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Effect of the Prostaglandin E_2, $F_2\alpha$ and Indomethacin on the Incorporation of ^{3}H-Methionine in Rat Blastocysts Development

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Abstruct: *The effect of the prostaglandin E_2, $F_2\alpha$ and indomethacin on the incorporation of ^{3}H-Methionine in rat blastocyst development was studied by the method of liquid scintillation technique. The eggs of intact pregnant rats when they were on the morula, early blastocyst, blastocyst, and late blastocyst stages were collected. Blastocysts of delayed implantation rats were also collected on the day 6 and 8. The rate of implantation of ^{3}H-Methinine in delayed blastocysts was smaller than in normal blastocysts. Addition of prostaglandin E_2 or $F_2\alpha$ had accelerated the incorporation of ^{3}H-Methionine in blastocysts at preimplantation and during delayed implantation ($P<0.05$). But addition of indomethacin inhibited the incorporation of ^{3}H-Methionine in blastocyst and delayed implantation on the day 6 and 8 ($P<0.05$).*

Key words: *Rat blastocyst, PGE_2, $PGF_2\alpha$, Indometacin, Methionine.*

In rats, differentiation of endometrial stromal cells into decidual cells occurs in response to the presence of the blastocyst [1]. An increase in endometrial vascular permeability always precedes the differentiation of decidual cells [1]. Large number of evidences have accumulated indicating that prostaglandins (PGs) have an obligatory role in the endometrial vascular permeability response and are also involved during the differentiation of decidual cells [2]. Indomethacin, an inhibitor of PG synthesis, prevents implantation in rats and mice and reduces the number of implantation sites and prevents the uterine blueing response [6, 7]. It is possible to reverse this effect by subcutaneous injections of PGE_2 or $PGF_2\alpha$ [3–5].

PG synthesis in blastocysts of rabbit [8, 9], hamster [10], mice [11], cows [12] and sheep [13] has been reported. In addition, PG synthesis by endometrium has been established for sheep [13], cows [12], humans [14], and rabbits [9]. It would appear that under a variety of conditions, both blastocysts and endometrium have a potential role for PG synthesis. For blastocyst growth, hatching, attachment and subsequent outgrowth of rat and mouse embryo *in vitro* there have some specific amino acid requirements [1, 19]. Protein synthesis by preimplantation rat embryos, as inferred from the incorporation of amino acid *in vitro* [16, 17]. On the other hand, it is indicated that maturational changes of blastocyst is usually dependent on protein synthesis from precursors in the environment [18, 20, 23]. Therefore, I have planned to explore the effect of PG E_2, $F_2\alpha$ and indomethacin on the incorporation of ^{3}H-Methionine in rat blastocysts.

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Materials and Methods

Adult virgin female rats of Wistar strain weighing from 190 to 250 g were used. They were maintained under controlled lighting condition (12 h light: 12 h darkness; lights on at 6:00 h). Animals were allowed to take pelleted-diet and water freely. Vaginal smears were taken in the morning for assessment of proestrus and graded. The animals were divided into two groups. One consisted of intact, pregnant animals and the other was experimental group. They were mated with the same strain males (which were to be fertile) on the evening of proestrus. The day when spermatozoa were detected in the vaginal smear was considered the day 1 of pregnancy.

Eggs were collected by flushing oviduct or uterus with culture medium at following stages: morula on the morning of day 4; early blastocyst on the early morning of day 5; blastocyst at noon of day 5; late blastocyst on the evening of day 5. The treatment group consisted of pregnant rats in which artificial delayed implantation was induced by the method described previously by Cochrane

and Meyer [15]. They were ovariectomized on the day 3, and then got injection of progesterone (2 mg/animal/day until autopsy). The blastocysts were collected by flushing uterine horns on the day 6 (about 126 h after fertilization) and on the day 8 (about 174 h after fertilization). BMOC-II, which contained polyvinylpyrrolidone (1 mg/ml) used as the basic medium.

The normal blastocysts were selected and transferred into 0.1 ml of culture medium in a microtube. I took out 20 blastocysts per tube. After preincubation at 37.5°C for 5 min, 0.1 ml of each BMOC-II, PGE_2, $PGF_2\alpha$ or indomethacin, and 18.5 kBq/ml (spec. act. 31.5 MBq/mM) ^{3}H-Methionine were added. The final concentrations of PGE_2, $PGF_2\alpha$ and indomethacin were 10, 1 and 10 μg/ml, respectively. Nine to ten tubes were observed in each case. These samples were incubated for 0 or 1 h. At the end of each incubation, cold TCA was added in each aliquot to a fluid concentration of 5% and it was heated at 80°C for 30 min. Acid-insoluble part was washed with 5% TCA and 90% ethyl alcohol. The filter was dried completely under an infrared lamp and incorporation of ^{3}H-Methionine in to protein were measured in a scintillation counter (Pakcard).

Data were analyzed by a Student's t-test. A value of $P \leq 0.05$ was considered statistically significant.

Results

Effect of PG E_2 and $F_2\alpha$ on the incorporation of ^{3}H-Methionine in morula, blastcyst and delayed blastocyst is shown in Fig. 1. The incorporation of ^{3}H-Methionine tended to increase according to the development of eggs from morula to blastocyst stage in intact rats. The addition of PG E_2 and $F_2\alpha$ had no effect on incorporation of ^{3}H-Methionine at morula and early blastocyst stage, but had the significant effect on incorporation of ^{3}H-Methionine at blastocyst, late blastocyst and delayed blastocyst on day 6 and day 8 stage ($P<0.05$).

Effect of indomethacin on the incorporation of ^{3}H-Methionine in morula, blastocyst and delayed blastocyst is shown in Fig. 2. Indomethacin when added to the medium slightly but not significantly decreased the ^{3}H-Methionine incorporation at morula and early blastocyst stages. But the addition of indomethacine inhibited the incorporation of ^{3}H-Methionine in late and delayed blastocyst (day 6, 8) ($P<0.05$).

Discussion

Incorporation of ^{3}H-Methionine was two times higher in late blastocyst than morula stage in intact rats. This result is in accordance with the previous reports [16, 17]. Incorporation of ^{3}H-Methionine in blastocyst on day 6 and 8 were also lower compared with each stage of normal rats but the difference was not significant. Similar results were showed by the incorporation of ^{14}C-glutamic acid in rat blastocysts during delayed implantation [20]. Weitlauf *et al.* [18, 19] have observed

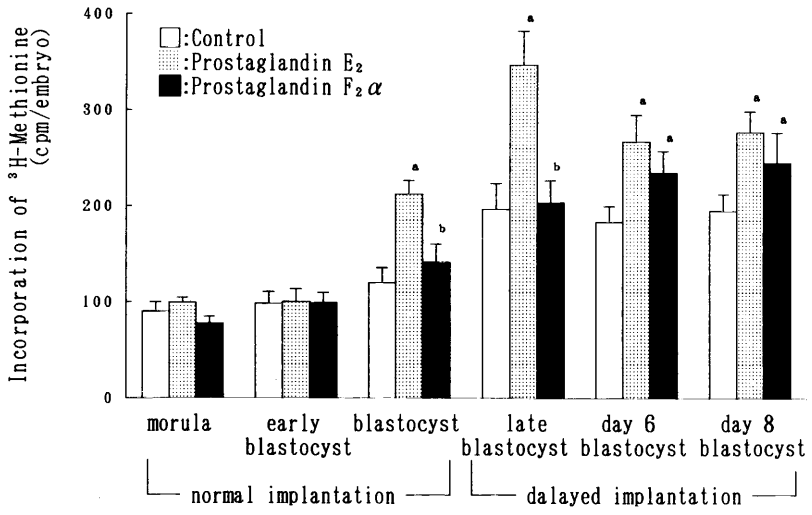

Fig. 1. Effect of prostaglandin E_2 and $F_2\alpha$ on the incorporation of ^{3}H-Mathionine in rat blastocyst during delayed implantation. Each value represents the mean ± S.D. [a]Significantly different from control in same stage ($P<0.05$). [b]Significantly different from PGE_2 in same stage ($P<0.05$).

that the incorporation of ^{35}S-methionine in mice blastocyst decreases greatly during delayed implantation. The tendency to decrease in the synthesis of DNA, RNA, and protein of rat blastocyst has also been observed during delayed implantation [20–23]. Blastocysts were considered to remain in a state of metabolic dormancy during delayed implantation.

The adminstration of PG E_2 and $F_2\alpha$ significantly increased the incorporation of ^{3}H-Methionine in blastocyst and late blastocyst in normal rats. Similarly, the incorporation of ^{3}H-Methionine was increased by PG E_2 and $F_2\alpha$ in blastocysts during delayed implantation. On the other hand, the effect of PG $F_2\alpha$ on the incorporation of ^{3}H-Methionine was significantly ($P<0.05$) decreased in blastocyst and late blastocyst when compared to PG E_2. The cause of this phenomenon is not clear. Although, PG E_2 is known to be the predominant product synthesized from the action of PG synthetase, and among the PG $F_2\alpha$ has frequently identified in significant amounts within the reproductive tract.

Jones and Harper [24] reported that rabbit blastocysts accumulated progressively more PG with the increase of age from 5 to 6.8 days. The amounts of PGE_2 and $PGF_2\alpha$ were reported to be approximately equal. Other reports indicated that PG synthesis occured at blastocyst stage. I suggest that rat blastocyst can synthesize and utilize PGs and that the incorporation of ^{3}H-Methionine as well as protein synthesis of blastocysts was accelerated by PG E_2 and $F_2\alpha$. These results were similar to the fact that a single dose of 4 μg estrone had an effect on the incorporation of ^{14}C-glutamic acid during delayed implantation, and it increased rapidly until 24 to 30 h after the treatment [23]. Uterine PG synthesis and/or metabolism is under the control of ovarian steriods, i.e. estrogen increases the uterine synthesis of PG. Administration of 17β-estradiol antiserum or antiestrogen is associated with a reduction in PG production. Progesterone, on the contrary, appears to be inhibitory in this regard, when given either alone or in combination with estrogen [25]. So, it might be suggested that PG synthesis of blastocyst is under the control of ovarian steriod.

At low physiological concentrations, PGE_2 and $PGF_2\alpha$ lowered cellular cAMP levels, and at higher concentrations, the production of cAMP was increased [26]. PG E_2 and $F_2\alpha$ when added to culture medium, modify the activities of enzymes, most of which are membrane-bound, such as PG cyclo-oxygenenase, PG dehydrogenase, thromboxane synthetase, adenylate cyclase, gunylate cyclase, and some of protein kinases [27]. We could assume that such compounds alter the activities of these enzymes through a common mechanism, such as an interaction with calmodulin, a calcium-dependent modulator protein [28].

Inhibition of the incorporation of ^{3}H-Methionine in blastocyst by indomethacin is known to suppress PG synthesis. In conclusion, the results presented in this paper suggest that PGE_2 and $PGF_2\alpha$ promote the meta-

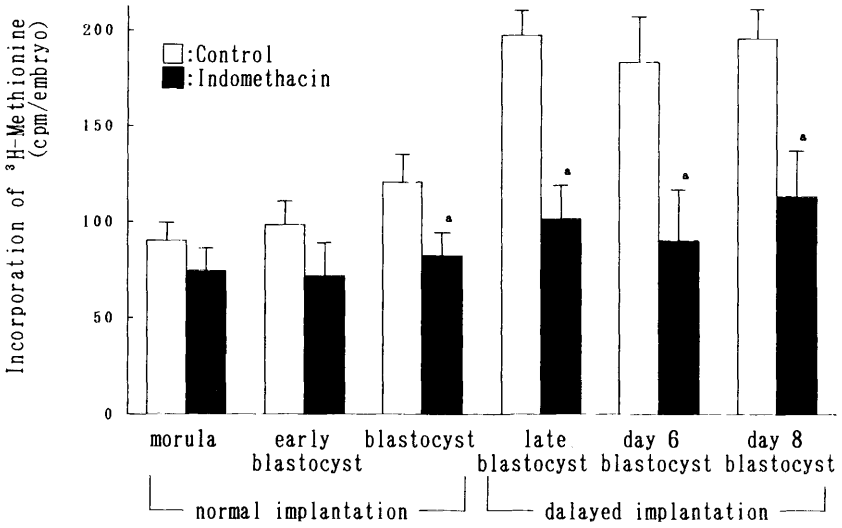

Fig. 2. Effect of indomethacin on the incorporation of ^{3}H-Methionine in rat embryo. Each value represents the mean ± S.D. [a]Significantly different from control in same stage ($P<0.05$).

bolic activity in blastocysts before and during delayed implantation. So, it may be suggested that PG E_2 or $F_2\alpha$ may be useful in culture medium for mammalian embryo transfer.

References

1) Psychoyos, A. (1973): Endocrine control of egg implantation. In: Handbook of Physiology, Section 7. Vol. 2. Part 2 (Greep, R.O., Astwood, E.G. and Geiger, S.R., eds.), pp. 187–215. American Physiological Society, Bethesda, M.D.
2) Kennedy, T.G. (1979): Prostaglandins and increased endometrial vascular permeability resulting from the application of an artificial stimulus to the uterus of the rat sensitized for the decidual cell reaction. Biol. Reprod., 20: 560–566.
3) Lau, I.F., Saksena, S.K. and Chang, M.C. (1973): Pregnancy blockage by indomethacin, an inhibitor of prostaglandin synthesis: its reversal by prostaglandins and progesterone in mice. Prostaglandins, 4: 795–803.
4) Kennedy, T.G. (1977): Evidence for a role for prostaglandins in the initiation of blastocyst implantation in the rat. Biol. Reprod., 16: 286–291.
5) Tsujii, H. and Moro, M. (1994): Effect of indomethacin and prostaglandins on decidual cell reaction and embryo implantation in mouse. Jpn. J. Fertil. Steril., 39: 314–321.
6) Hoffman, L.H. (1978): Antifertility effects of indomethacin during early pregnancy in the rabbit. Biol. Reprod., 18: 148–153.
7) Tsujii, H. (1984): Studies on the delayed implantation of blastocysts in the rat treated with indomethacin. J. Faculty Agri. Shinshu Univ., 21: 49–58.
8) Pakrasi, P.L. and Day, S.K. (1982): Blastocyst is the source of prostaglandins in the implantation site in the rabbit. Prostaglandin, 24: 73–77.
9) Harper, M.J.K., Norris, C.J. and Rajkumar, K. (1983): Prostaglandin release by zygotes and endometria of pregnant rabbit. Biol. Reprod., 28: 350–362.
10) Niimura, S. and Ishida, K. (1978): Histochemical studies of prostaglandin dehydrogenase in hamster eggs. Jpn. J. Anim. Reprod., 24: 16–18.
11) Niimura, S. and Ishida, K. (1987): Immunohistochemical demonstration of prostaglandin E_2 in preimplantation mouse embryos. J. Reprod. Fert., 80: 505–508.
12) Lewis, G.S., Thatcher, W.W., Bazer, F.W. and Curl, J.S. (1982): Metabolism of arachidonic acid *in vitro* by bovine blastocysts and endometrium. Biol. Reprod., 27: 431–439.
13) Hyland, J.H., Manns, J.G. and Humphrey, W.D. (1982): Prostaglandin production by ovine embryos and endometrium *in vitro*. J. Reprod. Fertil., 65: 299–304.
14) Tsang, B.K. and Ooi, T.C. (1982): Prostaglandin secretion by human endometrium *in vitro*. Am. J. Obstet. Gynecol., 142: 626–629.
15) Cochrane, R.L. and Meyer, R.K. (1957): Delayed nidation in the rat induced by progesterone. Proc. Soc. Exp. Med., 96: 155–159.
16) Tsujii, H., Sugawara, S. and Takeuchi, S. (1971): Incorporation of ^{14}C-L-Leucine in rat eggs of preimplantation stage. III Effects of addition of lactate and succinate. Tohoku J. Agri. Res., 22: 31–35.
17) Tsujii, H., Sugawara, S. and Takeuchi, S. (1972): Incorporation of ^{14}C-L-glutamic acid in rat eggs of preimplantation stages. Jpn. J. Zootech. Sci., 43: 91–96.
18) Weitlauf, H.M. and Greenwald, G.S. (1965): A comparison of ^{35}S-Methionine incorporation by the blastocysts of normal and delayed implanting mice. J. Reprod. Fert., 10: 203–208.
19) Weitlauf, H.M. (1969): Temporal changes in protein synthesis by mouse blastocysts transferred to ovariectromized recipients. J. Exp. Zool., 171: 481–486.
20) Tsujii, H., Sugawara, S. and Takeuchi, S. (1972): Effects of estrone on incorporation of ^{14}C-glutamic acid in rat blastocyst during delayed implantation. Jpn. J. Zootech. Sci., 43: 659–665.
21) Prasad, M.R.N., Dass, C.M.S. and Mohla, S. (1968): Action of oestrogen on the blastocyst and uterus in delayed implantation —an autoradiographic study. J. Reprod. Fert., 16: 97–104.
22) Jacobson, M.A., Sanyal, M.K. and Meyer, R.K. (1970): Effect of estrone on RNA synthesis in preimplantation blastocysts of gonadotrophin-treated immature rats. Endocrinology, 86: 982–987.
23) Sanyal, M.K. and Meyer, R.K. (1970): Effect of estrone on DNA synthesis in preimplantation blastocysts of gonadotrophin-treated immature rats. Endocrinology, 86: 976–981.
24) Jones, M.A. and Harper, M.J.K. (1984): Rabbit blastocysts accumulate [^{3}H]-prosta-glandins *in vitro*. Endocrinology, 115: 817–823.
25) Castracane, V.D. and Jordan V.C. (1975): The effect of estrogen and prosgesterone on uterine prostaglandin biosynthesis in the ovariectomized rat. Biol. Reprod., 13: 587–596.
26) Molnar, M., Asem, E. and Hertelendy, F. (1987): Differential effects of prostaglandin $F_2\alpha$ and of prostaglandin E_1 and E_2 on cyclic 3', 5'-monophosphate production and intracellular calcium mobilization in avian uterine smooth muscle cells. Biol. Reprod., 36: 384–391.
27) Needleman, P., Bryan, B., Wyche, A., Bronson, S.D., Eaking, K., Ferredelli, J.A. and Minkes, M. (1977): Thromoxane synthetase inhibitors as pharmacological tools: Differential biochemical and biological effects on platelet suspensions. Prostaglandin, 14: 897–907.