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Modulation of Uterine Phospholipase A_2 Activity by Estradiol During the Delayed Implantation Process in Rats

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ABSTRACT

The present study was performed to determine whether estradiol, via cAMP mediation, induces prostaglandin synthesis by modulating phospholipase A_2 activity which hydrolyzes phospholipids into arachidonic acids, a precursor for prostaglandin synthesis, during the implantation process in rats. Uterine phospholipase A_2 activity was elevated on day 5 of pregnancy when implantation normally occurs in rats. Moreover, phospholipase A_2 activity was higher in the implant sites than in the non-implant sites of uterus on day 6. In delayed implantation model, phospholipase A_2 activity was increased at 12 hrs after estradiol administration and at 8 hrs after dbcAMP administration. In addition, higher activity of phospholipase A_2 was induced by the treatment of estradiol plus theophylline, compared with estradiol-only treated group. The simultaneous treatment of indomethacin with estradiol or dbcAMP did not alter phospholipase A_2 activity compared with estradiol or dbcAMP-only treated group although significant suppression was observed in uterine PGE and $PGF_{2\alpha}$ concentrations. These results suggest that estradiol or cAMP stimulates uterine phospholipase A_2 activity, thereby increasing prostaglandin synthesis during the implantation process in rats.

Key Words: Estradiol, cAMP, Phospholipase A_2, Prostaglandins, Implantation

INTRODUCTION

Prostaglandins (PGs) are known to regulate several uterine functions including implantation of the blastocysts. It was demonstrated that uterine PGE and $PGF_{2\alpha}$ concentrations were sharply increased during the implantation period (Phillips and Poyser, 1981; Lee *et al.*, 1985; Yoon and Ryu, 1987) and levels were significantly greater in the implant sites than in the non-implant sites of uterus (Kennedy, 1977; Yoon and Ryu, 1987). In addition, estradiol appears to stimulate uterine PG synthesis during the implantation process (Dey *et al.*, 1982; Pakrasi *et al.*, 1983; Yoon *et al.*, 1991) and this process may be mediated by the elevation of uterine cAMP levels (Yoon *et al.*, 1991).

However, the mechanism by which estradiol or cAMP modulates uterine PG synthesis during the implantation process has not been clearly defined. Phospholipase A_2 (PLA_2) which hydrolyzes phospholipids into arachidonic acid is known to serve as a rate-limiting enzyme for PG synthesis. Evidence that PLA_2 activity can be modulated by ovarian steroids (Dey and Johnson, 1980; Dey *et al.*, 1982; Pakrasi *et al.*, 1983) suggests possible effects of estradiol and progesterone on this enzyme in uterine PG synthesis during the implantation process.

The present study was performed to determine whether estradiol, via cAMP mediation, induces uterine PG synthesis by modulating phospholipase A_2 activity during the implantation process in rats.

MATERIALS AND METHODS

Experimental groups

Female Sprague Dawley rats aged 2~3 months, weighing 200-250 g were used throughout this study. The morning in which sperm was found in the vagina was designated as day 1 of pregnancy.

Group 1: Uterine PLA_2 activity was measured during normal pregnancy which implan-tation occurs on day 5 of pregnancy. PLA_2 activity was also measured in the implant sites and the non-implant sites of uterus on days 6 and 7 of pregnancy.

Group 2: Uterine PLA_2 activity was measured during the delayed implantation period. Delayed implantation was induced by ovariectomy on day 3 of pregnancy, followed by a daily injection of progesterone (3 mg/0.2 ml in sesame oil) on days 3-7 and a single injection of estradiol (1 ug/0.1 ml in sesame oil) on day 8. Delayed implantation occurred by 24 hrs after estradiol treatment. Instead of estradiol on day 8 of pregnancy, 50 ul of 50 mM dbcAMP dissolved in PBS (0.01 M phosphate, 0.15 M NaCl, pH 7.0) was administered by an intrauterine instillation. To confirm the effect of cAMP, 100 mM theophylline (100 ul/uterine horn) was administered by an intrauterine instillation 2 hrs prior to estradiol administration during the delayed implantation process.

Group 3: Uterine PLA_2 activity was also measured after simultaneous treatement of indomethacin (2 mg/0.2 ml in sesame oil) with estradiol or dbcAMP on day 8 of pregnancy.

Just prior to intrauterine instillation, a cotton ligature was placed around the upper cervix to minimize the escape of fluid. At sacrifice, uterine tissues were immersed in cold saline containing 6 mM theophylline and indomethacin (10 ug/ml). Implant sites were identified at 24 hrs after the treatment of estradiol or dbcAMP and uteri were cut into the implant sites and the non-implant sites.

Determination of uterine PGs

Uterine tissues were homogenized (Polytron homogenizer, Janke & Kunkel, W. Germany) in 1 ml PBS and 3 ml ethyl acetate: isopropanol: 0.2 N HCl (3:1:1, V/V/V) solution. After vortexing for 15 sec twice, 2 ml ethyl acetate and 3 ml distilled water were added. After mixing, phases were separated by centrifugation. The organic phase (3-3.5 ml) was transferred to a test tube and dried by vortex evaporator. The residue was dissolved in gel Tris buffer. Concentrations of PGE and $PGF_2\alpha$ were measured by radioimmu-noassay (Clinical Assays, USA).

Determination of phospholipase A_2 activity

Uterine tissues were homogenized in ice-cold PBS (pH 7.4). The homogenate was centrifuged at 750×g (4°C) for 15 min and the supernatant was assayed for enzyme activity. Unlabeled dipalmitoyl-phosphatidylcholine and labeled dipalmitoyl-1-^{14}C-phosphatidylcholine (NEN, USA) were served as substrates.

100 ul of 80 nM unlabeled substrate, 100 ul of labeled substrate (10,000 cpm, 115.8 mCi/mmol) which were dissolved in 50 mM HEPES buffer containing 10 mM $CaCl_2$ and 100 ul of the same buffer were mixed and placed in a boiling water bath for 1 min. To this mixture, 300 ul of enzyme source (400 ug protein) was added and the reaction was run for 1 hr at 37°C. After incubation was stopped by addition of 2 ml Dole's reagent (0.33 N H_2SO_4: isopropanol: heptane=28:36:36), the mixture was heated to 60°C for 1 min and then cooled to room temperature. After 1 ml distilled water and 2 ml heptane were added, the mixture was centrifuged at 1,000×g for 15 min and the aqueous layer was frozen in an acetone-dry ice. The non-aqueous layer was decanted into a tube containing 100-150 mg of silicic acid (100~300 mesh) and content in a tube was mixed for 30 sec on vortex and centrifuged at 1,600×g for 15min. The radioactivity of liberated ^{14}C-palmitate from the top layer was counted in a β-counter. The activity was expressed as nmol of fatty acid released per hr per mg protein.

The differences between the experimental groups were analyzed by one-way ANOVA with t-test, and P values less than 0.05 were considered significant.

RESULTS

Uterine phospholipase A_2 activity during early pregnancy

Uterine PLA_2 activity was measured during early pregnancy (Fig. 1). PLA_2 activity was 9.03±1.82 nmol/mg protein/h on day 1 of pregnancy and decreased to 5.30±0.86 nmol/mg protein/h on day 2 of pregnancy, which is comparable to level of non-pregnant control (5.27±1.06 nmol/mg protein/h). PLA_2 activity was increased from day 3, reaching the maximum (15.00±1.66 nmol/mg protein/h) on day 5 when implantation normally occurs in rats.

Moreover, PLA_2 activity was greater in the implant sites than in the non-implant sites of uterus both on days 6 and 7 (Table 1), and especially PLA_2 activity in the implant sites (13.40±1.46 nmol/mg protein/h) was significantly greater than in the non-implant sites (7.70±0.73 nmol/mg protein/h) of uterus on day 6 ($p<0.05$).

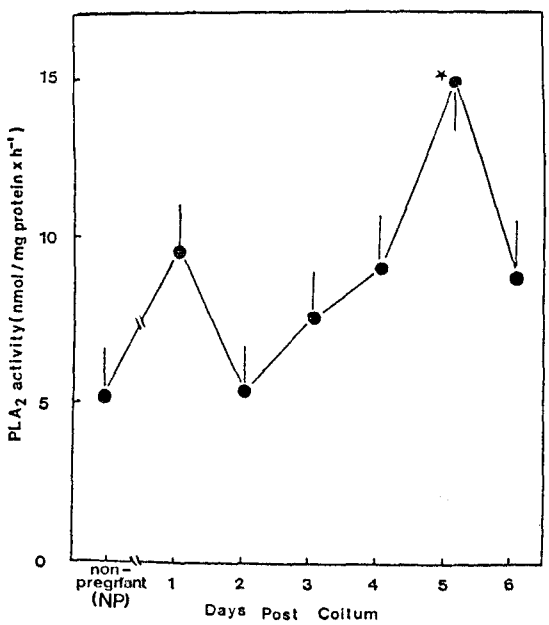

Fig. 1. Uterine PLA_2 activity during the early pregnancy in rat. Data are expressed as mean± SEM of 6 to 8 animaes. * $p<0.05$ as compared with the value of non-pregnant group.

Effects of estradiol or dbcAMP on uterine PLA_2 activity during the delayed implantation process

This experiment was performed to demonstrate whether estradiol stimulates uterine PGs synthesis by modulating phospholipase A_2 activity, an enzyme responsible for the arachidonic acid production which is a precursor for PGs synthesis (Fig. 2). During the delayed implantation process, the treatment of estradiol significantly increased PLA_2 activity at 12 hrs after estradiol administration (17.30±2.11 nmol/mg protein/h, $p<0.05$) as compared with progesterone

Table 1. Uterine PLA_2 activity in the implant and the non-implant sites of uterus on days 6 and 7 of pergnancy in rats

Day of pregnancy	PLA_2 activity(nmol/mg protein/h)	
	Implant sites	Non-implant sites
6	13.40±1.46*	7.70±0.73
7	9.25±1.03	7.24±1.20

*$p<0.05$ as compared with the value of PLA_2 activity in the non-implant sites on day 6 of pregnancy.

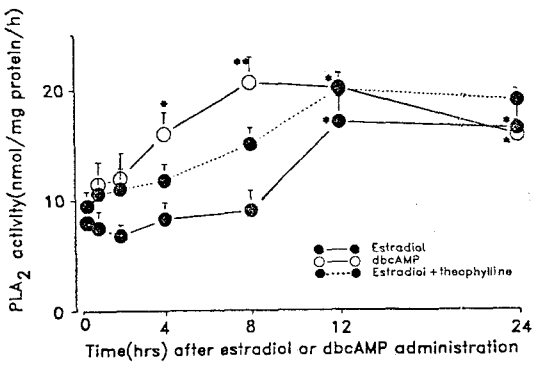

Fig. 2. Effects of estradiol, dbcAMP and theophylline on uterine PLA_2 activity during the delayed implantation process. Theophylline was pretreated 2 hrs prior to estradiol administration. Data are expressed as mean±SEM of 6 to 8 animals. *$p<0.05$. **$p<0.01$ as compared with the value at 0 hr.

only-treated group (8.55±0.54 nmol/mg protein/h). At this time, uterine PGs were also increased concomitantly.

Furthermore, the treatment of dbcAMP instead of estradiol during the delayed implantation process advanced an increase in PLA_2 activity 4hrs . PLA_2 activity was significantly increased at 8 hrs after dbcAMP treatment (21.16± 3.28 nmol/mg protein/h, $p<0.01$) as compared with progesterone only-treated group (8.55 ± 0.54 nmol/mg protein/h). PLA_2 activity was gradually decreased thereafter. The pretreatment of theophylline, phosphodiesterase inhibitor, 2 hrs prior to estradiol treatment resulted in an increase in PLA_2 activity as compared with estradiol-only treated group reaching the maximum at 12 hrs after estradiol administration.

Table 2 shows PLA_2 activity in the implant sites and the non-implant sites of uterus at 24 hrs following estradiol or dbcAMP administration during the delayed implantation process. PLA_2 activity was significantly greater in the implant sites than in the non-implant sites of uterus ($p<0.05$). With the treatment of estradiol, PLA_2 activity in the implant sites was 2.5 fold greater than in the non-implant sites while after dbcAMP administration PLA_2 activity in the implant sites was 3.4 fold greater than in the non-implant sites.

Table 2. Uterine PLA_2 activity in the implant sites and the non-implant sites at 24 hrs after estradiol or dbcAMP administration during the delayed implantation process

Treatments	PLA_2 activity(nmol/mg protein/h)	
	Non-implant sites	Implant sites
Estradiol	4.74±0.37	11.89±2.63*
dbcAMP	4.08±0.42	13.88±2.54*

Data are expressed as mean ± SEM of 6 to 8 animals.

* $p<0.05$ as compared with the value of PLA_2 activity in the non-implant sites

Effect of indomethacin on estradiol or dbcAMP-induced uterine phospholipase A_2 activity during the delayed implantation process

The simultaneous treatment of indomethacin with estradiol or dbcAMP significantly suppressed both PGs synthesis while estradiolor dbcAMP-induced uterine PLA_2 activity was not altered (Table 3). These data suggest that estradiol or cAMP modulates mobilization of arachidonic acid from phospholipids by activating PLA_2 activity but does not affect subsequent cyclooxygenation of arachidonic acid.

DISCUSSION

In the present study, it was demonstrated that estradiol or cAMP stimulates uterine PGs synthesis by modulating PLA_2 activity. PLA_2 activity was increased at 12 hrs when PGs levels were increased after estradiol administration. The treatment of dbcAMP induced an increase in PLA_2 activity as well as PG synthesis 4 hrs in

Table 3. Effect of indomethacin on estradiol or dbcAMP-induced uterine PLA_2 activity during the delayed implantation process

Treatments	PGs(ng/100 mg tissue)		PLA_2 activity
	PGE	$PGF_{2\alpha}$	(nmol/mg protein/h)
Estradiol	53.46±3.99	35.50±2.71	17.30±2.11
Estradiol+ID	4.32±1.93*	1.20±0.40*	17.55±2.74
dbcAMP	62.50±9.75	50.98±7.04	21.66±3.88
dbcAMP+ID	6.19±0.78*	4.68±0.77*	20.97±3.06

PLA_2 activity was measured at 12 hrs after estradiol and at 8 hrs after dbcAMP administration. Data are expressed as mean±SEM of 6 to 8 animals.

*$p<0.05$ as compared with the value of estradiol or dbcAMP-only treated group.

advance. In addition, higher PLA_2 activity was induced by the treatment of estradiol plus theophylline compared with estradiol-only treated group. According to Cox *et al.* (1982), uterine PLA_2 activity reached maximum just prior to implantation in rats, although the relative activity in the implant sites and non-implant sites of uterus was not examined. In rabbits, PLA_2 activity was greatly amplified on day 7 when trophoblast-endometrial apposition and attachment begin, and declined markedly within 24 hrs (Hoffman *et al.*, 1984). It was suggested that potential blastocyst-related stimuli for an increase in PLA_2 activity on day 5 in rats might include blastocyst-produced estrogen (Findlay, 1983: Kennedy, 1983) or histamine (Dey & Johnson, 1980). The effects of steroid hormones on uterine PLA_2 activity have been reported (Dey *et al.*, 1982: Pakrasi *et al.*, 1983), although little information is availables on modulation of uterine PLA_2 activity during the implantation process in rats. Implantation of silastic capsules containing estradiol-17β in hypophysectomized and progesterone-primed rats stimulated PLA_2 activity in endometrium (Dey *et al.*, 1982: Pakrasi *et al.*, 1983). These results support our results that estradiol might stimulate uterine PGs synthesis by way of modulating PLA_2 activity during the implantation process.

Exposure of cells to cAMP increased release of arachidonic acid, thereby stimulating PG production and this phenomenon has been described in several cell types (Dayal *et al.*, 1983; Baker *et al.*, 1985). Moreover, 8-bromo cAMP released arachidonic acid and increased PGE production in 3T3 fibroblasts (Lindgren *et al.*, 1978). In fat cell homogenates (Van den Bosh *et al.*, 1978) and human adherent synovial cells (Baker *et al.*, 1985), cAMP showed ability to stimulate PLA_2 activity. These results are consistent with our finding that dbcAMP increased uterine PLA_2 during the delayed implantation process. Higher activity of PLA_2 was induced by the treatment of estradiol plus theophylline as compared with estradiol-only treated group. The treatment of indomethacin suppressed estradiol- or dbcAMP-induced uterine PGs synthesis but did not change uterine PLA_2 activity. Since estradiol or dbcAMP stimulated PLA_2 activity, but did not stimulate PG synthesis in the presence indomethacin, it is unlikely that cyclooxygenase is a target for estradiol or cAMP to stimulate PG synthesis.

In conclusion, the present study has demonstrated that estradiol stimulates uterine PLA_2 activity via cAMP mediation, thereby increasing PG synthesis during the delayed implantation process in rats.

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= 국문초록 =

흰쥐의 착상기간중 Estradiol이 자궁의 Phospholipase A_2 활성도에 미치는 영향

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본 연구에서는 흰쥐의 수정란 착상시기에 estradiol이 prostaglandins(PGs) 합성의 전구체인 arachidonic acid를 생성하는데 관여하는 phospholipase A_2(PLA_2)의 활성도를 조절하므로써 PGs의 합성을 촉진하는가를 조사하여 다음과 같은 결과를 얻었다.

자궁의 PLA_2 활성도는 수정란이 착상하는 시기인 임신 제 5 일에 증가되었으며, 비착상부위에서 보다는 착상부위에서 더 높은 것으로 나타났다. Delayed implantation model에서, PLA_2 활성도는 estradiol을 투여한 지 11시간후에 증가되었으며, dbcAMP를 투여한지 8시간후에 증가되었다. 또한 estradiol을 투여하기 2시간전에 phosphodiesterase inhibitor인 theophylline을 투여하면 estradiol만 투여한것에 비하여 PLA_2 활성도가 증가되었다. Estradiol 또는 dbcAMP와 함께 indomethacin을 투여하면 자궁의 PGs합성은 억제되었으나 PLA_2 활성도에는 영향을 주지않았다.

이상의 결과로 보아 흰쥐의 착상시기에 estradiol은 cAMP를 매개로하여 자궁의 PLA_2 활성도를 촉진하므로써 PGs의 합성을 증가시키는 것으로 생각된다.