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cAMP Mediation in Estradiol-induced Uterine Prostaglandin Synthesis During the Delayed Implantation Process in Rats

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ABSTRACT

The present study was performed to elucidate the factors which modulate uterine prostaglandin synthesis during the implantation period in rats, by employing delayed implantation model. Administration of estradiol sharply increased uterine cAMP concentration 4 hrs later during the delayed implantation process. Concentrations of uterine PGE and PGF$_{2\alpha}$ were increased at 12 hrs after the estradiol treatment although an increase in PGF$_{2\alpha}$ was not statistically significant. The concomitant treatment of indomethacin with estradiol significantly suppressed estradiol-induced PGE and PGF$_{2\alpha}$ at 12 hrs, while uterine cAMP concentration was not suppressed. The treatment of dbcAMP without estradiol gradually increased uterine PGE and PGF$_{2\alpha}$ showing the maximum 8 hrs later, suggesting that cAMP minics estradiol effect on uterine prostaglandin synthesis during the implantation process. Furthermore, the pretreatment of theophylline, phosphodiesterase inhibitor, induced significantly greater concentrations of uterine PGE and PGF$_{2\alpha}$, compared with estradiol-only treated group. These results suggest that estradiol stimulates uterine prostaglandin synthesis and this process may be mediated by an elevation of cAMP during the delayed implantation process in rats.

Key Words: Estradiol, cAMP, Prostaglandin, Implantation

INTRODUCTION

Prostaglandins (PGs) are known to modulate several uterine functions including implantation of the blastocyst. During the implantation period, concentrations of uterine PGs were sharply increased in rats (Phillips and Poyser, 1981; Yoon and Ryu, 1987). Moreover, concentrations of PGE and PGF$_{2\alpha}$ were greater in the implant sites than in the non-implant sites of the uterus (Kennedy, 1977; Yoon and Ryu, 1987). Inhibition of uterine PGs synthesis by the treatment of indomethacin interfered with implantation in rats (Kennedy, 1977) and rabbits (Hoffman *et al.*, 1978; Lee *et al.*, 1985). The biochemical and physiological events leading to implantation seem to depend normally on a precise balance between estrogen and progesterone (Dey and Johnson, 1980). Therefore, a regulatory role of these hormones in PGs synthesis is likely to be operative in the uterus. Indeed, numerous reports have shown that ovarian steroid hormones modulate uterine production of PGs (Ham *et al.*, 1975; Thaler-Dao *et al.*, 1982; Schatz *et al.*, 1985; Gupta *et al.*, 1989).

The intraluminal instillation of cAMP (AMP or dbcAMP) induced blastocyst implantation (Holmes and Bergstrom, 1975; Webb, 1975, 1977; Fernandez-Noval and Leroy, 1978), suggesting that cAMP might be involved in PG synthesis. Furthermore, exposure of cells to cAMP results in increased release of arachidonic acid, culmi-

nating in PGs production and this phenomenon has been described in thyroid (Burke *et al.*, 1972), ovary (Marsh *et al.*, 1974) and platelets (Dayal *et al.*, 1983).

The present study was, therefore, attempted to elucidate the mechanism by which estradiol modulates uterine PGs synthesis during the implantation period in rats by employing delayed implantation model.

MATERIALS AND METHODS

Materials

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

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 μg/0.1 ml in sesame oil) on day 8. Until the treatment of estradiol on day 8, blastocysts did not implant and were floating in uterine lumen. Delayed implantation occurred by 24 hrs after estradiol treatment and this group was served as a control. Second group was treated with 50 μl of 50 mM dbcAMP dissolved in PBS (0.01 M phosphate, 0.15 M NaCl, pH 7.0) by an intrauterine instillation without estradiol treatment on day 8 of pregnancy. In third group, 100 mM theophylline (100 ul/uterine horn) was administered by an intrauterine instillation at 2 hrs prior to estradiol administration. Fourth group was treated with indomethacin (2 mg/0.2 ml in sesame oil) as well as estradiol 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 μg/ml). The 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.

Methods

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 (3:3:1, 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 transfered to a test tube and dried in vortex evaporator. The residue was dissolved in gel Tris buffer. Concentrations of PGE and $PGF_{2}\alpha$ were measured by radioimmunoassay (Clinical Assays, USA).

Determination of uterine cAMP: Uterine tissues were homogenized in 1 ml of 4 mM EDTA (pH 7.4) at 4°C and 1 ml of 6% TCA was added. The homogenate was centrifuged at 1,000 × g (4°C) for 25 min and supernatant was added to water-saturated ethyl ether. The mixture was stoot at room temperature for 30 min and stored at −70°C. The aqueous layer containing cAMP was freeze-dried and dried residue was dissolved in Tris/EDTA buffer (50 mM Tris-HCl, 4 mM EDTA, pH 7.5). cAMP concentration was determined by competitive protein binding assay (Amersham, UK).

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

Effect of estradiol on uterine prostaglandin synthesis during the delayed implantation process

To determine factors that regulate PGs production in uterus during the implantation period, we employed delayed implantation model. The treatment of progesterone-only after ovariectomy did not induce implantation but blastocysts were floating in the uterine lumen. If we injected estradiol to these rats on day 8, delayed implantation occurred within 24 hrs after estradiol treatment.

The administration of estradiol sharply increased uterine cAMP concentration 4 hrs later ($p<0.05$), decreasing cAMP concentration thereafter. Concentrations of both uterine PGE and $PGF_{2}\alpha$ were increased at 12 hrs after estradiol

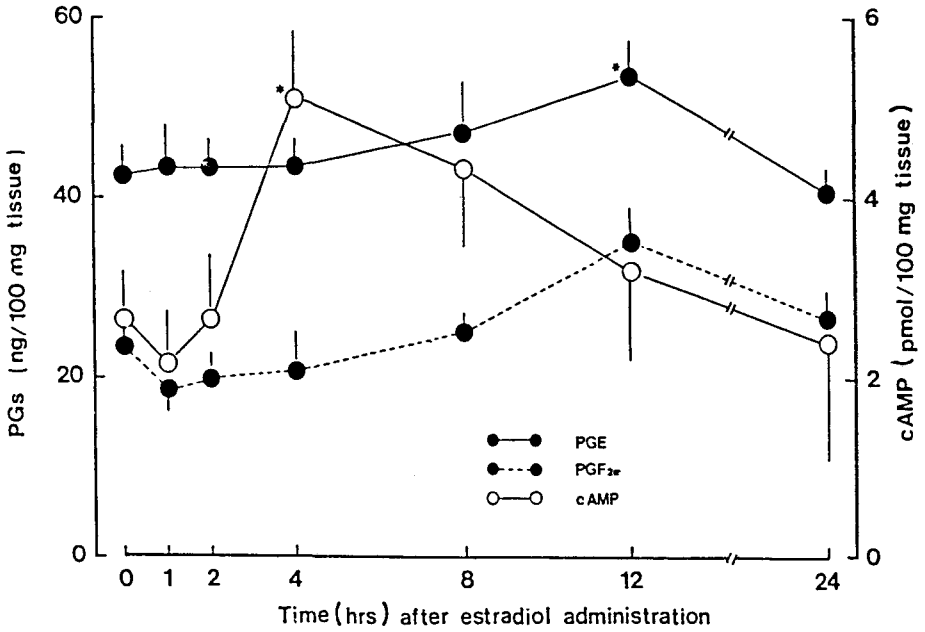

Fig. 1. *Uterine PG and cAMP concentrations during the delayed implantation process.* Data are expressed as mean ±SEM of 6 to 8 animals. *$p<0.05$ as compared with the value at 0 hr.

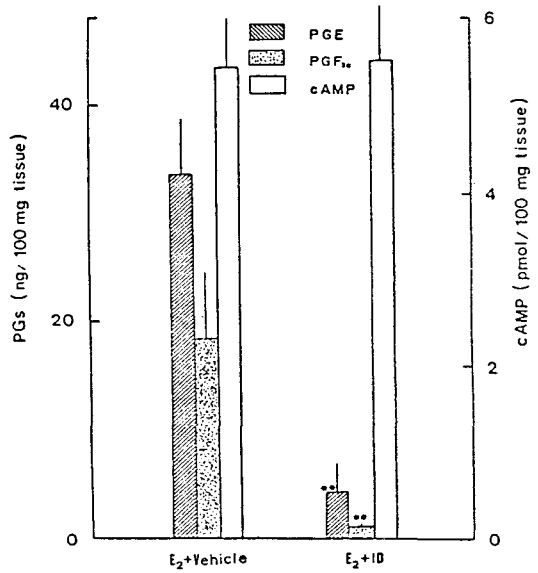

Fig. 2. Effects of indomethacin on uterine PGs and cAMP concentrations at 12 hrs following estradiol administration during the delayed implantation process. Data are expressed as mean±SEM of 6 to 8 animals. **$p<0.01$ compared with the value of E_2 plus vehicle treated group. E_2 (estradiol): ID (indomethacin).

Table 1. Induction of delayed implantation by estradiol-17β and dbcAMP

Treatments	N	Implantation incidence(%)	Implant sites
Estradiol-17β	6	67	7.8±1.4
Sesame oil	4	0	–
dbcAMP			
12.5 mM	4	50	4.6±1.1
25 mM	4	75	6.9±0.7
50 mM	4	100	8.2±0.7
100 mM	4	50	6.1±0.9
PBS	4	0	–

administration and an increase in PGE was statistically significant (Fig. 1). However, the simultaneous treatment of indomethacin with estradiol significantly suppressed both PGE and PGF$_{2\alpha}$ levels at 12 hrs after estradiol administration ($p<0.01$), while uterine cAMP concentration was not affected (Fig. 2).

Levels of PGE and PGF$_{2\alpha}$ were greater in the implant sites than in the non-implant sites of uterus at 24 hrs following estradiol adminis-

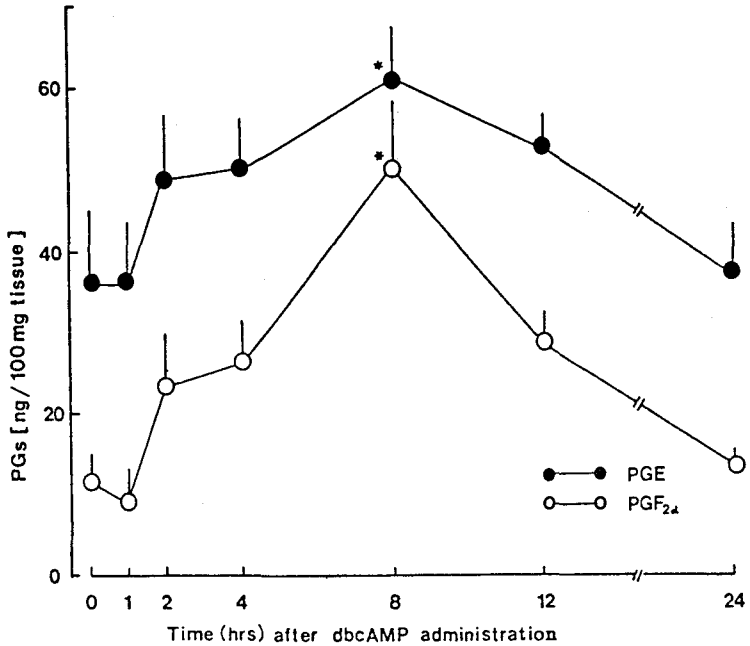

Fig. 3. Uterine PG concentrations during the delayed implantation process. Data are expressed as mean±SEM of 6 to 8 animals. *$p<0.05$ as compared with the value at 0 hr.

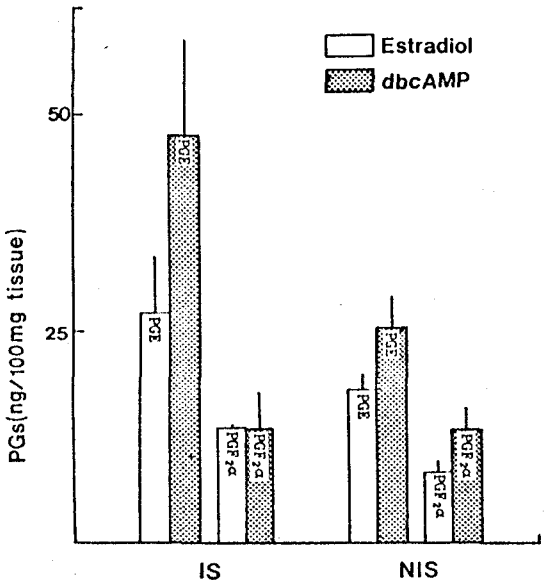

Fig. 4. PGs concentrations in the implant (IS) and the non-implant sites (NIS) of uterus at 24 hrs following estradiol or dbcAMP administration during the delayed implantation process. Data are expressed as mean±SEM of 6 to 8 animals. *$p<0.05$ as compared with the value of the non-implant sites.

tration (Fig. 4).

Modulation of uterine prostaglandin synthesis by cAMP during the delayed implantation process

To determine whether cAMP mediates the action of estradiol in uterine PG synthesis during the process of delayed implantation, different concentrations of dbcAMP were administered instead of estradiol (Table 1). The treatment of vehicle (sesame oil) for estradiol on day 8 did not induce implantation. When estradiol was administered on day 8, implantation incidence was 67 % and the number of the implant sites per uterus was 7.8. Administration of dbcAMP mimicked the effect of estradiol on implantation incidence and implant sites. While the treatment of vehicle (PBS) for dbcAMP showed no implantation incidence, 50 mM dbcAMP induced delayed implantation in 100% of animals.

To confirm if dbcAMP induced delayed implantation by stimulating uterine PGs synthesis, uterine PGs concentration were measured after dbcAMP administration instead of estradiol (Fig. 3). Concentrations of both PGE and $PGF_{2}\alpha$ were gradually increased, reaching the maximum at 8 hrs after dbcAMP treatment ($p<0.05$). This max-

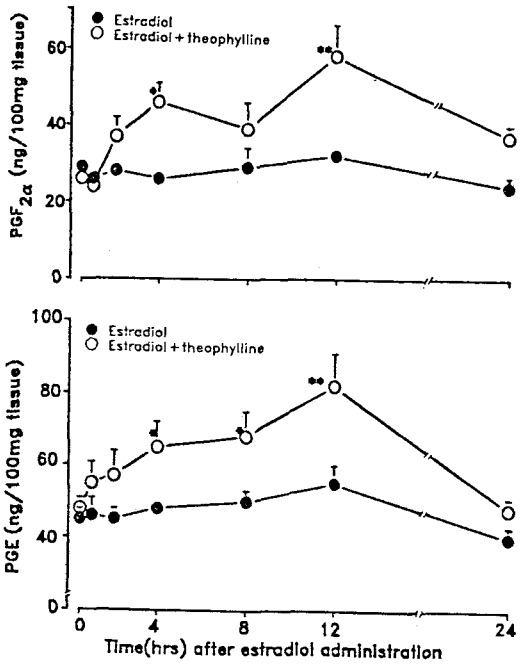

Fig. 5. Effect of theophylline on estradiol-induced PGs synthesis during the delayed implantation process. Theophylline was pretreated at 2 hrs prior to estradiol administration. Data are expressed as mean±SEM of 6 to 8 animals. *p <0.05, **p<0.01 as compared with the value of estradiol-only treated group.

imum increase occurred 4 hrs earlier, compared with estradiol-treated group. PGE concentration was significantly greater in the implant sites than in the non-implant sites of uterus at 24 hrs following dbcAMP administration (Fig. 4, $p < 0.05$). This result indicates that dbcAMP mimics the effect of estradiol on uterine PG synthesis, strongly suggesting that estradiol-dependent PGs synthesis is mediated by cAMP during the delayed implantation process.

Furthermore, the pretreatment of theophylline, phosphodiesterase inhibitor, at 2 hrs prior to estradiol administration significantly increased concentrations of uterine PGE and $PGF_{2}\alpha$ ($p < 0.01$) at 12 hrs after estradiol administration as compared with estradiol-only treated group during the delayed implantation process (Fig. 5).

DISCUSSION

In the present study, delayed implantation model was employed to determine factors that modulate PGs synthesis in uterus during the implantation process. Administration of estradiol on day 8 significantly increased uterine PGE concentration at 12 hrs after estradiol administration. This result is consistent with other reports that a single injection of estrogen following 2 days of progesterone priming increases PGE and $PGF_{2}\alpha$ concentrations (Dey *et al.*, 1982; Pakrasi *et al.*, 1983) and PGs output can be inhibited by the treatment of antiestrogen (Fenwick *et al.*, 1980; Schatz *et al.*, 1986). Estradiol administration increased uterine concentration of cAMP 8 hrs earlier than increases in PGs concentrations, suggesting that cAMP may be involved in the estradiol-induced uterine PGs synthesis. However, the simultaneous treatment of estradiol with indomethacin suppressed PGs synthesis but did not affect estradiol-induced cAMP increase, confirming that uterine PGs is newly synthesized by the treatment of estradiol.

Moreover, administration of dbcAMP without estradiol on day 8 showed similar effect to estradiol on implantation incidence and number of the implant sites. Uterine concentrations of both PGE and $PGF_{2}\alpha$ were gradually increased reaching the maximum at 8 hrs after dbcAMP administration. This increase in PGs concentrations was observed 4 hrs earlier than an increase induced by estradiol treatment. Levels of PGE and $PGF_{2}\alpha$ were greater in the implant sites than in the non-implant sites of uterus at 24 hrs following dbcAMP administration during the delayed implantation process. Furthermore, the pretreatment of theophylline prior to estradiol induced greater concentrations of uterine PGE and $PGF_{2}\alpha$ compared with estradiol-only treated group. These results indicate that dbcAMP mimics the effect of estradiol on uterine PGs synthe-sis. It was reported that administration of cAMP or dbcAMP into the uterine lumen stimulated metabolism and implantation of the experimentally delayed blastocyst (Holmes and Bergstrom, 1975; Webb, 1977; Fernandez-Noval and Leroy, 1978). Report that alloxan, an adenylate cyclase inhibitor, significantly reduced PGE and PGF

levels on days 5 and 6 of pregnancy in rats suggests a possible relationship between cAMP and PGs synthesis in the process of implantation in rats (Garg *et al.*, 1979). Moreover, it was reported that cAMP induced PGs synthesis in several cell types (Lindgren *et al.*, 1978; Dayal *et al.*, 1983; Baker *et al.*, 1985). The mechanism by which estradiol increases cAMP levels in uterine tissues has not been determined, but evidence has been accumulated that estradiol might act on plasma membrane, thereby activating adenylate cyclase via unknown mechanism (Finidori-Lepicard *et al.*, 1981; Sadler and Maller, 1981; Bergamini *et al.*, 1985).

In conclusion, the present study has demonstrated that estradiol stimulates uterine PGs synthesis via cAMP mediation during the delayed implantation process in rats.

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= 국문초록 =

흰쥐의 착상지연과정중 Estradiol에 의한 자궁내 Prostaglandin 생합성에 미치는 cAMP의 영향

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본 연구에서는 흰쥐의 착상지연을 유도하여 착상기간동안 자궁조직내 prostaglandin (PG) 생합성이 어떠한 인자에 의해서 조절되는가를 관찰하여 다음과 같은 결과를 얻었다.

흰쥐의 착상지연과정동안 estradiol을 처리하면 처리후 4시간만에 자궁조직내의 cAMP의 농도가 급격하게 증가하였다. PGE와 PGF$_{2\alpha}$의 농도는 estradiol을 처리한 후 12시간이 경과하였을때 증가하였으나 PGF$_{2\alpha}$의 증가는 통계적으로 유의하지는 않았다. 또한 indomethacin을 estradiol과 동시에 처리하면 estradiol 처리로 인한 PGE와 PGF$_{2\alpha}$의 농도 증가는 나타나지 않았으나 cAMP 농도는 증가하였다. dbcAMP를 처리하면 자궁내 PGE 및 PGF$_{2\alpha}$의 농도가 증가하기 시작하여 estradiol이 투여시에 비하여 4시간 빨리 8시간후에 최고치에 도달하였으며 phosphodiesterase inhibitor인 theophylline을 전처치하면 estradiol만 투여한 것에 비하여 자궁조직내 PGE 및 PGF$_{2\alpha}$의 농도가 유의하게 증가하였다. 이상의 결과로 보아 흰쥐의 착상지연과정동안 estradiol이 자궁의 prostaglandin 합성을 증가시키며 이러한 증가는 cAMP의 증가를 매개하는 것으로 생각된다.