EFFECT OF PREGNANT MARE SERUM GONADOTROPIN AND ESTRADIOL ON THE FORMATION OF 13,14-DIHYDROPROSTAGLANDIN $F_{2\alpha}$ IN RAT OVARY

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(Received January 30, 1981)

Effects of pregnant mare serum gonadotropin (PMS) and estradiol on the formation of 13,14-dihydroprostaglandin $F_{2\alpha}$ ($13,14H_2$ -PGF $_{2\alpha}$) from prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) and 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$ (15KD-PGF $_{2\alpha}$) in the ovarian homogenate of rats were examined. PMS and estradiol were given to the rats (s.c.) on the first day of diestrus, and the ovaries were removed 24 hours after treatment with each hormone. PMS stimulated the formation of $13,14H_2$ -PGF $_{2\alpha}$ from PGF $_{2\alpha}$ and 15KD-PGF $_{2\alpha}$, and estradiol markedly inhibited the formation of $13,14H_2$ -PGF $_{2\alpha}$ from PGF $_{2\alpha}$ and 15KD-PGF $_{2\alpha}$. However, the formation of 15KD-PGF $_{2\alpha}$ from PGF $_{2\alpha}$ did not change with PMS and estradiol. These results indicate that $13,14H_2$ -PGF $_{2\alpha}$ formed in rat ovary may play some important role in ovarian function and may be regulated by gonadotropins and ovarian steroids.

Keywords—ovary; 13,14-dihydro-PGF $_{2\alpha}$; gonadotropin; estrogen; PGF $_{2\alpha}$ catabolism

The catabolism of prostaglandin (PG) is initiated by two enzymes, 15-hydroxyprostaglandin dehydrogenase and Δ^13 -prostaglandin reductase; that is, the formation of 15-keto and 13,14-dihydro-15-keto compounds occurs. More recently, we have observed that an unidentified enzyme which converts 13,14-dihydro-15-keto PGF $_{2\alpha}$ (15KD-PGF $_{2\alpha}$) into 13,14-dihydro-PGF $_{2\alpha}$ ($13,14H_2$ -PGF $_{2\alpha}$) was present in rat ovary.¹⁾ Houlst and Moore²⁾ have also found that $13,14H_2$ -PGF $_{2\alpha}$ is converted from 15KD-PGF $_{2\alpha}$ in guinea-pig and pig kidney. It is known that PG metabolites, like $13,14H_2$ -PGF $_{2\alpha}$ which has the 15-hydroxy group, have an appreciable contractile activity on some smooth muscles, although the activity is weaker than that of the parent PG.²⁻⁴⁾ However, the effect of $13,14H_2$ -PG on ovarian function is not known entirely. It is very interesting to study the relationship between the formation of $13,14H_2$ -PG and ovarian function. In the present study, we investigated the effect of pregnant mare serum gonadotropin (PMS) and estradiol on the formation of $13,14H_2$ -PGF $_{2\alpha}$ from PGF $_{2\alpha}$ or

15KD-PGF $_{2\alpha}$ in rat ovary, and obtained an evidence that these hormones might play some role in the formation of $13,14H_2$ -PGF $_{2\alpha}$ in rat ovary.

Female rats of Wistar strain, weighing about 200 g, were used. They were housed in group cages (five rats per cage) under controlled conditions of light and temperature. Food and water were administered *ad libitum*. Vaginal smears were examined every morning, and rats exhibiting a regular 4-day estrous cycle were used for the experiments. Fifty IU of PMS was dissolved in 0.9% saline solution, and 20 μ g of estradiol in sesame oil, and each hormone was given to the rats (s.c.) on the first day of diestrus. Ovaries from each rat were removed 24 hours after the hormonal treatment and homogenized in Krebs-Ringer bicarbonate buffer (pH 7.4). The ovarian homogenate (ca. 3 mg protein) was incubated for 15 min with 0.2 μ Ci of [9 - 3 H]-PGF $_{2\alpha}$ (10.9 Ci/mmol, New England Nuclear) or 0.2 μ Ci of [5 , 6 , 8 , 11 , 12 , 14 - 3 H]- $13,14$ -dihydro-15-keto PGF $_{2\alpha}$ (75 Ci/mmol, *ibid*) in a test tube containing NAD (4 mM final concentration, Sigma

TABLE I. Effect of PMS and Estradiol on the Catabolism of $\text{PGF}_{2\alpha}$ to 15KD- $\text{PGF}_{2\alpha}$ and 13,14 H_2 - $\text{PGF}_{2\alpha}$ in Rat Ovary

	dpm $\times 10^4$ /ovary/15min		
	Remaining $\text{PGF}_{2\alpha}$	15KD- $\text{PGF}_{2\alpha}$	Formed 13,14 H_2 - $\text{PGF}_{2\alpha}$
Control	2.4 $\pm$ 0.09	13.8 $\pm$ 0.70	1.6 $\pm$ 0.05
PMS	2.0 $\pm$ 0.08*	12.2 $\pm$ 0.11	5.3 $\pm$ 0.14*
Control	1.5 $\pm$ 0.04	11.4 $\pm$ 0.28	2.9 $\pm$ 0.12
Estradiol	2.4 $\pm$ 0.11*	11.6 $\pm$ 0.35	1.7 $\pm$ 0.22*

PMS (50 IU) was dissolved in 0.9% saline solution, and estradiol (20 μ g) in sesame oil. Both hormones were given to the rats (s.c.) on the first day of diestrus, and the ovaries were removed 24 hours after treatment with each hormone. The ovarian homogenate with 0.2 μ Ci of ^3H - $\text{PGF}_{2\alpha}$ was incubated in a test tube containing NAD at 37°C in 95% O_2 -5% CO_2 for 15 min. Control: vehicle alone, PMS: pregnant mare serum gonadotropin, $\text{PGF}_{2\alpha}$: prostaglandin $\text{F}_{2\alpha}$, 15KD- $\text{PGF}_{2\alpha}$: 13,14-dihydro-15-ketoprostaglandin $\text{F}_{2\alpha}$, 13,14 H_2 - $\text{PGF}_{2\alpha}$: 13,14-dihydroprostaglandin $\text{F}_{2\alpha}$. Each value shows the mean $\pm$ S.E. of 5 rats.

* $p < 0.01$: Significantly different from control.

TABLE II. Effect of PMS and Estradiol on the Conversion of 15KD- $\text{PGF}_{2\alpha}$ into 13,14 H_2 - $\text{PGF}_{2\alpha}$ in Rat Ovary

	dpm $\times 10^4$ /ovary/15 min	
	Remaining 15KD- $\text{PGF}_{2\alpha}$	Formed 13,14 H_2 - $\text{PGF}_{2\alpha}$
Control	20.7 $\pm$ 0.18	5.5 $\pm$ 0.12
PMS	16.8 $\pm$ 0.24*	10.7 $\pm$ 0.25*
Control	19.7 $\pm$ 0.48	6.7 $\pm$ 0.12
Estradiol	22.5 $\pm$ 0.12*	3.6 $\pm$ 0.26*

PMS (50 IU) was dissolved in 0.9% saline solution, and estradiol (20 μ g) in sesame oil. Both hormones were given to the rats (s.c.) on the first day of diestrus, and the ovaries were removed 24 hours after treatment with each hormone. The ovarian homogenate with 0.2 μ Ci of ^3H -15KD- $\text{PGF}_{2\alpha}$ was incubated in a test tube containing NAD at 37°C in 95% O_2 -5% CO_2 for 15 min. Each value shows the mean $\pm$ S.E. of 5 rats.

* $p < 0.01$: Significantly different from control. Abbreviations are the same as in Table I.

the addition of 2 ml of acidified alcohol, and $\text{PGF}_{2\alpha}$ and its metabolites were extracted with ethyl ether. The ether extract was applied on a silica-gel thin-layer plate and developed in a solvent system consisting of chloroform-methanol-acetic acid-water (90:9:1:0.65, v/v).⁵⁾ Radioactivity on the plate was located by a thin-layer chromatogram scanner. Each radioactive spot was scraped off and placed in a scintillation vial containing 10 ml of Aquasol-2 scintillator solvent (New England Nuclear). The radioactivity was counted by a liquid scintillation spectrometer.

Effects of PMS and estradiol on the formation of 15KD- and 13,14 H_2 - $\text{PGF}_{2\alpha}$ from $\text{PGF}_{2\alpha}$ are shown in Table I. Administration of PMS stimulated $\text{PGF}_{2\alpha}$ catabolism and especially increased the formation of 13,14 H_2 - $\text{PGF}_{2\alpha}$. Remaining ^3H - $\text{PGF}_{2\alpha}$ was increased to 165.2% of control level and ^3H - $\text{PGF}_{2\alpha}$ was reduced to 80.4% of control level, and ^3H -13,14 H_2 - $\text{PGF}_{2\alpha}$ formed was greatly increased (339.0%) above control level. In contrast, administration of estradiol inhibited the catabolism of $\text{PGF}_{2\alpha}$ in rat ovary. Remaining ^3H - $\text{PGF}_{2\alpha}$ was increased to 165.2% of control level and ^3H -13,14 H_2 - $\text{PGF}_{2\alpha}$ formed declined to 57.7% of control level. On the other hand, no pro-

Chemical Co.) at 37°C in an atmosphere of 95% O_2 -5% CO_2 with constant shaking (100 cycles/min). The incubation was terminated by

minent change in the formation of ³H-15KD-PGF_{2α} was found with either hormone. Table II shows the effect of PMS and estradiol on the conversion of 15KD-PGF_{2α} into 13, 14H₂-PGF_{2α} in rat ovary. The percentage of remaining ¹H-15KD-PGF_{2α} added as substrate was significantly decreased (81%) below control level by the treatment with PMS, and ³H-13,14H₂-PGF_{2α} formed was significantly increased to 194.4% of control level. However, the treatment with estradiol inhibited the conversion of 15KD-PGF_{2α} into 13,14H₂-PGF_{2α}. The percentage of remaining ³H-15KD-PGF_{2α} was increased to 114.4% of control level, and ³H-13,14H₂-PGF_{2α} formed declined to 54.3% of control level.

We have recently reported that both PMS and estradiol increased PGF level in rat ovary.⁶⁾ On the other hand, there was a significant difference between PMS and estradiol on the formation of 13,14H₂-PGF_{2α} in rat ovary. These results indicate that gonadotropins and ovarian steroids may regulate not only PG level but also PG catabolism in rat ovary. Therefore, 13,14H₂-PG may be involved in the action of gonadotropins and ovarian steroids as well as PGE and PGF which cause an ovulation or luteolysis in some species of animals.⁷⁻¹¹⁾ Although there are some reports about the biological inactivation of PG in uterus and placenta of rat^{12,13)} and in uterus and oviduct of rabbit,¹⁴⁾ it is considered that 13,14H₂-PGF_{2α} formed in rat ovary may play some important role in ovarian function. This compound could not be produced in rat uterus¹⁾ and has an appreciable contractile activity on smooth muscle preparations.²⁻⁴⁾ We have more recently reported that 13,14H₂-PGF_{2α} *in vitro* increased ovarian steroidogenesis in rat¹⁵⁾ and suggested a significant role of 13,14H₂-PGF_{2α} in ovarian function. The discovery of the effect of PMS and estradiol on the formation of 13,14H₂-PGF_{2α} in the present study may be useful in clarifying the action of 13,14H₂-PGF_{2α} in ovulatory process or luteolytic mechanism.

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