Induction of germinal vesicle breakdown activity in *Xenopus laevis* oocytes by Triton X-100

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ABSTRACT

Induction of germinal vesicle breakdown (GVBD) in *Xenopus laevis* oocytes is triggered by progesterone. This substance acts on the oocyte surface to induce GVBD. In the present study, Triton X-100 (0.01%), a non-ionic detergent, was found to artificially trigger GVBD in follicle-free oocytes in *Xenopus laevis* as in the case of progesterone when it was applied alone for a short period externally *in vitro*. The result suggests that the interaction of Triton with the oocyte surface causes some change which induces GVBD activity. In addition, it was determined by the trypan blue exclusion test that this treatment was mild enough to maintain oocyte survival. Thus, unstained oocytes were regarded to be alive for GVBD determination.

It is known that progesterone acts at the surface of *Xenopus laevis* oocytes to induce germinal vesicle breakdown (GVBD) (5, 8). Using *Xenopus laevis* oocytes *in vitro*, it has been demonstrated that other steroids (1), and numerous non-steroidal agents, which operate at the membrane level and/or induce changes of intracellular Ca^{2+} distribution, such as organomercurials (2), lanthanum chloride (13), amphiphilic cationic drugs (1, 14) and insulin (4), are able to mimic the progesterone action. On the other hand, the inhibition of progesterone-induced activation by theophylline (3, 12), papaverine (3) and gam-mexane (14) has been attributed to an effect at the membrane level. Consequently, it was assumed that various external treatments, which favour the oocyte surface, would influence GVBD activity. With respect to this, we here report that a brief treatment of *Xenopus laevis* follicle-free oocytes with 0.01% Triton X-100, a non-ionic detergent, mimics progesterone action in inducing GVBD activity.

We prepared follicle-free oocytes as follows. Ovaries were obtained after decapitation from sexually mature *Xenopus laevis* females, and

kept at 20°C in our laboratory until use. Ovarian fragments from isolated ovaries were cut into small pieces and stored in a modified Barth's medium, pH 7.6, which contains 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO_3 , 0.33 mM $\text{Ca}(\text{NO}_3)_2$, 0.41 mM CaCl_2 , 0.82 mM MgSO_4 , 7.5 mM Tris-HCl, and 10 $\mu\text{g/ml}$ each of benzylpenicillin potassium and streptomycin sulfate (6). Small pieces of fragments were subjected to collagenase (Sigma Chemical Co., type I) treatment (0.1%, for 2 h), and then the follicles around the full-grown oocytes were removed manually with watchmaker's forceps under a dissecting microscope with immersion in CMF-medium, pH 7.6, which contains 127 mM NaCl, 2.9 mM KCl, 3.6 mM NaHCO_3 and 3 mM Tris-HCl (10) on all occasions. The follicle-free oocytes obtained in this manner were stored in ice-cold Barth's medium until use. These oocytes responded to progesterone (Sigma Chemical Co.) (10 μM) on 20 min-exposure, but not to human chorionic gonadotropin (Teikoku Zōki Pharmaceutical Co.) (50 IU/ml), indicating the absence of functional follicle cells, the target for the gonadotropin action. Spontaneous induction was about 10% in the absence of progesterone, which served as controls in our experimental systems. The experiments were

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done at about 20°C and finished within four days after ovariectomy.

In all cases, pools of follicle-free oocytes from the same female were divided into two groups (each of about 150 oocytes) and separately incubated in 50 ml of Barth's medium containing

0.01% Triton X-100 (Beckman Instruments) (Fig. 1). At the indicated time, each of about 20 oocytes was transferred to Triton-free Barth's medium (about 50 ml). After further washing twice with Barth's medium, they were incubated in 2 ml of Barth's medium for a final 18 h. In the

Fig. 1 Time course of induction of GVBD activity by Triton X-100 in *Xenopus laevis* oocytes. Two pools of about 150 follicle-free oocytes from the same female were incubated separately in 50 ml of Barth's medium containing 0.01% Triton X-100. At the given times, each of about 20 oocytes was washed three times in 50 ml of Triton-free Barth's medium. After placing in 2 ml of Barth's medium for a subsequent 18 h, the percentage of surviving oocytes by trypan blue exclusion test (A) and GVBD activity of survival oocytes (B), respectively, were measured. Open circles are the averages of six experiments estimated from three different females. □, Control oocytes without Triton treatment. Vertical bars represent the standard error of the mean.

initial experiment, the survival rates of oocytes subjected to the Triton treatment were determined by the trypan blue exclusion test (9) as a measure of the integrity of the oocyte surface in order to remove doubtful results caused by cell death prior to fixation for GVBD determination. Oocytes were exposed to Barth's medium containing 0.3% trypan blue (Wako Pure Chemical Industries) for several minutes and then fixed by adding 25% trichloroacetic acid after washing with dye-free Barth's medium. The unstained oocytes were judged to be alive and stained ones to be dead under a microscope. As shown in Fig. 1A, the time course of stainability displayed an initial lag of approximately 60 min-exposure and oocytes remained more viable after 18 h. After the treatment for more than 80 min, the survival rates gradually decreased. Triton, as expected, is more toxic than progesterone, suggesting progressive time-dependent cytolysis by penetrating the oocytes.

On the other hand, 0.05% Triton led to a complete loss of oocyte viability (data not shown). We therefore routinely used Triton at a lower concentration, *i.e.* 0.01%. This treatment was mild enough to maintain the normal resting potential of the oocyte surface.

Unstained, *i.e.* viable oocytes were examined for GVBD determination (Fig. 1B). It was unexpectedly found that the oocytes typically responded by undergoing GVBD, and thus Triton alone in the absence of progesterone gave sufficient expression of GVBD activity. The period of Triton treatment required for the induction of a high percentage (about 80%) of GVBD activity was found to be 80 min where a plateau level was reached. The breakdown of the germinal vesicle was induced after about the same period of incubation as for the progesterone (0.1 μ M)-induced action (1, 16). While the nature of secondary toxic actions after prolonged exposure is not clear, this result suggests that Triton affects induction of GVBD activity largely via action at the level of the oocyte surface. In contrast with other non-steroid agents, *e.g.* organomercurials and insulin (2, 4), Triton proved effective in inducing GVBD activity within a much shorter period of contact with oocytes.

In pronase-treated starfish oocytes, 0.01% Triton that acted for 1 h failed to induce GVBD, and rather abolished their responsiveness to 1-methyladenine at the surface (11). We do not know how to explain the difference between the *Xenopus* oocytes and starfish oocytes with respect to the Triton action, though subtle differences in the surface environment are likely

to play an important role. In summary, Triton is expected to serve as a new promising tool in investigating the membrane site of action of progesterone to induce GVBD activity in *Xenopus* oocytes, despite our ignorance of the mechanism of action of this nonionic detergent.

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