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EFFECTS OF EXOGENOUS PROSTAGLANDINS, FSH AND LH ON OVARIAN PROGESTINS IN HYPOPHYSECTOMIZED RATS: POSSIBLE DIFFERENCE IN THEIR ATTACK POINT

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SUMMARY

The purpose of this study is to determine whether or not prostaglandins (PGs) exert a stimulatory or inhibitory influence on the biosynthesis of ovarian progestins and to determine if PGs (E_1, E_2, $F_2\alpha$) have a different locus of action from the pituitary gonadotropins (FSH, LH) on the biosynthesis by hypophysectomized rats *in vivo*. Female rats of the Wistar strain aged 10–11 weeks were housed in a constant temperature (23 ± 1°) and artificially lit (6:00 a.m.–6:00 p.m.) room. They were hypophysectomized at various stages of the estrous cycle. PG E_2 (cyclodextrine, ONO, 10 μg/0.1 ml saline, i.v.), FSH (NIH-S-3, 10 μg/0.1 ml saline, i.v.) or LH (NIH-S-10, 10 μg/0.1 ml saline, i.v.) was injected into the saphenous vein without anesthesia on day 6 after the operation. Rats were decapitated at 5, 15, 30 and 60 min after the injection. In the group receiving PG E_2, a highly significant increase ($P < 0.001$) was found in 20α-OH-P (20α-hydroxy-4-pregnen-3-one) content and concentration at 5 min after the injection as compared with hypophysectomized controls. There were, however, no significant differences between those two groups at 15, 30 and 60 min after the injection. Progesterone levels showed no significant changes following PG E_2 injection at any experimental times (5, 15, 30 and 60 min). Levels of progesterone and 20α-OH-P in the FSH injected group showed a significant increase at all experimental times after the injection as compared with hypophysectomized controls. Similar results were found in the group receiving LH. PG E_1, E_2 and $F_2\alpha$ at a dose of 20 μg/0.1 ml, increased ovarian 20α-OH-P significantly ($P < 0.01$) at 5 min after the injection. There was no significant change in progesterone levels. The results indicate that the locus of action of the PG E_2 may be different from that of gonadotropins (FSH LH) and PGs may not be an essential intermediate in the action of tropic hormones on ovarian steroidogenesis.

INTRODUCTION

A valid physiological explanation for the cyclic regression of corpus luteum in mammals has proved to be elusive. Since a luteolytic factor appears to regulate the life span of the ovarian corpus lureum (in rabbit and guinea pig, Loeb, 1923 [1], 1927 [2]; in sheep, Anderson, 1969 [3]; in mare, Ginther, 1971 [4]), and this factor was assumed to be prostaglandin $F_2\alpha$ (PG $F_2\alpha$) (McCrachen, 1971) [5], it has been suggested that PGs may be affected in biosynthesis of ovarian progestins (Channing, 1971a [6]; Hirai *et al.*, 1974a [7]). On the other hand, the interaction among FSH (follicle stimulating hormone), LH (luteinizing hormone) and PGs to regulate steroidogenesis was introduced by Kolena (1972) [8]. However, the precise nature of the physiological relationship among ovarian function, gonadotropins and PGs is not yet known. In the early study, elevation of the ovarian cyclic AMP (CAMP) level was attributed to the interaction between gonadotropins and PGs, and CAMP was assumed to stimulate the steroid-synthesizing cell. Recently it was reported that PGs were not an essential intermediate in the action of LH upon adenyl cyclase (Marsh, 1971) [9] or that PGs themselves accelerated the biosynthesis of steroid (Behrman, 1971) [10]. On the other hand, PGs (E, A and F) can cause luteinization of monkey and pig granulosa cell cultures (Kolena, 1971) [11], while many conversely claimed that it was PG $F_2\alpha$ which played the role of luteolysin (Pharriss and Wyngarden, 1969) [12].

During short term *in vitro* incubation of corpus luteum most investigators found a stimulatory influence of PGs upon progesterone secretion (Marsh, 1971 [9]; Sperrof, 1970 [13]). In contrast, investigators have shown that intra-arterial infusion of high doses of PG $F_2\alpha$ will suppress progesterone production in nonpregnant monkeys (Alletta, 1973 [14]; Kirton, 1970 [15]). However, PG $F_2\alpha$ infused into the systemic circulation of sheep with transplanted ovaries does not induce luteal regression (McCrachen, 1971) [16]. This is explained partly by a dilution effect and partly by the rapid metabolism of PG $F_2\alpha$ in the circulation, particularly by the lungs (Piper and Vane, 1971 [17]; Piper *et al.*, 1970 [18]). Thus, it seems likely that the luteolytic factor (tentatively PG $F_2\alpha$) acts primarily in a local manner rather than as a systemic hormone.

It was the purpose of this study to determine whether PGs act specifically on biosynthesis of ovarian progestins by the hypophysectomized rats and, if so, to differentiate the effects of FSH, LH and PGs on such biosynthesis.

MATERIALS AND METHODS

1. *Experimental animals*

Mature, virgin, Wistar female rats (from Nihon Rat Farms), aged 10–11 weeks, were bred in an air conditioned room (15–17 times/h) at 23°, humidity 55% and lighting from 6:00 a.m.–6:00 p.m. They were housed five per cage and maintained on CLEA Laboratory Chow, CE-2 and water *ad libitum*. All rats were hypophysectomized through the paraphyaryngeal approach. They were used in the experiment on the 6th postoperative day [19].

2. *Prostaglandins*

Since PG E_1, E_2 and $F_2\alpha$ were all hardly water-soluble, PG E_1 cyclodextrine, PG E_2 cyclodextrine and PG $F_2\alpha$ cyclodextrine (ONO Co. Ltd. Japan) were used, each in a saline solution containing 10 μg in absolute molecular weight of PG per 0.1 ml of saline. Twenty μg/0.1 ml saline solution was used in some experiments. PGs were given intravenously.

3. *Pituitary gonadotropins*

A 10 μg/0.1 ml saline solution of FSH (NIH-S-3) and LH (NIH-S-10) were prepared immediately before use. A mixture of FSH and LH was prepared in such a way as to contain 10 μg of each per 0.1 ml of saline.

4. *Procedure and experimental schedule*

(1). *Time-course after the drug injection.* Hypophysectomy was performed in each stage of the estrous cycle, and operated animals were divided into 4 groups to inject PG E_2, FSH, LH and FSH + LH, respectively. Intact control and hypophysectomized-untreated control (hypox control) were used. The injection was made without anesthesia into the vena saphenus of the hind limb. At 5, 15, 30 and 60 min after the injection, the animals were killed by decapitation without anesthesia. Laparotomy was carried out on all rats and the ovary, uterus and adrenal were removed, and individually weighed. The ovary was rapidly frozen and stored at −20° until extraction. The number of animals ranged from 35–32 in each group and 8–6 for each point of the injection. The injection and decapitation were performed from 8:00 to 10:30 a.m.

(2). *Effects of PGs (E_1, E_2, $F_2\alpha$) on ovarian progestins.* The initial experiment described above revealed that when PG was systematically given to hypox rats through the intravenous route, the progestin (20α-OH-P) level attained a peak in 5 mins (the details are given later). On the basis of these results, each 20 μg/rat of PG E_1, PG E_2 and PG $F_2\alpha$ was given intravenously to the hypophysectomized animals, and they were sacrificed 5 min after the injection to compare ovarian progestin levels among the groups.

5. *Extraction, purification and estimation of ovarian progestins*

The ovary was individually homogenized and then extracted with dichloromethane. After removing cholesterol and lipid by centrifugation at (−20°), the neutral fraction was purified by the quantitative t.l.c. to isolate 20α-hydroxypregn-4-en-3-one (hereafter referred to as 20α-OH-P) and progesterone. The former was determined by the microfluorometric technique [19], and the latter was estimated by enamine-formation-microfluorometric technique [20]. The details were described elswhere [19, 20].

RESULTS

1. *Change in body weight and organ weight (Table 1)*

On the 6th day after hypophysectomy, each treated group showed body weight loss, ranging 15.9–17.5% as compared with the initial value. This tendency was observed also in the hypox control, and there was no significant difference between the treated and non-treated groups. The body weight loss is attributed to insufficient food intake and disturbance of protein biosynthesis or metabolic disorder, resulting from the operative stress. Significant change in body weight was not observed in any of PG E_2 group, FSH group, LH group and FSH + LH group at any experimental time point (5, 15, 30 and 60 min) after the injection. In the ovarian weight, no significant change appeared in any group and at any experimental time point except that a significant increase was observed in FSH + LH group over the value in the hypox control. It can only be said that the PGE_2 group showed relatively low values as a whole. Comparison between the treated groups, however, reveals that the ovarian weight in PG E_2 group was significantly lower than that of any other treated group at each experimental time point. The uterus weight was significantly higher in LH group than in the hypox control, and relatively higher also in FSH group and FSH + LH group. In PG E_2 group, however, it conversely tended to decrease. When compared between the treated groups, it was significantly higher in LH group than in the others, and next in order came FSH group, FSH + LH group and PG E_2 group. In this last, the uterus weight was significantly lower than in the others. In the adrenal weight, no significant change appeared.

2. *Change in ovarian 20α-OH-P (Fig. 1)*

a. *In PG E_2-injected group.* As seen in Fig. 1, ovarian 20α-OH-P was significantly higher in both content and concentration ($P < 0.001$) in PG E_2 group than in the hypox control at 5 min after the injection. At 15 min, however, the level declined abruptly, and there was already no significant difference from the

Table 1. Changes in body weight and organ weight in rats after systemic administration of PG E_2, FSH, LH and FSH + LH on the 6th day following the hypophysectomy

Group	Time after injected	No. of rat	Body weight Before hypox	Body weight 6th day after* hypox	Ovarian W. mg	Uterine W. mg	Adrenal W. mg
Hypox	—	12	194.7 ± 4.75	160.7 ± 4.66	35.8 ± 3.26	135.7 ± 6.16	28.5 ± 1.86
PG E_2	5 min	8	184.2 ± 5.08	145.4 ± 5.73	29.4 ± 2.94	115.0 ± 6.90	30.4 ± 1.57
	15 min	7	188.4 ± 5.96	148.2 ± 7.33	35.0 ± 1.30	127.0 ± 7.92	30.0 ± 1.38
	30 min	8	185.2 ± 3.34	156.2 ± 3.40	33.0 ± 1.92	120.8 ± 12.55	33.0 ± 1.00
	60 min	7	194.2 ± 11.8	160.2 ± 7.68	37.0 ± 2.30	162.2 ± 18.46	36.6 ± 3.44
FSH	5 min	7	194.0 ± 4.99	173.0 ± 6.16	39.0 ± 3.13	187.3 ± 11.00	32.0 ± 1.73
	15 min	8	211.8 ± 7.62	181.0 ± 7.99	47.5 ± 2.99	155.5 ± 12.02	29.8 ± 7.99
	30 min	6	184.2 ± 4.72	162.4 ± 5.63	36.8 ± 1.83	138.4 ± 4.34	32.8 ± 1.99
	60 min	7	195.0 ± 5.31	154.0 ± 6.72	46.5 ± 4.03	152.8 ± 11.40	35.0 ± 1.96
LH	5 min	8	193.4 ± 3.53	162.2 ± 5.77	40.8 ± 1.70	221.5 ± 17.12	30.5 ± 2.33
	15 min	8	192.2 ± 4.21	159.6 ± 2.98	42.6 ± 2.48	194.8 ± 11.36	26.2 ± 0.58
	30 min	6	189.8 ± 3.71	156.3 ± 1.49	46.3 ± 1.32	219.3 ± 16.60	27.5 ± 1.32
	60 min	7	199.0 ± 4.78	172.5 ± 7.03	42.5 ± 2.90	221.3 ± 5.68	29.3 ± 2.50
FSH + LH	5 min	7	214.5 ± 4.21	178.3 ± 2.39	57.3 ± 6.26	148.0 ± 14.23	34.8 ± 1.89
	15 min	8	202.8 ± 0.75	184.5 ± 6.55	45.3 ± 1.18	155.5 ± 16.99	35.0 ± 1.68
	30 min	8	218.6 ± 3.83	186.4 ± 4.92	53.2 ± 5.21	144.6 ± 3.83	31.4 ± 1.99
	60 min	7	212.3 ± 3.59	181.0 ± 2.12	51.3 ± 2.29	153.8 ± 17.72	30.8 ± 1.62

* Day injected

Body weight decreased by 15.9–17.5% in both control and treated groups. Any treated group did not show significant difference in body weight from the hypox control at 5, 15, 30 and 60 min after the administration. Ovarian weight was relatively low in PG E_2 group as a whole. It was significantly lower in this group than in the other treated groups at each time point. Uterine weight tended to decrease in PG E_2 group as a whole. It was significantly lower in this group than in the other treated groups at each time point. No significant change was observed in adrenal weight.

control. At 30 min, the rise was only slight but the difference was not statistically significant. At 60 min, no significant change was observed.

b. *In FSH-injected group.* Ovarian 20α-OH-P was significantly higher in this group than in the hypox control ($P < 0.01$) at 5, 15, 30 and 60 min after the injection. This was significant both in content and concentration (Fig. 1).

c. *In LH-injected group.* As in FSH-injected group, ovarian 20α-OH-P was significantly higher ($P < 0.01$) in LH-injected group than in the hypox control at 5, 15, 30 and 60 min after the injection, in both content and concentration (Fig. 1).

d. *In FSH + LH-injected group.* Ovarian 20α-OH-P was significantly higher ($P < 0.01$) in this group than in the hypox control at 5 min after the injection, and markedly higher ($P < 0.001$) at 15, 30 and 60 min. This effect was regarded as potentiation by the synergic action of FSH and LH at 60 min.

3. *Change in ovarian progesterone (Fig. 2 and 3)*

a. *In PG E_2 injected group.* Ovarian progesterone tended to decrease compared with the hypox control at 5 min after the injection (both in content and concentration), but the change was not statistically significant. It remained without any significant change at 15 min, but tended to increase at 30 min. Also at 60 min, no significant change was observed as compared with the hypox control.

b. *In FSH-injected group.* Ovarian progesterone significantly increased over the value of hypox control ($P < 0.01$) at 5 min after the injection, and, still more at 15, 30 and 60 min ($P < 0.01$). The significancy was confirmed both in content (Fig. 2) and concentration (Fig. 3).

c. *In LH-injected group.* Ovarian progesterone significantly increased over the value of the hypox control ($P < 0.01$) already at 5 min after the injection, and this was maintained at 15, 30 and 60 min. Thereafter, the slope of increase became less sharp. These increases were all significant either in content (Fig. 2) or in concentration (Fig. 3).

d. *In FSH + LH-injected group.* Ovarian progesterone level was significantly higher in this group than in the hypox control ($P < 0.001$) at 5, 15, 30 and 60 min after the injection. It should be noted that significant increases had already occurred at 5 min ($P < 0.001$) as compared with the value of the intact control. And these increases were observed in content as well as in concentration.

4. *Effects of various PGs (E_1, E_2 and $F_2\alpha$) on ovarian progestins (Fig. 4 and 5)*

In this experiment all the animals were decapitated at 5 min after PGs injection, and ovarian progestins of individual animals were separately assayed.

a. *Effects on ovarian 20α-OH-P.* Ovarian 20α-OH-P level in PG E_1 group was significantly higher ($P < 0.01$) than in the hypox control both in content and concentration. Furthermore, in concentration, the level in PG E_1 group was significantly higher even than in the intact control ($P < 0.05$).

In PG E_2 group, ovarian 20α-OH-P level was significantly higher than in the hypox control both in content and concentration ($P < 0.01$). And in concentration, the level in PG E_2 group was even higher

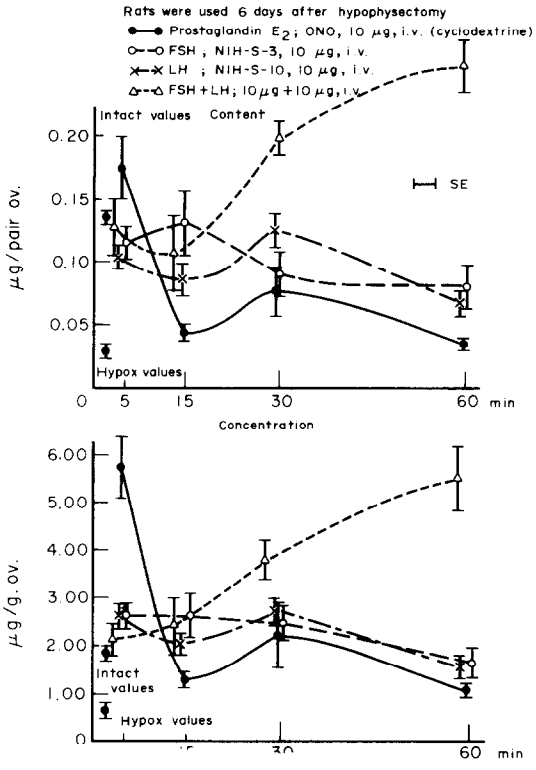

Fig. 1. Alteration of ovarian 20α-OH-P at various times after the PG E_2 or pituitary gonadotropin treatment by the hypophysectomized rats. It was markedly increased ($P < 0.001$) in PG E_2 group at 5 min, but abruptly decreased at 15 min, and tended to increase slightly at 30 min. Significant changes were not seen in 15, 30 and 60 min. It was markedly higher in FSH group than in hypox control ($P < 0.01$) at each of 5, 15, 30 and 60 min. Also in LH group similar significant increase was observed ($P < 0.01$). On 20α-OH-P level, synergic action of the FSH + LH was shown. Note: Difference in time course of effect between PG E_2 and tropic hormones.

than in the intact control ($P < 0.05$), as seen in PG E_1 treatment.

Ovarian 20α-OH-P level in PG $F_2\alpha$ group was higher than in the hypox control, and the difference was statistically significant both in content and concentration. In the concentration, the level was even higher than in the intact control ($P < 0.05$), as seen in PG E_1 or PG E_2 treatment.

b. *Effects on ovarian progesterone.* The intravenous injection of PG E_1, PG E_2 or PG $F_2\alpha$ did not show any significant change in ovarian progesterone level. It is of interest that despite the significant fall of ovarian 20α-OH-P level induced by hypophysectomy, any marked fall of ovarian progesterone level was not observed until as late as 6 days after the operation.

DISCUSSION

The present experiment, in which PGs were systematically given to rats through the intravenous route on the 6th day after hypophysectomy revealed: (1). 20α-OH-P showed a marked increase ($P < 0.001$) at 5 min after PG E_2 injection, but then abruptly decreased. At 15 min, no significant difference from the hypox control was seen, and this level continued at 30 and 60 min. (2). Progesterone levels tended to decrease at 5 min after PG E_2 injection but tended to increase at 15 and 30 min. However, a statistically significant change was not observed in any case. (3). When FSH or LH alone was given intravenously instead of PG E_2, both ovarian 20α-OH-P and progesterone levels were significantly higher ($P < 0.01$ or more) than in the hypox non-treated at any experimental time (5, 15, 30 and 60 min) after the injection. [4]. In further observation, which referred to these results, experiments were designed in which PGs (E_1, E_2 and $F_2\alpha$) were intravenously given to hypox rats, and observed at 5 min after the injection. It was found that ovarian 20α-OH-P level was significantly increased in all the cases, while ovarian progesterone level did not shown any significant change in these experiments.

From the basis of these four findings possible discussion was made concerning the attack points of PGs, which were given systematically.

Exactly where or how tropic hormones (FSH or LH) interacts with PGs in controlling the steroido-

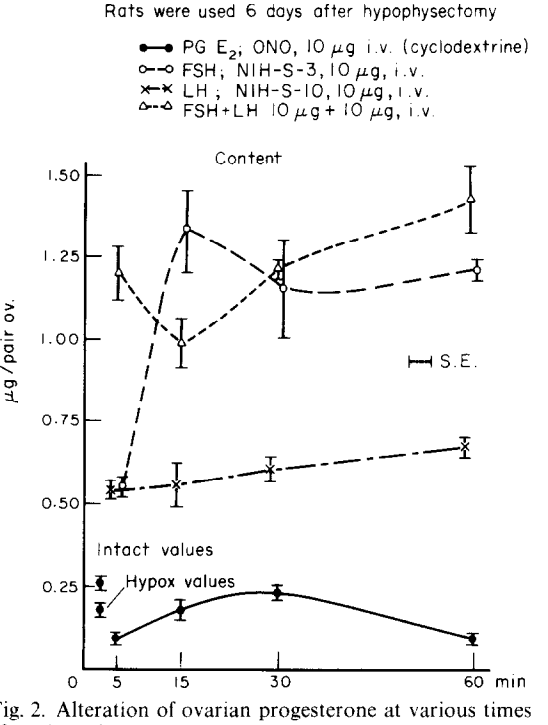

Fig. 2. Alteration of ovarian progesterone at various times after the PG E_2 or pituitary gonadotropin treatment by the hypophysectomized rats. It tended to be lower in PG E_2 group than in hypox control at 5 min, and to increase slightly at 15 and 30 min, then again decreasing at 60 min. Significant changes were not seen. In the FSH group it abruptly increased at 5 min, keeping the high level at 15, 30 and 60 min. Difference from the control value was significant at each time point ($P < 0.01$–$P < 0.001$). Same results were shown in LH group. In the FSH + LH group, progesterone was increased dramatically. Note: Marked difference between PG E_2 and tropic hormones in that the former was ineffective while the latter induced a dramatic increase.

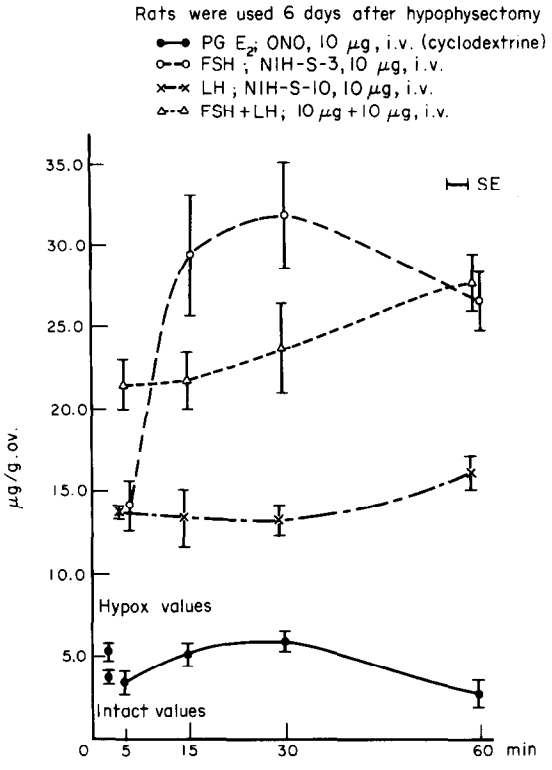

Fig. 3. Alteration of ovarian progesterone at various times after the PG E_2 or pituitary gonadotropin treatment by the hypophysectomized rats. Ovarian weight was significantly lower in hypox rat than in intact rat (at 6 postoperative days), whereas progesterone content was not significantly decreased by hypox. As the consequence, progesterone concentration was higher, in the hypox than in the intact. However, 20α-OH-P content was markedly diminished by hypox, and consequently its concentration was also lower significantly. In PG E_2 group, no significant change was seen in progesterone concentration in comparison with hypox control. Progesterone was shown markedly increasing as content after FSH + LH group.

genesis is difficult to say at this time. The tropic hormone, for example, LH, is known as the first messenger to interact with a receptor in the membrane to stimulate adenylate cyclase, thus converting ATP into CAMP (the second messenger), which in turn stimulates luteal cells or ovarian granulosa cells to accelerate the release of progesterone from them. CAMP stimulates the conversion of cholesterol to pregnenolone, the customary rate limiting reaction in the synthesis of progesterone in corpus luteum [21]. The intracellular site of action of CAMP is not known for certain. There is a view that CAMP may stimulate the protein kinase enzyme to phospholylation of histone, [22], which, through the intermediate of DNA, would induce new enzymes to increase the transcription of the messenger RNA molecule. According to another view, CAMP would stimulate ribosomal activity td enhance the biosynthesis of enzyme at the translation level [23]. There is also a report that CAMP may act at some unknown site [21].

The locus of action of PGs still remains unknown. PGs are not an essential intermediate in the action of LH upon adenyl cyclase since in bovine corpus luteum and in porcine granulosa cells [8], a homogeneous cell system, the stimulatory effects of saturating doses of LH and PGs on CAMP levels are more than additive. In contrast to this, Kuehl *et al.* (1970) [24], who carried out an experimental study on the mouse ovary from the standpoint of PG-blocking agent, concluded that PGs were an essential intermediate in LH action. Against this, however, it was pointed out [25] that, it would be difficult for Kuehl *et al.* to represent the action mechanism of PGs exactly since they used more than its toxic dose. Most *in vitro* studies in the ovary have demonstrated that PGs are not the sole mediator of the steroidogenic

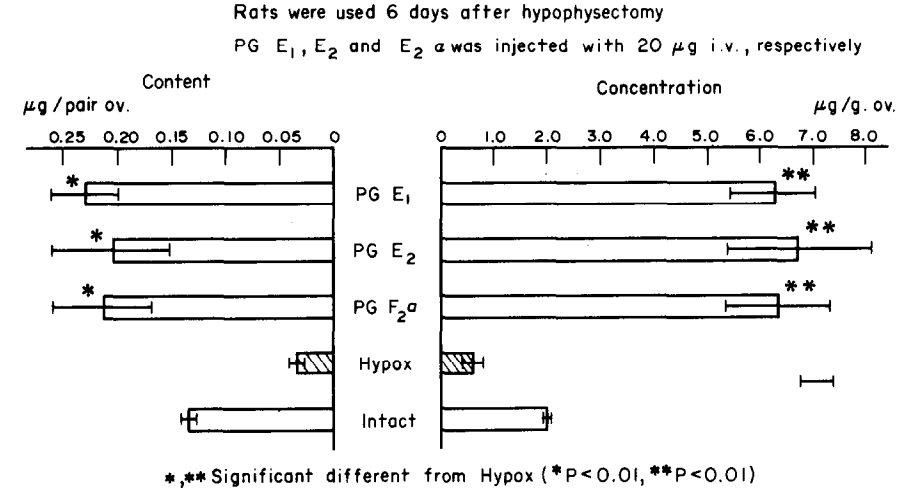

Fig. 4. Influence of PGs on ovarian 20α-OH-P by the hypophysectomized rats. At 5 min after intravenous injection, all the animals were decapitated. Ovarian 20α-OH-P content was significantly larger in PG E_1, PG E_2 and PG $F_2\alpha$ group than in hypox control. This also the case with the concentration.

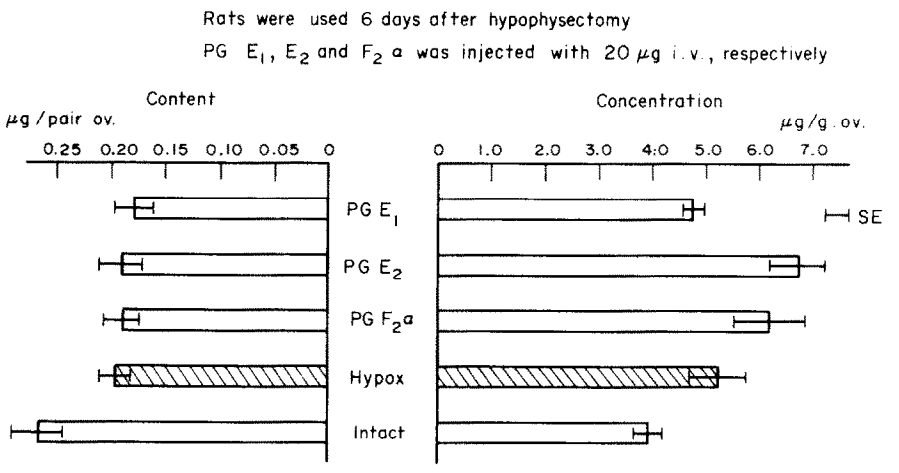

Fig. 5. Influence of PGs on ovarian progesterone by the hypophysectomized rats. Decapitation at 5 min. No significant change elicited by any of PGs (E_1, E_2, $F_2\alpha$). Hypox induced marked fall of 20α-OH-P, but no significant drop of progesterone level.

effect of LH. In rabbit [26] and bovine ovary [9], combinations of maximally stimulating amounts of LH and PGs produce a greater stimulation in adenylate cyclase or progestin secretion than either agent alone does. During short term *in vitro* incubations of ovary, particularly corpus luteum, most investigators found a stimulatory effect of PGs upon progesterone secretion [9], [13]. If, however, incubations are prolonged for up to 6 h, as in the case of rabbit corpus luteum incubated in organ culture [27], it is possible to get some *in vitro* inhibitory effects of PG $F_2\alpha$ upon progesterone secretion. Whether or not PGs exert a stimulatory or inhibitory influence upon progestin secretion probably depends upon what step in steroidogenesis is rate limiting. Evidence for the role of PG $F_2\alpha$ in inducing luteal regression is not clear. Originally Pharriss and Wyngarden[12] suggested that PG $F_2\alpha$ might induce luteolysis by constricting the utero-ovarian vein. No overall drop in ovarian blood flow following the administration of physiological quantities of PG $F_2\alpha$ occurs in the sheep [5] or the rat [28]. However, the very fact that PGs stimulate progesterone synthesis *in vitro* but depress it *in vivo* would underline the importance of the microcirculation as a possible control mechanism. Pretreatment of ovarian tissue with PGs *in vivo* did not affect the basal levels of CAMP, whereas there was a significant reduction in the ability of LH to stimulate higher levels of CAMP, suggesting that PGs may work in its early stages of progesterone depression by interference with CAMP production [29]. In addition to the direct effects of decreased progesterone production by the corpus luteum, PGs have been shown to cause a gradual decrease in the number of luteal binding sites for LH. Kinetic analysis has shown that 24 h after administrations of PGs, LH binding capacity decreases 72%, whereas LH binding affinity remains unchanged [28]. Thus, the actual number of binding sites for LH is diminished and the sites that remain are structurally and functionally intact.

The present experiment showed that PGs alone stimulated the biosynthesis of ovarian progestins, especially 20α-OH-P. However, at which time, ovarian progesterone level was not significantly changed by PG administration. On the other hand, when LH or FSH was alone injected, both ovarian progestins, that is, 20α-OH-P and progesterone, were significantly increased. It is known that PGs themselves can stimulate adenylate cyclase which then acts within the cell to stimulate steroidogenesis [30]. On the other hand, it is also known that hormones (FSH or LH)–receptor (in the membrane) interaction results in a stimulation of the enzyme adenylate cyclase which converts ATP to CAMP [11], and then steroidogenesis was consequently stimulated. As mentioned before, however, CAMP stimulates the conversion of cholesterol to pregnenolone in steroidogenesis. According to the present result, it is possible to say that the attack point of PGs in steroidogenesis in part is assumed to be at the activation of 20α-hydroxysteroid dehydrogenase or the stimulation of the production of this enzyme. The interconversion route of progesterone ⇄ 20α-OH-P has been established since, Wiest *et al.* (1968) [31] and Zander *et al.* (1959) [32]. In the present results, ovarian progesterone level was not changed by PG E_1, PG E_2 and PG $F_2\alpha$, while 20α-OH-P was significantly increased by any of them. Hirai *et al.* (1975) [33], who performed *in vivo* infusion of rat ovary by the newer technique of IUOA method (intrauterine–oviduct approach of ovarian infusion techniques), proposed cholesterol → pregnenolone → 20α-OH-pregnenolone (5-pregnen-3β,20α-diol) → 20α-OH-P route. The present results may support the hypothesis, and suggests PG E_2 acts to stimulate the activity of 20α-hydroxysteroid dehydrogenase on the biosynthesis of ovarian progestins *in vivo*. On the other hand, the significant increase in both 20α-OH-P and progesterone after LH or FSH seemed to follow the above mentioned pattern of adenylate cyclase → CAMP → cholesterol → pregnenolone (general steroidogenesis), which was pro-

posed by Kolena and Channing[11] as mentioned above.

From these results it can be seen that PGs are probably not an essential intermediate in the actions of tropic hormones (LH or FSH) and that the two agents do not act in the same place. Furthermore when PGs (E_1, E_2, $F_2\alpha$) are systematically given to the hypox rats, these were all effective in producing or releasing ovarian 20α-OH-P. This is in agreement with the fact that the PGs acted primarily in a local manner rather than as a systemic hormone, [5], [17], however, their actions cannot necessarily be considered to be effectively local. It seems likely that in the presence of the hypophysis, the effect of systemic administration of PGs may be masked by the endogenous tropic hormones (LH or FSH) or that exogenous PGs in circulating blood may be affected by some factor which is dependent on the presence or absence of the hypophysis.

Additionally, it should be noted, from previous reports [19] that the results induced two distinct facts; one is the that endogenous progestin-genesis seems to last at least 4 or 5 days in the ovary after pituitary removal and, the other that, in the adrenal gland corticoidogenesis may immediately decline within 60 min after the operation. That the most appropriate time after hypophysectomy must be 6 day when the ovary is no longer secreting significant quantities of ovarian steroid but when some sensitivity to gonadotropin appears to have been lost. In contrast, the appropriate time for ACTH assay is at least one hour after hypophysectomy. This is why rats 6 days after hypophysectomy have been used in this experiment. In agreement with these results, a particular, problem is whether the ovary has a complete steroidogenesis after hypophysectomy but when some sensitivity to gonadotropin appears to have been lost. FSH is also capable of altering serum steroid levels and of inducing ovulation in hypophsectomized immature female rats. Beginning 7 days after hypophysectomy the rats were injected with FSH or LH [34]. Ovulation can be induced by either LH or FSH and the resultant corpus lutea synthesizes progesterone, as in the hypophysectomized hamster [35] and other animals [36], [37]. Further evidence in male animals was found in males hypophysectomized at 28 days of age and hormone (FSH, LH) injections initiated 2 months later. Both steroidogenesis and spermatogenesis were found [38].

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This paper is dedicated to the late Dr. Gregory Pincus on the 10th anniversary of his death in homage to his devotion to reproductive endocrinology and his help in the development of general science.

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