performed by the specific double-isotope dilution derivative assay [2].

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METHODS

The foetuses of 10 cross-bred Merino ewes with known mating dates, were cannulated between 99 and 103 days of gestation as previously described [9]. The animals were allowed to recover from the stress of surgery for at least 6 days, during which time the well-being of the foetuses was routinely monitored as described in

an earlier paper, with daily blood gas and urine osmolality measurements [8]. Thereafter, experiments were only performed if the urine osmolality was within the normal unstressed range, as previously established [10].

Five foetuses (mean gestational age 107 ± 0.9 days) received a continuous ACTH (Synacthen, Ciba-Geigy, Switzerland) infusion ($n = 3$ at 0.5 μg/h, $n = 2$ at 5 μg/h) into the venous cannula for 24 h. Five foetuses (mean gestational age 109 ± 0.2 days) received a lower dose (0.05 μg/h). Immediately prior to the start of the ACTH infusion, 3 ml foetal blood was taken for cortisol analysis via radioimmunoassay, and 1 ml for ACTH assay. Thereafter, foetal blood samples for cortisol analysis were collected every 6 h for the duration of the infusion. In all but 2 foetuses, sampling continued for 24 h following cessation of the ACTH infusion. Blood samples were also collected from the ewes, via an indwelling jugular vein catheter, contemporaneously with the foetal samples, for cortisol assay, in the latter protocol.

Blood samples were spun immediately and the plasma stored at $-20°$ until assayed.

Blood gases and haemoglobin were measured on a Ciba-Corning 278 Blood Gas System and a Corning 2500 Co-oximeter (Australian Diagnostics Corp., Melbourne). Urine osmolality was measured on an Advanced Osmometer (Advanced Instruments, MA, U.S.A.).

Plasma immunoreactive ACTH was measured as described previously [4, 11] using the Sorin Biomedica RIA kit (Oris Industrie, St Quentin-Yvelines, France). Intra-assay coefficients of variation were 9.1 and 18% at high and low values, respectively. Foetal plasma, with high immunoreactive values of ACTH, diluted linearly with plasma from dexamethasone suppressed sheep. The limit of sensitivity in these assays was 2 pmol/l.

Radioimmunoassay of cortisol

Quantitation of cortisol in foetal blood was determined by a recently developed radioimmunoassay. To 2 ml of the plasma, [^{3}H]cortisol (approx. 1400 cpm) is added for the internal recovery marker. 1 ml is then extracted with 7 ml dichloromethane (Ajax Chemicals, Aubur, NSW, Australia), centrifuged at 3000 rpm for 5 min and the aqueous phase discarded. After drying in a water bath at 37°C, 1 ml sample diluent is added, vortexed, and allowed to stand at room temperature for 1 h. Sample diluent contains, in 1 l water, 16.84 g $Na_2HPO_4 \cdot 7H_2O$, 4.74 g $Na_2EDTA \cdot 2H_2O$, and 2.0 ml 2% NaN_3. To 100 ml of this is added a freshly prepared mixture of 0.5 g bovine gamma globulin, and 0.1 g ANS (8-anilino-1-6880-napthalene sulphonic acid, Kodak, Eastman). An aliquot of the assay sample and one of the unextracted plasma are taken for counting on a liquid scintillation counter for recovery measurements.

From the assay sample 2 × 150, 30 and 10 μl aliquots were assayed. The antibody (kindly provided by Dr P. Vecsei, Pharmakologisches Inst. der Universitat, Heidelberg, Germany) is a polyclonal antibody, raised in rabbits and used at a dilution of $1/3.6 \times 10^{-6}$. It cross-reacts 0.68% with cortisone, 1.73% with corticosterone, 30% with prednisolone, 0.21% with dexamethasone, 7.43% with 11-desoxycortisol, 0.009% with progesterone and $<0.001\%$ with androstenedione, androsterone, oestriol, oestradiol, oestrone and pregnenolone.

The [^{125}I]-cortisol tracer (2000 Ci/mmol) was from Amersham (Bucks., England), used at approx. 3000 cpm/50 μl. The samples are incubated overnight at room temperature, and separated using 1 ml 20% PEG (polyethylene glycol, Kochlight Ltd, Haverhill, Sulfolk, England). The cortisol standard was supplied by Steraloids Inc. (NH, U.S.A.).

The standard curve range is 3.9 to 1000 pg, and with a 2 ml sample 0.2–139 nmol/l can be measured accurately. 50% displacement of bound radiolabel was at 60.7 pg/tube.

The intra-assay coefficient of variation was 10.3% at 50 pg/tube. The inter-assay variation for the controls was 13.1% at 30 pg/tube ($n = 20$) and 10.7% at a value of 200 pg/tube ($n = 19$).

The double isotope dilution derivative assay has already been described and validated for use with foetal sheep blood [2].

Sixteen samples of foetal, lamb and adult blood taken over a period of months, were analysed for cortisol concurrently, using the double isotope dilution derivative assay and the radioimmunoassay. The line of symmetry, describing the relationship between the two assays over a wide range of blood cortisol concentrations, is presented in Fig. 1. The correlation coefficient was $r = 0.949$.

Statistics and calculations

The results for the values of blood gases, haemoglobin, haematocrit and foetal urine

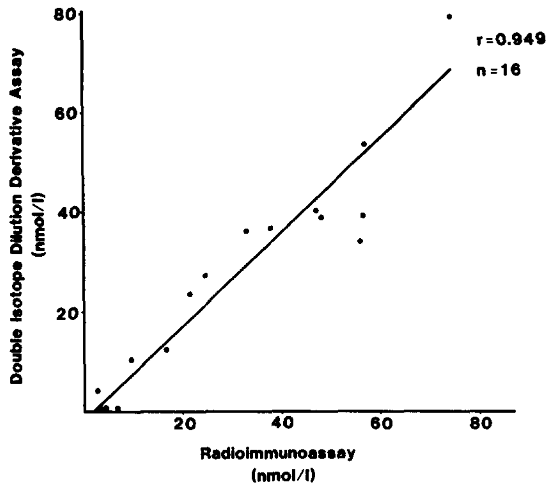

Fig. 1. The calculated line of symmetry indicating the relationship between cortisol concentrations measured using the double-isotope dilution derivative assay and the radioimmunoassay. Blood samples were collected from foetuses, lambs and adult sheep.

osmolality in each of the two groups, before and after ACTH infusion, were analysed by paired student's *t*-test. In group 1 cortisol values at each time point were tested against initial values by the Dunnett test.

In group 2, all foetal cortisol values < 10 nmol/l, and the concurrent foetal plasma ACTH and maternal cortisol values were averaged for each foetus. The same procedure was applied to all foetal cortisol values > 10 nmol/l, with their concurrent maternal cortisol values. The differences in foetal plasma cortisol and ACTH in the two sub-groups were analysed by unpaired student's *t*-test.

The mean values in maternal plasma cortisol concentrations were used to calculate a foetal plasma cortisol concentration which might have arisen by placental transfer of cortisol from ewe to foetus, using the figures obtained previously by Hennessy *et al.* [5].

$$\text{Calculated foetal cortisol derived from mother} = \frac{i_m \times BCR_m \times 1.4}{BCR_f \times 100}$$

where i_m = measured plasma cortisol in mother; BCR_m = blood clearance rate in mother (77 l/h); BCR_f = blood clearance rate in foetus (8.2 l/h); and 1.4% = mean % of maternal production of cortisol transferred to foetus.

RESULTS

There was no significant difference between the values for blood gases and urine osmolality prior to, and at the end of the ACTH infusions (student's paired *t*-test, $P > 0.1$). All foetuses were unstressed with values in the normal range as shown in Table 1.

In the foetuses receiving either 0.5 or 2.5 μg ACTH per h, the cortisol responses were identical and have been grouped together. The plasma IR-ACTH values for the first 12 h of infusion were 51.8 ± 4.9 pmol/l ($n = 3$) in those receiving the 0.5 μg/h dose and > 267 pmol/l in the two receiving the highest dose.

Foetal blood cortisol concentrations in the presence and absence of ACTH stimulation are presented in Table 2. Foetal blood cortisol concentration had increased significantly by 12 h after commencement of ACTH infusion. Thereafter, cortisol levels continued to rise, until the infusion was terminated. Following cessation of the ACTH infusion, cortisol levels in foetal blood declined by 6 h, and returned to basal levels by 12 h. In one animal which became stressed (foetal urine osmolality = 365 mosmol/kg H_2O) and died within 48 h after termination of the infusion, the blood cortisol concentration had increased markedly at the final sampling, to 31.9 nmol/l. The endogenous ACTH was 129 pmol/l in this sample, compared with a value of < 4 pmol/l in the previous sample, 18 h after cessation of the ACTH infusion.

In the five foetuses given the very lowest dose (0.05 μg/h) the increase in plasma ACTH values was more variable. The cortisol results were divided into two groups, those < 10 nmol/l, and those > 10 nmol/l; the concurrent ACTH values

Table 1. Blood gases, haemoglobin (Hb), haematocrit (PCV), and foetal urine osmolality (FUO) in two groups of immature ovine foetuses, before (i) and after (ii) infusion of ACTH for 24 h at 0.5–5.0 μg/h (group 1) or 0.05 μg/h (group 2)

	pH	pCO_2 (mmHg)	pO_2 (mmHg)	Hb (g/dl)	PCV (%)	FUO (mOsm/kg H_2O)
Group 1 (i)	7.483 ± 0.01	39.0 ± 1.0	23.5 ± 0.8	7.8 ± 0.2	27.0 ± 1.0	146 ± 17
(ii)	7.456 ± 0.01	37.8 ± 1.7	23.7 ± 1.0	7.9 ± 0.4	27.0 ± 1.0	179 ± 18
Group 2 (i)	7.460 ± 0.01	42.6 ± 1.6	23.6 ± 1.0	7.3 ± 0.5	25.0 ± 1.5	150 ± 17
(ii)	7.460 ± 0.01	40.4 ± 1.7	23.6 ± 1.0	6.7 ± 0.6	24.0 ± 1.6	140 ± 17

Values are mean ± SEM; $n = 5$ in each group.

Table 2. Effect of infusion of ACTH (0.5–5.0 μg/h) on plasma cortisol concentrations in 5 ovine foetuses (mean gestational age 107 ± 0.9 days)

Sampling time	Plasma cortisol concentration (nmol/l)
Basal	3.9 ± 1.0
6 h ACTH	7.0 ± 1.3
12 h ACTH	17.8 ± 1.9*
18 h ACTH	31.6 ± 3.6**
24 h ACTH	48.5 ± 6.0**
6 h post-ACTH	11.3 ± 5.2
12 h post-ACTH	2.7 ± 0.2
18 h post-ACTH	2.5 ± 0.1
24 h post-ACTH	2.3, 5.4, 31.9

Values are mean ± SEM; $n = 5$ basal and ACTH, $n = 3$ post-ACTH. *$P < 0.05$; **$P < 0.001$.

for the cortisol values in each group, are shown in Table 3.

There was a statistically significant difference between the cortisol and ACTH values in the two groups (Student's t-test; $P < 0.05$).

In this table is also shown an approximation of the plasma cortisol concentration in the foetus due to cortisol crossing the placenta from the ewe. It is evident that whereas maternally-derived cortisol may make a significant contribution to the foetal plasma cortisol concentration in the foetuses without ACTH infusion, the increase seen in the infused foetuses was most likely of foetal adrenal origin.

DISCUSSION

The double-isotope dilution derivative assay [2] has the required specificity, accuracy and sensitivity for the measurement of cortisol in ovine foetal blood, in the period 100–120 days of gestation, when the values are <5 nmol/l (1.8 ng/ml). However, it requires a sample volume of 10 ml. Thus repetitive sampling, over 24–48 h is impractical. The radioimmunoassay described in detail in this paper has been rigorously validated by comparison with the DIDDA, and gives answers in the same range as those previously published [2–4]. It requires an extraction step, as found in other cortisol radioimmunoassays [12, 13] and is the most sensitive radioimmunoassay yet described for use with ovine foetal plasma. By using this radioimmunoassay it has been possible to explore the time-course and ACTH sensitivity of activation of foetal adrenal cortisol production using many fewer animals than would have been required if animals had had to be sacrificed at given time points for measurement of messenger RNA concentration.

Table 3. Foetal cortisol and corresponding ACTH and maternal cortisol values grouped according to whether foetal cortisol values were (A) <10 nmol/l or (B) >10 nmol/l, in 5 immature ovine foetuses treated with a 24 h infusion of ACTH (0.05 μg/h)

	Foetal (measured)		Maternal	Foetal (calculated)
	ACTH (pmol/l)	Cortisol (nmol/l)	cortisol (nmol/l)	cortisol (nmol/l)
A.	4.6 ± 0.6	3.9 ± 0.2	12.7 ± 1.4	1.7 ± 0.2
B.	8.4 ± 0.9*	17.4 ± 3.7*	17.4 ± 3.7	2.8 ± 0.7

Values are mean ± SEM, $n = 5$ fetuses. *$P < 0.05$.

The period between 100 and 120 days of gestation in the ovine foetus is a period during which the adrenal gland continues to grow, although the rates of steroidogenic cell multiplication and hypertrophy are lower at this stage than after 130 days [14]. The volume of smooth endoplasmic reticulum in each cell is also minimal at 100 days of gestation [15]. Infusion of large doses of ACTH (5 IU/h, for 90 min) did not significantly increase plasma cortisol concentrations [2]. Basal and stimulated cortisol outputs of adrenocortical cells, *in vitro*, are lower than those of tissue from either earlier or later in gestation [1, 6, 16].

During this period the amounts of mRNA coding for the steroidogenic enzymes, side-chain cleavage ($P450_{scc}$) and 17α-hydroxylase ($P450_{17\alpha}$) are much lower than either earlier or later in gestation [7]. Although the biosynthetic step catalysed by $P450_{scc}$, the conversion of cholesterol to pregnenolone, is regarded as the principal rate limiting step in cortisol biosynthesis in the adult adrenal [17], the provision of cholesterol to the mitochondrial enzyme $P450_{scc}$, rather than the amount of activity of $P450_{scc}$, is the step which responds rapidly (within 5 min) when ACTH is added to cells *in vitro* [18]. However, 24 h after hypophysectomy in the adult rat, plasma corticosterone is immeasurable and the mRNA for $P450_{scc}$ is decreased to 20% of control values [19]. Within 3 h of treating hypophysectomized rats with i.m. ACTH (5 IU/100 g body weight) the relative concentration of mRNA for $P450_{scc}$ had doubled, which was sufficient to allow plasma corticosterone values to increase back to pre-hypophysectomy values [19].

It was shown previously [8] that when ovine foetuses were infused with relatively low doses of ACTH (0.5 μg/h), *in vivo*, for 24 h, there was a marked increase in the amount of mRNA for both $P450_{scc}$ and $P450_{17\alpha}$. The present study indicates that by 12 h of ACTH infusion at this rate, the relatively quiescent ovine foetal adrenal of 100–120 days of gestation, can acquire the

capacity to increase plasma cortisol concentrations significantly, implying that sufficient quantities of both enzymes are present. The fact that a 10-fold higher dose does not increase foetal plasma cortisol concentrations any earlier suggests that the concentration of ACTH achieved at the lower dose (52 pmol/l) was not the limiting factor. The long time period required to increase plasma cortisol values in the foetus at this age contrasts with the short time period (3–10 min) required in the adult sheep, *in vivo* [20]. However, the *in vitro* studies with bovine or human foetal adrenal cells, a period of 24 h was necessary to increase mRNAs of either or both $P450_{scc}$ and $P450_{17\alpha}$ [21, 22]. Within 12 h of cessation of exogenous ACTH, the plasma cortisol values were back down to <3 nmol/l, reflecting, no doubt, the decrease in plasma ACTH values to those insufficient to stimulate cortisol synthesis.

During the period 90–120 days of gestation the endogenous plasma ACTH values, in chronically cannulated unstressed foetal sheep have been shown to be about 4 pmol/l, or 0.5×10^{-11} M [3, 23, 24]. Similar values were recorded in the current study. The fact that a relatively small increase in plasma ACTH to 8 pmol/l, on average, if present for an adequate time, was sufficient to activate the foetal adrenal lends weight to the previous hypothesis [8] that the adrenal normally lacks sufficient endogenous stimulation at this time period in gestation. When small increments in endogenous plasma ACTH begin to occur, after 130 days of gestation, the maturation and growth of the foetal adrenal accelerate. The most pressing question now raised is why the endogenous ACTH is so low at this particular time period in gestation.

Acknowledgements—This work was supported by grants to the Howard Florey Institute of Experimental Physiology and Medicine from the National Health and Medical Research Council of Australia. The authors would like to thank Dr Richard MacIsaac and Karen Moritz for help with the 24 h sampling.

REFERENCES

1. Wintour E. M., Brown E. H., Denton D. A., Hardy K. J., McDougall J. G., Oddie C. J. and Whipp G. T.: The ontogeny and regulation of corticosteroid secretion by the ovine foetal adrenal. *Acta Endocr.* (*Copenh.*) **79** (1975) 301–316.
2. Brown E. H., Coghlan J. P., Hardy K. J. and Wintour E. M.: Aldosterone, corticosterone, cortisol, 11-deoxycortisol and 11-deoxycorticosterone concentration in the blood of chronically cannulated ovine foetuses: effect of ACTH. *Acta Endocr.* (*Copenh.*) **88** (1978) 364–374.
3. Hennessy D. P., Coghlan J. P., Hardy K. J. and Wintour E. M.: Development of the pituitary–adrenal axis in chronically cannulated ovine fetuses. *J. Dev. Physiol.* **4** (1982) 339–352.
4. Wintour E. M., Coghlan J. P., Hardy K. H., Hennessy D. P., Lingwood B. E. and Scoggins B. A.: Adrenal corticosteroids and immunoreactive ACTH in chronically cannulated ovine foetuses with bilateral adrenalectomy. *Acta Endocr.* **95** (1980) 546–552.
5. 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.
6. Glickman J. A. and Challis J. R. G.: The changing response pattern of sheep fetal adrenal cells throughout the course of gestation. *Endocrinology* **106** (1980) 1371–1376.
7. Tangalakis K., Coghlan J. P., Connell J., Crawford R., Darling P., Hammond V., Penschow J. and Wintour E. M.: Tissue distribution and levels of gene expression of three steroid hydroxylases in ovine fetal adrenal glands. *Acta Endocr.* (*Copenh.*) **120** (1989) 225–232.
8. Tangalakis K., Coghlan J. P., Crawford R., Hammond V. E. and Wintour E. M.: Steroid hydroxylase gene expression in the ovine fetal adrenal gland following ACTH infusion. *Acta Endocr.* (*Copenh.*) **123** (1990) 371–377.
9. Lingwood B. E., Hardy K. J., Horacek I., Scoggins B. A. and Wintour E. M.: The effects of antidiuretic hormone on urine flow and composition in the chronically cannulated ovine fetus. *Q. J. Exp. Physiol.* **63** (1978) 315–330.
10. Wintour E. M., Bell R. J., Congiu M., MacIsaac R. J. and Wang X.: The value of urine osmolality as an index of stress in the ovine fetus. *J. Dev. Physiol.* **7** (1985) 347–354.
11. MacIsaac R. J., Carson R. S., Horvath A. P. and Wintour E. M.: Premature birth of live lambs induced by pulsatile infusion of ACTH. *J. Endocr.* **124** (1990) 99–107.
12. Magyar D. M., Buster J. E., Elsner C. W., Nathanielsz P. W., Oliveros R. and Abraham G. E.: Validation of a specific radioimmunoassay to measure plasma cortisol in the fetal sheep. *Steroids* **33** (1979) 45–53.
13. Magyar D. M., Elsner C. W., Nathanielsz P. W., Lowe K. C. and Buster J. E.: Comparison of two rapid cortisol radioimmunoassays for use in fetal sheep. *Steroids* **34** (1979) 111–119.
14. Boshier D. P. and Holloway H.: Morphometric analyses of adrenal gland growth in fetal and neonatal sheep. I. The adrenal cortex. *J. Anat.* **167** (1989) 1–14.
15. Boshier D. P. and Holloway H.: Morphometric analyses of adrenal gland growth in fetal and neonatal sheep. III. Volumes of the major organelles within the zona fasciculata steroidogenic cells. *J. Anat.* (1991). In press.
16. Manchester E. L. and Challis J. R. G.: The effects of adrenocorticotropin, guanylylimidodiphosphate, dibutyryl adenosine 3′5′-monophosphate, and exogenous substrates on corticosteroid output by ovine fetal adrenal cells at different times in pregnancy. *Endocrinology* **111** (1982) 889–895.
17. Stone D. and Hechter O.: Studies on ACTH action in perfused bovine adrenals: the site of action of ACTH in corticosteroidogenesis. *Archs Biochem. Biophys.* **51** (1954) 457–469.
18. Jeffcoate C. R., DiBartolomeis M. J., William C. A. and Mcnamara B. C.: ACTH regulation of cholesterol movement in isolated adrenal cells. *J. Steroid Biochem.* **27** (1987) 724–729.
19. Imai T., Seo H., Murata Y., Ohno M., Satoh Y., Funahashi H., Takagi H. and Matsui N.: Alteration in

the expression of genes for cholesterol side-chain cleavage enzyme and 21-hydroxylase by hypophysectomy and ACTH administration in the rat adrenal. *J. Molec. Endocr.* **4** (1990) 239–245.
20. Blair-West J. R., Coghlan J. P., Denton D. A., Scoggins B. A., Wintour E. M. and Wright R. D.: The onset of effect of ACTH, angiotensin II and raised plasma potassium concentration on the adrenal cortex. *Steroids* **15** (1970) 433–448.
21. Lund J., Faucher D. J., Ford S. P., Porter J. C., Waterman M. R. and Mason J. I.: Developmental expression of bovine adrenocortical steroid hydroxylases. Regulation of $P\text{-}450_{17\alpha}$ expression leads to episodic fetal cortisol production. *J. Biol. Chem.* **263** (1988) 16,195–16,201.
22. Di Blasio A. M., Voutilainen R., Jaffe R. B. and Miller W. M.: Hormonal regulation of messenger ribonucleic acids for $P450_{scc}$ (Cholesterol side-chain cleavage enzyme) and $P450_{c17}$ (17α-hydroxylase/17,20-lyase) in cultured human fetal adrenal cells. *J. Clin. Endocr. Metab.* **65** (1987) 170–175.
23. MacIsaac R. J., Bell R. J., McDougall J. G., Tregear G. W., Wang X. and Wintour E. M.: Development of the hypothalamic–pituitary-axis in the ovine fetus: ontogeny of action of ovine corticotropin-releasing factor. *J. Dev. Physiol.* **7** (1985) 329–338.
24. Norman L. J., Lye S. J., Wlodek M. E. and Challis J. R. G.: Changes in pituitary responses to synthetic ovine corticotrophin releasing factor in fetal sheep. *Can. J. Physiol. Pharmac.* **63** (1985) 1398–1403.