Progesterone treatment abolishes exogenously expressed ionic currents in *Xenopus* oocytes

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Shcherbatko, Anatoly D., Christopher M. Davenport, Joan C. Speh, Simon R. Levinson, Gail Mandel, and Paul Brehm. Progesterone treatment abolishes exogenously expressed ionic currents in *Xenopus* oocytes. *Am J Physiol Cell Physiol* 280: C677–C688, 2001.—Fully grown oocytes of *Xenopus laevis* undergo resumption of the meiotic cycle when treated with the steroid hormone progesterone. Previous studies have shown that meiotic maturation results in profound downregulation of specific endogenous membrane proteins in oocytes. To determine whether the maturation impacts the functional properties of exogenously expressed membrane proteins, we used cut-open recordings from *Xenopus* oocytes expressing several types of Na⁺ and K⁺ channels. Treatment of oocytes with progesterone resulted in a downregulation of heterologously expressed Na⁺ and K⁺ channels without a change in the kinetics of the currents. The time course of progesterone-induced ion channel inhibition was concentration dependent. Complete elimination of Na⁺ currents temporally coincided with development of germinal vesicle breakdown, while elimination of K⁺ currents was delayed by ~2 h. Coexpression of human β₁-subunit with rat skeletal muscle α-subunit in *Xenopus* oocytes did not prevent progesterone-induced downregulation of Na⁺ channels. Addition of 8-bromo-cAMP to oocytes or injection of heparin before progesterone treatment prevented the loss of expressed currents. Pharmacological studies suggest that the inhibitory effects of progesterone on expressed Na⁺ and K⁺ channels occur downstream of the activation of cdc2 kinase. The loss of channels is correlated with a reduction in Na⁺ channel immunofluorescence, pointing to a disappearance of the ion channel-forming proteins from the surface membrane.

sodium channels; potassium channels; maturation; internalization; cdc2 protein kinase

FOR MANY YEARS *Xenopus* oocytes have served as an excellent system for elaborating intricate mechanisms of cell cycle control. In vivo, stage VI immature oocytes are physiologically arrested in the first meiotic prophase at the G₀/M border and resume meiosis when gonadotropins stimulate surrounding follicle cells, causing them to secrete the steroid hormone progesterone (46). The progesterone binds to surface membrane receptors (8, 43) and initiates oocyte maturation, a crucial process transforming the immature oocyte into a fertilizable egg. This stimulation of progesterone receptors is followed by a decrease in cAMP-dependent protein kinase activity and activation of a cascade of multiple protein kinases: Mos, Raf, mitogen-activated protein kinase, and activation of cdc25 phosphatase (13, 20). These events lead to activation of a universal cytoplasmic maturation promoting factor (MPF) (39), a heterodimer composed of a regulatory subunit (cyclin B) and a catalytic subunit (cdc2 protein kinase) (34). Activated MPF then induces nuclear envelope disassembly (germinal vesicle breakdown; GVBD), comprising chromosome condensation accompanied by spindle formation and profound cytoskeletal reorganization (6, 38). During progesterone-induced maturation, oocytes undergo remarkable structural reorganization. The early events include progressive size reduction of the microvilli, flattening of the plasma membrane, movement of cortical granules away from the plasma membrane, and a significant decrease in the density of intramembrane particles. After several hours of progesterone treatment, changes in transmembrane fluxes of Cl⁻, Na⁺, K⁺, and Ca²⁺ have been detected as well as membrane potential depolarization, an increase in resistance, and a decrease in membrane capacitance (31). A striking phenomenon of selective endocytosis of native membrane-bound proteins that accompanies meiotic maturation has been well established in oocytes (44, 45, 51, 60).

The process of internalization has been implicated in the modulation of voltage-operated Ca²⁺ channels in rat pituitary cells (36) and human neuroblastoma cells (49) and of Na⁺ channels in fetal rat brain neurons (17). Such a process has also been reported for agonist-induced regulation of adrenoreceptors (27) and cystic fibrosis transmembrane conductance regulator chloride channel (52).

Our studies utilized the fact that a variety of cloned ion channels can be heterologously expressed in *Xenopus* oocytes and that their functional properties can be

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monitored during progesterone-induced maturation. The results presented in this paper indicate that exogenously expressed Na\(^+\) and K\(^+\) channels undergo complete loss during progesterone-induced maturation. The present study indicates that the progesterone-triggered signal transduction pathway downstream of cdc2 kinase activation is responsible for the channel loss.

MATERIALS AND METHODS

Obtaining and handling Xenopus oocytes. Sections of ovary were surgically isolated from anesthetized Xenopus frogs (Nasco, Fort Atkinson, WI). After extensive washing, the follicle cell layer was mechanically removed from stage V–VI oocytes without any enzymatic pretreatment. Enzymatic digestion was avoided because of the possible modification of membrane proteins by collagenase, usually employed to free oocytes from follicle cells. Cells were allowed to recover overnight in a nutrient OR-3 medium containing 50% L-15 medium, 100 μg/ml gentamicin, 4 mM glucose, and 1 mM Na-HEPES, all from GIBCO BRL (Grand Island, NY), with pH adjusted to 7.6 with NaOH. The next day, oocytes were individually injected with 100–140 ng of RNA coding for one of the following α-subunits: rat skeletal muscle (SKM1; Ref. 65), rat peripheral nerve (rPN1; Ref. 64), human peripheral nerve (hPN1; Ref. 32; kindly provided by Dr. F. Hoffman), rat brain type IIα (RBIIα; Ref. 4; kindly provided by Dr. A. Goldin), rat Kv1.4 (Ref. 54; generous gift of Dr. Lily Jan), or rat DRK1 (Ref. 21; kindly provided by Dr. Rolf Joho). In the experiments in which the effect of human βα-subunit (Ref. 37; kindly provided by Dr. A. George) was tested, the oocytes were injected with a mixture of 75 ng of α RNA and 25 ng of βα RNA. The methods used to synthesize the RNA were identical to those previously published (47). Microinjection was performed with a Drummond Nanoject (Drummond Scientific, Broomall, PA). Injected oocytes were maintained at 18°C in OR-3 medium for up to 5 days until the desired expression level was achieved. However, oocytes could be routinely maintained for at least 2 wk in OR-3 medium without signs of deterioration.

Progesterone-treated oocytes were judged to be undergoing meiotic maturation by the appearance of a white spot in the oocyte animal hemisphere. This white spot is a visible indication of the GVB (6, 61). The time required for the GVB to occur varied from oocyte to oocyte, and in general it took 6–9 h for a distinct white spot to develop.

Drugs. Progesterone, heparin, taxol, and phalloidin were obtained from Sigma. Roscovitine, brefeldin A, and Ro 20-1724 were purchased from Calbiochem, and 8-bromo-cAMP (8-Br-cAMP) was from RBI. The stock solutions of progesterone, roscovitine, olomoucine, brefeldin A, and Ro 20-1724, thapsigargin, and calpain inhibitor were prepared in di-methyl sulfoxide (DMSO).

Electrophysiology. Na\(^+\) and K\(^+\) currents were measured by using a standard cut-open voltage-clamp technique (63) within 2–5 days after the RNA injection. Currents were generally recorded from the animal side of the oocyte at 21–22°C. For the cut-open oocyte voltage-clamp recordings, the three-compartment chamber provided with the CA-1 voltage clamp (Dagan, Minneapolis, MN) was used. Both top and guard chamber solutions contained 110 mM NaCH\(_3\)SO\(_3\), 2 mM Ca(CH\(_3\)SO\(_3\))\(_2\), and 10 mM Na-HEPES at pH 7.2. The bottom chamber contiguous with the cell interior contained a solution composed of 120 mM KCH\(_3\)SO\(_3\), 1 mM K-EGTA, and 10 mM K-HEPES at pH 7.2. Agar bridges filled with 120 mM NaCH\(_3\)SO\(_3\) and containing a black platinized Platinum wire were used to pass current and control the chamber potential. An intracellular micropipette filled with 3 M KCl (~100 kΩ) measured the membrane potential. Currents were acquired with the use of a CA-1 oocyte clamp amplifier (Dagan). Oocytes that showed an obvious lack of proper voltage control were discarded.

Data analysis was performed by using HEKA PulseFit software (Instrutech, Great Neck, NY) and IGOR Pro (WaveMetrics, Lake Oswego, OR). Cumulative data are presented as means ± SD. Where appropriate, changes were tested with Student’s t-test. Statistical significance was accepted at a level of P < 0.04.

Immunochemistry. Immunohistochemical staining of Xenopus oocytes was performed to determine whether progesterone treatment led to the disappearance of Na\(^+\) from the plasma membrane. Twenty oocytes were injected with 100 ng of SkM1, and an additional ten oocytes were sham injected with distilled water as a control. After injection, the oocytes were kept at 18°C for an additional 48 h to allow for maximal expression of functional Na\(^+\) channels. At this time, 10 of the SkM1 injected oocytes were treated with 20 μM progesterone for 10 h. All of the oocytes were fixed for 2–4 h at 4°C in 0.1 M phosphate-buffered saline (PBS) at pH 7.3 containing 4% paraformaldehyde. After a 1-h rinse in PBS, the oocytes were cryoprotected through a series of sucrose phosphate buffers, embedded in Lipshaw embedding compound, and frozen on dry ice. Sections were cryostat cut at 8-μm thickness, thaw mounted onto slides, and stored at −20°C. The sections from sham-injected, SkM1-injected, and progesterone-treated oocytes were pretreated for 1 h in PBS containing 4% goat serum, 2% bovine γ-globulin, and 0.3% Triton X-100 (PBS-GBT) at 21°C. After a brief rinse in PBS, the sections were treated overnight with a 1:100 dilution in PBS-GBT of a primary antibody directed against a highly conserved Na\(^+\) channel epitope (18). Control sections were preblocked with primary antibody along with a 100 M excess of the peptide antigen for 3 h at 21°C. After three rinses in PBS, the sections were incubated for 1 h in a PBS-GBT containing 1:100 dilution of goat anti-rabbit secondary antibody conjugated to Alexa 568 (Molecular Probes, Eugene, OR). After three more rinses in PBS, the sections were air dried and coverslip mounted with Vectashield anti-fade medium (Vector Laboratories). Sections were viewed with a Zeiss LSM 510, version 2.5, confocal laser scanning microscope by using a helium-neon laser with a 543-nm emission wavelength. Optical images of 0.4 μm were collected of each oocyte preparation. Images were processed with Adobe Photoshop 5.0.

RESULTS

Progesterone treatment leads to the complete suppression of currents associated with expressed Na\(^+\) and K\(^+\) channels. Cut-open oocyte voltage-clamp recordings were used to test the effects of progesterone treatment on the amplitude of currents associated with exogenously expressed Na\(^+\) and K\(^+\) ion channels. In the cut-open mode of voltage clamp, Na\(^+\) and K\(^+\) currents were stable over time (>30 min) without any noticeable change in amplitude or kinetics. Groups of oocytes were incubated with progesterone in external solution, and amplitudes of expressed currents were measured at different times in randomly selected oocytes during the progesterone application. Incubation of oocytes
with 1 μM progesterone for up to 10 h did not affect ionic currents, and no signs of maturation were detected (data not shown). In contrast, treatment with 10 μM progesterone resulted in almost 50% inhibition of Na⁺ currents after 6 h and the appearance of a white spot in 10% of oocytes (data not shown). The most uniform oocyte response in terms of current suppression and maturation was detected with 20 μM progesterone incubation. Control experiments with long-lasting DMSO treatment showed no effect on current amplitude and did not trigger maturation. The Na⁺ currents resulting from expression of α-subunit of rat skeletal muscle (SkM1) Na⁺ channels consistently displayed a reduction of peak amplitude during treatment with 20 μM progesterone (Fig. 1). On closer examination of the data, the inhibition was found to be insignificant during the first 4 h of progesterone treatment. However, after 4 h, the progesterone-induced block of the Na⁺ current became profound and eventually resulted in a complete suppression. Additionally, coexpression of the human β₁-subunit with SkM1 α-subunit in Xenopus oocytes led to fast-inactivating Na⁺ currents that also disappeared during treatment with progesterone (Fig. 2). Almost total elimination of Na⁺ currents coincided with the time of GVBD as judged by the appearance of a white spot in the oocyte animal hemisphere. Progesterone treatment uniformly diminished Na⁺ current peak amplitude at all voltages (Fig. 1, B and C), and inhibition of Na⁺ currents was not accompanied by any substantial change in kinetics of

![Bar chart](http://example.com/bar_chart.png)

**Fig. 1.** Effects of progesterone treatment on Na⁺ currents in *Xenopus* oocytes. **A:** time course of progesterone-induced inhibition of Na⁺ currents resulting from expression of rat skeletal muscle α-subunit (SkM1). Oocytes were incubated at time 0 with 20 μM progesterone. A random sample of several oocytes was removed for current assay every hour after the progesterone treatment was started. Values represent means ± SD of peak Na⁺ current amplitude; n = no. of oocytes tested. The peak current amplitudes measured during progesterone treatment were normalized to the peak current amplitudes recorded from control oocytes incubated with DMSO. *P < 0.04 vs. control (t-test).** B: representative current-voltage relationships of SkM1 Na⁺ channels formed by the α-subunit in a control, untreated oocyte (○) and after incubation with 20 μM progesterone for 8 h (●). C: the same current-voltage relationship of SkM1 Na⁺ channels as in B shown on an expanded scale in oocytes incubated with 20 μM progesterone for 8 h. D: cut-open voltage-clamp recordings of SkM1 Na⁺ currents from control, untreated oocytes. The voltage was stepped from a holding potential of −100 mV in increments of 5 mV, starting from −40 mV. E: cut-open voltage-clamp recordings of SkM1 Na⁺ currents from oocytes incubated with 20 μM progesterone for 7 h. The voltage was stepped from a holding potential of −100 mV in increments of 5 mV, starting from −35 mV.
inactivation or voltage dependence of activation of the current (Fig. 1, D and E). We also observed a similar time course of progesterone-induced inhibition of Na\(^+\) currents resulting from expression of \(\alpha\)-subunits of human peripheral nerve (hPN1), rat peripheral nerve (rPN1), and rat brain type IIa (RBIIa) Na\(^+\) channels.

The effect of 20 \(\mu\)M progesterone treatment on Na\(^+\) channels formed by the \(\alpha\) and \(\beta\)-subunits in control, untreated oocytes (○) and after incubation with 20 \(\mu\)M progesterone for 7 h (●). D: the same current-voltage relationship of SKM1 Na\(^+\) channels as in C shown on an expanded scale in oocytes incubated with 20 \(\mu\)M progesterone for 7 h.

Involvement of cAMP in progesterone-induced effects on expressed ion channels. In a complex cascade of progesterone-induced early signaling events, the decrease in oocyte cAMP level is well established and is thought to be critical in the meiotic resumption process (6, 61). On binding to its plasma membrane receptors, progesterone leads to a G protein-mediated sharp decrease in adenylyl cyclase activity (23). Decline in cAMP levels causes a decrease in protein kinase A activity that, in turn, leads to dephosphorylation of putative maturation-inhibiting proteins and subsequent activation of MPF. On the other hand, it is known that cAMP-dependent phosphorylation can also modulate activity of voltage-gated ion channels (12). To test whether cAMP mediates the effect of progesterone on expressed Na\(^+\) and K\(^+\) channels, we incubated oocytes with 2 mM 8-Br-cAMP, a membrane-permeable analog of cAMP. A relatively high concentration of 8-Br-cAMP was selected in light of previous reports that Xenopus oocytes have very low permeability to cAMP and high endogenous activity of cAMP phosphodiesterase (9). Treatment with 8-Br-cAMP did not show any substantial effect on the level of Na\(^+\) and K\(^+\) channel expression but effectively prevented the progesterone-induced inhibition of expressed currents (Fig. 5A). Moreover, progesterone treatment (up to 10 h) did not result in the appearance of a distinct white spot in the oocyte animal hemisphere, a sign of GVBD, when 8-Br-cAMP was present in the incubation medium. This observation suggests that elevated level of cAMP in oocytes inhibits the development of proges-
terone-induced maturation and confirms similar earlier findings (9). In contrast, we were unable to confirm other reports that inhibitors of cAMP phosphodiesterase block oocyte maturation (9). Incubation of oocytes with 50 μM Ro 20-1724, a selective inhibitor of cAMP phosphodiesterase (53), did not prevent progesterone-induced inhibition of expressed currents (Fig. 5B) or maturation as scored by the appearance of an animal hemisphere white spot.

**Involvement of the inositol 1,4,5-trisphosphate-sensitive pathway in progesterone-induced inhibition of expressed channels.** Studies have clearly implicated the phospholipid signaling pathway in meiotic resumption of *Xenopus* oocytes (42). To test whether inositol 1,4,5-trisphosphate (IP₃)-stimulated events play a role in progesterone effect on expressed ion channels, we used heparin, a potent antagonist of IP₃ receptors (24, 11). Microinjection of 40 nl of 200 μM heparin into oocytes immediately before progesterone treatment inhibited the progesterone-induced effects (Fig. 5C). Microinjection of heparin per se did not induce significant changes in the level of the expressed currents. It is established that intracellular Ca²⁺ levels are regulated by IP₃, and it is likely that IP₃-sensitive Ca²⁺ stores are responsible for the transient increase of free calcium associated with progesterone-induced maturation (24). We found that oocyte incubation with 200 μM thapsigargin, which causes a depletion of IP₃-sensitive Ca²⁺ depots by inhibiting Ca²⁺-ATPase responsible for maintaining high Ca²⁺ concentration in intracellular...
organelles (28), did not induce maturation or inhibition of expressed channels (Fig. 5D).

Free intracellular Ca\(^{2+}\) can activate an array of Ca\(^{2+}\)-binding proteins. Among them are Ca\(^{2+}\)-regulated proteases that are thought to play a key role in the regulation of the cell cycle. Specifically, the Ca\(^{2+}\)-activated cysteine protease calpain was shown to be involved in the control of the meiotic cycle in *Xenopus* oocytes (57, 58, 70). Incubation of oocytes with 100 \(\mu\)M calpain inhibitor did not affect the time course of progesterone-induced maturation as well as inhibition of expressed channels (data not shown).

Disruption of cytoskeleton or cytoplasmic protein traffic is not involved in progesterone-induced downregulation of expressed ion channels. It is well established that all three major filamentous systems of the cytoskeleton (i.e., actin filaments, intermediate filaments, and microtubules) undergo a profound reorganization during meiotic resumption in *Xenopus* oocytes (6). Moreover, it is also known that the cytoskeleton is involved in the regulation of the function of endogenous (22, 67) and expressed voltage-activated channels (59). It is likely that cytoskeletal rearrangements associated with maturation underlie the progesterone-induced inhibition of expressed ion channels. To test the potential role of the cytoskeleton, we incubated oocytes with 100 \(\mu\)M taxol or 100 \(\mu\)M phalloidin for 30 min before and during progesterone treatment. Taxol promotes the formation of highly stable microtubules that resist depolymerization (25, 29), and phalloidin is an F-actin stabilizer (15, 26). We found that neither taxol (Fig. 6A) nor phalloidin affected the progester-

Fig. 4. Effect of progesterone on Kv1.4 K\(^+\) currents and on currents resulting from coexpression of Na\(^+\) and K\(^+\) channels in the same oocyte. A: cut-open voltage-clamp recordings of Kv1.4 K\(^+\) currents from control, untreated oocytes. The voltage was stepped from a holding potential of \(-100\) mV in increments of 10 mV, starting from \(-50\) mV. B: cut-open voltage-clamp recordings of Kv1.4 K\(^+\) currents from oocytes incubated with 20 \(\mu\)M progesterone for 7 h. The voltage was stepped from a holding potential of \(-100\) mV in increments of 10 mV, starting from \(-50\) mV. C: cut-open voltage-clamp recordings of currents from control, untreated oocytes coexpressing SkM1 Na\(^+\) and DRK1 K\(^+\) channels. The voltage was stepped from a holding potential of \(-100\) mV in increments of 10 mV, starting from \(-60\) mV. D: cut-open voltage-clamp recordings of currents from oocytes coexpressing SkM1 Na\(^+\) and DRK1 K\(^+\) channels after incubation with 20 \(\mu\)M progesterone for 7 h. The voltage was stepped from a holding potential of \(-100\) mV in increments of 10 mV, starting from \(-60\) mV.
one-induced maturation and associated inhibition of expressed channels.

It has also been established that during maturation the Golgi breaks down (14) and intracellular membrane transport becomes blocked (35). It is conceivable that these maturation-induced events could underlie the inhibitory effect of progesterone on expressed channels. To address this issue, we treated SkM1 Na\(^+\) channel-expressing oocytes with 100 \(\mu\)M brefeldin A, a fungal metabolite that disrupts protein traffic through the Golgi apparatus (50). Brefeldin A failed to produce an inhibitory effect on expressed Na\(^+\) channels (Fig. 6B). This result ruled out the block of protein traffic between the Golgi and the plasma membrane as the plausible mechanism of progesterone triggered suppression of ion channels.

Involvement of the cdc2 kinase in progesterone-induced inhibition of expressed ion channels. Progesterone triggers the transduction process that culminates in activation of cyclin-dependent protein kinase cdc2. It is well established that this event is universal and common in all eukaryotic cells (48). To clarify the role of cdc2 kinase in progesterone-induced inhibition of expressed ion channels, we took advantage of the recent discovery of several specific inhibitors of cyclin-dependent kinases (1, 40). We used olomoucine and roscovitine, two highly specific inhibitors of cdc2/cyclin B kinase. Oocytes were incubated with 50 \(\mu\)M Ro 20-1724 in the surrounding media during progesterone treatment. C: protective effect of heparin against progesterone-induced inhibition of Na\(^+\) currents. Heparin was microinjected (40 nl of 200 \(\mu\)M stock solution) into oocytes before incubation with progesterone. D: thapsigargin treatment had no effect on progesterone-induced inhibition of Na\(^+\) currents. Oocytes were incubated with 200 \(\mu\)M thapsigargin in the surrounding media during progesterone treatment.

Fig. 5. Effects of 8-bromo-cAMP (8-Br-cAMP), Ro 20-1724, heparin, and thapsigargin on progesterone-induced inhibition of SkM1 \(\alpha\)-subunit Na\(^+\) currents. Representative data were obtained from untreated oocytes, oocytes treated with 20 \(\mu\)M progesterone for 6–7 h, and oocytes incubated with 20 \(\mu\)M progesterone for 7–8 h plus treatment with the indicated drugs. The peak current amplitudes measured during progesterone treatment were normalized to those recorded from control oocytes. Values represent means \(\pm\) SD of peak Na\(^+\) current amplitude; \(n = \) no. of oocytes tested. A: 8-Br-cAMP prevents progesterone-induced inhibition of Na\(^+\) currents. Oocytes were incubated with 2 mM 8-Br-cAMP in the surrounding media during progesterone treatment. B: treatment with Ro 20-1724 had no effect on progesterone-induced inhibition of Na\(^+\) currents. Oocytes were incubated with 50 \(\mu\)M Ro 20-1724 in the surrounding media during progesterone treatment. C: protective effect of heparin against progesterone-induced inhibition of Na\(^+\) currents. Heparin was microinjected (40 nl of 200 \(\mu\)M stock solution) into oocytes before incubation with progesterone. D: thapsigargin treatment had no effect on progesterone-induced inhibition of Na\(^+\) currents. Oocytes were incubated with 200 \(\mu\)M thapsigargin in the surrounding media during progesterone treatment.
channels (Fig. 6, C and D). Furthermore, progesterone treatment did not result in the appearance of a white maturation spot in the oocytes incubated with olomoucine or roscovitine in contrast to the control group of oocytes (data not shown).

**Progesterone-induced inhibition of expressed currents is due to a decrease in surface membrane expression of ion channels.** The time course of progesterone-triggered downregulation of exogenously expressed currents suggests that channels may be lost. To address this issue, we assayed Na$^+$ channels in oocyte surface membranes by using the antibodies raised to an epitope in the internal loop linking domains III and IV of pore-forming $\alpha$-subunit (18). Immunocytochemical experiments were carried out as described in MATERIALS AND METHODS, and the results are shown in Fig. 7. We found Na$^+$ channel immunofluorescence clearly present in the plasma membrane of oocytes injected with SkM1 $\alpha$-subunit (Fig. 7B), while no Na$^+$ channel-related staining was seen in the surface membrane region of progesterone-treated oocytes (Fig. 7C). Thus these results lead to the conclusion that progesterone-induced downregulation of Na$^+$ currents could be attributed to a reduction of cell surface expression of channel-forming proteins.

**DISCUSSION**

Gonadal steroids exert profound effects on both the central nervous system and the peripheral nervous system. It has been accepted that steroid hormones produced by peripheral glands can cross the blood-brain barrier and affect a variety of important brain and spinal cord functions. Moreover, a recent study
found that glial cells in the brain and other parts of the nervous system can synthesize steroids de novo from cholesterol (30). In addition to activity in glial cells, neurosteroidogenesis has been established in Purkinje cells, pyramidal neurons in the hippocampus, neurons in the retinal ganglion, and sensory neurons in the dorsal root ganglia (66). There is a growing interest in the effects of steroid hormones at the level of the cell membrane in contrast to their “classic” intracellular role as regulators of transcription (3, 56, 71). Recently, nongenomic steroid action has been widely recognized and has been implicated in altering neuronal excitability (75), synaptic functioning (73), cell membrane ultrastructure, and endoexocytotic activity (3). In this regard, progesterone, in particular, is considered to be one of the most active steroids. Nongenomic electrophysiological effects of progesterone include inhibition of a Ca\(^{2+}\) current in human intestinal smooth muscle cells (7), a broad spectrum of K\(^+\) channels in human T lymphocytes (19), several cloned \(K_v\) channels expressed in cell lines (19), K\(^+\) channels in the plasma membrane of cultured renal epithelioid Madin-Darby canine kidney cells (62), \(K_v\) channels in hepatocytes (68), the native voltage-activated tetraethylammonium and 4-aminoypyridine-sensitive K\(^+\) channels in \(Bufo\) oocytes (74), and K\(^+\) currents in starfish oocytes (60). In addition, a recent study reports that progesterone treatment leads to selective downregulation of rat \(eag\)
K⁺ but has no comparable effect on either Shaker H4, Drosophila eag, or Kv1.4 K⁺ channels heterologously expressed in Xenopus oocytes (10). In contrast, our results demonstrate that progesterone-induced maturation in Xenopus oocytes is associated with functional inhibition of exogenously expressed SkM1, hPN1, rPN1, and RBIIa Na⁺ channels as well as Kv1.4 and DRK1 K⁺ channels. It is possible that faster disappearance of Na⁺ channels vs. K⁺ channels is a result of the differential spatial distribution of those channels. Clustering of specific ion channels in the surface membrane is well known and is thought to be based on the differential association of ion channel proteins with cytoskeletal elements.

Previous studies have shown that the Drosophila K⁺ channel β-subunit homologue Hyperkinetic (Hk) associates with eag α-subunits when coexpressed in Xenopus oocytes and protects against a downregulation in eag current amplitude that was otherwise observed in response to treatment with progesterone (72). Furthermore, studies of an activity-induced internalization of the native Na⁺ channels in immature brain tissues such as cultured fetal rat forebrain neurons or early postnatal hippocampal slices demonstrated that low expression of Na⁺ channels αβ-subunit complexes in immature brain neurons correlates appropriately with the ability of Na⁺ channels to be internalized (2). To test whether auxiliary subunits would affect maturation-associated downregulation of exogenous Na⁺ channels, we coexpressed human β₁-subunit with α-subunit in Xenopus oocytes. Comparison of the time courses of progesterone-induced inhibition of Na⁺ currents associated with α-subunit alone or αβ-subunit complexes did not reveal any substantial differences. Thus functional association of auxiliary β₁-subunit with pore-forming α-subunit is unable to protect tested Na⁺ channels from progesterone-induced downregulation.

Several previous studies demonstrated rapid and reversible inhibition of K⁺ and Ca²⁺ channels on progesterone application (7, 19, 62). It was suggested that in some cases progesterone might affect ion channels directly or through membrane-delimited pathways (19). Our study shows that the slow onset of maturation-induced downregulation is incompatible with the direct effect of progesterone on the ion channels and implicates a complex signaling cascade involved in the progesterone-triggered process of meiotic resumption. In a search for second messengers that mediate the observed inhibition of ion channels, we found that preventing progesterone-induced drop in cAMP concentration or blocking IP₃ binding would inhibit the maturation process and associated downregulation of expressed channels. It has been generally accepted that progesterone-induced modulations of two well-known signaling pathways, the adenylyl cyclase and phosphatidylinositol pathways, are the initial early steps implicated in meiotic resumption (6, 61). Our observation that currents expressed up to 4 h after the start of progesterone treatment were not affected dramatically leads us to suggestion that early events by themselves contribute little to inhibition of currents. In this respect, early events in progesterone-induced signaling cascade should proceed unobstructed to ensure proper activation of the cytoplasmic MPF and the following functional downregulation of expressed channels. Indeed, we demonstrate that two highly specific inhibitors of protein kinase cdc2, olomoucine and roscovitine, effectively prevent progesterone-induced effects. Therefore, we speculate that biochemical events subsequent to protein kinase cdc2 activation in the pathway of maturation are responsible for downregulation of expressed channels.

There are some apparent contradictions in the implication of Ca²⁺ in the regulation of meiosis. Some studies report a progesterone-triggered transient increase in intracellular Ca²⁺ found by using Ca²⁺-sensitive microelectrodes and the Ca²⁺-sensitive luminescent protein aequorin (41, 69), while other studies utilizing identical Ca²⁺-detection techniques found no increase in intracellular free Ca²⁺ (5, 16, 55). However, despite these obvious inconsistencies, other sufficient evidence exists to indicate that intracellular Ca²⁺ is important for regulating progesterone-induced maturation (24, 33, 58). In an attempt to test the role of Ca²⁺ in progesterone-induced effects, we used thapsigargin, which is known to induce a transient increase in intracellular free Ca²⁺ by depleting IP₃-sensitive stores. We found that thapsigargin treatment did not affect functional properties of expressed channels and did not prevent progesterone-induced downregulation of ion channels. Our experiments with thapsigargin confirmed previous reports that an increase in intracellular Ca²⁺ per se is not sufficient to induce maturation (61). In contrast, heparin effects indicated that IP₃-dependent events are obligatory steps in progesterone-induced oocyte maturation. These results together imply that the precise timing of specific steps in a progesterone-triggered sequence of events is very important for successful progression of maturation and the associated downregulation of ion channels.

The antibody labeling experiments have indicated that progesterone treatment leads to elimination of a pore-forming protein of the SkM1 Na⁺ channel from oocyte surface membrane. Interestingly, the retrieval of membrane proteins is known to be associated with maturation. Previous studies have demonstrated that three distinct membrane proteins, β₁-integrin (45), U-cadherin (44), and Na⁺-K⁺-ATPase (51), undergo similar internalization during oocyte maturation. This finding points to an evidently common mechanism for maturation-associated control of membrane proteins. On the basis of the existing body of evidence, Muller et al. (45) postulated that at least three well-distinguished major events occur in a coordinate way to regulate plasma membrane proteins during maturation: 1) the oocyte plasma membrane is cleared from the proteins by endocytosis, 2) the insertion of the proteins into the oocyte plasma membrane is inhibited, and 3) the production of the membrane proteins increases. Apparently, the observed disappearance of exogenously expressed Na⁺ channels is rather a part of
a general regulatory phenomenon that serves to build up the maternal store of membrane proteins in the egg cytoplasm for subsequent development. The precise mechanism governing ion channels retrieval in matur- ing oocytes remains to be elucidated. Given the great physiological significance of ion channel modulation, the experimental approach utilized in this study provides a valuable system to elucidate molecular determinants governing the excitability of cells during the cell cycle.

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PROGESTERONE INDUCES DOWNREGULATION OF ION CHANNELS


