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Fluctuation of fetal rat hepatic histidine decarboxylase activity through the glucocorticoid-ACTH system

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In 1960, Kahlson and coworkers [1] reported that large amounts of histamine are produced and released into the general circulation by the action of hepatic L-histidine decarboxylase (HDC) in fetal rat near the approach to parturition. In an attempt to determine the physiological action of histamine, Taguchi *et al.* first purified and characterized fetal rat hepatic HDC [2], and very recently Joseph *et al.* followed the characterization and expression of cDNA for this enzyme [3]. However, the mechanism regulating HDC synthesis, as well as HDC fluctuation, in fetal rat liver is unknown. We previously reported that glucocorticoids stimulate a *de novo* synthesis of HDC from mastocytoma P-815 cells [4] and glandular stomachs of rats [5]. Furthermore, Liggins *et al.* [6] reported that the fluctuation of the cortisol concentration in fetal lamb plasma is ACTH dependent in late pregnancy. These results encouraged us to determine whether the observed fluctuation of fetal rat HDC was associated with a change

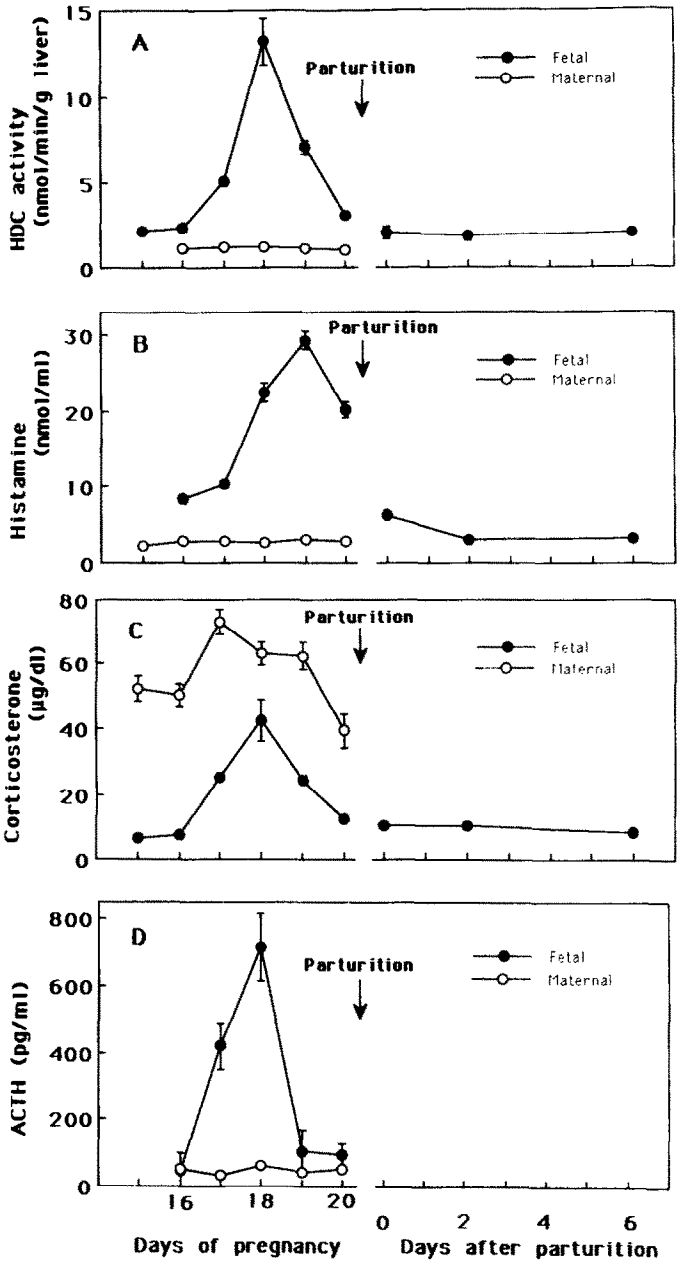

Fig. 1. Age-dependent changes in HDC activity (A) and in plasma levels of histamine (B), corticosterone (C) and ACTH (D) during development of fetal, neonatal and maternal rats. Each value is the mean ± SEM of four samples (A, B and C), or three samples (D).

of fetal ACTH-glucocorticoid activity. Thus, plasma levels of corticoid and ACTH, and hepatic HDC activity, were measured in fetuses and neonates of various ages.

Materials and Methods

Animals. Pregnant Wistar rats were purchased from the Shizuoka Experimental Animals and Agricultural Cooperative (Hamamatsu, Japan) on day 14 of gestation. Successful mating was determined by observation of a vaginal plug the morning following mating. This day was counted as day 0 of gestation. Rats were housed individually in plastic cages with food (Oriental Laboratory chow) and water *ad lib.*

Preparation of livers and plasma from fetus and neonate. Pregnant rats were decapitated under ether anesthesia, and fetuses were removed and washed with saline. Fetal blood was collected with a micropippet through an incision made in the axillar region. After combining blood from two fetuses, the blood was allowed to clot at room temperature and was centrifuged to collect the plasma. Fetal and neonatal livers were homogenized with 0.1 M potassium phosphate buffer (pH 6.8) containing 10 μM pyridoxal 5'-phosphate, 0.2 mM dithiothreitol and 1% polyethylene glycol 300 with a Teflon–glass homogenizer.

Assays for L-histidine decarboxylase activity and histamine. The HDC activity was measured essentially by the

method of Watanabe *et al.* [7] with a slight modification [5]. Briefly, aliquots of the supernatants obtained from the tissue homogenates were dialyzed against the same buffer. Then the dialysates were incubated at 37° for 1.5 hr in a reaction mixture (1 mL) containing 0.25 mM histidine in the same buffer. The reactions were terminated by adding 2.1 mM $HClO_4$, and clear supernatants were obtained from the mixture by centrifugation. Histamine formed during the incubation was separated from histidine on a column of Amberlite CG-50 (Type I, Na^+ form), and measured by the *o*-phthalaldehyde method. The recovery of histamine through these steps was more than 90% in all cases. Histamine levels in plasma and liver homogenates were assayed by an HPLC system using a column of histamine pak (JASCO, Tokyo, Japan) and a fluorometer.

Assays for ACTH and corticosterone in plasma. Plasma ACTH levels were assayed by using a radioimmunoassay kit, the ACTH Kit (Diagnostic Products Corp., Los Angeles, CA, U.S.A.). Plasma corticosterone levels were determined by using a modified method as described by Dawson *et al.* [8]. Plasma corticosterone was extracted on a SEP-PAK C_{18} column (Waters, Milford, MA, U.S.A.) and separated by reverse phase HPLC column chromatography.

Cell cultures of fetal or neonatal hepatic cells. Liver cells were obtained from 18-day-old fetuses and 5-day-old neonates according to the method of Yeoh *et al.* [9]. Isolated hepatic cells were cultured with RPMI 1640 medium containing 10% fetal bovine serum.

Results and Discussion

As shown in Fig. 1A, HDC activity increased greatly in fetal liver at late pregnancy and reached the maximum level after 18 days of gestation, followed by a rapid decrease at near parturition. Corresponding to these changes, histamine levels of fetal plasma (Fig. 1B) also fluctuated during development of the fetus with a peak at day 19 of gestation, suggesting that the source of fetal plasma histamine is the fetal liver. On the other hand, maternal hepatic HDC activity and histamine level did not change during this period. The concentrations of corticosterone (Fig. 1C) and ACTH (Fig. 1D) in fetal plasma fluctuated during development of the fetus with a peak at day 18 of gestation, which closely resembled that of hepatic HDC activity. The fluctuation experiments show that the pituitary-adrenal system may operate in the rat fetus, resulting in the fluctuation of HDC in fetal liver. In fact, the hypothalamus-pituitary-adrenal system in lambs was reported to be completed a couple of days before parturition [6]. The transfer of maternal steroid through the placenta may be reflected in part in the increase of the fetal level, as suggested in the case of lambs [6]. However, neither plasma corticosterone nor hepatic HDC activity in the fetus was varied by adrenolectomy of the pregnant rats (data not shown). Since maternal hepatic HDC activity was not affected in spite of the high level of corticosterone in maternal plasma (Fig. 1A), the rapid augmentation of fetal liver HDC activity can be explained by the change in the molecular or kinetic properties of inducing HDC. However, in our preliminary experiments no practical differences in the molecular and catalytic properties were observed between the partially purified enzymes extracted from mastocytoma P-815 cells treated with or without dexamethasone (data not shown). Therefore, it is conceivable that glucocorticoid-responsive cells in fetal liver alter the response to steroid in inducing HDC, or they disappear from the liver during the development process of fetus to neonate. To clarify these points, we examined the *in vitro* effect of dexamethasone on HDC activity in cultured liver cells isolated from fetus, neonate and adult. When liver cell suspensions were incubated with dexamethasone for 18 hr, HDC activity in the suspension from the fetus, but not from the neonate and adult, was increased (Table 1). The basal enzyme activity was 10-fold higher in the fetal liver cell suspension than in either the neonatal or the adult liver cell suspension. The increase induced by dexamethasone was repressed completely by the addition of cycloheximide or actinomycin D. Furthermore, we found that administration of hydrocortisone phosphate (2 mg/kg) to newborn rats 8 days after birth, had no effect on hepatic HDC activity (data not shown). These results indicate that change of the number of histamine-forming cells may cause the deficiency of glucocorticoid-induced stimulation in HDC activity in neonate or adult. The available evidence indicates that fetal liver, the principal hematopoietic organ in late embryonic life, contains mast cell precursor cells which can differentiate into metachromatic mast cells present in the subcutaneous tissue [9–13]. Kitamura *et al.* demonstrated that the number of mast cell precursors in the fetal liver cells of mice increases rapidly from day 15 to day 18 of gestation [12], at which time inducing HDC begins to appear, as shown in Fig. 1A. Watanabe *et al.* [14] demonstrated that before parturition embryos from genetically mast cell deficient W/W^V mice lacked the increase of hepatic HDC activity shown in the normal fetus (Fig. 1A). Since we previously found that dexamethasone does not stimulate HDC synthesis in peritoneal cavity mast cells [4], it is likely that the glucocorticoid-stimulated histamine-forming type of cells in fetal liver may be mast cell precursors but not mast cells. However, it is necessary to identify histamine-forming cells in order to perform the immunocytochemical localization of HDC in a variety of fetal liver cell types.

Table 1. Effect of dexamethasone on HDC activity in the liver cell suspension from fetal, neonatal and adult rats

Additions	Hepatic HDC activity (pmol/min/10^7 cells)		
	Fetal cells	Neonatal cells	Adult cells
None	2.26 ± 0.35	0.226 ± 0.082	0.105 ± 0.009
Dexamethasone	7.30 ± 0.46*	0.105 ± 0.033	0.112 ± 0.010
+ Cycloheximide	1.48 ± 0.30		
+ Actinomycin D	2.06 ± 0.20		
Cycloheximide	1.66 ± 0.23		
Actinomycin D	1.92 ± 0.31		

The liver cell suspensions were prepared from fetal, neonatal and adult rats, and cultured with or without dexamethasone (1 μM) and/or cycloheximide (5 μg/mL) or actinomycin D (0.5 μg/mL) for 18 hr. The cells were harvested and assayed for HDC activity. Each value is the mean ± SEM of three samples.

* $P < 0.05$ vs other fetal cell values.

The physiological function of histamine production in hematopoietic cells of fetal liver is not yet defined. However, histamine has been reported to promote the maturation of granulocyte precursors in hematopoietic stem cells (CFU) to granulocytes via H_2 receptors [15]. This may be favorable for the neonatal defense mechanism.

In summary, our studies suggest that the fluctuation of HDC activity in fetal liver in late gestation is regulated by the plasma glucocorticoid level through the pituitary-adrenal system. Taken together, these results support the conclusion that glucocorticoid promotes a rapid increase in HDC synthesis in fetal liver histamine-forming cells, as well as in mouse mastocytoma P-815 cells [4] and rat glandular stomachs [5].

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