THE JOURNAL OF EXPERIMENTAL ZOOLOGY 277:155–165 (1997)

Differential Effects of Gonadotropin and Orthovanadate on Oocyte Maturation, Ovulation, and Prostaglandin Synthesis by *Rana* Ovarian Follicles In Vitro

KYUNG J. CHANG,[1] JUNG W. KIM,[1] WOOK-BIN IM,[1] HAE M. KANG,[2] AND HYUK B. KWON[1*]

[1]*Department of Biology and Hormone Research Center, Chonnam National University, Kwangju 500-757, Republic of Korea*

[2]*Department of Genetic Engineering, Chongju University, Chongju 360-764, Republic of Korea*

ABSTRACT Effects of gonadotropin and sodium orthovanadate on oocyte maturation, ovulation, prostaglandin, and progesterone synthesis were examined during in vitro culture of *Rana* ovarian follicles obtained from hibernating animals. Frog pituitary homogenates (FPH, 0.05 gland/ml) effectively induced oocyte maturation (germinal vesicle breakdown, GVBD) and ovulation (>90%) in ovarian follicles obtained in mid-hibernation (mid-December through late January). In contrast, orthovanadate induced a limited amount (<45%) of ovulation of some oocytes without concomitant induction of maturation during mid-hibernation. In late hibernation (February to early March), considerable spontaneous maturation and ovulation occurred in cultured ovarian follicles. During this period both FPH (0.0005–0.05 gland/ml) or orthovanadate (0.01–1 mM) treatment markedly increased the incidence of ovulation and accelerated the onset of ovulation in a dose dependent manner. When examined after 12 h of culture, few (< 5%) oocytes ovulated in response to orthovanadate had undergone GVBD whereas most (> 90%) of those ovulated in response to FPH had matured. However, the majority of oocytes ovulated (72%) within 6 h of exposure to FPH had intact GVs, indicating that GVBD is also not a prerequisite for ovulation induction in response to FPH. Orthovanadate stimulated $PGF_{2\alpha}$ in a dose dependent manner but failed to stimulate progesterone production whereas FPH stimulated secretion of both $PGF_{2\alpha}$ and progesterone. The amount and time course of $PGF_{2\alpha}$ secretion in response to orthovanadate were similar to results produced with FPH stimulation. Treatment of $PGF_{2\alpha}$ to follicles obtained in late hibernation also accelerated the ovulation of the oocytes. Taken together, the data suggest that orthovanadate enhanced ovulation of immature oocytes was mediated via enhanced $PGF_{2\alpha}$ production and that oocyte maturation is not essential or prerequisite for in vitro oocyte ovulation in *Rana. J. Exp. Zool. 277:155–165, 1997.*

It has been well established that various gonadal hormones (steroids, prostaglandins) produced by the ovarian follicles in response to gonadotropins play key roles in the ovulatory process in most vertebrates including fish and mammals (Jones, '87; Guillette et al., '91; Murdoch et al., '93). Typically, oocyte maturation and ovulation occur more or less synchronously in response to gonadotropin stimulation in vivo or in vitro and it is believed that the two events are closely linked to each other (Masui and Clarke, '79; Schuetz, '85).

It was demonstrated in previous studies that progesterone, 12-*O*-tetradecanoylphorbol-13-acetate (TPA, a protein kinase C activator), and frog pituitary homogenate (FPH) induced ovulation of *Rana* oocytes during ovarian fragment or follicle culture (Wright, '61; Schuetz, '71; Kwon et al., '89, '92). In these studies nearly all oocytes ovulated in response to hormone or ovulation agonist treatment underwent GVBD when oocytes were examined at the end of the culture period (18–24 h). Furthermore, agents (cycloheximide, cAMP, aminogluthethimide) that inhibited oocyte maturation were found to strongly suppress in

Contract Grant sponsor: Korea Science and Engineering Foundation; Contract Grant number HRC-95-0103; Ministry of Education of Korea, Contract Grant number BSRI-94-4425.

*Correspondence to: Dr. Hyuk B. Kwon, Department of Biology, Chonnam National University, Kwangju 500-757, Republic of Korea.

Received 22 May 1996; Revision accepted 11 September 1996

vitro ovulation of oocytes (Schuetz, '86; Kwon et al., '92). Such results suggest that oocyte maturation may be a prerequisite for oocyte ovulation in *Rana*.

However, Schuetz ('86) found that immature oocytes were ovulated following prostaglandin treatment of ovarian follicles. We also observed that a considerable number of oocytes ovulated with intact GVs in vitro in response to gonadotropin or other ovulation agonists in *Rana* (unpublished data).

In recent studies with fish, orthovanadate was found to induce oocyte ovulation and to stimulate prostaglandin synthesis in vitro (Ranjan and Goetz, '90; Hsu and Goetz, '91, '93). Preliminary studies carried out with *Rana* also indicated that orthovanadate induced oocyte ovulation, although less efficiently than FPH, without concomitant oocyte maturation induction. Thus, studies were carried out to more closely assess and compare the effects of FPH and orthovanadate on oocyte maturation and ovulation as well as the secretion of prostaglandin and progesterone. These studies were carried out using ovarian follicles obtained at the different periods of hibernation. Our results indicate that the selective effects of orthovanadate on ovulation are closely correlated with its ability to stimulate prostaglandin but not progesterone synthesis.

MATERIALS AND METHODS

Animals

Frogs (*Rana dybowskii*) were collected in late autumn or early winter from streams in southwestern Korea. Animals were kept in artificial hibernation in a dark room without heating in plastic tanks containing various sizes of stones under which they hibernate. Cold tap water was supplied continuously through the tank. Hibernation periods for this frog were arbitrarily divided into three periods: October to mid-December (early hibernation), mid-December to January (mid-hibernation), and February to early March (late hibernation).

Culture of ovarian fragments

Animals were killed by decapitation, ovaries were removed immediately and placed in amphibian Ringer (AR) (Kwon and Schuetz, '85). Ovarian fragments containing about 20 follicles each were dissected from the ovary using watchmaker's forceps. All experimental manipulation was conducted at room temperature (18–22° C). Routine in vitro culture was carried out using multiwell culture dishes (24 wells/dish) with one ovarian fragment cultured in 2 ml AR per well (Kwon et al., '92). In time course studies, five ovarian fragments were cultured in a flask containing 10 ml of AR. Cultures were maintained in a shaking incubator at 24°C and agitated at 80 oscillations per min for various periods of time. Sodium orthovanadate (Na_3VO_4), purchased from Sigma Chemical Co. (St. Louis, MO), was dissolved in AR in a stock of 200 mM. FPH was prepared from female frogs as described elsewhere (Kwon and Schuetz, '85). Different concentrations of vanadate were prepared by diluting stock solutions with AR. At designated time points, oocytes liberated from the ovarian fragments were collected, counted, and fixed with trichloroacetic acid (TCA, 5%) to assess the presence or absence of follicle cells and oocyte GVBD. After fixation, only oocytes having a smooth, shiny surface and devoid of follicle cells were counted as ovulated oocytes. Culture media were saved and kept in a deep freezer (–20°C). The absence of follicle cells on oocytes was monitored by comparing the oocyte surface with follicle cell enclosed oocytes (defolliculated oocyte) and denuded oocytes. Defolliculated oocytes were obtained by peeling away the theca/epithelium layer from intact ovarian follicles with microforceps. Attached follicle cells turned white following TCA treatment (Schuetz and Lessman, '82). Denuded oocytes were prepared by shaking defolliculated oocytes in calcium-free AR (Schuetz and Lessman, '82). Our previous studies showed that the oocytes attached with follicle cells (defolliculated oocytes) produced progesterone as efficiently as intact follicles while denuded oocytes produced nearly no detectable amount of progesterone in response to FPH (Kwon and Ahn, '94). The ovulated oocytes in response to vanadate or FPH exhibited no white follicle cells in their surface after TCA fixation and had no capacity to produce progesterone (data not shown). All oocytes in ovarian follicles were found to have intact GVs at the time of isolation regardless of the season. Normally follicular oocytes undergo GVBD and ovulate in vitro in response to FPH from mid-hibernation. However, in late hibernation follicular oocytes spontaneously undergo GVBD and ovulate without FPH treatment (Kwon et al., '89, '92).

Prostaglandin and progesterone radioimmunoassay (RIA)

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and progesterone secreted into the culture medium by ovarian follicles during culture were measured by RIA. General

assay procedures for $PGF_{2\alpha}$ were adapted from those described by Cetta and Goetz ('82), whereas progesterone was measured as described in earlier work (Kwon et al., '89). Medium samples were assayed directly without extraction. Validation procedure for $PGF_{2\alpha}$ RIA with amphibian follicle culture was described by Chang et al., ('95) and that for progesterone by Kwon et al., ('89). Antiserum for $PGF_{2\alpha}$, which was purchased from Sigma Chemical Co., was reported to crossreact 60% with prostaglandin $F_{1\alpha}$, less than 0.1% with prostaglandin E1 and E2, and less than 0.01% with prostaglandin A1, A2, B1. The crossreactivity of antiserum for progesterone has been described in a previous report (Kwon et al., '89). The sensitivity for $PGF_{2\alpha}$ RIA was 5 pg $PGF_{2\alpha}$/tube and that for progesterone was 10 pg/tube. Labeled $PGF_{2\alpha}$ (5,6,8,9,11,12,14,15 [n]-^{3}H-prostaglandin $F_{2\alpha}$; 209 Ci/mmol) and labeled progesterone (1,2, 6,7-^{3}H-progesterone; 99 Ci/mmol) were obtained from Amersham (Buckinghamshire, UK). Each sample was quantified for tritium using a Packard (Meriden, CT) Tri-Carb 1500 liquid scintillation analyzer. Duplicate hormone standards (5–1,000 pg for $PGF_{2\alpha}$; 10–2,000 pg for progesterone) were included in each assay. The between and within assay coefficients of variation for $PGF_{2\alpha}$ were 7.3 and 6.5%, and those for progesterone were 11.6 and 7.7%, respectively.

Statistics

Ovulation or maturation data (percent) were transformed using arcsin transformation before statistical analysis. Ovulation or maturation data and $PGF_{2\alpha}$ or progesterone levels were analyzed by one- or two-way analysis of variance (ANOVA) or Student's *t*-test.

RESULTS

Effect of orthovanadate on the ovulation of oocytes in vitro

Initial experiments were carried out to ascertain whether orthovanadate stimulated ovulation of cultured *Rana* ovarian follicles. Ovarian fragments obtained in mid-hibernation (mid-January) were incubated in AR in the presence or absence of various doses of sodium orthovanadate (0.01–1 mM) or FPH (0.05 gland/ml) for 24 h and the incidence of ovulation and GVBD were determined (Fig. 1). All concentrations of orthovanadate induced oocyte ovulation but the average ovulation rate was significantly lower (<45%) than occurred in FPH treated group (>90%) ($P < 0.01$ by *t*-test). Ovulation induced with orthovanadate appeared

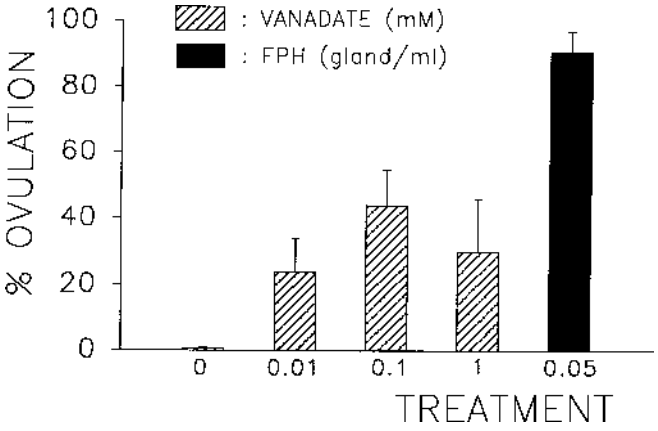

Fig. 1. In vitro effect of orthovanadate and FPH on the ovulation of *Rana dybowskii* oocytes obtained in mid-hibernation. Ovarian fragments were cultured for 24 h in the presence or absence of various doses of orthovanadate (0.01–1 mM) or FPH (0.05 gland/ml) and examined for ovulation after culture. Each bar in the figure represents the average % ovulation (mean ± SEM) based on data obtained from four animals.

to be delayed about 3 h from that seen with FPH during the period of ovulation initiation but most oocytes ovulated around 9 h as observed with FPH (Fig. 2). All oocytes ovulated in response to orthovanadate had intact GVs

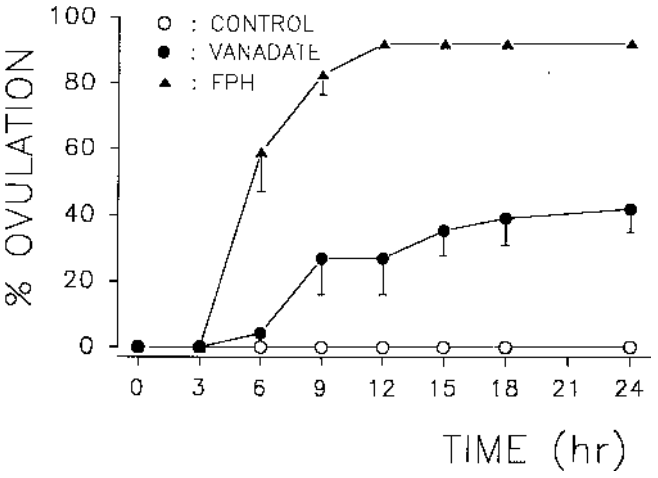

Fig. 2. Time course of vanadate- and FPH-induced ovulation of ovarian follicles obtained from frogs collected in mid-hibernation. Ovarian fragments were cultured for up to 24 h in the presence or absence of orthovanadate (0.1 mM) or FPH (0.05 gland/ml). At designated time points, the accumulated ovulated oocytes were counted. The % ovulation was obtained by dividing the total number of ovulated oocytes by the total number of follicles originally present in ovarian fragments. Each point in the figure represents the average % (mean ± SEM) ovulation based on data obtained from three animals.

while those ovulated in response to FPH had undergone GVBD (>90%) when examined after 24 h by cracking the oocytes after fixing (data not shown). There were no evident differences in the characteristics of the surface of oocytes ovulated in response to orthovanadate and FPH and no granulosa cells were observed on the oocyte surface. Thus orthovanadate induced oocyte ovulation without concomitant induction of maturation of frogs obtained in mid-hibernation.

Effect of orthovanadate and FPH on oocyte ovulation and maturation in vitro: Dose-response and time course study

The effects of different doses of orthovanadate and FPH on the onset and incidence of ovulation were also examined using ovarian fragments obtained in late hibernation (mid-February). At designated time periods of FPH or orthovanadate treatment, ovulated oocytes were collected, counted (Fig. 3), and examined for GVBD (Figs. 4 and 5). Consistent with previous observations (Kwon et al., '92), approximately half of the oocytes ovulated spontaneously in the absence of gonadotropin during the 24 h culture period (Fig. 3). Treatment of fragments with orthovanadate or FPH increased the incidence of ovulation and accelerated the time course of ovulation in a dose-dependent manner (Fig. 3A, B). The lowest dose of orthovanadate (0.01 mM) increased the incidence of ovulation (32%) significantly by 6 h of culture when compared to control (7%) ($P < 0.01$, by t-test) (Fig. 3). In general, the incidence and time course of ovulation stimulated with orthovanadate (Fig. 3A) resembled that produced by FPH (Fig. 3B). The high dose of FPH (0.05 gland/ml) markedly stimulated ovulation by 6 h as compared to control (69 and 7%, respectively) ($P < 0.01$, by t-test) (Fig. 3). Ovulation of most oocytes occurred between 6–12 h of culture in response to both treatments as previously reported (Kwon et al., '92).

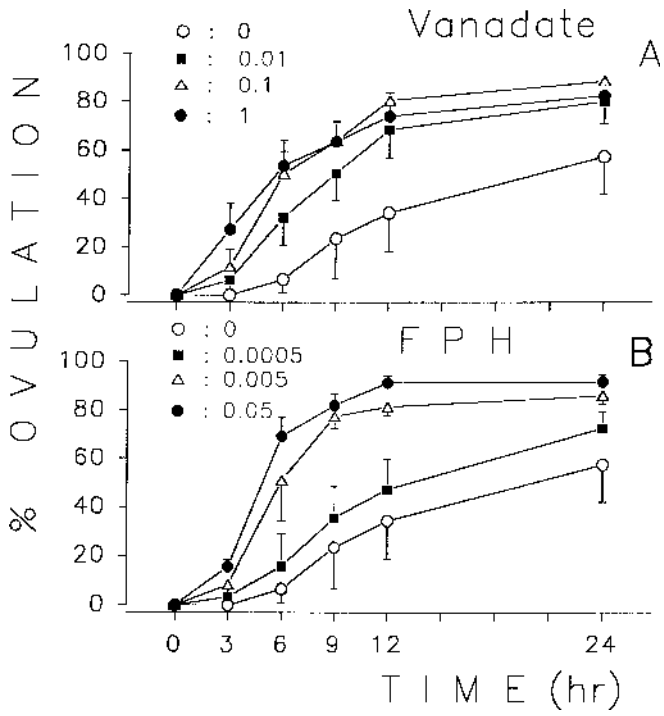

Fig. 3. Time course of vanadate- and FPH-induced ovulation of *R. dybowskii* ovarian follicles in vitro. Five ovarian fragments from each animal, containing about 20 follicles each, were obtained in February and cultured in a flask containing 10 ml of AR in the presence or absence of various doses of vanadate (0.01–1 mM) or FPH (0.0005–0.05 gland/ml). At designated time points, the accumulated ovulated oocytes were counted. The % ovulation was obtained by dividing the total number of ovulated oocytes by the total number of follicles originally present in ovarian fragments. Each point in the figure represents the average % (mean ± SEM) ovulation based on data obtained from five animals.

In order to ascertain whether oocyte ovulation and maturation occur simultaneously, GVBD of oocytes ovulated in the above experiment were examined. Ovulated oocytes were collected and fixed at designated culture periods (0–3, 3–6, 6–9, 9–12, and 12–24 h) and examined for GVBD. In control cultures, essentially all oocytes (81/84) that ovulated spontaneously within 9 h had intact GVs while most of those that ovulated spontaneously after 12 h exhibited GVBD (127/146) (Fig. 4). Orthovanadate accelerated the onset of ovulation and increased the incidence of ovulation, although most of those oocytes that ovulated within 12 h had intact GVs (Fig. 4). For example, at the dose of 0.1 mM of vanadate, 80% (402/500) of the oocytes ovulated but only 1% (4/402) had matured within 12 h, while in control 34% (171/500) of oocytes ovulated and 13% (22/171) had matured. However, about half of the oocytes (76/141, 53%) that ovulated during the later part of culture (12–24 h) underwent GVBD in the presence of orthovanadate (0.01–1 mM) (Fig. 4). Thus, orthovanadate appeared to accelerate oocyte ovulation but not induction of oocyte maturation.

In contrast, FPH treatment enhanced the incidence of ovulation and accelerated the time of GVBD during culture (Fig. 5). A high proportion of the oocytes ovulated after 6 h had undergone GVBD (543/625, 87%) whereas only 38% of oocytes ovulated before 6 h had undergone GVBD (264/690). In controls, 52% (149/284) of oocytes that ovulated after 6 h of culture and all oocytes that ovulated within 6 h (33/33) had intact GVs. Thus, FPH appeared to simultaneously accelerate ovulation and oocyte maturation.

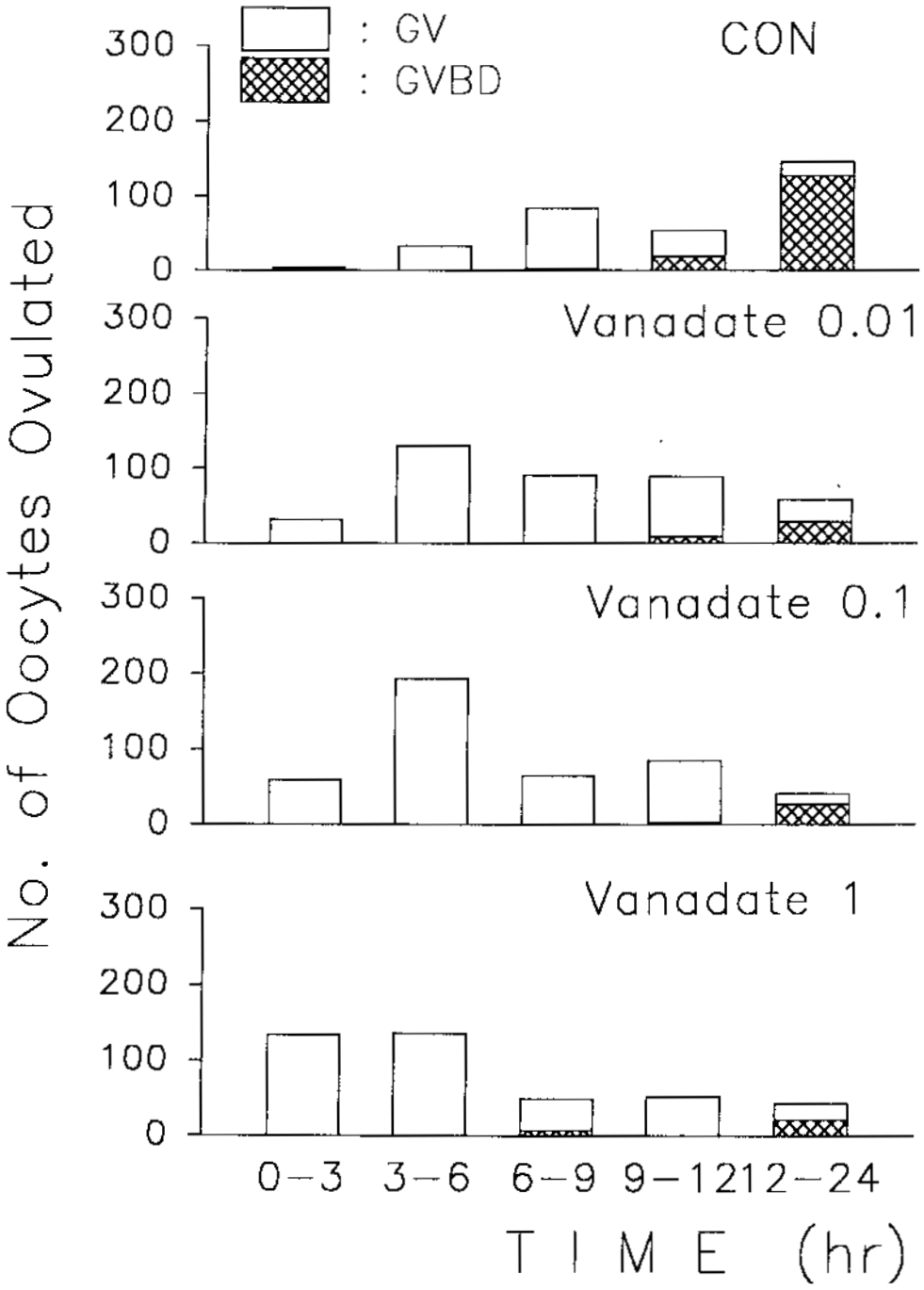

Fig. 4. Maturation of oocytes ovulated at different times of culture in response to vanadate. Oocytes ovulated in response to various doses of vanadate were obtained from the time-course experiment (Fig. 2). At designated time points, ovulated oocytes were removed from the culture flask and checked for GVBD after fixation with TCA. Each bar in the figure represents the total number of oocytes ovulated at the designated culture periods. The total number of oocytes with intact GV are depicted with blank bars whereas oocytes with GVBD are represented by the hatched bars. Data were obtained using ovarian fragments from 5 animals.

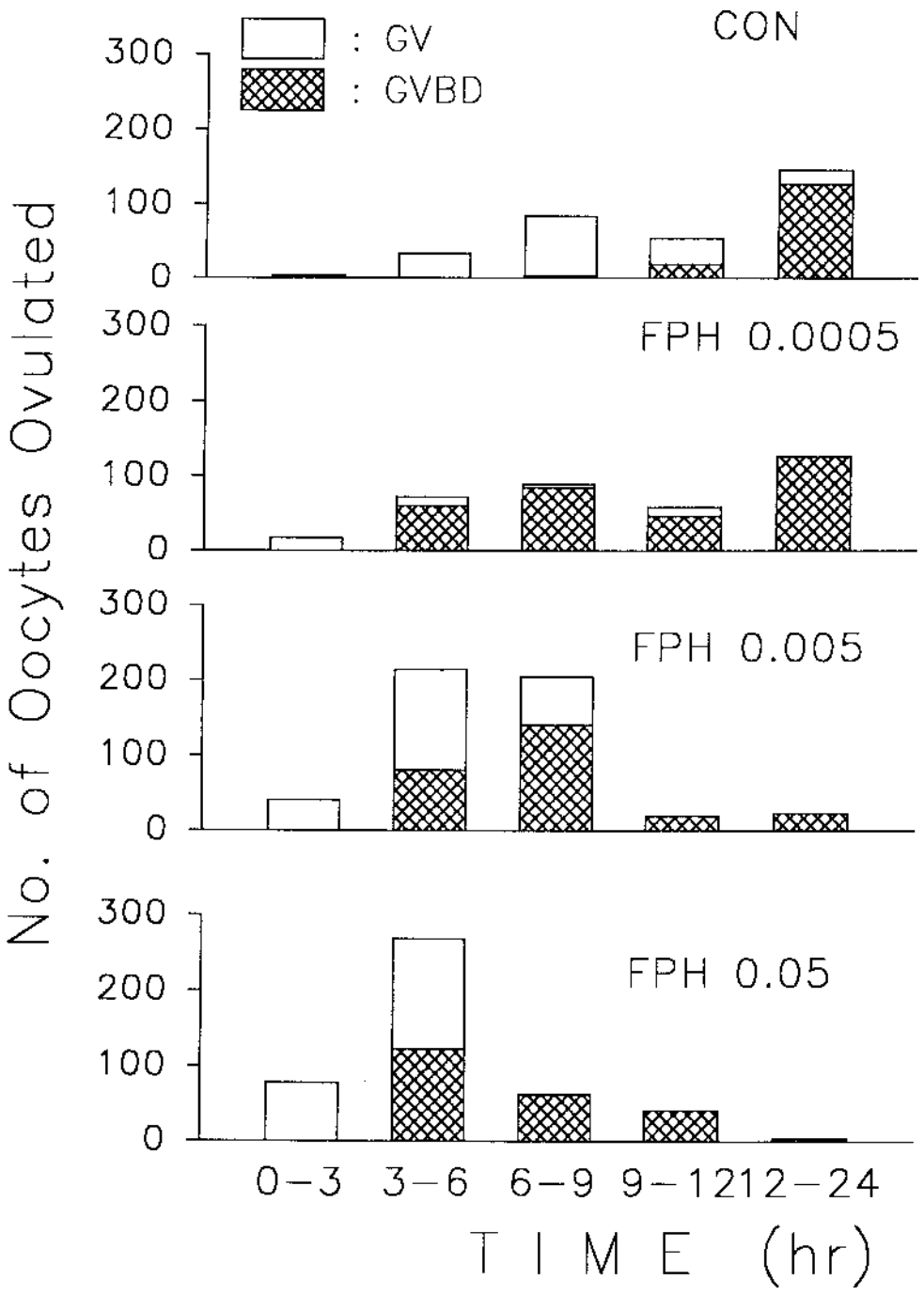

Fig. 5. Maturation of oocytes ovulated at different duration of culture in response to FPH in vitro. Ovulated oocytes from ovarian follicles in response to various doses of FPH were also obtained from a time-course study (Fig. 2). At designated time points, ovulated oocytes were removed from culture flask and their oocyte maturation (GVBD) was examined. Each bar in the figure represents the total number of oocytes ovulated at the designated culture periods. The number of oocytes with intact GV was depicted with blank bars and those oocytes with GVBD with hatched bars. Data obtained from 5 animals are represented.

Effect of orthovanadate on the prostaglandin synthesis by ovarian fragments

Experiments were carried out to ascertain whether orthovanadate stimulate ovarian fragments to synthesize prostaglandin during culture. Ovarian fragments obtained in mid-hibernation (January) were cultured for 9 or 24 h in the presence or absence of various doses of orthovanadate (0.01–1 mM) and amounts of $PGF_{2\alpha}$ secreted into the medium were measured. Orthovanadate significantly increased the levels of $PGF_{2\alpha}$ above controls at 9 and 24 h in a dose related manner ($P < 0.05$, by ANOVA) (Fig. 6).

Time course of $PGF_{2\alpha}$ production by ovarian fragments in response to FPH and orthovanadate

Ovarian fragments obtained in mid- and late-hibernation were cultured in AR for up to 24 h in the presence or absence of orthovanadate (1 mM) or FPH (0.05 gland/ml). At designated time points, levels of $PGF_{2\alpha}$ in media were measured. In mid-hibernation, orthovanadate and FPH stimulate fragments to secrete significant amounts of $PGF_{2\alpha}$ (40–157 pg/follicle, and 5–138 pg/follicle, respectively) ($P < 0.01$, when compared to basal levels by two-way ANOVA) during the 24 h culture period whereas in controls basal levels remained very low (5–23 pg/follicle) (Fig. 7). However, fragments obtained in late-hibernation secreted consistently high levels of $PGF_{2\alpha}$ (4–140 pg/follicle) even in the absence of FPH or orthovanadate during the various culture periods (Fig. 7).

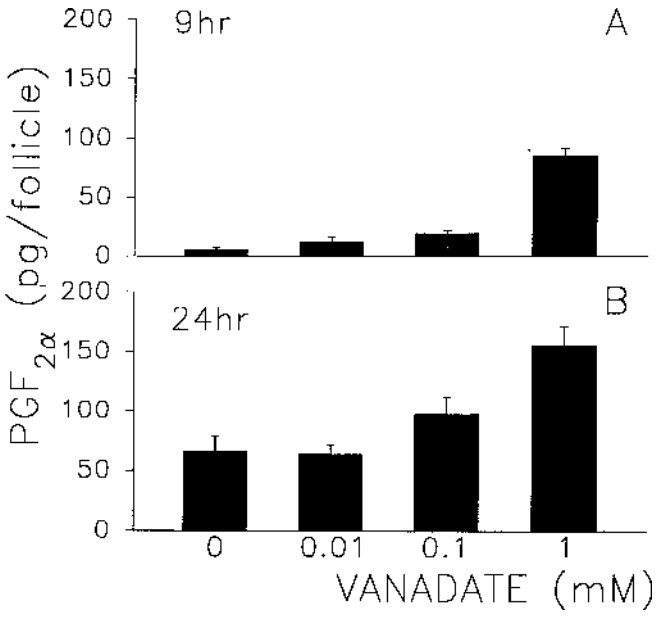

Fig. 6. Effect of orthovanadate on the production of prostaglandin $F_{2\alpha}$ by ovarian fragments following in vitro culture. Ovarian fragments obtained in mid-hibernation period were cultured for 9 and 24 h in the presence or absence of various doses of orthovanadate (0.01–1 mM). The amounts of prostaglandin $F_{2\alpha}$ secreted into medium by the fragments during culture were measured by RIA. Prostaglandin $F_{2\alpha}$ levels secreted by the follicles during 9 h culture are depicted in **A** and those during 24 h culture in **B**.

Effect of prostaglandin $F_{2\alpha}$ on the oocyte ovulation and maturation by ovarian fragments in vitro

The effects of $PGF_{2\alpha}$ on the oocyte ovulation and maturation in vitro were examined using ovarian fragments obtained in the mid- and late-hibernation periods. During mid-hibernation, only a small portion of the oocytes ovulated (18 ± 5%, n = 11 animals) without GVBD in response to $PGF_{2\alpha}$ (5 μg/ml) during a 24 h culture period. In contrast most of the oocytes ovulated and underwent GVBD (>90%) in response to FPH (0.05 gland/ml). When ovarian fragments were obtained in late hibernation, most of the oocytes ovulated (222/300) without GVBD within 6 h of culture in response to $PGF_{2\alpha}$ (5 μg/ml) (Table 1). Likewise, most of the oocytes ovulated without GVBD in response to FPH (0.05 gland/ml) and to vanadate (1 mM) during the initial 6 h of culture (240/300 and 193/300, respectively) (Table 1).

Effect of orthovanadate on the progesterone production by ovarian fragments

To ascertain the effects of orthovanadate on follicular progesterone production, ovarian fragments were obtained in mid-hibernation and cultured in the presence or absence of vanadate (1 mM) or FPH (0.05 gland/ml), and at designated time points levels of progesterone were measured. Orthovanadate failed to increase progesterone levels (<12 pg/follicle) throughout the culture period (Fig. 8). In contrast, FPH markedly stimulated progesterone secretion and maximum levels were present at 9 h and decreased subsequently. Clearly, orthovanadate had no stimulating effect on progesterone production by ovarian follicles.

DISCUSSION

The present results demonstrate that orthovanadate acts on ovarian follicles to induce ovulation of immature oocytes while stimulating

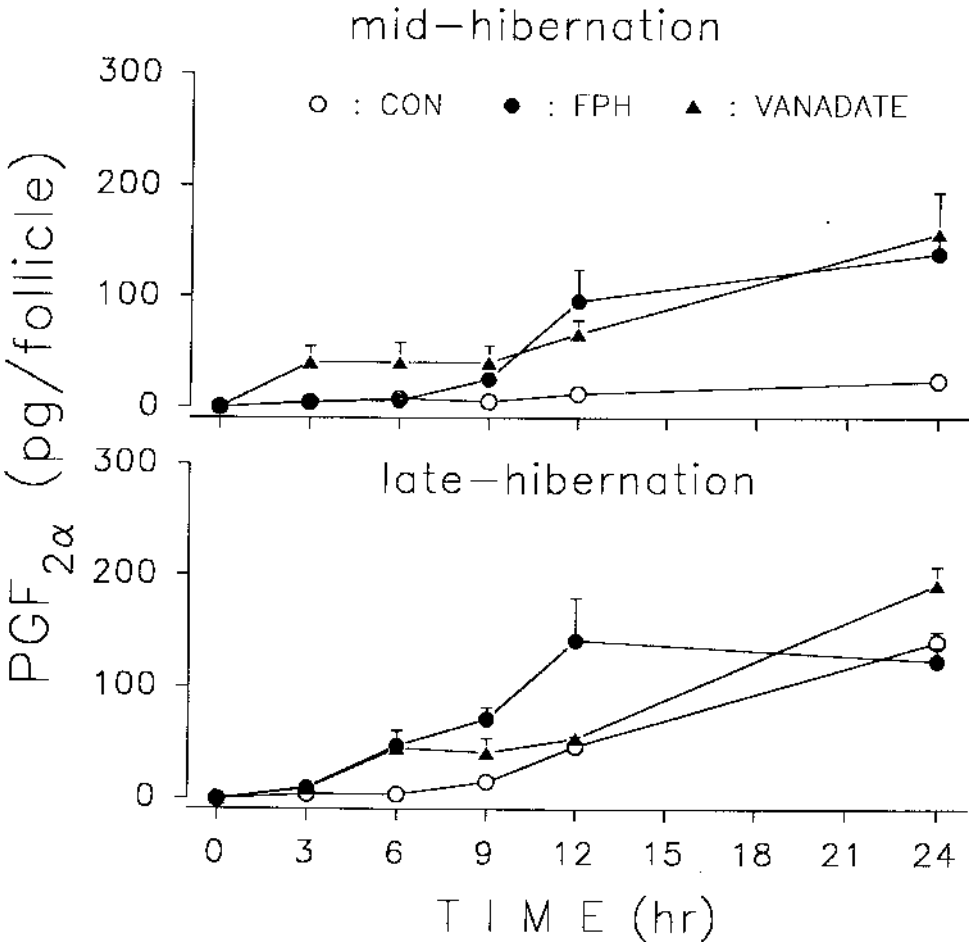

Fig. 7. Time course of prostaglandin $F_{2\alpha}$ by ovarian fragments collected at different hibernation periods in response to vanadate and FPH stimulation. Ovarian fragments were obtained from frogs collected at mid- and late-hibernation periods, and cultured in the presence or absence of orthovanadate (1 mM) or FPH (0.05 gland /ml) for up to 24 h. At designated time points, amounts of prostaglandin secreted by the fragments during culture were measured by RIA. Each point in the figure represents the average pg prostaglandin $F_{2\alpha}$ secretion (mean ± SEM) of data obtained from three animals.

prostaglandin but not progesterone synthesis. Thus orthovanadate effectively dissociates oocyte ovulation and maturation in *Rana* ovarian follicles as has been observed earlier with prostaglandin treatment (Schuetz, '86).

The effects of orthovanadate on ovarian follicles collected during mid- and late-hibernation period were examined since earlier studies showed that seasonal differences in responses to FPH and culture conditions appeared in this frog. All oocytes obtained in mid-hibernation and most oocytes obtained in late-hibernation had intact GVs when they ovulated in response to vanadate while most of oocytes obtained in the same period underwent GVBD and ovulated in response to FPH (Figs. 4 and 5). Oocytes ovulated by vanadate look normal and healthy as judged by microscopic observations. The oocyte surface (vitelline membrane) was devoid of granulosa cells and the characteristics of the animal and vegetal poles appeared identical to those seen in oocytes ovulated in response to FPH. Approximately half the oocytes ovulated during the later part of culture (12–24 h) matured in the presence of vanadate (Fig. 4), thus indicating that vanadate has no adverse effect on oocyte maturation. Thus, it is likely that vanadate induced ovulation is normal and the ovulation is not linked to oocyte maturation. From the fact that vanadate was consistently less efficient than FPH in the induction of ovulation (Figs. 1 and 2), it is also evident that vanadate stimulates prostaglandin secretion like FPH but does not mimic all the actions of FPH, particularly the secretion of progesterone (Fig. 8). In earlier stud-

TABLE 1. Effect of prostaglandin $F_{2\alpha}$ on the oocyte ovulation and maturation in vitro by Rana *ovarian follicles obtained in early February*[1]

	Ovulation (GVBD)			Total no. of oocytes ovulated/no. of follicles cultured
	Duration of culture (h)			
	0–6	6–12	12–24	
Control	29 (0)	41 (2)	35 (26)	105/300
FPH (0.5 gland/ml)	240 (0)	34 (25)	2 (2)	276/300
Vanadate (1 mM)	193 (0)	70 (2)	12 (6)	275/300
$PGF_{2\alpha}$ (5 μg/ml)	222 (0)	25 (2)	19 (5)	266/300

[1]Oocytes liberated from ovarian fragments at designated culture periods were counted and examined for GVBD.

ies it was suggested that oocyte GVBD or maturation promoting factor (MPF) are involved in the separation of connections between oocyte and granulosa cells during the ovulation process (Schuetz, '85). However, at present, the molecular mechanisms that mediate separation of the granulosa cells from oocytes in response to orthovanadate treatment without progesterone production remain unresolved.

A considerable number of oocytes ovulated within 6 h in response to FPH also had intact GVs (Fig. 5). In particular, high dose of FPH (0.05 gland/ml) accelerated ovulation without oocyte GVBD (Fig. 5, Table 1). This may be due to a transient increase in intrafollicular cAMP levels induced by a high dose of FPH, which can adversely affect oocyte maturation (Kwon and Schuetz, '85). This idea is consistent with the fact that immature oocytes ovulated in response to a high dose of FPH eventually matured after prolonged culture (18–24 h) which would allow time for cAMP metabolism to occur.

Both orthovanadate and FPH stimulated $PGF_{2\alpha}$ production by ovarian follicles (Figs. 6 and 7),

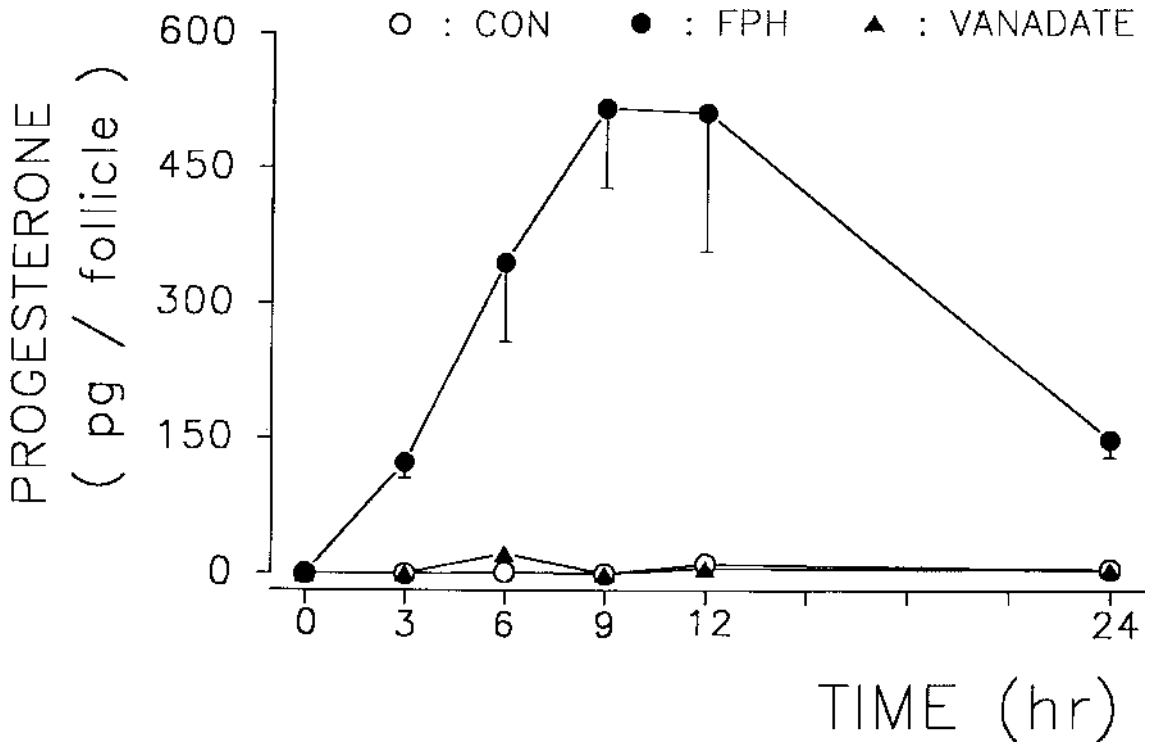

Fig. 8. Effect of orthovanadate on the production of progesterone and prostaglandin $F_{2\alpha}$ by ovarian fragments during in vitro culture. Ovarian fragments obtained in mid-hibernation period were cultured in the presence or absence of orthovanadate (1 mM) or FPH (0.05 gland/ml) for up to 24 h. At designated time points, the amounts of progesterone or prostaglandin $F_{2\alpha}$ secreted into the medium by the fragments were measured by RIA. Each point in the figure represents average (mean ± SEM) pg progesterone per follicle obtained from three animals.

which is known to play a role in the ovulation process in amphibians (Gobbetti and Zerani, '92; Chang et al., '95). Significantly, $PGF_{2\alpha}$ is not produced by oocytes devoid of follicle walls (unpublished data) indicating that the follicle wall rather than the oocyte is responsible for the production of $PGF_{2\alpha}$ and induction of ovulation. Treatment of ovarian follicles obtained in late hibernation with $PGF_{2\alpha}$ stimulated oocyte ovulation without oocyte maturation (Table 1). In contrast, $PGF_{2\alpha}$ treatment of cultured ovarian fragments induced a much lower incidence of ovulation (<20%) than FPH (>90%) in the mid-hibernation period. Furthermore, in earlier studies, we demonstrated that the marked increases in $PGF_{2\alpha}$ by ovarian follicles in response to FPH or TPA were not always followed by ovulation. Likewise, suppression of $PGF_{2\alpha}$ production by ovarian follicles with indomethacin failed to completely inhibit FPH or TPA induced ovulation in this frog (Chang et al., '95). Such results strongly suggest that $PGF_{2\alpha}$ is not the sole mediator for ovulation but one of several other ovarian factors such as other eicosanoids as well as steroids (Espey et al., '86). Alternatively, vanadate may directly act on the follicle wall to stimulate muscle contraction and ovulation independent of prostaglandin production (Anselmi et al., '87).

As orthovanadate is known to be an activator of G protein(s) (Krawietz et al., '82; Combest and Johnson, '83), G protein(s) may be involved in the signal transduction pathway for oocyte ovulation and prostaglandin synthesis in amphibian ovarian follicles. However, orthovanadate has also been shown to have diverse effects on the various enzymes in biological systems (Wilisky, '90).

In summary, these experiments demonstrate that orthovanadate induces oocyte ovulation without maturation and progesterone production. Prostaglandins produced in response to vanadate seem to play a role at least in part in the ovulation process independent of oocyte maturation. The evidence strongly support the view that the actions of vanadate are mediated in large part due to the production of selective prostaglandins that can act independent of and in the absence of maturational steroids (progesterone) to induce ovulation of immature oocytes.

ACKNOWLEDGMENTS

This work was supported by grants awarded to Dr. H.B. Kwon from Korea Science and Engineering Foundation through Hormone Research Center (HRC-95-0103) and from the Ministry of Education of Korea (BSRI-94-4425). We thank Professor A.W. Schuetz for his helpful comments and suggestion in preparing the manuscript.

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