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In Vitro Ovulation and Prostaglandin Synthesis by Ovarian Follicles of *Rana dybowskii*

Hye Young Kong, Kyungja Chang, Wook-Bin Im, and Hyuk Bang Kwon*

Hormone Research Center and Division of Life Science, College of Natural Sciences, Chonnam National University, Kwangju 500-757, Korea

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Changes in the levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2) in culture medium during *in vitro* ovulation of *Rana dybowskii* follicles were examined. The ovulation was induced by frog pituitary homogenate (FPH) or TPA (12-*O*-tetradecanoylphorbol-13-acetate, a protein kinase activator) and the levels of PGs were measured by radioimmunoassay. When the ovarian follicles were cultured, only a few oocytes were ovulated by 12 h, but half of them were ovulated by 24 h in response to FPH, whereas around 30% of oocytes were ovulated by 12 h and maximum ovulation (around 50%) occurred by 24 h in response to TPA. Without any stimulation (control), no ovulation occurred. TPA elevated the level of $PGF_{2\alpha}$ to high levels when compared to control (basal levels), but the increase by FPH was less evident. Likewise, the levels of PGE_2 increased markedly in response to TPA, but rather decreased by FPH treatment. Interestingly, $PGF_{2\alpha}$ induced ovulation but PGE_2 suppressed FPH- or $PGF_{2\alpha}$-induced oocyte ovulation. Basal levels of PGs increased steadily during culture. When theca/epithelium (THEP) layer and granulosa cell-enclosed oocytes (GCEOs) were separated by microdissection and cultured independently, higher levels of both PGs were secreted by THEP than by GCEOs. Synthesis of PGs by follicle or follicular components was strongly suppressed by exogenous cAMP or indomethacin. These results suggest that: 1) $PGF_{2\alpha}$ plays an important role in *Rana* ovulation, 2) protein kinase C is involved in PGs production, and 3) theca/epithelium layer is responsible for the PGs production in *Rana*.

The preovulatory surge of luteinizing hormone (LH) induces a series of ovulatory changes culminating in the rupture of the follicle in mammals (Richards and Hedin, 1988; Irianni and Hodgen, 1992; Erickson, 1996). This process has been compared to an inflammatory response, in which the key regulators appear to be prostaglandins (PGs), plasminogen activators, steroids, and collagenase (Espey, 1980; Irianni and Hodgen, 1992). Various steroids and prostaglandins are produced by ovarian follicles in association with gonadotropin-induced ovulation in vertebrates (Cetta and Goetz, 1982; Tsang et al., 1988; Gobbetti and Zerani, 1992a, b; Hsu and Goetz, 1992). Although PGs play a role in ovulation, different types of PGs are known to act differently in mediating the ovulation process in various vertebrates. For example, in fishes, F type PGs stimulate in vitro oocyte ovulation but E type PGs do not (Goetz and Theofan, 1979), while E type PGs play important roles in ovulation in mammals (Murdoch et al., 1993). It was also reported that PGE_2 and $PGF_{2\alpha}$ acted antagonistically each other for luteal function in fishes and mammals (Michael et al., 1993; Murdoch et al., 1993). Recent reports also indicate that protein kinase C (PKC) mediates LH action in ovulation, while cAMP acts as a negative regulator in the ovulation process in various vertebrates (Goetz et al., 1982; Goetz, 1993; Morris and Richards, 1995; Chang et al., 1995, 1996). However, the ovulation process is a very complex event and the precise role and action mechanism of PGs in the ovulation process is not clearly understood (Morris and Richards, 1993; Shoham et al., 1995; Hedin and Erickson, 1997).

In the present study, using the frog ovarian follicle culture system, we examined the different roles of PGE_2 and $PGF_{2\alpha}$ during *in vitro* ovulation of frog follicles and determined which type of tissues are responsible for the synthesis of PGE_2 and $PGF_{2\alpha}$ in ovarian follicles. In addition, the role of PKC and cAMP in the ovulation process in the frog was examined.

Materials and Methods

Animals

Frogs (*R. dybowskii*) were collected from the stream in

* To whom correspondence should be addressed.
Tel: 82-62-530-3391, Fax: 82-62-530-0500
E-mail: kwonhb@chonnam.chonnam.ac.kr

the Chonnam area, a southwestern part of the Korea peninsular from October to March. Animals were kept in a state of artificial hibernation in glass tanks containing various sizes of stones in a cold room. Cold underground water or dechlorinated tap-water was supplied continuously through the tank (Kwon et al., 1992). Frogs were used for experiments within a week after collection.

Hormones and Reagents

TPA was dissolved in 8 mM of dimethylsulfoxide. cAMP was dissolved in amphibian Ringer (AR) in a stock of 2 mg/ml. A potent cyclooxygenase inhibitor, indomethacin (IM), prostaglandin E_2, and prostaglandin $F_{2\alpha}$ were dissolved in absolute ethanol in 2 mg/ml, 10 mg/ml, and 10 mg/ml, respectively. Frog-pituitary homogenate (FPH) was prepared from female frogs. Glands were homogenized in AR with an ultrasonic homogenizer (Ultrasonic W-380, U.S.A.). The homogenate was centrifuged (4℃, 15,000 g, 20 min) to remove debris and the supernatant was frozen (-20℃) in aliquots until needed.

All the reagents in stocks were diluted with corresponding vehicle before use. Vehicle concentration was maintained at 0.25% or 0.55% (v/v) of AR. All the concentration of reagents treated were chosen based on previous reports (Goetz et al., 1989; Kwon et al., 1992; Chang et al., 1995).

Preparation of follicular tissues

Frogs were killed by decapitation and their ovaries were carefully removed and transferred to petri-dishes containing AR. Ovarian fragments containing about 20 follicles were dissected from the ovary by using watchmaker's forceps under stereomicroscope. Ovarian fragment was used in the experiment for ovulation because ovulation can be achieved more readily from ovarian fragments than from isolated ovarian follicles (Kwon et al., 1992).

Granulosa cell-enclosed oocytes (GCEOs), theca/epithelium (THEP) layer and denuded oocytes (oocytes) were separated from ovarian fragments by manual dissection under a stereomicroscope (Kwon and Ahn, 1994). Using watchmaker's fine forceps, the THEP layer with blood vessels, was peeled away from the follicles in ovarian fragments, and simultaneously the GCEOs were separated from ovarian fragments. Usually, most granulosa cells remained on the membrane of the oocyte rather than on the THEP layer. In order to obtain denuded oocytes, GCEOs were further processed by washing 3 times with calcium-free ARs with 15 min shaking. Only those oocytes that have a smooth oocyte surface without any attached granulosa cells were considered as oocytes.

Culture of follicular components

In vitro culture was carried out using multiwell culture dishes (24 wells/dish; Nunc, Denmark). Ovarian fragments containing 20 follicles or different follicular components were cultured for various durations of time (0, 3, 6, 9, 12 h) in AR in the presence or absence of FPH (0.05 gland/ml), TPA (10 μM), cAMP (2.5 mM), indomethacin (5 μg/ml), PGE_2, or $PGF_{2\alpha}$ (5 μg/ml). The duration of follicle culture and the doses of FPH or chemicals were chosen based on a previous report (Kwon et al., 1992).

Ovarian fragments were transferred to each well of the culture plate containing 2 ml of AR and test agents were added to each well later. These plates were then incubated in a shaking incubator (80 oscillations per minute) at 22℃ for various periods of time. After culture, oocytes liberated from the ovarian fragment were counted and culture media were saved and kept in a deep freezer (-20℃) until assayed for prostaglandin radioimmunoassay.

Different types of follicular components obtained from 20 follicles each were also incubated in wells containing 2 ml of AR per well with or without test agents. Thus, THEP layers peeled from 20 follicles, 20 individual GCEOs and 20 individual oocytes were placed in each well and various test agents such as FPH (0.05 gland/ml), TPA (10 μM), cAMP (2.5 mM), indomethacin (5 μg/ml), PGE_2, and $PGF_{2\alpha}$ (5 μg/ml), were added later and cultured for 24 h.

Radioimmunoassay (RIA) of Prostaglandins

The amount of PGE_2 or $PGF_{2\alpha}$ secreted into the medium by ovarian follicles or follicular components during culture were measured by RIA. The general assay procedure for prostaglandin was adopted from Cetta and Goetz (1982) and our previous work (Chang et al., 1995). Prostaglandin $F_{2\alpha}$ antiserum (Sigma) was reported to crossreact 66% with prostaglandin F1α, less than 0.1% with prostaglandin F_1 and E_2, and less than 0.01% with prostaglandin A1, A2 or B1. The sensitivity for $PGF_{2\alpha}$-RIA was 5 pg of $PGF_{2\alpha}$/tube. PGE_2 antiserum (Sigma) was reported to crossreact 65% with PGE_1, 5% with $PGF_{1\alpha}$, 1.5% with $PGF_{2\alpha}$, 13% with PGB1, 6% with PGB2, 28% with PGA1 and 7% with PGA2. The sensitivity for PGE_2-RIA was 15 pg of PGE_2/tube. Labeled $PGF_{2\alpha}$([5, 6, 8, 9, 11, 12, 14, 15] n-^{3}H-prostaglandin $F_{2\alpha}$; 203 Ci/mmol) and PGE_2 ([5, 6, 8, 11, 12, 14, 15] n-^{3}H- prostaglandin; 187 Ci/mmol) was obtained from Amersham (Buckingamshire, U.K.). Each sample was quantified for tritium using a Packard Tri-Carb 2300, liquid scintillation analyzer. Duplicate hormone standards ($PGF_{2\alpha}$; 5-1,000 pg/100 μl, PGE_2; 25-2,000 pg/100 μl) were included in each assay. The between and within assay coefficients of variation for $PGF_{2\alpha}$ were 7.7% (n=22) and 4.3% (n=10) and those for PGE_2 were 7.5% (n=28) and 4.0% (n=10), respectively.

Statistical Analysis

The ovulation rate was calculated and data was pre-

sented as percent (%) ovulation. Experimental treatments were done in duplicate or triplicate using ovarian fragment and follicular components obtained from an individual frog. The average ovulation rates obtained from replicates were transformed using arcsin-square root transformation before statistical analysis. The ovulation data and PGs levels were analyzed by one- or two-way analysis of variance (ANOVA) or Student's t-test.

Results

Time course of FPH or TPA induced ovulation during ovarian fragment culture

Initially, experiments were carried out to determine the time course of *in vitro* oocyte ovulation in the frog. Ovarian fragments containing 20 follicles obtained from frogs collected in February were cultured for up to 24 h in the presence or absence of FPH (0.05 gland/ml) or TPA (10 μM), and ovulated oocytes were counted at designated time points (0, 3, 6, 9, 12, and 24 h) (Fig. 1). Only a small number of oocytes ovulated by 12 h, but around half of the oocytes (48%) ovulated by 24 h in response to FPH. However, TPA induced ovulation began to occur by 6 h and significantly increased by 9-12 h ($P<0.05$, when compared to control) and consistently increased until cultured for 24 h (51%). Without addition of hormone or phorbol ester, the oocyte ovulation was not observed throughout the culture period (control). Thus, it is evident that TPA stimulates ovulation more effectively than FPH by 9-12 h of culture ($P<0.05$, when compared to FPH treated group), but not by 24 h of culture.

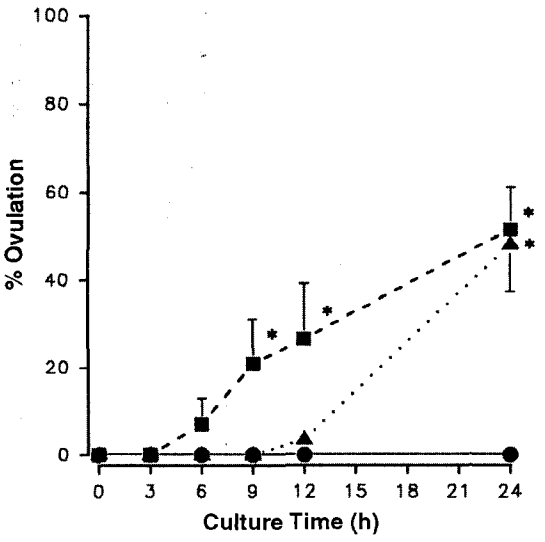

Fig. 1. Time course of ovulation by ovarian fragments obtained from frogs collected in February. Ovarian fragments were cultured in the absence (●) or presence of FPH (▲, 0.05 gland/ml) or TPA (◆, 10 μM) for up to 24 h. At designated time points, the ovulation rate was examined under a microscope. Each point represents the average of percent ovulation (x̄±SE) per fragment (5 animals). *P<0.01.

Effects of FPH and TPA on the secretion of $PGF_{2\alpha}$ and PGE_2 by the ovarian fragments following in vitro culture

To determine whether FPH or TPA stimulate synthesis of $PGF_{2\alpha}$ and PGE_2 by ovarian follicles, ovarian fragments were cultured for up to 24 h in the presence of FPH (0.05 gland/ml) or TPA (10 μM) and, at designated time points, PGs levels in culture medium were measured by RIA (Fig. 2). The amounts of $PGF_{2\alpha}$ in medium increased markedly by 9 h and increased consistently until 24 h in response to TPA ($P<0.05$, when compared to control or FPH treated group). The level of $PGF_{2\alpha}$ also increased steadily to higher levels by 24 h ($P<0.05$, when compared to basal level) in response to FPH. Basal levels of $PGF_{2\alpha}$ also steadily increased throughout the culture period ($P<0.01$, by one-way ANOVA) although less evident than those observed in TPA or FPH treated groups (Fig. 2). Levels of PGE_2 increased markedly by 9 h and reached the most by 24 h in response to TPA ($P<0.05$, when compared to basal or FPH treated groups). Interestingly, however, PGE_2 levels were much lower throughout the culture hour in the presence of FPH than those observed in basal levels ($P<0.05$, when compared to control at 24 h). Thus, it is evident that FPH rather suppressed PGE_2 synthesis by follicles while TPA stimulated it. It is also of interest that absolute amounts of PGE_2 secreted by the follicles were much higher

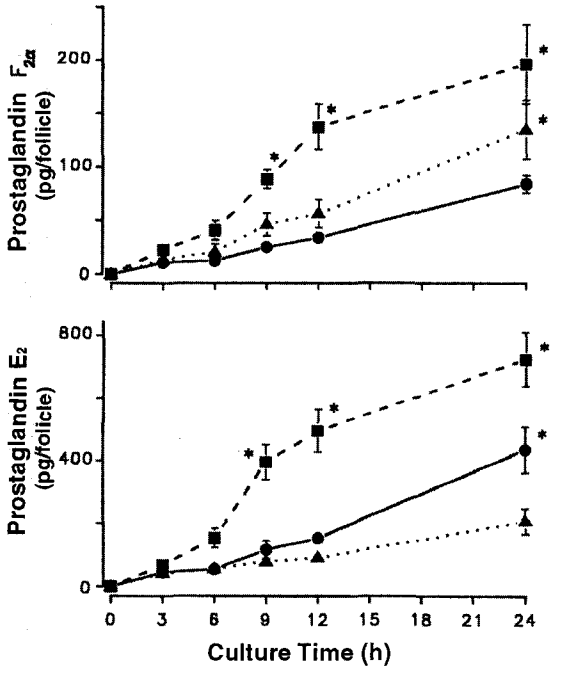

Fig. 2. Time course of prostaglandin secretion by ovarian fragments obtained from frogs collected in February. Ovarian fragments were cultured in the absence (●) or presence of FPH (▲, 0.05 gland/ml) or TPA (■, 10 μM) for up to 24 h. At the designated time points, the culture medium was collected and the concentration of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 in the medium were measured by RIA. Each point represents the average picogram of $PGF_{2\alpha}$ and E_2 (Mean±SE) per follicle (5 animals). *P<0.01.

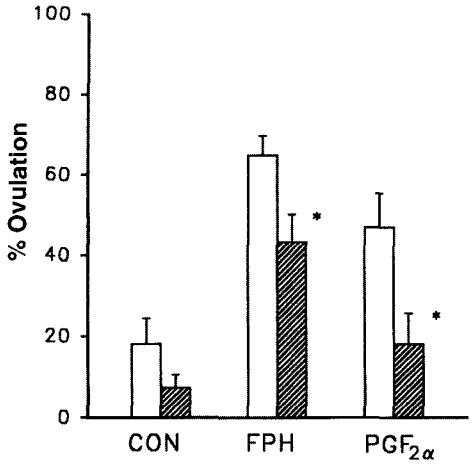

Fig. 3. Effect of prostaglandin E_2 on FPH- and $PGF_{2\alpha}$-induced ovulation *in vitro*. Ovarian fragments obtained frogs collected in February and early March were cultured for 24 h in the presence or absence of FPH (0.05 gland/ml) or $PGF_{2\alpha}$ (5 μg/ml) with or without PGE_2 (5 μg/ml). Ovulation rate was examined after 24 h of culture. Each bar in the figure represents the average percent ovulation (x ± SEM) per fragment in 11 frogs. *P<0.05.

than those of $PGF_{2\alpha}$ throughout the culture period (Fig. 2).

Effect of PGE_2 on FPH or $PGF_{2\alpha}$ induced ovulation

In preliminary experiments, we found that treatment of $PGF_{2\alpha}$, but not PGE_2, to cultured ovarian follicles induced oocyte ovulation *in vitro*. Experiments were carried out to assess the effect of PGE_2 on FPH- or $PGF_{2\alpha}$-induced ovulation. The ovarian fragments harvested during February and early March were cultured in the presence of FPH (0.5 gland/ml) or $PGF_{2\alpha}$ (5 μg/ml) with or without PGE_2 (5 μg/ml). Ovulated oocytes were counted after 24 h of culture. As expected, treatment of FPH or $PGF_{2\alpha}$ induced ovulation effectively by follicles, but simultaneous addition of PGE_2 suppressed the FPH- or $PGF_{2\alpha}$-induced ovulation significantly *($P<0.05$, by* t-test*)* (Fig. 3). Treatment of PGE_2 alone to the follicles did not induce ovulation and rather suppressed the ovulation which occurred spontaneously (control) (Fig. 3). Thus it is evident that PGE_2 acts negatively on oocyte ovulation *in vitro*.

Productions of PGE_2 and $PGF_{2\alpha}$ by different types of follicular components

Experiments were carried out to determine which components, THEP layers or GCEOs, are responsible for producing $PGF_{2\alpha}$ and PGE_2 in ovarian follicles. Intact follicles, GCEOs, THEP layers, or denuded oocytes were obtained from ovarian fragments by microdissection. The follicular components were cultured for 24 h in the presence or absence of FPH or TPA. The levels of PGE_2 and $F_{2\alpha}$ in the medium were determined by RIA (Fig. 4). In the presence of FPH, much higher levels of $PGF_{2\alpha}$ were produced by intact follicles (323 pg/follicle) and the THEP layer (192 pg/follicle) than

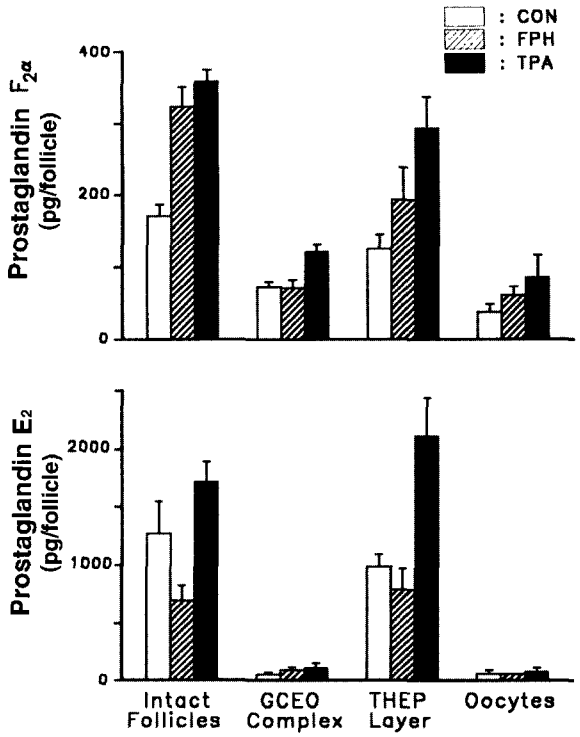

Fig. 4. The production of $PGF_{2\alpha}$ and PGE_2 by different types of follicular components in *R. dybowskii*. Intact follicles, granulosa cell-enclosed oocytes (GCEOs), theca/epithelium (THEP) layer, and denuded oocytes were obtained from frogs collected in January to March and cultured for 24 h in the presence or absence of FPH (0.05 gland/ml) or TPA (10 μM). The concentrations of $PGF_{2\alpha}$ and PGE_2 in medium were measured by RIA after 24 h of culture. Each bar in the figure represents the average concentration (x ± SE) of PGs per follicle (3-5 animals). *P<0.01.

those produced by GCEOs (70 pg/follicle) or denuded oocytes (59 pg/follicle) (*$P<0.01$*, when compared THEP layer with GCEOs by two-way ANOVA). Likewise, TPA stimulated $PGF_{2\alpha}$ production by intact follicles (359 pg/follicle) and THEP layer (294 pg/follicle), but much less by GCEOs (121 pg/follicle) and oocytes (86 pg/follicle) (*$P<0.01$* between THEP and GCEOs). Even in the absence of TPA or FPH, considerable amount of $PGF_{2\alpha}$ was produced by intact follicles (170 pg/follicle) and the THEP layer (124 pg/follicle), but not by GCEOs (72 pg/follicle) and oocytes (37 pg/follicle). Similarly, very high levels of PGE_2 were produced by intact follicles (1,725 pg/follicle) and THEP (2,116 pg/follicle) while negligible levels of the hormone were produced by GCEOs or oocytes in the presence of TPA. Interestingly, FPH did not stimulate PGE_2 production by intact follicles or THEP and produced lower levels than basal levels (Fig. 4). It is also of interest that considerable amounts of PGE_2 were produced by intact follicles (1274 pg/follicle) and THEP (985 pg/follicle) without any stimulation (basal levels).

Effects of cAMP on FPH-induced $PGF_{2\alpha}$ and PGE_2 synthesis in ovarian follicular tissues

Previously, we observed that cAMP suppressed the

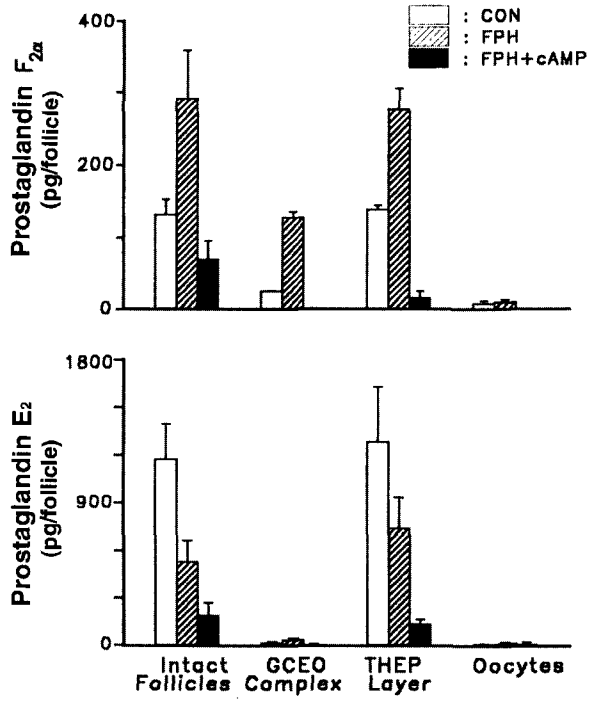

Fig. 5. Effects of cAMP on FPH- and TPA-stimulated production of PGE_2 and $PGF_{2\alpha}$ by follicular components in *R. dybowskii* collected in March. Intact follicles, GCEOs complex, THEP layer, and oocytes were obtained by microdissection and cultured in the presence or absence of FPH (0.05 gland/ml) with or without cAMP (2.5 mM) for 24 h. After 24 h of culture, concentrations of $PGF_{2\alpha}$ and PGE_2 in medium were measured by RIA. Each bar in the figure represents the average concentration ($\bar{x} \pm$ SE) of PGs per follicle (3 animals). *P<0.05.

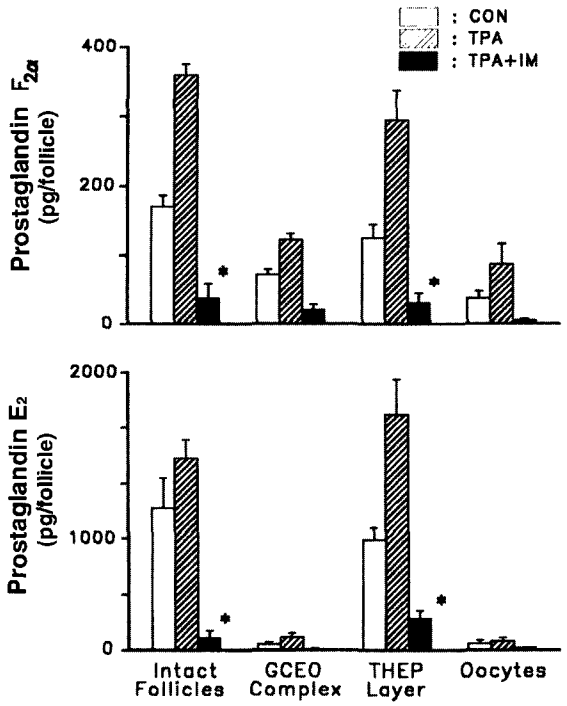

Fig. 6. Effect of IM on TPA-stimulated production of $PGF_{2\alpha}$ and PGE_2 by follicular components. Intact follicles, GCEO complex, THEP layer, and oocytes were isolated from ovarian follicles and the follicular components were cultured in the presence or absence of TPA (10 μM) with or without IM (5 μg/ml) for 24 h. After culture, the amounts of $PGF_{2\alpha}$ or PGE_2 in medium were measured by RIA. Each bar in the figure represents the average concentration ($\bar{x} \pm$ SE) of $PGF_{2\alpha}$ or PGE_2 per follicle (3 animals). *P<0.01.

production of $PGF_{2\alpha}$ by intact follicles in culture. In order to ascertain whether cAMP acts differently on PGs production by different tissues, THEP layer and GCEOs were separated from ovarian follicles and cultured in the presence or absence of FPH (0.05 gland/ml) with or without cAMP (2.5 mM). $PGF_{2\alpha}$ or PGE_2 levels in the medium were measured for 24 h of culture (Fig. 5). Higher levels of $PGF_{2\alpha}$ were produced by intact follicles (290 pg/follicle) and THEP layer (276 pg/follicle) than by GCEOs (136 pg/follicle) *($P<0.01$,* when compared THEP with GCEOs). Addition of exogenous cAMP, suppressed the FPH stimulated $PGF_{2\alpha}$ production by intact follicles (70 pg/follicle) and THEP layer (16 pg/follicle) (*$P<0.01$*) and the levels were lower than basal levels (Fig. 5). Even in the absence of any stimulation, considerable amounts of PGE_2 were produced by intact follicles (1,172 pg/follicle) and THEP (1,282 pg/follicle) (Fig. 5). Treatment of FPH decreased the basal production of PGE_2 to lower levels by intact follicles (527 pg/follicle) and THEP (737 pg/follicle). Simultaneous addition of FPH and cAMP produced the lowest levels of PGE_2 by intact follicles (190 pg/follicle) and THEP (137 pg/follicle). GCEO's and oocytes produced almost non-detectable levels for PGE_2. Thus, it is evident that cAMP suppressed the synthesis of $PGF_{2\alpha}$ and PGE_2 by ovarian components and did not exhibit any distinguishing effect on different tissues.

Effects of Indomethacin on TPA-induced $PGF_{2\alpha}$ and PGE_2 synthesis by different types of follicular compartments

Experiments were carried out to ascertain whether IM suppressed TPA-induced PG synthesis by different ovarian components. Various follicular components were cultured for 24 h in the presence or absence of TPA (10 μM) with or without IM (5 μg/ml) and then PGs levels in media were measured. As seen in Fig. 6, TPA stimulated $PGF_{2\alpha}$ production by intact follicles and THEP layers and PG productions were inhibited significantly by the presence of indomethacin (*$P<0.01$,* by t-test) in both groups. IM also strongly suppressed TPA-induced production of PGE_2 by intact follicles or THEP layers significantly (*$P<0.01$*).

Discussion

The data presented here demonstrate that $PGF_{2\alpha}$, not PGE_2, is associated with ovulation. We also note that the theca-epithelium layer is a major site of prostaglandins syntheses, and protein kinase C mediates LH action on prostaglandin synthesis in amphibian ovarian follicles.

It is of interest that protein kinase C activation is more effective than frog gonadotropin in inducing ovulation and stimulating $PGF_{2\alpha}$ and PGE_2 production *in vitro* (Figs. 1 and 2). Particularly, it appeared that FPH consistently suppressed PGE_2 production by ovarian follicles while protein kinase C activation stimulated it (Figs. 2, 4, and 5). This suggests that protein kinase C activation is not the only signal transduction pathway for gonadotropin action and other signal transduction pathway may be present in follicles which is responsive to gonadotropins. Possibly, PGE_2 production is suppressed by some factors present in this alternative pathway which is triggered by gonadotropins.

Since PGE_2 suppressed $PGF_{2\alpha}$- and FPH-induced ovulation, it is likely that PGE_2 acts as a negative regulator of ovulation in amphibian follicles (Fig. 3). In several fishes, it was reported that the levels of F type prostaglandins were high at the time of ovulation and the levels of E type prostaglandins were high just before oocyte maturation and low at the time of ovulation (Cetta and Goetz, 1982). Since oocyte maturation occurred prior to ovulation in fish as observed in other vertebrates, PGE_2 is not directly linked to ovulation in this animal (Goetz et al., 1989). Various steroids, and PKC activators were known to induce $PGF_{2\alpha}$ synthesis and ovulation during *in vitro* culture of ovarian follicles in fishes (Ranjan and Goetz, 1987; Berndtson et al., 1989; Goetz et al., 1991; Ballou et al., 1992). In addition, PGE_1 and PGE_2 were known to increase intracellular cAMP production and inhibit spontaneous ovulation in brook trouts and gold fish (Goetz and Nagahama, 1985). Taken together, as observed in the data presented here, $PGF_{2\alpha}$ plays a positive role and PGE_2 a negative role in ovulation of fishes. In contrast to this, in mammals, gonadotropin or hCG induces PGE_2 as well as $PGF_{2\alpha}$ synthesis and ovulation (Higuchi et al., 1995) and the levels of PGE_2 are much higher than those of $PGF_{2\alpha}$ during *in vitro* ovulation (Murdoch et al., 1993). Thus, in mammals, PGE_2 as well as $PGF_{2\alpha}$ seem to play an important role in the ovulation process (Wang and Leung, 1989; Higuchi et al., 1995)

Although there is much knowledge about the site of prostaglandin synthesis in other animals, there was no report about the site of prostaglandins synthesis in amphibian ovarian follicles. In the present studies, we succeeded to separate the theca/epithelium layer from granulosa cell enclosed oocytes using a microdissection technique and make it possible to examine which components of follicle are responsible for the production of prostaglandins by follicles. Data presented here clearly show that the theca-epithelium layer is a major site of prostaglandins synthesis in follicles (Figs. 4 and 5).

There have been a number of investigations on the role of cAMP in ovulation or prostaglandin synthesis by ovarian follicles in fishes. Exogenous cAMP was known to suppress $PGF_{2\alpha}$ synthesis and ovulation in some fishes (Goetz et al., 1982, 1991). Data presented here also indicated that cAMP suppresses TPA or FPH induced prostaglandin production in *Rana* follicles (Fig. 5). It was also reported that treatment of cAMP analog suppressed $PGF_{2\alpha}$ synthesis and ovulation in rabbit ovaries in culture (Yoshimura et al., 1994). Thus it seems likely that cAMP plays a negative role in ovulation in various vertebrates including mammals. However, considering the fact that a pulse increase in cAMP levels commonly occurs *in vivo*, we can not completely exclude the possibility of the positive action of a cAMP surge in the ovulation process.

Previously, we have shown that TPA- or FPH-induced ovulation and $PGF_{2\alpha}$ production by ovarian follicles were inhibited by indomethacin (Chang et al., 1996; Espey et al., 1986). The present data also demonstrated that TPA-induced prostaglandins syntheses were drastically inhibited by indomethacin in various follicular tissues (Fig. 6). Thus, it is evident that IM suppressed the synthesis of PGE_2 as well as $PGF_{2\alpha}$ regardless of the type of tissues.

In summary, the present study demonstrates that prostaglandin $F_{2\alpha}$ plays a key role in ovulation in amphibians and protein kinase C mediates gonadotropin action in inducing ovulation and stimulating $PGF_{2\alpha}$ synthesis. In addition, it is demonstrated, for the first time, that prostaglandins are synthesized predominantly by the theca-epithelium layer in frog follicles.

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References

Berndtson AK, Goetz FW, and Duman, P (1989) *In vitro* ovulation, prostaglandin synthesis, and proteolysis in isolated ovarian components of yellow perch (*Perca flavescens*): effects of 17α-20β-dihydro-4-pregnen -3-one and phorbol ester. *Gen Comp Endocrinol* 75: 454-465.

Ballou LR, Chao CP, Holness MA, Barker SC, and Raghow R (1992) Interleukin-1-mediated PG E_2 production and sphingomyelin metabolism. *J Biol Chem* 267: 20044-20050.

Cetta P and Goetz PW (1982) Ovarian and plasma Prostaglandin E and F levels in brook trout (*Salvelinus fontinalis)* during pituitary induced ovulation. *Biol Reprod* 27:1216-1221.

Chang KJ, Kim JW, Lee J, Im WB, Kwon HB, and Schuetz AW (1995) Prostaglandin production and ovulation during exposure of amphibian ovarian follicles to gonadotropin or phorbolester *in vitro*. *Gen Comp Endocrinol* 100: 257-266.

Chang KJ, Ra CH, Soh JM, Lee WK, and Kwon HB (1996) Induction of oocyte ovulation and prostaglandin synthesis by gonadotopin and phorbol ester *in vitro* in amphibian (*Rana nigromaculata*) ovarian follicles. *Korean J Zool* 39: 266-272.

Erickson GF (1996) Physiologic basis of ovulation induction. *Semin Reprod Endocrinol* 14: 287-297.

Espey LL (1980) Ovulation as an inflammatory reaction-a hypothesis. *Biol Reprod* 22: 73-106.

Espey LL, Norris C, and Saphire D (1986) Effect of time and dose of indomethacin of follicular prostaglandins and ovulation in the rabbit. *Endocrinology* 119: 746-754.

Gobbetti A and Zerani M (1992a) $PGF_{2\alpha}$, PGE_2, and sex steroids from the abdominal gland of the male crested newt *Thiturus carnifx* (Laurd). *Prostaglandins* 43:101-109.

Gobbetti A and Zerani M(1992b) A possible involvement of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in *Rana esculenta* ovulation: effects of mammalian gonadotropin releasing hormone on *in vitro* $PGF_{2\alpha}$ and 17β-estradiol production from ovary and oviduct. *Gen Comp Endocrinol* 87: 163-170.

Goetz FW (1993) Involvement of protein kinase C in agonist-stimulated goldfish ovulation. *Biol Reprod* 48: 846-850.

Goetz FW, Duman P, Ranjan M, and Herman CA (1989) Prostaglandin F and E synthesis by specific tissue components of the brook trout *(Salvelinus fontinalis)* ovary. *J Exp Zool* 250: 196-205.

Goetz PW and Nagahama Y (1985) The *in vitro* effects of cyclicnucleotides on prostaglandin-induced ovulation of gold fish (*Carassius auratus*). *Zool Sci* 2: 225-228.

Goetz PW, Hsu S-Y, and Selover A (1991) Stimulation of prostaglandin synthesis in fish follicles by a phorbol ester and calcium ionophore. *J Exp Zool* 259: 355-364.

Goetz FW, Smith DC, and Krickl SP (1982) The effects of prostaglandins, phosphodiesterase inhibitors and cyclic AMP on ovulation of brook trout (*Salvelinus fontinalis*) oocytes. *Gen Comp Endocrinol* 48: 154-160.

Goetz FW and Theofan G (1979) *In vitro* stimulation of germinal versicle breakdown and ovulation of yellow perch (*Perca flavescens*) oocytes. Effects of 17α-hydroxy-20β-dihydroprogesterone and prostaglandin. *Gen Comp Endocrinol* 37: 273-285.

Hedin L and Erikson, A. (1997) Prostaglandin synthesis is suppressed by progesterone in rat preovulatory follicles *in vitro*. *Prostaglandins* 53: 91-106.

Higuchi Y, Yoshimura T, Tanaka N, Ogino H, Sumiyama M, and Kawakami, S (1995) Different time-course production of peptidic and nonpeptidic leukotrienes and prostaglandins E_2 and $F_{2\alpha}$ in the ovary during ovulation in gonadotropin-primed immature rats. *Prostaglandins* 49: 131-140.

Hsu SY and Goetz PW (1992) The effect of E and F prostaglandin on ovarian cAMP production and follicular contraction in brook trout (*Salvelinus fontinalis*). *Gen Comp Endocrinol* 88: 434-443.

Irianni F and Hodgen GD (1992) Metabolism of ovulation. *Endocrinol Metab Clin North Am* 21: 19-38.

Kwon HB and Ahn RS (1994) Relative roles of theca and granulosa cells in ovarian follicular steroidogenesis in the amphibian, *Rana nigromaculata*. *Gen Comp Endocrinol* 94: 207-214.

Kwon HB, Chang KJ, Yoo YR, Lee CC, and Schuetz AW (1992) Induction of ovulation and oocyte maturation of amphibian (*Rana dybowskii*) ovarian follicles by protein kinase C *in vitro*. *Biol Reprod* 47: 169-176.

Michael AE, Abayasekara DRE, and Webley GE (1993) The luteotrophic actions of prostaglandins E2 and F2α on dispersed marmoset luteal cells are differentially mediated via cyclic AMP and protein kinase C. *J Endocrinol* 138: 291-298.

Morris JK and Richards JS (1993) Hormone induction of luteinization and prostaglandin endoperoxide synthase-2 involves multiple cellular signaling pathways. *Endocrinology* 133: 770-779.

Morris JK, and Richards, JS (1995) Luteinizing hormone induces prostaglandin endoperoxide synthase-2 and luteinization *in vitro* by A-kinase and C-kinase pathways. *Endocrinology* 136: 1549-1558.

Murdoch WJ, Hansen T, and Mcpherson LA (1993) A review-role of eicosanoids in vertebrate ovulation. *Prostaglandins* 46: 85-115.

Ranjan M and Goetz FW (1987) Protein kinase C as a possible mediator of gold fish(*Carassius auratus*) ovulation. *J Exp Zool* 242: 355-361.

Richards JS and Hedin L (1988) Molecular aspects of hormone action in ovarian follicular development, ovulation, and luteinization. *Annu Rev Physiol* 50: 441-463.

Shoham Z, Schacter M, Loumaye E, Weissman A, MacNamee M, and Insler V (1995) The Luteinizing hormone surge the final stage in ovulation induction: modern aspects of ovulation triggering. *Fertil Steril* 27: 237-251.

Tsang BK, Arodi J, Li M, Anisworth L, Srikandakumar A, and Downey BR (1988) Gonadotropic regulation of prostaglandin production by ovarian follicular cells of the pig. *Biol Reprod* 38: 627-635.

Wang J and Leung PC (1989) Synergistic stimulation of prostaglandin E2 production by calcium ionophore and protein kinase C activator in rat granulosa cells. *Biol Reprod* 40: 1000-1006.

Yoshimura Y, Hosoi Y, Atlas SJ, Ghodgaonkar R, Dubin NH, Dharmarajan AM, and Wallach EE (1994) Inhibition of gonadotrophin-induced ovulation in rabbits by perfusion with dibutyryl cAMP via reduction of ovarian prostaglandin production. *J Reprod Fertil* 101: 207-212.

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