Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 777–780. Pergamon Press. Printed in Great Britain

EFFECT OF PROSTAGLANDINS ON THE *IN VITRO* BIOSYNTHESIS OF ESTRONE, ESTRADIOL AND PROGESTERONE BY RABBIT OVARIAN FOLLICLES

VASANT V. PATWARDHAN and ANDRÉ LANTHIER
Laboratoire d'Endocrinologie, Hôpital Notre-Dame et Département de Médecine, Université de Montréal, Montréal, Canada

(*Received* 18 *December* 1976)

SUMMARY

These experiments were done to determine the effect of prostaglandins on steroid biosynthesis in the rabbit ovarian follicles. Whole follicles were incubated *in vitro* with or without added prostaglandins. Estrone, estradiol and progesterone content of the incubated follicles was determined by radioimmunoassays. $PGF_2\alpha$ had no effect on the biosynthesis of the three steroids. Estrogen formation was stimulated by both PGE_1 and PGE_2. Progesterone biosynthesis, however, was stimulated by only PGE_1. These results are discussed in relation to the conditions existing *in vivo* in the LH stimulated rabbit follicles.

INTRODUCTION

In view of the well established luteolytic action of prostaglandins their effect on steroidogenesis in the corpus luteum has been extensively studied[1–3]. There are also reports on the *in vitro* effects of prostaglandins on progesterone production by mouse ovaries maintained in culture[4, 5] and by cultured granulosa cells obtained from ovaries of different species[6–8]. Their effects on steroidogenesis in the whole follicles of the ovary have not received equal attention. Recently, prostaglandins PGE and PGF have been reported to be present in the ovarian follicles of the rabbit[9] and the rat[10]. Further, their intrafollicular concentrations have been shown to be considerably augmented under the influence of LH[9, 10]. Therefore, the possible effects of prostaglandins on follicular steroidogenesis are of some interest.

Prostaglandin PGE_2 has been shown to stimulate the *in vitro* progesterone formation by isolated rat[11] ovarian follicles. However, a possibility of species differences in this regard was indicated to us by our earlier observation that in the case of human ovarian follicles PGE_2 and $PGF_2\alpha$ inhibited the *in vitro* conversion of ^3H labeled pregnenolone to progesterone and androgens by that tissue[12]. Further, in their studies on cultured rabbit granulosa cells, Erickson and Ryan[8] reported that in contrast to observations with granulosa cells from spontaneous ovulators[6] both PGE_2 and $PGF_2\alpha$ failed to stimulate the production of either progesterone or estradiol in their system. In view of these considerations we have studied the effect of exogenously added prostaglandins PGE_1, PGE_2 and $PGF_2\alpha$ on the *in vitro* biosynthesis of estrone, estradiol and progesterone by the isolated rabbit ovarian follicles.

MATERIALS AND METHODS

Tissue preparation and incubation: Sexually mature female white New Zealand rabbits (3.5–5.0 kg) were used in these experiments. Animals were sacrificed by injection of air into the ear vein. Each animal yielded 10–12 ovarian follicles (1 mm or more) by dissection with instruments used for ophthalmologic surgery. In any one experiment follicles obtained from the same animal were used. Groups of 2–3 follicles were randomly distributed in incubation vessels containing 2 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, with added glucose (200 mg %). In each experiment one or two groups served as control while to the others various amounts of prostaglandins (dissolved in 0.02 ml ethanol) were added. The control vessels contained equal amounts of added ethanol. The vessels were then incubated for 3 h at 37° in an atmosphere of 95% O_2 and 5% CO_2. The incubation technique was essentially as described by Mills[13].

Estradiol, estrone and progesterone assays. These steroids were assayed as follows: After incubation, approximately 1000 c.p.m. each of tritiated estradiol (S.A. 114.0 Ci/mmol), estrone (S.A. 54.3 Ci/mmol) and progesterone (S.A. 48.0 Ci/mmol) were added to each vessel and the follicles were homogenized in the incubation medium. The homogenate was then extracted with diethyl ether (8 ml, 3 times) with centrifugation after each extraction. The ether extracts of each vessel were pooled and evaporated to dryness. Estradiol, estrone and progesterone fractions from the residue were isolated by column chromatography on Sephadex LH-20. The fractions were then each dissolved in 1 ml ethanol and suitable aliquots were taken for determination of recovery of the added tracers and for radioimmunoassays. The procedures followed for chromatographic separation and

Table 1. Effect of prostaglandins on the estrone and estradiol content of rabbit ovarian follicles after *in vitro* incubation (estrogen measurements were done on the follicles + incubation medium)

Prostaglandin added per ml	Estrone (ng/follicle)		Estradiol (ng/follicle)	
	Control	Exptl.	Control	Exptl.
PGE_1 (5 μgs)	1.44 ± 0.41	4.97 ± 2.81**	4.22 ± 3.08	10.13 ± 4.80(1)
PGE_1 (10 μgs)	1.44 ± 0.41	8.06 ± 2.60***	4.22 ± 3.08	15.43 ± 7.71*
PGE_2 (5 μgs)	2.71 ± 0.16	4.79 ± 1.25**	2.78 ± 0.26	5.85 ± 1.25***
PGE_2 (10 μgs)	2.61 ± 0.52	5.67 ± 1.28***	3.67 ± 0.48	5.87 ± 1.85*
$PGF_2\alpha$ (5 μgs)	2.18 ± 0.63	2.18 ± 0.84	3.45 ± 2.20	3.99 ± 2.30
$PGF_2\alpha$ (10 μgs)	1.68 ± 0.43	1.41 ± 0.25	3.10 ± 1.59	4.97 ± 2.72
LH (0.1 μg)	1.19 ± 0.24	4.92 ± 0.95****	5.40 ± 1.25	24.28 ± 10.25****

In Tables 1 and 2: Each value is the mean of 4–6 observations ± S.D. (1) $P < 0.1$, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$, Vs respective control.

radioimmunoassays of estradiol, estrone and progesterone fractions have been described by us in a recent publication[14]. The steroid values in each vessel were expressed as ng/follicle.

RESULTS AND DISCUSSION

Utilizing the above technique, the effect of PGE_1, PGE_2 and $PGF_2\alpha$ on the biosynthesis of estrone, estradiol and progesterone by the rabbit follicles was tested in a series of experiments. The three prostaglandins were tested at concentrations of 1, 2, 5 and 10 μg/ml of incubation medium. At the lower two concentrations the compounds showed no effect on steroid biosynthesis. The effects that were observed with 5 and 10 μg concentrations are shown in Tables 1 and 2.

The steroid content of a series of unincubated rabbit ovarian follicles was found by us to be: estrone, 0.13–0.28 ng/follicle; estradiol, 0.30–0.54 ng/follicle and progesterone, 1.8–4.7 ng/follicle. The values for different control incubations in Table 1 show that the follicles were synthesizing appreciable amounts of estrone and estradiol in the course of incubation. The progesterone content per follicle found after incubation (control values in Table 2) was variable and in comparison with values for unincubated follicles would indicate that only small amounts of progesterone were being synthesized or after synthesis the compound was being further metabolized. Addition of 0.1 μg/ml of LH (NIH-LH-B9), a known stimulator of steroid biosynthesis in the rabbit follicle[13], to the incubating follicles resulted in significant increase in the estrone, estradiol and progesterone content measured in the follicles together with the medium (Tables 1 and 2). This indicates that measurement of the estrone, estradiol and progesterone content of the incubated follicles together with the medium would provide an appropriate index of the formation of these steroids during the course of the incubation. However, we would like to point out that there is one limitation to the interpretation of the data thus obtained. The steroids that are formed during the incubation (especially progesterone[15]) may also be metabolized to a certain extent by the follicle and their final accumulation may reflect the combined result of biosynthesis and metabolism. Therefore, in the following discussion although the effects of prostaglandins on the estrone, estradiol and progesterone content of incubated follicles have been interpreted as being on steroid formation, the observed effects might also be in part due to altered metabolism of the formed steroids.

Table 2. Effect of prostaglandins on the progesterone content of rabbit ovarian follicles after *in vitro* incubation (progesterone measurements were done on follicles + incubation medium)

Prostaglandin added per ml	Progesterone (ng/follicle)	
	Control	Exptl.
PGE_1 (5 μgs)	4.96 ± 2.68	10.77 ± 3.36*
PGE_1 (10 μgs)	4.96 ± 2.68	22.77 ± 8.34***
PGE_2 (5 μgs)	5.71 ± 1.41	5.08 ± 1.44
PGE_2 (10 μgs)	3.31 ± 0.81	2.61 ± 0.49
$PGF_2\alpha$ (5 μgs)	1.94 ± 0.97	2.31 ± 1.14
$PGF_2\alpha$ (10 μgs)	1.52 ± 0.77	1.97 ± 0.70
LH (0.1 μg)	3.18 ± 2.40	28.13 ± 16.4****

* $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$.

In both control and experimental incubations there was considerable variation in values obtained from different follicles. This is reflected in the rather large standard deviations for many of the mean values in Tables 1 and 2. However, when the data were analyzed for statistical significance it showed interesting differences in the effects of the three prostaglandins. $PGF_2\alpha$ had no significant effect on estrogen or progesterone formation in the follicles. On the other hand, both PGE_1 and PGE_2 stimulated the *in vitro* biosynthesis of estrone and estradiol (Table 1). Progesterone formation was, however, stimulated by PGE_1 only while PGE_2 had no effect (Table 2). The levels of statistical significance of stimulation by PGE_1 and PGE_2 are shown in the two tables. It is difficult to explain why the biosynthesis of progesterone was not stimulated by PGE_2 while that of estrogens was.

After the completion of our studies Erickson and Ryan[16] have reported that testosterone production in isolated rabbit follicular thecal tissue was stimulated by both PGE_2 and $PGF_2\alpha$. Granulosa cells from the same follicles produced little testosterone *in vitro* and the prostaglandins had no effect on that production. Earlier they had observed[8] lack of any effect of PGE_2 and $PGF_2\alpha$ on the *in vitro* production of progesterone or estrogens by isolated rabbit granulosa cells. Our experiments were done with the whole follicles and thus one cannot surmise which cell type PGE_2 and PGE_1 act on. The lack of stimulatory effect of $PGF_2\alpha$ in our experiments as compared to its stimulatory effect on testosterone production by the isolated thecal tissue[16] may be due to the difference in steroids that were measured or due to a difference in the response of whole follicles as compared to that of isolated tissue.

Studies with corpora lutea[1–3], cultured granulosa cells[6, 7] and ovaries maintained in culture[4, 5] have shown that prostaglandins of the PGE series in general stimulate *in vitro* progesterone production by these tissues. $PGF_2\alpha$ is either less active or has inhibitory action on progesterone production by these ovarian tissues under *in vitro* conditions[3, 17]. Our results and those reported by Lindner *et al* on the rat follicles[11] show that prostaglandins of the PGE series also have a stimulatory effect on the *in vitro* production of progesterone and estrogens by the whole follicle. However, within the different compounds of the PGE series there might be species specificities regarding their stimulatory effects. Thus, in contrast to its effect on progesterone production by the rat follicles[11], PGE_2 did not stimulate progesterone production in our experiments with the rabbit follicles. As noted earlier, that compound also had no effect on progesterone formation by cultured rabbit granulosa cells[8]. $PGF_2\alpha$ when tested at doses comparable to those used for PGE_1 seems to have no effect on *in vitro* estrogen and progesterone formation in the whole follicle.

Whether the *in vitro* stimulatory effects of PGEs on the production of progesterone and estrogens by isolated ovarian follicles have any physiological significance under *in vivo* conditions is not certain. LeMaire *et al*[9], have found the endogenous follicular PGE concentrations to be 256 ± 125 pg per follicle in estrous rabbits with 5–15 times elevated concentrations in the follicles of HCG treated animals. In comparison with these endogenous levels, the amounts (5 and 10 μgs/ml) that were required in the present experiments to produce the *in vitro* stimulatory effects on steroid biosynthesis do not seem to be physiological. There are also other considerations which indicate that prostaglandins may not be involved in physiologic regulation of steroid biosynthesis in the rabbit follicle. In the female rabbit LH stimulation *in vivo* (endogenously released by coitus or exogenously administered) causes increased follicular biosynthesis of progesterone and estrogens[14, 18] and also of the prostaglandins PGE and PGF[9]. However, the rise in prostaglandin concentrations is detected considerably (about 4 h) after the LH induced rise in steroid concentrations. This seems to preclude the possibility of prostaglandins having an intermediary role in the LH induced stimulation of follicular steroidogenesis. This is also indicated by the recent observation of Armstrong *et al.*[19] that in the rat, indomethacin treatment 1 h before LH, prevented the LH induced increase in ovarian PGF levels but produced no significant inhibition of the LH induced rise in ovarian progesterone levels. Although levels of ovarian PGE were not measured in these experiments, indomethacin is known to be an inhibitor of biosynthesis of both PGF and PGE[20]. Another possibility for a regulatory role for prostaglandins on steroidogenesis in the rabbit follicle was suggested by the fact that the time period (5–10 h after LH stimulation[9]) of the rise in prostaglandin concentrations in the LH stimulated follicles corresponds to a period of declining levels of progesterone and estrogens in the follicles[14, 18]. Further, at 9–10 h after LH stimulation the prostaglandins reach their peak concentrations in the follicles while the progesterone and estrogen contents of such follicles[14, 18] as well as the amounts of these steroids released into ovarian vein[21] are found to be even lower at this time period than those in the case of unstimulated animals. These observations suggested the possibility that prostaglandins may have an inhibitory action on steroidogenesis in the rabbit follicles. Our *in vitro* results indicate that this may not be the case.

Acknowledgements—This work was supported by grant no. MA-1788 of Medical Research Council of Canada. We are grateful to the Upjohn Company, Kalamazoo, Michigan, for a gift of prostaglandins and to NIAMDD, NIH, Bethesda, for a gift of LH. We acknowledge the excellent technical assistance of Mr. Yvon Labelle and Mrs. Françoise Boulanger.

REFERENCES

1. Labhsetwar A. P.: *Fed. Proc.* **33** (1974) 61–77.
2. Marsh J. M. and LeMaire W. J.: *J. clin. Endocr. Metab.* **38** (1974) 99–106.
3. Rao Ch. V.: *Prostaglandins* **7** (1974) 209–213.
4. Neal P., Baker T. G., McNatty K. P. and Scaramuzzi R. J.: *J. Endocr.* **65** (1975) 19–25.
5. Neal P., Baker T. G. and McNatty K. P.: *J. reprod. Fertil.* **47** (1976) 157–159.
6. Channing C. P.: *Prostaglandins* **2** (1972) 331–349.
7. McNatty K. P., Henderson K. M. and Sawers R. S.: *J. Endocr.* **67** (1975) 231–240.
8. Erickson G. F. and Ryan K. J.: *Endocrinology* **97** (1975) 108–113.
9. LeMaire W. J., Yang N. S. T., Behrman H. R. and Marsh J. M.: *Prostaglandins* **3** (1973) 367–376.
10. LeMaire W. J., Leidner R. and Marsh J. M.: *Prostaglandins* **9** (1975) 221–229.
11. Lindner H. R., Tsafriri A., Lieberman M. E., Zor U., Koch Y., Bauminger S. and Barnea A.: *Recent Prog. Horm. Res.* **30** (1974) 79–138.
12. Patwardhan V. V. and Lanthier A.: *Prostaglandins* **6** (1974) 385–388.
13. Mills T. M.: *Endocrinology* **96** (1975) 440–445.

14. Patwardhan V. V. and Lanthier A.: *Acta endocr., Copenh.* **82** (1976) 792–800.
15. YoungLai E. V.: *Acta endocr., Copenh.* **73** (1973) 775–782.
16. Erickson G. F. and Ryan K. J.: *Endocrinology* **99** (1976) 452–458.
17. Behrman H. R. and Hichens M.: *Prostaglandins* **12** (1976) 83–95.
18. YoungLai E. V.: *J. Reprod. Fertil.* **30** (1972) 157–159.
19. Armstrong D. T., Dorrington J. H. and Robinson J.: *Can. J. Biochem.* **54** (1976) 796–802.
20. Yang N. S. T., Marsh J. M. and LeMaire W. J.: *Prostaglandins* **4** (1973) 395–404.
21. Hilliard J. and Eaton L. W.: *Endocrinology* **89** (1971) 522–527.