THE ROLE OF UTERINE TISSUE IN CHANGES OF PROSTAGLANDIN CONTENT IN THE RAT OVARY

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Abstract—The role of uterine tissue in the changes of rat ovarian prostaglandin (PG) content was investigated. PGE and PGF levels in rat ovary were decreased to about one-fourth of control levels by hysterectomy, when the contents were determined on the day of estrus 4 days after surgery. Both the PGE and PGF contents in diestrous ovary 10 to 14 days after hysterectomy tended to be decreased, as compared with those in intact control (diestrus), but no significant difference was observed. Administration of estradiol to hysterectomized rats on the day of diestrus increased neither PGE nor PGF levels. Also with the administration of pregnant mare serum gonadotropin, no increase in the PG contents was seen in the ovary of hysterectomized rats. The incorporation of 3H-arachidonic acid into the 3H-PGE and 3H-PGF fractions, expressed in terms of the organ, was much greater in the uterus than in the ovary. The incorporation of 3H-arachidonic acid into the 3H-PGE and 3H-PGF fractions in the ovary was higher on the day of estrus than on the day of diestrus. These results strongly suggest that the changes in the PG content in rat ovary may be regulated, at least in part, by uterine tissue.

It is well known that prostaglandin (PG) derived from uterus, especially PGF2α, causes luteal regression in many species of animals (1–4). It has been observed that the PGE and PGF contents in uterus were significantly increased by the administration of estrogen to ovariectomized rats (5, 6) and that the PGF content in the uterus of intact rats was increased on the day of proestrus when endogenous estrogen showed high levels (7). Gengenbach et al. (8) reported that the simultaneous treatment with estrogen and PGF2α brought about complete luteal regression in sheep. Furthermore, it has been found that the estradiol level in rat ovary was gradually increased when PGF2α was injected into uterine lumen (9). These results suggest that estrogen and PG may interact in reproductive function.

We recently reported that the PGF content in rat ovary was significantly increased after treatment with estradiol and pregnant mare serum gonadotropin (10). In the present study, the role of uterine tissue in the changes of rat ovarian PG content was investigated and its importance was confirmed.

MATERIALS AND METHODS

Female Wistar strain rats, weighing about 200 g and with a 4-day estrous cycle, were housed under constant temperature (24°C) and the lighting regimen of 12 hr light and 12 hr dark. Water and food were always
available. Rats hysterectomized on the day of estrus were sacrificed on the day of estrus 4 days after surgery. Rats hysterectomized on the day of diestrus were randomly assigned to the experimental or control groups 10 to 14 days after surgery. Twenty µg of estradiol was dissolved in sesame oil and 50 IU of pregnant mare serum gonadotropin (PMS, Teikoku Zoki Pharmaceutical Co.) was dissolved in 0.9% saline. Each hormone was given to the hysterectomized rats s.c. on the first day of diestrus. Ovaries from each rat were removed 24 hours after each hormonal injection. The ovarian PG was extracted and separated into PGE and PGF, as previously described (11, 12); that is the ovaries were homogenized in acidified alcohol, and the homogenate was centrifuged at 3,000 rpm for 15 min. The total PG fraction in the supernatant was extracted with ethyl ether, and then the ether phase was evaporated to dryness under a stream of nitrogen. The residue was separated into the PGE and PGF fractions by silica-gel column chromatography. Each fraction was determined by bioassay using rat stomach fundus (13).

The incorporation of 3H-arachidonic acid ([5, 6, 8, 9, 11, 12, 14, 15-3H]-arachidonic acid, 64 Ci/m mol, New England Nuclear) into the 3H-PG fraction in the ovary and uterus of the same rat was compared by using two diestrous rats and three estrous rats, and then these tissues of each rat were prepared into 8 fragments. The incorporation of 3H-arachidonic acid into the PG fraction in the ovary was compared between diestrous rats and estrous rats. The tissue fragments were incubated in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1 µCi of 3H-arachidonic acid at 37°C for 60 min under 95%O2 - 5%CO2. After incubation, each tissue was homogenized in 1 ml of water and washed with petroleum ether, and then the total PG fraction was extracted with 2 ml of acetonitrile. The acetonitrile phase containing the total PG fraction was evaporated to dryness under a stream of nitrogen (14). The residue was separated into the PGE and PGF fraction by thin-layer chromatography (15). Each spot on the plate corresponding to the authentic PGE2 and PGF2α was visualized by exposure to iodine vapour, scraped off and placed in a scintillation vial containing 10 ml of Aquasol-2 scintillator solvent (New England Nuclear). The radioactivity of each spot was counted using a liquid scintillation spectrometer.

RESULTS

Influence of hysterectomy on PG content in rat ovary: Figure 1 shows the effect of hysterectomy on PG content in rat ovary. While the PGE level in the ovary of intact control (estrous) was 2.5±0.4 ng/ovary and the PGF level 4.2±1.7 ng/ovary, the PGE level in the estrous ovary of hysterectomized rat was 0.7±0.05 ng/ovary and the PGF level 1.2±0.2 ng/ovary. Both the PGE and PGF levels were decreased to about one-fourth of control levels (p<0.01 and p<0.05.
respectively).

On the other hand, the PGE level in the ovary of intact control (diestrus) was 0.6±0.1 ng/ovary, and that of the hysterectomized group was 0.3±0.04 ng/ovary (Fig. 2). However, there was no significant difference, although the PGE level in the ovary of hysterectomized rat tended toward a decrease. The PGF level in the ovary of intact control (diestrus) was 0.7±0.05 ng/ovary, and that of the hysterectomized group was 0.5±0.1 ng/ovary (Fig. 3). There was no significant difference between intact control and the hysterectomized group, with regard to PGE levels.

Effects of estrogen and PMS on PG content in hysterectomized rat ovary: The PGE and PGF levels in the hysterectomized ovary of rats treated with estradiol and PMS are shown in Figs. 2 and 3. Although the PGE level in the ovary of hysterectomized rat treated with estradiol was increased about 1.8 fold as compared with that of hysterectomized control, there was no statistically significant difference 24 hours after treatment (Fig. 2). Estradiol also had no effect on the PGF level in the ovary of hysterectomized rat; that is, PGF level in the treated group was 0.5±0.07 ng/ovary and PGF level in control group 0.5±0.1 ng/ovary (Fig. 3). Both the PGE and PGF levels in the ovary of hysterectomized rat treated with estradiol were lower than in the intact control. The treatment with PMS tended to decrease both the PGE and PGF levels as compared with those of the non-medicated hysterectomized group. The PGE level in the ovary of hysterectomized rat treated with PMS was 0.2±0.02 ng/ovary and PGF level was 0.3±0.06 ng/ovary.

Incorporation of arachidonic acid into PG fraction in rat ovary and uterus: Figure 4 shows the incorporation of 3H-arachidonic acid into the 3H-PGE and 3H-PGF fractions in rat ovarian and uterine tissues. The incorporation of arachidonic acid into the PGE fraction was higher both in ovary and in uterus than into the PGF fraction. The incorporation of arachidonic acid into the PGE and PGF fraction expressed in terms of the organ was much higher in uterus than in ovary.

Incorporation of arachidonic acid into PG fraction in diestrous and estrous rat ovary: Figure 5 shows the results of the incor-
poration of 3H-arachidonic acid into the 3H-PGE and 3H-PGF fractions in the diestrous and estrous ovary of rats. The incorporation into both the PGE and PGF fractions on the day of estrus was higher than on the day of diestrus, and the incorporation into the PGE fraction on the day of estrus was significantly different from that in diestrus (p<0.05); that is, the incorporation on the day of estrus was 379±35 dpm/ovary, while that on the day of diestrus was 268±29 dpm/ovary.

**DISCUSSION**

We recently reported that the PGF content in the ovary of intact rat was significantly increased by treatment with estrogen, and suggested that uterine tissue may play an important role in the changes of the PG content in rat ovary (10). In the present study, hysterectomy was carried out on the day of estrus when both the PGE and PGF levels in the ovary of intact rat were high, and on the day of diestrus when both the levels were the lowest (10). As expected, the PGE and PGF levels in the ovary of hysterectomized rat were decreased to one-fourth of intact control levels (Fig. 1), suggesting that the changes in ovarian PG levels may be regulated by the uterine tissue. Ham et al. (7) found that the uterine PGF level was highest on the day of proestrus when the excretion of estrogen reached the maximum, and we have also confirmed that the uterine PG in the ovariectomized rat was increased by a single injection of estrogen (6). On the basis of these data, estradiol and PMS were given on the day of diestrus to hysterectomized rats, exhibiting a regular 4-day estrous cycle after the surgery. As estradiol and PMS could not increase the PG content in the ovary of hysterectomized rat (Figs. 2 and 3), and as Blatchley et al. (16) reported that PGF in utero-ovarian vein could not be detected in the hysterectomized guinea-pig, the increasing effect of these hormones on the PG content previously observed in the ovary of intact rat (10) is thought to be due to an increase in the uterine PG levels.

Bauminger et al. (17) reported that gonadotropins in vitro significantly increased the accumulation of PG in rat Graafian follicles after incubation for 5 hours. The system of PG biosynthesis, therefore, may be activated by the alteration of ovarian sensitivity to gonadotropins. Although the incorporation of arachidonic acid into the
PGE and PGF fractions, expressed in terms of 100 mg tissue wet weight, was slightly lower in the ovary than in the uterus, the incorporation expressed in terms of the organ was much greater in the uterus than in the ovary. Thus, the ovary may have the capacity to synthesize PG from arachidonic acid, and this capacity may be influenced by gonadotropins and/or ovarian steroids.

Our results indicate that changes of PG content in rat ovary may be mainly regulated by the uterine tissue, although the possibility of production by the ovary itself cannot be ruled out.

REFERENCES

1) Flint, A.P.F. and Hillier, K.: Prostaglandins and reproduction. Edited by Karim, S.M.M., p. 271–308, MTP Press, Lancaster (1975)
2) Hasel, W., Shemesh, M., Hixon, J. and Lukaszewska, J.: Extraction, isolation, and identification of a luteolytic substance from bovine endometrium. Biol. Reprod. 13, 30–37 (1975)
3) Horton, E.W. and Poyser, N.L.: Uterine luteolytic hormone: a physiological role for prostaglandin F2α. Physiol. Rev. 56, 595–651 (1976)
4) Hoffman, L.H.: Antifertility effects of indomethacin during early pregnancy in the rabbit. Biol. Reprod. 18, 148–153 (1977)
5) Castracane, V.D. and Jordan, V.C.: Consideration into the mechanism of estrogen-stimulated uterine prostaglandin synthesis. Prostaglandins 12, 243–251 (1976)
6) Kogo, H., Yamada, K. and Aizawa, Y.: Effect of estradiol on prostaglandin metabolism in rat uterus. Prostaglandins 13, 785–794 (1977)
7) Ham, E.A., Cirillo, V.J., Zanetti, M.E. and Kuehl, F.A.: Estrogen-induced synthesis of specific prostaglandins in uterus. Proc. natn. Acad. Sci. U.S.A. 72, 1420–1424 (1975)
8) Gengenbach, D.R., Hixon, J.E. and Hansel, W.: A luteolytic interaction between estradiol and prostaglandin F2α in hysterectomy of ewes. Biol. Reprod. 16, 571–579 (1977)
9) Takahashi, M. and Aizawa, Y.: Effect of uterine prostaglandin F2α on the level of ovarian steroids in rats. Folia pharmacol. jap. 76, 93–97 (1980) (Abs. in English)
10) Inazu, N., Kogo, H. and Aizawa, Y.: Effect of gonadotropin and estrogen on changes in levels of prostaglandin in rat ovary. Japan. J. Pharmacol. 31, 247–251 (1980)
11) Takeuchi, K., Yamada, K. and Aizawa, Y.: Biological assay of prostaglandin using rat uterus. Folia pharmacol. jap. 71, 675–682 (1975) (Abs. in English)
12) Aral, Y., Takeuchi, K., Yamada, K., Kogo, H. and Aizawa, Y.: Influence of carragenin inflammation on prostaglandin release from rat synovial membrane and action of anti-inflammatory drugs. Folia pharmacol. jap. 75, 765–769 (1979) (Abs. in English)
13) Takeuchi, K., Kogo, H. and Aizawa, Y.: Biological assay of prostaglandin in urine using fundus of rat stomach. Folia pharmacol. jap. 76, 179–184 (1980) (Abs. in English)
14) Kogo, H. and Aizawa, Y.: Effect of estrogen on interconversion of prostaglandin F and E in rat uterus. Folia pharmacol. jap. 73, 871–876 (1977) (Abs. in English)
15) Grén, K. and Samuelsson, B.: Prostaglandin and related factors. XIX. Thin-layer chromatography of prostaglandins. J. Lipid Res. 5, 117–120 (1964)
16) Blatchley, F.R., Donovan, B.T., Poyser, N.L., Horton, D.W., Thompson, C.J. and Los, M.: Identification of prostaglandin F2α in the utero-ovarian blood of guinea-pig after treatment with oestradiol. Nature 230, 243–244 (1971)
17) Bauminger, S., Lieberman, M.E. and Lindner, H.R.: Steroid-independent effect of gonadotropin on prostaglandin synthesis in rat Graafian follicles in vitro. Prostaglandins 9, 753–763 (1975)