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Membrane permeability changes during *Rana* oocyte maturation

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Abstract. A transition from an open system to a closed one must occur during the complex process of meiotic maturation of the amphibian oocyte. Membrane permeability to urea in *Rana* oocytes following progesterone stimulation was determined, and the largest decrease was found to coincide with germinal vesicle breakdown. These findings suggest that the timing of the disappearance of membrane permeability correlates with developmental events that prepare the oocyte for a hostile environment.
Key words. Oocyte maturation; urea permeability; germinal vesicle breakdown; progesterone; *Rana pipiens*.

Meiotic maturation in amphibian oocytes involves a complex series of biochemical and physiological changes which prepare cells that are maintained in a suitable environment (the ovary) for expulsion into a hypotonic and nutrient-deficient environment (pond water). Prior to maturation, ovarian oocytes possess a plasma membrane with selective permeability properties that are similar to those of somatic cells. After maturation, membrane permeabilities to amino acids[1] and Na^2 are dramatically reduced while K^+ and Cl^- currents[3] disappear and electrical resistance increases[4]. In effect, these cells are transformed into self-sufficient, nearly closed systems.

Most studies of membrane shut-down during maturation have dealt with substrates that are actively transported and metabolized. For the Na^+-K^+ pump and some Na^+-coupled transport pathways, the time course correlating membrane changes with maturational events and the mechanisms involved have been investigated in *Xenopus* oocytes[5–8]. In general, transport activities decrease around the time of germinal vesicle breakdown but the detailed time course is not clear and may vary for different transporters. We present here data on changes in *Rana* oocyte membrane permeability to urea (a substrate which is neither actively transported[9,10] nor metabolized) during progesterone-induced maturation. The data show that urea permeability decreases during maturation in a multiphasic manner, with the largest change coinciding with germinal vesicle breakdown.

Materials and methods

Full grown ovarian *Rana pipiens* oocytes (stage Y5 of Kemp[11]), were isolated in ice-cold Ringer's solution (containing in mM: 104.5 NaCl, 2.5 KCl, 1.2 Mg SO_4, 6.6 $NaHCO_3$, 1.2 Na_2HPO_4, 2.0 NaH_2PO_4, and 0.7 $CaCl_2$ at pH 7.2) as previously described[12]. Cells were handled using glass transfer pipettes and were defolliculated manually using watchmaker's forceps. For experiments, cells were warmed to 20 °C and transferred to Ringer's containing 1 μg/ml progesterone (Calbiochem; solution prepared by drying ethanol stock solution in vial and redissolving in Ringer's) for 0 (control) to 12 h. For urea uptake studies, control and progesterone-treated oocytes were transferred to Ringer's solution containing 1 mM unlabelled and ^{14}C-labelled urea (New England Nuclear Corp; ~30 μCi/ml medium, 50.5 mCi/mmole). After 1–5 min, cells were removed from the medium, rinsed briefly in ice-cold urea-free Ringer's, transferred to 5 ml distilled water and boiled for 10 min. One ml aliquots of the boiled extracts were assayed using standard liquid scintillation procedures.

Preliminary experiments indicated that urea was neither metabolized nor actively accumulated in *Rana* oocytes: 1) ^{14}C-urea moved as a single spot on thin layer chromatograms of cell extracts and media from control and progesterone cells (97% recovery) and 2) after 1–5 h incubations in Ringer's containing 0.01–100 mM urea, the mean urea cell water-to-medium concentration ratio for 17 cells was 0.99 ± 0.01 (SE).

Results

Figure 1 shows the time course of urea uptake by control and progesterone-treated oocytes. Uptake was a linear function of incubation time over the period studied. Thus, the slopes of these lines measure the initial rate of urea uptake, which is directly proportional to urea permeability. For controls, urea influx was 7.8×10^3 cpm/cell. After 5 h exposure to progesterone, influx had decreased by 25%; after 9.5 h, the influx was only 14% of control value. Clearly, a dramatic change in urea permeability occurred during exposure to progesterone.

To determine the relationship between the reduction in urea permeability and the progression of meiotic maturation, we correlated initial urea uptakes with the breakdown of the oocyte nucleus (germinal vesicle) in

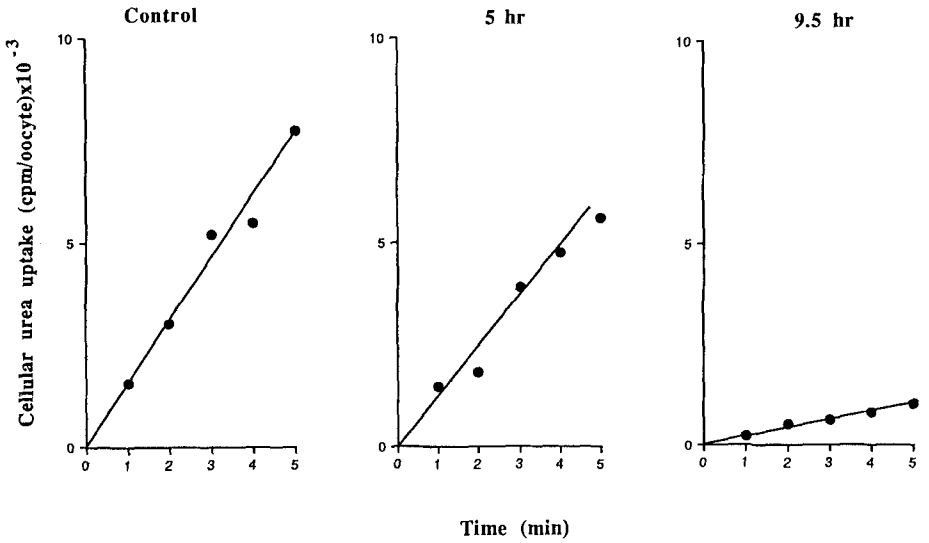

Figure 1. Time course of urea uptake in control and progesterone-exposed oocytes. The incubation medium contained 1 mM unlabelled and labelled (6×10^7 cpm/ml) urea. Each point represents the mean of 8 oocytes with the magnitude of SE less than the symbol size. Solid lines were obtained by linear regression analysis; for each time course the regression coefficient was greater than 0.98.

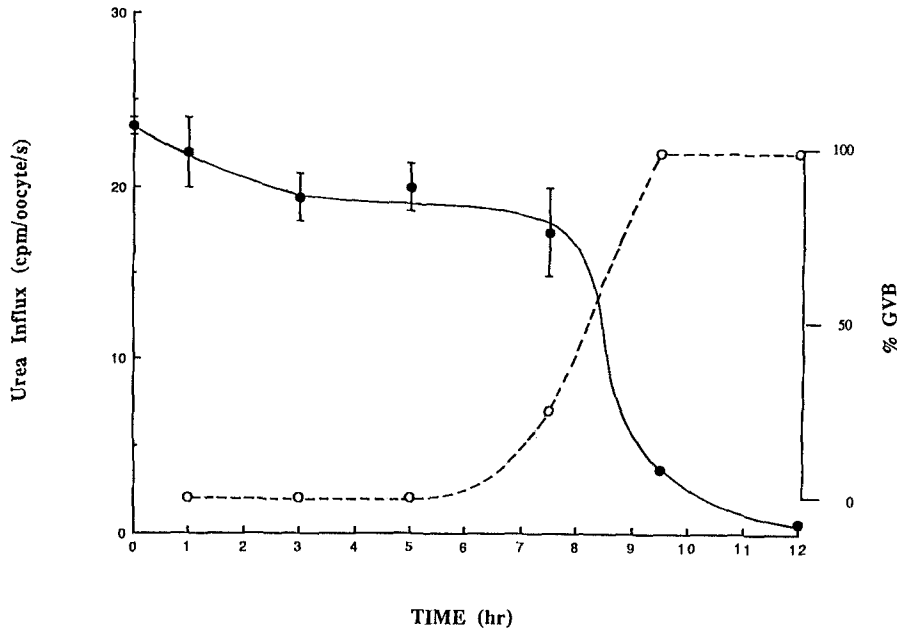

Figure 2. Urea influx (solid circle) and GVB (open circle) of oocytes obtained from a single donor as a function of progesterone exposure time. Urea influx measured over 5 min. Influx (solid line) data given as mean ± SE for 5 oocytes; GVB (dashed line) was scored for 10 oocytes at each time.

control and progesterone-treated cells. To do this, cells were manually dissected at various times to determine the presence of a germinal vesicle. Germinal vesicle breakdown (GVB) is one widely used marker in the progression of cellular events that comprise meiotic maturation[13,14]. As shown in figure 2, progesterone exposure caused multiphasic changes in urea influx. Influx decreased by about 20% during the first 3 h of exposure, was level for the next 4 h and then declined rapidly from 7 to 9.5 h. After 12 h of progesterone exposure, the influx had been reduced 42-fold from the control value. In contrast, the time course of GVB was simple, with a single large increase occurring between 5 and 9 h exposure. Thus, the greatest decrease in urea influx occurred during the time that oocytes underwent GVB. Similar results were obtained in two other experiments with different donor frogs.

Discussion

The results of the present study show that the apparent urea permeability of the oocyte membrane decreased

42-fold over 12 h of exposure to progesterone. This decrease occurred in 3 phases: 1) an early phase, which accounted for about 20% of the change occurred before any cells exhibited GVB, 2) a major phase coincided with GVB, and 3) a late phase, which accounted for about 20% of the change and occurred after all cells had undergone GVB.

These findings were consistent with observation of the reductions in oocyte leucine uptake both during and after GVB[1] (phases 2 and 3 in the present study). These changes in membrane permeability appear to be related to meiotic maturation rather than progesterone exposure per se since medium-sized oocytes do not exhibit GVB or permeability changes after progesterone exposure (data not shown). Furthermore, many transport systems including alanine transport[5,6], passive fluxes of K^+ and Na^+ (refs 2, 15), and active transport of K^+ (ref. 6) show different patterns of temporal relationships of the regulatory decrease of transport rates during meiotic maturation. Recent work indicates that the regulation of Na^+-K^+-ATPase isozymes during meiotic maturation may play a role in the development of the early embryo[8]. Thus, the membrane changes are likely to be part of the complex program for maturational transformation.

What is the nature of the changes that occur in the oocyte plasma membrane during maturation? For substances that are not actively transported, membrane permeability is a function of the intrinsic permeability per unit of cell surface area and the actual surface area. The surface of unstimulated (control) oocytes is characterized by numerous microvilli[16]. The effective amplification of cell surface area by microvilli has not been determined for *Rana* ooctyes, but for *Bufo* Dick et al.[17] estimated an amplification factor of about 5-fold. Assuming that *Rana* oocytes are spheres 1500 µm in diameter and that the surface amplification factor is also 5, we calculate a urea permeability for control oocytes of 7.3×10^{-5} cm/s. This value is two orders of magnitude higher than that for *Xenopus* oocyte[10], but lower than those reported for human erythrocytes[18]. Methodological differences in obtaining both the defolliculated oocyte (manual dissection in this study vs collagenase incubation) and the urea permeability (isotope uptake vs efflux kinetics) may contribute to the observed difference in oocyte studies. The difference may be also due to the difference in species since *Xenopus*, *Rana*, and human oocytes exhibit different water permeabilities[10,19,20] and voltage-dependent currents[3,21]. During maturation, oocyte microvilli are retracted and surface structures become flattened and smooth[22]. The detailed time course of this process is unknown, but it is unlikely that microvillus contraction can account entirely for the 42-fold decrease in urea permeability found here. Intrinsic membrane permeability is likely to have changed. This view is consistent with the findings in membrane electrical properties of Xenopus oocytes where a marked (3-fold) increase in specific resistance (with normalized surface area) begins at 50 to 60% of the time between the application of progesterone and GVB[4]. Furthermore, the membrane potential depolarization following progesterone-induced maturation is inhibited by cycloheximide[15], suggesting that membrane properties interact with cytoplasmic factors (e.g., new protein) which may be involved in the regulation of membrane permeability.

If we use the surface amplification value for *Bufo*, then we calculate that maturation caused at least an 8-fold decrease in the intrinsic permeability of the oocyte membrane to urea. This one order of magnitude of decrease in urea permeability is comparable to the magnitude of increase in urea permeability elicited by antidiuretic hormone (ADH) in certain transporting epithelia[5,23]. The analogy between progesterone action in oocytes and ADH action in epithelia is closer since progesterone reduces oocyte cyclic-AMP[3,13] and membrane permeability while ADH increases epithelial cell cyclic-AMP and membrane permeability. Before mechanistic links between cyclic-AMP and membrane permeability changes can be defined in progesterone-treated oocytes, additional data are needed on temporal relationships between apparent permeability changes, surface area changes and cyclic-AMP levels.

Since the loss of Na^+-K^+ pump activity at GVB is achieved by the internalization of pumps[7,8], the inhibition of the Na^+ cotransport systems appears to result from membrane potential depolarization[24], and surface area reduction and intracellular messengers (e.g. cAMP) may be involved in decreased urea transport, the oocyte apparently employs different mechanisms to modify its membrane transport properties during maturation.

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