Effect of Labor and Prostaglandins on Phenylethanolamine N-Methyltransferase in Human Fetal Membranes

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Abstract. Biochemical investigations have shown the presence of the enzyme phenylethanolamine N-methyltransferase (PNMT), which converts norepinephrine (NE) to epinephrine (E) in human pregnant tissues, e.g. myometrium and fetal membranes. The enzymatic activity of PNMT in myometrium is known to decrease during labor. In the present study, PNMT activity in chorio-decidua was measured and the effect of labor and prostaglandins (PG) on PNMT activity was determined. A cytosol fraction of chorio-decidua was incubated with [^{14}C]-S-adenosyl-L-methionine and normetanephrine for 60 min to measure PNMT activity. Fetal membranes were obtained at elective Cesarean section and normal vaginal delivery from full term pregnant women, and the activity of PNMT in chorio-decidua was compared. Significantly lower activity in chorio-decidua obtained from normal vaginal delivery than that from elective Cesarean section was observed. The concentration of 13, 14-dihydro-15-keto-prostaglandin $\text{F}_{2\alpha}$ (DHK-PGF $_{2\alpha}$) in amniotic fluid obtained at normal vaginal delivery were measured by RIA, and the relationship of the DHK-PGF $_{2\alpha}$ level to PNMT activity was studied. There was a significantly negative correlation between the concentration of DHK-PGF $_{2\alpha}$ in amniotic fluid and PNMT activity in chorio-decidua. When various concentrations of PGF $_{2\alpha}$ were pre-incubated with minced chorio-decidua for 2 h, PNMT activity was decreased in a dose dependent manner. These results indicated that PGF $_{2\alpha}$ might affect the bioconversion of NE to E in fetal membranes. The presence of this enzyme in fetal membranes may play an important role in regulating the concentration of catecholamines during pregnancy and parturition.

Key words: Phenylethanolamine N-methyltransferase (PNMT), Fetal membranes, Prostaglandins, Catecholamine.
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THE ENZYME phenylethanolamine N-methyltransferase (PNMT, EC 2.1.1.28) which catalyzes the conversion of norepinephrine (NE) to epinephrine (E) is known to be highly localized in the adrenal medulla [1]. Recently, the presence of this enzyme in human pregnant tissues, e.g. myometrium and fetal membranes, has been demonstrated [2, 3, 4]. Hobel *et al.* reported that PNMT in human myometrium might act to maintain uterine quiescence during pregnancy [2].

It is known that the concentrations of the

catecholamines (CA) and prostaglandins (PG) in amniotic fluid increase with gestation and are markedly increased during labor [5, 6]. Casimili *et al.* speculated that the enzymatic activity of PNMT in fetal membranes might modulate PG secretion, which provided a stimulus for the onset of labor [3, 7, 8].

In the present study, the effects of labor and PG on PNMT activity in fetal membranes were investigated.

Materials and Methods

Fetal membranes obtained at elective Cesarean section or normal vaginal delivery from twenty

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healthy women at term were subjected to the study. The membranes were removed from the placenta immediately after delivery and washed several times with physiological saline and chorio-decidual tissue was separated from the amnion layer. Amniotic fluid was also obtained from 13 cases of normal vaginal delivery to measure the concentration of amniotic 13, 14-dihydro-15-keto-prostaglandin F₂ α (DHK-PGF₂ α). Indomethacin and EDTA-2Na were added to amniotic fluid and centrifuged. The supernatant of amniotic fluid and chorio-decidea were kept frozen at -80°C until assayed. [^{14}C]-S-adenosyl-L-methionine ([^{14}C]-SAM, SA; 1.6 GBq/mmol) and [^3H]-SAM (SA; 407.6 GBq/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Other chemicals were of reagent grade and obtained commercially. Quantitative determination of radioactivity was carried out in an ALOCA LSC-3500 Liquid Scintillation Counter. Protein content was estimated with a commercial dye reagent (Tonein TP kit, Otsuka Pharmaceuticals, Tokyo) according to the procedure of Bradford [9]. The concentration of DHK-PGF₂ α was measured with a DHK-PGF₂ α RIA Kit (Advanced Magnetics Inc, Cambridge, MA).

The purpose of the study was explained and agreement of the ethical committee of Showa University was obtained. Informed consent was obtained from all patients.

The results were subjected to statistical analysis by Student's *t*-test.

Measurement of PNMT activity

Chorio-decidual tissue were homogenized in isotonic (1.19%) potassium chloride and centrifuged at 105,000 g for 60 min at 4°C , to yield a cytosol fraction containing the enzyme. Incubation was performed by a modification of the method originally described by Axelrod [1]. The incubation procedure is briefly as follows. Approximately 100 μg protein of cytosol fraction was incubated with 0.55 μmol of normetanephrine as substrate, and 0.78 nmol of [^{14}C]-SAM (8,000 dpm) for methyl donor at 37°C for 60 min. For the blank incubation, boiled enzyme was used. The reaction was terminated by adding 1 ml of 0.1 M borate buffer (pH = 10). Tritiated metanephrine (15,000 dpm), synthesized by incubation of cytosol fraction of bovine adrenal medulla with [^3H]-SAM were

added for recovery. The radiolabeled reaction products were extracted by shaking with 5 ml of toluene: isoamylalcohol = 4:1 for 30 min. Metanephrine in the organic layer was back extracted with 500 μl of 0.1 N acetic acid [10]. The water fraction was evaporated under N₂ gas and reconstituted with ethanol and subjected to thin layer chromatography (TLC, SILICA GEL 60F-254, Merck). Radiolabeled products were developed on TLC with the solvent system of n-butanol: acetic acid: water = 4:1:1 (Rf value for metanephrine of 0.27). The metanephrine spots were isolated under ultraviolet light (254 nm wavelength) and scraped. The metanephrine was eluted from silica gel with 1 ml of 0.1 N HCl, then mixed with 9 ml of Aquasol, and counted after 12 h. The amount of [^{14}C]-metanephrine formed was expressed as PNMT activity.

RIA of DHK-PGF₂ α

One thousand cpm of tritiated DHK-PGF₂ α was added to amniotic fluid to correct the recovery loss and acidified to pH 3 to 4 with 0.1 N hydrochloride. PG were extracted with ethyl acetate on a preactivated Amprep Octadecyl C₁₈ column (Amersham, UK), and measured by RIA. The mean intra-assay coefficient of variation was 9.6%.

Results

Evaluation of measurement of PNMT activity

A liner relationship between the duration of incubation and the rate of metanephrine formed was observed until 60 min. A liner relation was also obtained when the protein concentrations from 50 μg to 200 μg were added into incubation medium.

Effect of labor on PNMT activity in chorio-decidea

PNMT activities in chorio-decidea collected at normal vaginal delivery and elective Cesarean section at term were compared to study the effect of labor. Significantly lower PNMT activity in chorio-decidea was observed at normal vaginal delivery (42.6 ± 7.1 pmoles/mg protein/60 min, mean $\pm$ SEM, $n=10$) than at elective Cesarean section (74.6 ± 7.3 pmoles/mg protein/60 min, $n=10$) (Fig. 1).

Fig. 1. Activity of PNMT in chorio-decidua obtained at elective Cesarean section (without labor) and normal vaginal delivery (with labor). Data are expressed as the mean $\pm$ SEM. *, $P < 0.05$.

Fig. 2. Correlation of the concentration of DHK-PGF2α in amniotic fluid and chorio-decidua which were collected in 13 cases of normal vaginal delivery. $r = -0.587$, $P < 0.05$.

Concentration of amniotic DHK-PGF2α and PNMT activity in chorio-decidua at delivery

In 13 cases of normal vaginal delivery, amniotic fluid and fetal membranes were obtained at delivery. The concentration of DHK-PGF2α in amniotic fluid at delivery was 3.78 ± 0.45 ng/ml (mean $\pm$ SEM) and a significantly negative correlation ($P < 0.05$) between the amniotic DHK-PGF2α

Fig. 3. The effect of PGF2α on PNMT activity in chorio-decidua. Procedure for incubation described in the text. Data are expressed as the mean $\pm$ SEM. *, $P < 0.01$ vs. control.

level and chorio-decidua PNMT activity was observed (Fig. 2).

Effect of PGF2α on PNMT activity in chorio-decidua

To elucidate the effect of PGF2α on PNMT activity in chorio-decidua, minced chorio-decidua tissue was incubated with various concentrations of PGF2α for 2 h and the tissue was homogenized to measure PNMT activity. As shown in Fig. 3, PNMT activity was decreased by the addition of PGF2α in a dose dependent manner. A significant decrease ($P < 0.01$) in PNMT activity was observed when more than $2 \mu\text{M}$ of PGF2α was added to the incubation medium.

Discussion

CA and PG are thought to be closely related to uterine contraction during labor [8, 11, 12, 13], and it is well known that the concentrations of PG and CA in human amniotic fluid are markedly increased during labor [5, 6]. In the present study, an attempt was made to measure PNMT activity in chorio-decidua and the effect of labor and PG on this enzyme activity was demonstrated.

PNMT activity in fetal membranes was reported to be localized in amnion and chorion [3]. In our recent study, PNMT activity in fetal membranes was higher in chorio-decidua than in amnion. Intense immunopositive staining for PNMT was

observed in trophoblast cells in fetal membranes at term [14].

It is interesting to note that PNMT activity in chorio-decidua obtained at normal vaginal delivery was significantly lower than that at elective Cesarean section. This is the first report to demonstrate the effect of labor on PNMT activity in fetal membranes. In human myometrium, Hobel *et al.* reported that human myometrial PNMT activity was significantly decreased during labor, and they speculated that the relative accumulation of myometrial NE promoted the uterine contraction during labor [2]. Our findings suggested that fetal membranes as well as myometrium may also be involved in the mechanism of uterine contraction. The factors which regulate PNMT activity in myometrium and fetal membranes are unclear.

The administration of NE into the amniotic cavity caused uterine contraction during therapeutic abortion [15]. The addition of a physiological concentration of NE significantly enhanced the release of PGE₂ and PGF₂α in human fetal membranes *in vitro* [12]. Renzo *et al.* reported the presence of β-adrenergic receptors in human amniotic membrane, and Su *et al.* speculated that CA in amniotic fluid might stimulate the release of PG from amniotic membrane by utilizing cyclic adenosine monophosphate as a second messenger [8, 13]. However, the relation between biosynthesis and PG in chorio-decidua has not been clearly established.

The relationship of amniotic DHK-PGF₂α to PNMT activity in chorio-decidua at delivery was determined in the present study. The concentration of DHK-PGF₂α which is known to be a stable

metabolite of PGF₂α in amniotic fluid [16] and PGF₂α increases as labor progresses at normal vaginal delivery [17, 18]. Our finding of a negative correlation between the amniotic DHK-PGF₂α concentration and PNMT activity in chorio-decidua appeared to provide new information as to the effect of PG on PNMT in physiological conditions.

In the next experiment, therefore, the effect of PG on PNMT activity in chorio-decidua was studied *in vitro*. The addition of PGF₂α to the incubation medium suppressed PNMT activity in a dose dependent manner. The concentration of PGF₂α added to the incubation medium was at a pharmacological level, but the possibility that part of the PGF₂α in the incubation medium was converted to an inactive form by PGDH in chorio-decidua would exist [19]. It is always appropriate to exercise caution concerning the extrapolation of *in vitro* studies to *in vivo* situations.

These results obtained in *in vivo* and *in vitro* studies indicated that amniotic PG secreted from fetal membranes modulates the conversion of NE to E in chorio-decidua. They also indicated the positive interaction of PG production and CA synthesis in fetal membranes. The question is raised whether intrinsic DHK-PGF₂α may affect PNMT activity.

However, the presence of monoamine oxydase (MAO) which catalyzes the inactive form of CA was well documented in fetal membranes [3, 20]. Further study is necessary to elucidate the relative importance of both enzymes, PNMT and MAO, on the regulation of uterine contraction during pregnancy and labor.

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