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FETAL-MATERNAL ADRENOCORTICAL ACTIVITIES APPROACHING PARTURITION IN MICE

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The temporal profile of fetal-maternal adrenocortical activities, including blood corticosterone concentration and adrenal expression of cytochrome P45011ß, approaching parturition was investigated in mice. The fetal serum corticosterone concentration reached a peak on gestational day (G) 17 and maternal serum corticosterone reached a highest value on G15 and then subsequently decreased. Fetal and maternal adrenal expression of cytochrome P45011ß increased with advancing pregnancy before the blood corticosterone concentrations reached each peak but then did not change substantially until the term. The results suggest that the increases of blood fetal-maternal corticosterone concentrations before peak are contributed by the increased adrenocortical activities, whereas the subsequent drops after peaks are not adrenocortical dependent. Other possible factors responsible for the drop such as placental catabolism, tissue utilization and liver metabolism are discussed.

Key words: corticosterone, cytochrome P45011ß, adrenal cortex, maternal fetoplacental unit, mouse

INTRODUCTION

Fetal adrenal glucocorticoids function as an initiating signal in the cascade of parturition in primates, sheep and goats (1-5). Nevertheless, in rats and mice, whether or not the fetal adrenocortical products play the same role remains unclear. To answer this question, knowledge of fetal adrenocortical activities during this period is essential. Unlike the findings in primates, sheep and goats, in which fetal cortisol levels keep increasing before term and fall after labor (1-5), many studies demonstrated that corticosterone concentrations in rat fetuses reach a highest value on gestational day (G) 19, but fall precipitously G20 afterward until term (6-9). In mice, however, fundamental data are almost lacking on changes of fetal corticosteronc concentrations during the pregnancy due to the difficulties of collecting blood. Dalle et al. reported fetal plasma corticosterone changes during the last three days of gestation in Swiss CD1 strain mice, determined with the classical protein-binding method (10). Therefore, it is apparently necessary to confirm this only result in mice using a more advanced method. Our previous study suggsted a substantially lower corticosterone level on G18 than that on G16 (11, and unpublished data) in mouse fetuses.

Thus, purposes of this study were to systematically characterize in mice the temporal profile of corticosterone concentration in the fetal blood near term and analyze the possible factors which contribute to the changes of fetal corticosterone concentration. We therefore examined the blood corticosterone level in dams and changes of corticosterone-synthesizing enzyme, cytochrome P45011ß (P45011ß), in both fetal and maternal adrenals.

Mice have become an important experimental animal species for molecular genetic studies on mammalian development and reproduction because of the feasibility of conducting genetic manipulations as well as the established genetic and molecular basis in this species (12). Therefore, we attempted not only to supplement mouse data to the previous studies in primates, sheep and rats, but also to provide the basic information for our further molecular and genetic studies on roles of fetal hypothalamic-pituitary-adrenal axis on mammalian pregnancy and parturition.

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MATERIALS AND METHODS

Animals

Jcl:ICR mice (CLEA Japan, Tokyo) were used. All animals were handled according to the guidelines of the Institute of Experimental Animals, Shimane Medical University. The presence of a vaginal plug on the morning after mating indicated G0. The gestation period was 19-20 days.

Bleeding and RIA for corticosterone

All of samples were taken at 14:00h. Dams (4-6 for each stage) from G14 to G18 were anesthetized with ether and their trunk blood was collected as serum for corticosterone assay. Embryos from G16 to G18 were bleeded by cervical veins and/or heart puncture with a micropipet as previously described (11). To obtain enough sample for the assay and to minimize the artificial error and variation, it was necessary to pool the blood from several embryos (8-11 for an assay sample; 4 assays for each gstational stage) of several different litters. Serum corticosterone was assayed by RIA as described previously (11). Data were analyzed with the Mann-Whitney U test. Values are shown as the mean±SEM.

Immunohistochemical detection of adrenal P45011ß

Expression of P45011ß in both fetal and maternal adrenals was detected with the improved method of immunogold-silver staining using a specific antibody as previously described (11). Briefly, the part of embryos containing the adrenal was excised and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight. Following being processed and embedded in paraffin, the specimens were serially cut into 5 mm-thick sections. The sections containing the central medullary tissue were selected and immunocytochemicall stained with the improved method of immuogold-silver staining (13) using the primary antibody for P45011ß (rabbit anti-rat P45011ß; provided by Drs. Yuzuru Ishimura and Fumiko Mitani, Department of Biochemistry, Keio University School of Medicine), the secondary antibody conjudated with gold (Amersham International, Aylesbury, UK) and silver enhancing kit (British BioCell International, Cardiff, UK).

RESULTS

Fetal corticosterone concentrations

The pooled serum corticosterone concentration in mouse embryos increascd from 382.00±17.58 ng/ml on G16, reached a peak of 625.33 ± 23.86 on G17 ($p<0.01$ for G17 vs. G16) and abruptly fell to 134.00 ± 5.29 on G18 ($p<0.01$ for G18 vs. G17) (Fig. A).

Maternal corticosterone concentrations

The maternal corticosterone levels in dams increased from 320.50 ± 34.35 ng/ml on G14, reached a highest value of 664.48 ± 16.54 ng/ml on G15 ($p <0.05$ for G15vs. G14) and gradually decreased to 438.06 ± 40.85 ng/ml on G18 ($p <0.05$ for G18 vs. G15) (Fig. B).

Fetal and maternal adrenal P45011ß expression

In the mouse fetal adrenal, the cortex and its outer and inner zones could be distinctively observed under a light microscope from G15 (11). On G14, a limited number of P45011ß-positivc cells could be first detected and were present throughout the gland, although the cortical and medullary cells were not yet clearly separated (data not shown). From G15, the staining became gradually apparent (data not shown) and the positive staining area corresponded to the inner zone (11) of the fetal adrenocortex (Fig. A). It was reproducible that the number of P45011ß-positive cells markedly increased from G16 to G17 (Fig. A) and that it did not show an apparent change from G17 to G18 (Fig. A).

In mothers, P45011ß-positive cells were present in the zona fasciculata and reticularis of the adrenal (Fig. B). We reproducibly found that both the number of positive cells and the staining intensity in the maternal adrenal increased from G14 to G15 (Fig. B) and maintained basically unchanged until parturition (Fig. B).

DISCUSSION

In the present study, the serum corticosterone concentration in fetal ICR mice reached a peak on G17 and then abruptly fell until term, as is similar to the changes in fetal rats (6-9). Our results showed the

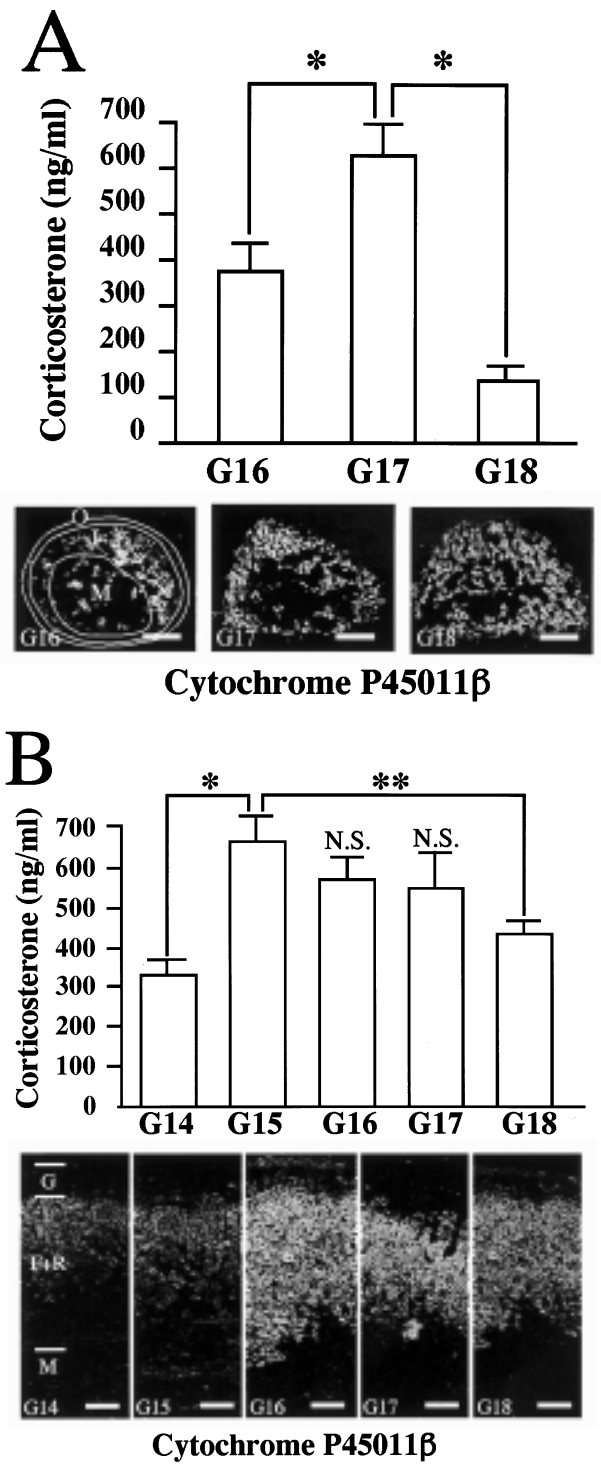

Fig. The serum corticosterone levels by RIA and the adrenal expression of P45011ß by immunohistochemistry in mouse fetuses and mothers. (A) Fetal serum corticosterone levels (upper panel) and representative photos from multiple observations with similar results illustrating the P45011ß expression in the fetal adrenal (lower panel). (B) Maternal serum corticosterone levels (upper panel) and representative photos from multiple observations with similar results illustrating the P45011ß expression in the maternal adrenal (lower panel). *P <0. 01; **P <0. 05; N.S.: not significantly different as compared to G15. O: outer zone; I: inner zone; M: medulla; G: glomerulosa; F: fasciculata; R: reticularis. Bars= 100 μm (A) and 200 μm (B).

same tendency of blood corticosterone changes as that in Swiss CDI strain mice by Dalle et al. (10), which was determined by a protein-binding method. However, the peak in our results is one day earlier than that of Dalle et al. (10) (adjusted to their count of gestational day). Dupouy et al. (6) found that circulating corticosterone in rat dams reaches a peak earlier than that in rat fetuses without subsequent falls prior to the term. Our results in mice also showed the peak one day earlier in dams than that in fetuses, but followed by a progressively decrease until the parturition. We found the same peak for blood corticosterone level in ICR mouse mothers as that in ICI mice by Barlow et al. (14), but the subsequent drop was not as precipitous as theirs (14). In addition, we found that a mother to fetus ratio for serum corticosterone of ICR mice was 1.5 on G16, 0.9 on G17 and 3.3 on G18, respectively. This ratio is very similar to that of rats (15), but differs from that of Swiss CD1 mice (10) which remained stable at about 5 during the last three days of gestation (10). Above disparities in mice between ours and others (10, 14) may be due to the differences of mouse strains and/or experimental systems, such as different times of blood collection : 14:00 in this study vs. 10:00 in Dalle et al.(10).

The increase of fetal-maternal adrenal P45011ß expression matched the change of the circulating corticosterone before the peaks and can account for the corresponding increases of the circulating corticosterone. However, the subsequent drops of corticosterone concentrations in fetuses and mothers were unlikely adrenocortex-dependent, since the corresponding adrenal P45011ß expression remained stable in spite of the drop of corticosterone levels after the peak. To interpret the fall of blood corticosterone level, extra-adrenal factors should be considered.

Because the placenta is permeable to corticosterone and this transplacental passage is mainly from maternal side to fetal one (16), the change of the fetal corticosterone level at least partially results from that of maternal corticosterone via the placenta. Further, as an important component of maternal-fetoplacental unit, the placenta is not simply permeable to corticosteroids, but, by metabolism (reduction or oxidation), regulates the qualitative and quantitative pattern of these corticosteroids arriving at the fetus (17-19). It has been believed that a major role of the placenta in

primates is to protect the fetus from the relatively high concentrations of cortisol in the maternal circulation by converting biologically active cortisol to inactive cortisone (17-19). Therefore, the placenta by its permeability and metabolism may play a regulatory role in the concentration in fetuses during the late pregnancy.

On the other hand, the stage approaching parturition is critical for both the fetal and maternal compartments, and therefore the tissue utilization and liver metabolism of glucocorticoids in fetuses and mothers increase during this stage (2, 20).

Any one or a combination of these factors may lead to the decrease of blood corticosterone concentrations in fetuses and mothers. In fetuses, the precipitous drop in the serum corticosterone level on G18, compared to the relatively mild drop in mothers, may be partly attributed to the abrupt increase of the tissue utilization due to the rapid growth of the body and functional maturation of the tissues in addition to the placental factor. However, to date, there is no direct evidence on placental catabolism, tissue utilization and liver metabolism of glucocorticoids in mice. Therefore, these possible factors responsible for the changes in corticosterone level in mice obviously warrant further study.

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REFERENCES

1) Lanman JT (1977) Parturition in nonhuman primates. *Biol Reprod* 16: 28-38.
2) Pepe GJ and Albrecht ED (1995) Actions of placental and fetal adrenal steroid hormones in primate pregnaney. *Endocrine Rev* 16: 608-648.
3) Bassett JM and Thorburn GD (1969) Foetal plasm corticosteroids and the initiation of parturition in sheep. *J Endocrinol* 44: 285-286.
4) Murphy BEP (1982) Human fetal serum cortisol levels related to gestational rise, independent of sex or model of delivery. *Am J Obste Gynecol* 144: 276-282.
5) Thorburn GD (1978) Hormonal control of parturition in the sheep and goat. *Sem Perinatol* 2: 235-245.
6) Dupouy JP, Coffigny H and Magre S (1974) Maternal and foetal corticosterone levels during late pregnancy in rats. *J Endocrinol* 65: 347-352.
7) Dupouy JP and Dubois MP (1975) Ontogenesis of the α-MSH, ß-MSH and ACTH cells in the foetal hypophysis of the rat. Correlation with the growth of the adrenals and adrenocirtical activity. *Cell Tiss Res* 161: 373-384.
8) Cohen A (1973) Plasma corticosterone concentration in foetal rats. *Horm Metab Res* 5: 66.
9) Holt PG and Pliver IT (1968) Plasm corticosterone concentrations in the perinatal rat. *Biochem J* 108:339-341.
10) Dalle M, Giry J, Gay M and Delost P (1978) Perinatal changes in plasma and adrenal corticosterone and aldosterone concentrations in the mouse. *J Endoerinol* 76: 303-309.
11) Zhang H, Hatta T, Udagawa J, Moriyama R, Hashimoto R and Otani H (1998) Induction of ectopic corticotropic tumor in mouse embryos by exo utero cell transplantation and its effects on the fetal adrenal gland. *Endocrinology* 139: 3306-3315.
12) Marth JD (1996) Molecular medicine in genetically engineered animals. *J Clin Invest* 97: 1999-2002.
13) Holgate GS, Jackson P, Cowen PN and Bird CC (1983) New method of immunostaining with enhanced sensitivity. *J Histochem Cytochem* 31: 983-944.
14) Barlow SM, Morrison PJ and Sullivan FM (1974) Plasma corticosterone levels during pregnancy in the mouse: the relative contributions of the adrenal glands and foetal-placental units. *J Endocrinol* 60: 473-483.
15) Martin CE, Cake MH, Hartmann PE and Cook IF (1977) Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. *Acta Endocrinologica* 84: 167-176.
16) Arishima K, Nakama, S Morikawa, Hashimoto Y and Eguchi Y (1977) Maternal-foetal interrelations of plasma corticosterone concentrations at the end of gestation in the rat. *J Endocrinol*

72: 239-240.

17) Pepe GJ and Albrecht ED (1984) Transuteroplacental metabolism of cortisol and cortisone during mid-and late gestation in the baboon. *Endocrinology* 115: 1946-1951.

18) Murphy BEP, Clark SJ, Donald IR, Pinsky M and Vedady D (1974) Conversion of maternal cortisol to cortisone during placental transfer to the human fetus. *Am J Obstet Gynecol* 118: 538-541.

19) Mitchell BF, Seron-Ferre M and Jaffe RB (1982) Cortisol-cortisone interrelationship in the late gestation rhesus monkey fetus in utero. *Endocrinology* 111: 1837-1842.

20) Griffing GT, Melby JG (1994) The Maternal Adrenal Cortex. In: Maternal-Fetal Endocrinology, 2nd ed.(Tulchinsky D and Little AB eds) pp.155-159, Saunders, Philadelphia.