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Progesterone induces a rapid increase in $[Ca^{2+}]_{in}$ of *Xenopus laevis* oocytes

(steroid stimulation/calcium activity increase/photoprotein aequorin/meiosis resumption)

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ABSTRACT **Progesterone causes a rapid increase in the intracellular free calcium level in fully grown amphibian oocytes. When albino *Xenopus laevis* oocytes were microinjected with the Ca-specific photoprotein aequorin, the calcium-induced luminescence from this protein increased at 40–60 sec after the addition of progesterone and returned to the control level within 5–6 min. No further change in the aequorin glow could be detected throughout the remainder of the maturation period. This transient increase in cytoplasmic free calcium may be involved in controlling the resumption of oocyte maturation.**

Full-grown amphibian oocytes undergo meiotic maturation when exposed to progesterone (1). During this period, altered ion fluxes, increased protein synthesis and phosphorylation, the appearance of a "mitotic" factor, and the meiotic divisions can be detected (2). All of these events, except the meiotic divisions, occur in enucleated oocytes that are subsequently exposed to progesterone (1, 2). Thus, the steroid can act at an extranuclear site in these target cells. Additional evidence suggests that the initial site of progesterone action is at or near the cell surface (2, 3) and is probably mediated by ionic changes within the oocyte. For example, O'Connor *et al.* (4) reported that oocytes preloaded with ^{45}Ca exhibited a marked increase in the rate of calcium efflux soon after exposure to progesterone. Moreover, exposure of the oocyte to Ca^{2+} and calcium ionophore A23187 (5) or iontophoresis of Ca^{2+} into the oocyte cortex (6) can induce maturation in the absence of steroid. These observations have led to the suggestion that a progesterone-stimulated increase in intracellular free calcium may be a necessary first step in the resumption of meiosis (4).

In the current report, we directly demonstrate that progesterone causes an increased cytoplasmic calcium activity in these cells. The method used involved monitoring changes in the light emission from progesterone-stimulated oocytes that were injected with the calcium-specific photoprotein aequorin.

Bellé *et al.* (7) had previously reported that single *Xenopus laevis* oocytes injected with aequorin did not emit any detectable resting glow or any light output in response to progesterone stimulation. However, these investigators used wild-type oocytes which are heavily pigmented. Such pigmentation could have masked the aequorin light emission and might account for their negative results. To circumvent this problem, we utilized albino oocytes from the mutant *Xenopus laevis* described by Bluemink and Hoperskaya (8).

MATERIALS AND METHODS

Animals and Oocytes. Wild-type *X. laevis* were purchased from Nasco (Fort Atkinson, WI). Mutant albino *Xenopus* were obtained from G. Nace (University of Michigan) and from R. Briggs (Indiana University). None of the animals received gonadotropin injections. Ovarian fragments were surgically removed from animals that were anesthetized by hypothermia. Ovarian tissue and manually defolliculated stage VI oocytes (9), 1.2–1.4 mm in diameter, were cultured in OR-2 medium at pH 7.6 (10).

Aequorin Injections. Defolliculated oocytes were transferred to an injection dish that contained calcium-free OR-2 medium. Oocytes were microinjected with 40 nl of 1% aequorin solution and then returned to regular OR-2 medium for 3 hr to allow for healing of the injection wound and equilibration of the injected aequorin within the oocyte cytoplasm.

Monitoring of Aequorin Luminescence. Albino oocytes previously injected with aequorin were mounted individually in a dish with a transparent bottom and placed on the stage of a Leitz compound microscope (fitted with a lens of 0.6 N.A., with a collection efficiency of 8%). OR-2 solutions were flowed through the dish at a rate (1 ml/min) sufficient to completely exchange the solution in 30 sec. The optics of the microscope cast an image of the oocyte upon the cathode of a photomultiplier (EMI 9781A, quantum efficiency ≈20%) that was maintained in an insulated housing (Pacific Photometric 3377-D) at −70°C with dry ice in order to decrease thermionic emissions in the dark (dark noise) to less than two per second. Photoelectrons were simultaneously monitored on an oscilloscope and counted over 10-sec intervals with a digital counter.

At the end of an experiment, each aequorin-injected oocyte was scored for successful maturation by the criteria described elsewhere (1, 2).

RESULTS

A wild-type oocyte injected with aequorin exhibited no resting aequorin luminescence nor could any glow be detected in response to progesterone stimulation. These results confirm the earlier studies by Bellé *et al.* (7). A control albino oocyte injected with aequorin and exposed only to the culture medium exhibited a resting glow of 1–2 photoelectrons per sec above the dark noise. After an appropriate measuring interval, to establish that the control resting glow was constant, a valve was actuated that caused OR-2 medium containing progesterone at 1 or 5 μg/ml to enter the culture dish. In 14 oocytes of a total of 30 injected with aequorin (all of which subsequently underwent maturation), at approximately 40–60 sec after progesterone was introduced, the light output started to increase and reached its highest level by 80–120 sec. Representative examples of this response, which was quite variable for different oocytes, are shown in Fig. 1. A total of three oocytes from two albino frogs gave the most pronounced light emissions (111–190 photoelectrons per sec) in response to progesterone (Fig. 1*A*; Table 1). Intermediate responses (10–24 photoelectrons per sec) were obtained with four oocytes from three animals (Fig. 1*B*) and very low responses (3–7 photoelectrons per sec) were measured in seven oocytes from five animals (Fig. 1*C*). In most cases, the

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Abbreviation: CDR, calcium-dependent regulator (calmodulin).

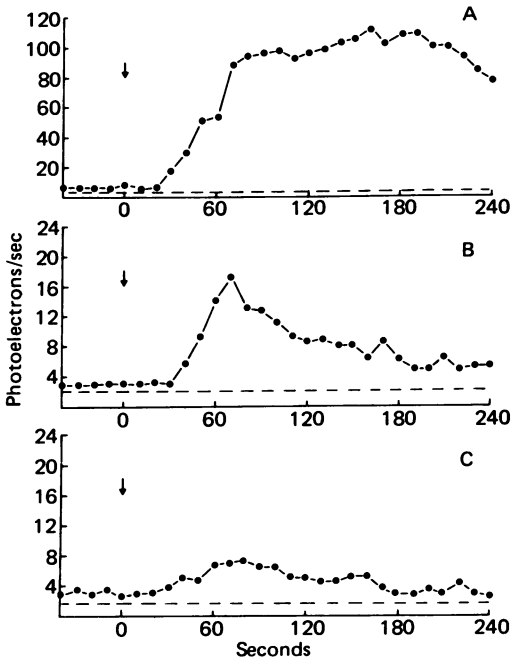

FIG. 1. Aequorin luminescence from single albino *X. laevis* oocytes stimulated by progesterone. Each oocyte was injected with 40 nl of 1% aequorin, allowed to equilibrate for 3 hr, and then monitored. Progesterone (1 or 5 μg/ml) was introduced at the time indicated by the arrow. (*A*) Frog II oocyte no. 3; (*B*) frog II oocyte no. 5; (*C*) frog I oocyte no. 3 (Table 1). The dark noise (mean rate of thermionic emission of the photomultiplier tube) is represented by the dashed line.

light emission slowly declined to the resting value within 5–6 min after the initial exposure to the steroid. No further change in the aequorin glow was detected throughout the remainder of the maturation period. In contrast to the above results, an increased aequorin glow was not observed in any of 10 additional oocytes (from three animals) that failed to undergo maturation after progesterone administration.

Treatment of oocytes with 1% Triton X-100 at any point during maturation, which allowed calcium to enter the oocyte or aequorin to diffuse out, produced a light glow of 10^4 to 10^5 photoelectrons per sec that lasted for several minutes. In addition, when aequorin-injected oocytes were allowed to reach metaphase II of meiosis and were activated with calcium ionophore A23187, the light output increased approximately 10,000-fold (unpublished data), a response comparable to the results obtained during the fertilization of aequorin-injected medaka and sea urchin eggs (11, 12). Consequently, the decline of the light emission after the initial increase after exposure to progesterone was not due to the exhaustion of the injected aequorin.

DISCUSSION

We have demonstrated in this study that progesterone stimulation causes a rapid, but only transient, increase in the level of cytoplasmic free calcium in full-grown amphibian oocytes. This increase in free calcium, indicated by an increased aequorin glow, was never detected in oocytes that failed to undergo meiotic maturation in response to progesterone administration.

There are at least two possible reasons why we did not detect an increased glow from all maturing oocytes. (*i*) The calcium activity that increases after progesterone exposure may be restricted to only a portion of the oocyte cytoplasm. For example,

Table 1. Aequorin luminescence from maturing albino *Xenopus* oocytes*

Frog	Oocyte	Mean resting glow†	Mean prog. resp.‡	Peak prog. resp.§
I	1	1.0 ± 0.1	10.2 ± 1.3	16
	2	1.0 ± 0.1	132.2 ± 9.3	190
	3	1.1 ± 0.1	3.7 ± 0.4	7
	4	1.2 ± 0.1	3.0 ± 0.3	5
II	1	1.0 ± 0.1	2.3 ± 0.2	4
	2	1.1 ± 0.1	3.3 ± 0.2	6
	3	1.0 ± 0.1	57.8 ± 6.2	118
	4	1.0 ± 0.1	9.6 ± 1.4	24
	5	1.0 ± 0.1	4.8 ± 0.6	15
	6	2.1 ± 0.2	70.9 ± 5.5	111
III	1	1.0 ± 0.1	2.8 ± 0.2	6
	2	1.0 ± 0.1	6.3 ± 0.6	10
IV	1	1.0 ± 0.1	2.1 ± 0.2	3
V	1	1.3 ± 0.1	2.0 ± 0.2	4

* For each frog the ratio of the number of oocytes exhibiting an increased aequorin glow to the total oocytes examined that underwent maturation were: I, 4/5; II, 6/9; III, 2/5; IV, 1/4; V, 1/4; and VI, 0/3.

† Photoelectrons per sec as mean (±SEM) averaged over a 2-min counting interval. The dark noise (1–2 photoelectrons per sec) has been subtracted from all values in the table.

‡ Mean response to progesterone photoelectrons per sec as mean (±SEM) averaged over the time period when the response was higher than the mean resting glow.

§ Photoelectrons per sec for the highest measured rate per 10-sec counting interval during the response period.

it has been reported that progesterone is more effective in inducing maturation when applied to the animal hemisphere (13); increases in calcium activity might also be primarily restricted to this location. However, there are no external landmarks on the albino oocyte that enables one to distinguish the animal from the vegetal hemisphere. Thus, the injection of aequorin into these oocytes was random with respect to the animal–vegetal pole axis, and the orientation of the injected oocyte to the optical axis of the microscope was also random. Therefore, in several instances we may have been monitoring the nonresponsive portion of the oocyte. Furthermore, the opacity of the cytoplasm is such that it would not allow light to pass through the nonreactive part of the oocyte to the photomultiplier tube.

(*ii*) The state of hormonal stimulation of the albino oocyte prior to our experiments may have been variable. Wild-type animals ovulate in the laboratory only after gonadotropin injections. However, mutant albino frogs occasionally ovulate spontaneously (i.e., without gonadotropin injections). A "stimulated" wild-type female frog is considered to be one that has been induced to ovulate within 1 week prior to an experiment. Many of the so-called early events of maturation that occur in response to progesterone in oocytes from unstimulated frogs are either bypassed or have already taken place prior to progesterone exposure in oocytes from stimulated animals (2). Thus, albino frogs that exhibit spontaneous meiosis and ovulation within a day or two after an experiment may be considered to be "stimulated." No oocyte taken from such an animal ever gave an increased aequorin glow in response to progesterone [six oocytes from two frogs (data not shown)]. Oocytes from such animals may have already undergone the change in free calcium prior to progesterone treatment *in vitro*.

In view of these problems, there appear to be good reasons why we did not detect an increased aequorin glow from all of the maturing albino oocytes. Therefore, we suggest that the initial progesterone–oocyte interaction, which leads to the resumption of meiosis, involves the transient increase of calcium located in or near the oocyte surface. A similar rapid increase in intracellular free calcium has been detected in invertebrate starfish oocytes that have been exposed to 1-methyladenine, a compound that induces maturation in these oocytes (14). The increased aequorin luminescence in these oocytes was detected in less than 2 sec after the application of the adenine derivative. Our observations with a vertebrate oocyte system suggest that progesterone could have a second mode of action in other steroid target cells—i.e., altering intracellular calcium levels—in addition to its well-documented mode of action at the level of gene transcription.

The mechanism by which calcium might be involved in regulating oocyte maturation remains obscure. However, if calcium does function in some way to mediate the progesterone signal, then increased calcium activity must affect certain pathways within the oocyte that ultimately lead to the resumption of meiosis. Transient increases in the level of free calcium could affect certain cellular processes directly (15). Alternatively, free calcium could act via the mediation of a calcium-binding protein, such as the protein initially identified as an activator of cyclic AMP phosphodiesterase in the mammalian brain (16). This regulatory protein has been referred to as calcium dependent regulator (CDR) or calmodulin (16, 17). Recently, we successfully isolated a protein from *Xenopus* ovarian oocytes (18) that is similar, if not identical, to the CDR protein found in rat testis (19) and sea urchin eggs (20). The presence of CDR within the oocyte suggests a potential link between an increase in free calcium and the eventual meiotic division.

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