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Original Article

Changes in membrane properties during *in-vitro* meiotic maturation of the limpet *Patella vulgata*

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ABSTRACT Changes in the membrane properties of the oocyte of the mollusk, *Patella vulgata*, were analyzed following the induction of meiosis reinitiation by paleopedial ganglia extract or by the weak base ammonia. During maturation it was possible to distinguish between an early phase characterized by an initial hyperpolarization and a late phase consisting of a depolarization which triggers an action potential with a long-term overshoot (20 minutes) of the membrane potential. Major changes in individual ionic permeabilities were studied using both current and voltage clamp conditions. The depolarizing phase appears to depend on decreases in K^+ membrane permeability. Finally we observed that the overshoot did not appear to be directly related to germinal vesicle breakdown (GVBD) since it was absent in Na-deprived artificial sea water and could be ellicited in the presence of TEA bromide, which did not induce maturation. This last observation suggests that it may result from a change in specific K^+ ion permeability due to the possible activation of stretch channels.

KEY WORDS: *oocyte, meiosis, mollusk, ionic permeability, Patella*

Introduction

Patella vulgata (Fig. 1) oocytes remain arrested in the first prophase stage of meiosis in the ovary. The release from prophase block involves a change in intracellular pH, whereas release of the second block in metaphase 1 depends on a rise in intracellular free calcium (Guerrier *et al.*, 1986a, b, c). The exact nature of meiosis inducers in *Patella* oocytes are still unknown. Shirai *et al.* (1987), presented evidence for the involvment of a gonad stimulating substance (GSS) localized in paleopedial structures which would act on the follicular cells to trigger maturation *via* the action of a postulated relay meiosis stimulating substance (MIS).

Meiosis can be triggered artificially using NH_4Cl in the external medium, known to alkalinize the cytoplasm of the oocyte. Transduction of the signal at the membrane level may involve ionic channels.

The change in ionic permeabilities accompanying meiotic maturation have been extensively studied in echinoderm (Miyazaki *et al.*, 1975a, b; Shen and Steinhardt, 1976; Moreau *et al.*, 1978a, b, c; Charbonneau *et al.*, 1983; Guerrier *et al.*, 1983; Moody and Bosma, 1985; David *et al.*, 1985, 1988) and amphibian oocytes (Moreau *et al.*, 1976, 1985; Baud *et al.*, 1982; Miledi, 1982; Barish, 1983; Peres and Bernardini, 1983; Schlichter, 1983; Rodeau and Vilain, 1987; Vilain *et al.*, 1989). In contrast, little is known about the changes in ionic permeabilities elicited during the maturation of molluskan oocytes except for *Barnea* and *Spisula* (Finkel and Wolf, 1978; Dubé and Guerrier, 1982; Guerrier *et al.*, 1986a, b; Dubé, 1988). In these two species, increasing external K^+ concentration in the presence of Ca^{2+} induced the reinitiation of meiosis, but this treatment was inefficient when applied to *Patella* oocytes. This observation raises the problem of the role of K^+ permeability during meiosis. In fact, in echinoderms, a selective loss of K^+ current was described after GVBD (Miyazaki, *et al.*, 1975a, b; Moody and Bosma, 1985; Simoncini and Moody, 1990). In amphibians, a reduction of the K^+ permeability has also been reported by different laboratories (Morill and Watson, 1966; O'Connor *et al.*, 1977; Ziegler and Morill, 1977; Morill and Ziegler, 1980; Rodeau and Vilain, 1987; Vilain *et al.*, 1989).

In the present study, we have analyzed the evolution of the membrane conductance of *Patella vulgata* oocytes during meiosis. We report original data obtained while using both current clamp and voltage clamp recordings. We observed a dramatic change in K^+ permeability, which seems to be responsible for a typical response triggering an overshoot of the membrane potential. Different hypotheses accounting for such a variation in K^+ conductance are discussed.

Results

Immature oocytes

Resting potential

Immediately after inserting the microelectrode into an immature oocyte suspended in ASW, the resting potential was between -5 and

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A

B

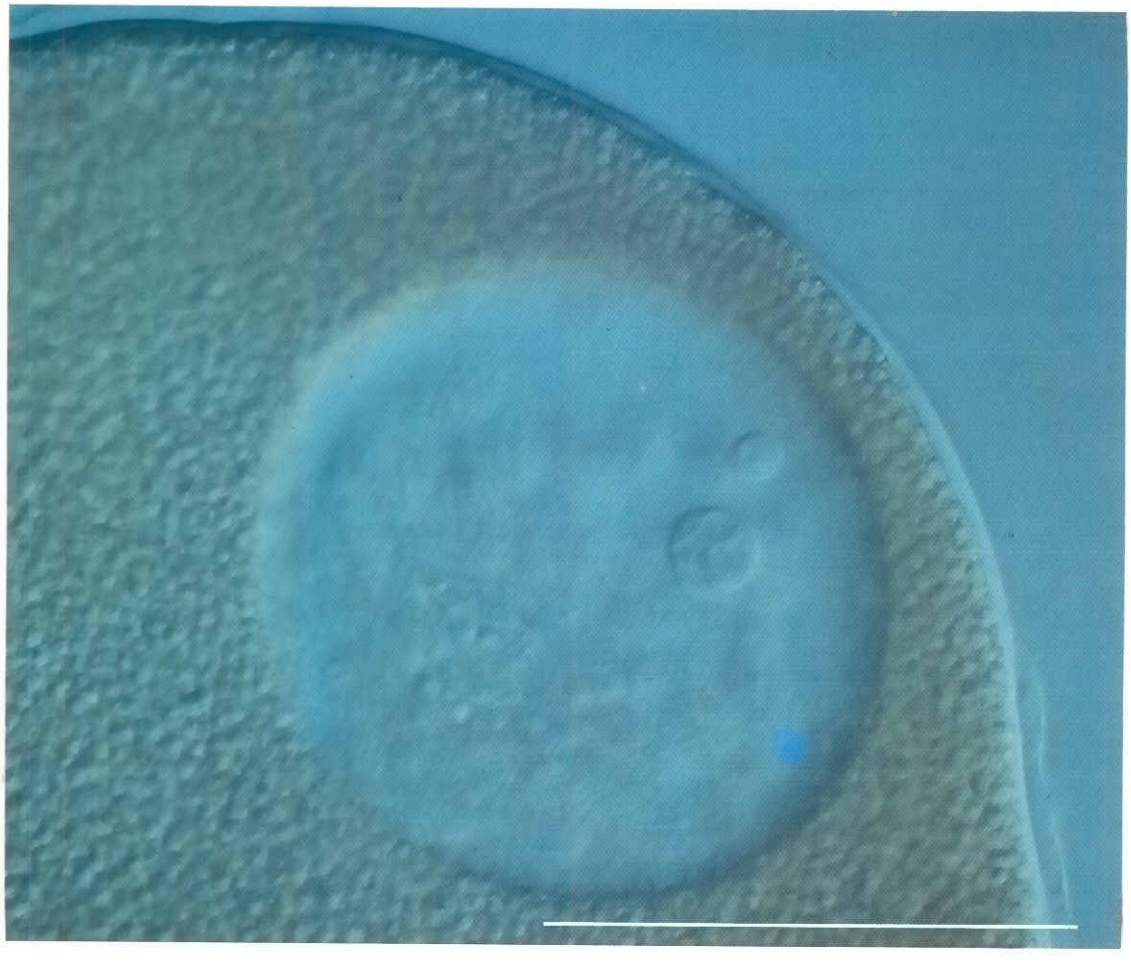

Fig. 1. The marine mollusk *Patella vulgata*. (A) *External morphology in situ, attached to the rocks, in Roscoff (see arrows). The animal lives in the intertidal zone. The diameter of the shell is about 2.5 cm.* **(B)** *Immature oocyte of* Patella vulgata, *observed in vivo in Normarski interferential contrast. Inside the large nucleus (germinal vesicle) 2 nucleoli can be seen. Bar = 50 μm.*

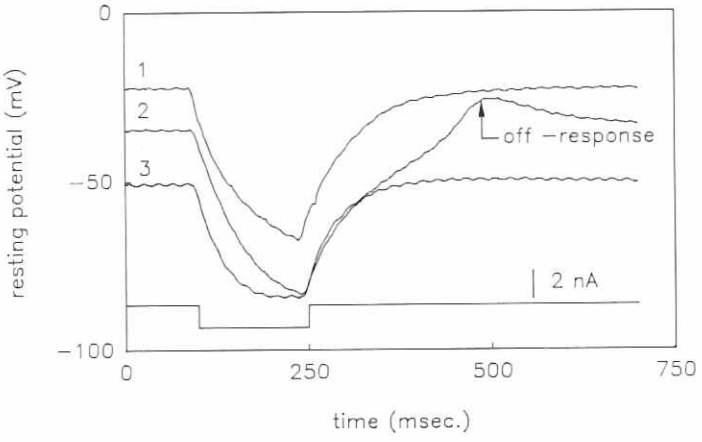

Fig. 2. Evolution of resting potential and membrane resistance, after impalement of the immature *Patella* oocyte by a microelectrode. *1,2,3, correspond respectively to 1, 4 and 10 min after penetration by the microelectrode. The evolution of membrane resistance was measured by applying a hyperpolarizing current. Note that an «off-response» can be obtained as shown at 4 min (curve n°2).*

-20 mV (inside negative). In nearly 75% of the penetrated oocytes, the resting potential shifted toward -30 and -40 mV and then slowly to -60, -70 mV. No intermediate values between -30 and -60 mV were observed. Hyperpolarizing pulses facilitate the transition toward -60 mV. These oocytes were divisible in two groups: with and without «off response» to hyperpolarizing pulses (an «off response» is the spike generated at cessation of hyperpolarizing current; Fig. 2). Oocytes with an «off response» had high membrane resistance and non-linear current voltage relation. Oocytes without the «off response» had lower membrane resistance and linear I/V plots. These membrane potentials were considered to be a result of poor electrode penetration, and the batches were discarded. In these conditions the resting membrane potential of an immature oocyte with an «off-response» was -61 ± 7 mV, n= 82.

The predominant ion contributing to the membrane potential is K^+ ion. A tenfold increase in the external potassium concentration (10 to 100 mM) depolarizes the membrane by 53 ± 3 mV (n=7). Changes in Na^+ concentration had only a small effect upon the resting potential – a tenfold decrease in Na^+ concentration triggers a small hyperpolarization that never exceeds 5 mV. A tenfold increase in Ca^{2+} concentration depolarizes the oocyte by 7 mV ± 2 (n=4). Halving the external Cl^- concentration has no effect on membrane potential.

In order to determine the relative permeability of the membrane to K^+ and Na^+ ions, equation (1) was used (Shen and Steinhardt, 1976)

$$\exp(VF/RT) = [K^+]_o + (P_{Na}/P_K)[Na^+]_o/[K^+]_i \quad (1)$$

Where V_m is the resting membrane potential of the cell, P_K and P_{Na} the permeability of the cell for K^+ and Na^+ respectively, $[K^+]_o$, $[Na^+]_o$, $[K^+]_i$, $[Na^+]_i$ are respectively the internal and external ionic activities and R,T,F the usual values. The equation (1) represents a linear relation between exp(VF/RT) and the external K^+ concentration if we assume that, in the absence of external Cl^-, the effect of Cl^- ion flux upon the membrane potential is minimized, and if the relative permeability of the membrane to Na^+ and K^+ remains constant while varying the external K^+ concentration. The reciprocal of the slope is an estimate of internal K^+ concentration and

$$\text{y intercept} = (P_{Na^+}/P_K{}^+)[Na^+]_o/\,[K^+]_i.$$

In the case of immature oocytes $P_{Na}/P_K = 0.0394 \pm 0.006$, n=7. The estimated internal K^+ concentration was 287 mM ± 14, n=7.

Action potentials

Depolarizing current pulses of 1.5 nA, *i.e.* 0.8 to 1 μA/cm^2 and 70 ms duration induced all or no action potentials as shown in Fig. 3. Threshold potential was observed between -44 to -38 mV. The peak of action potential was -19 ± 4 mV. Overshoots were rarely observed. The total duration of the action potential, from the initiation of the stimulus, was 372 msec. ± 20 (n= 94). The duration of the action potential evoked by the first stimulus was longer (about 450 ms).

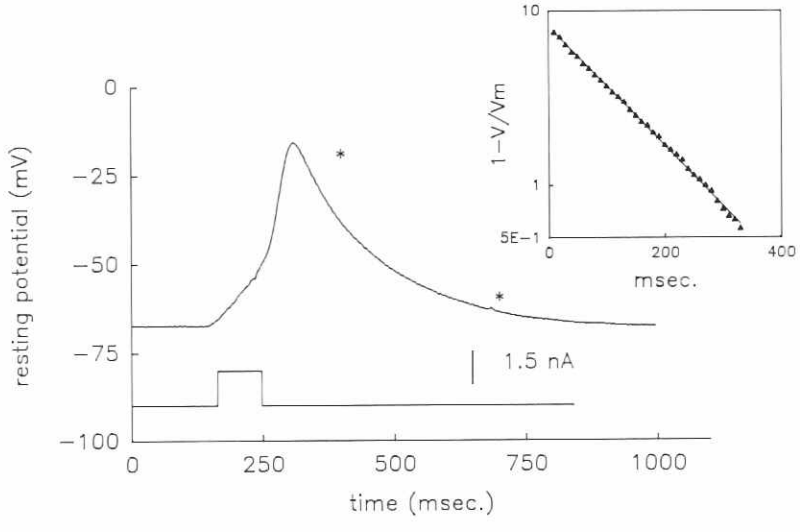

Fig. 3. Action potential of an immature *Patella* oocyte obtained with a threshold depolarizing current. *The inset represents the semi-log plot of 1-V/V_m vs time of the repolarizing phase of the action potential corresponding to the portion of curve between (*).*

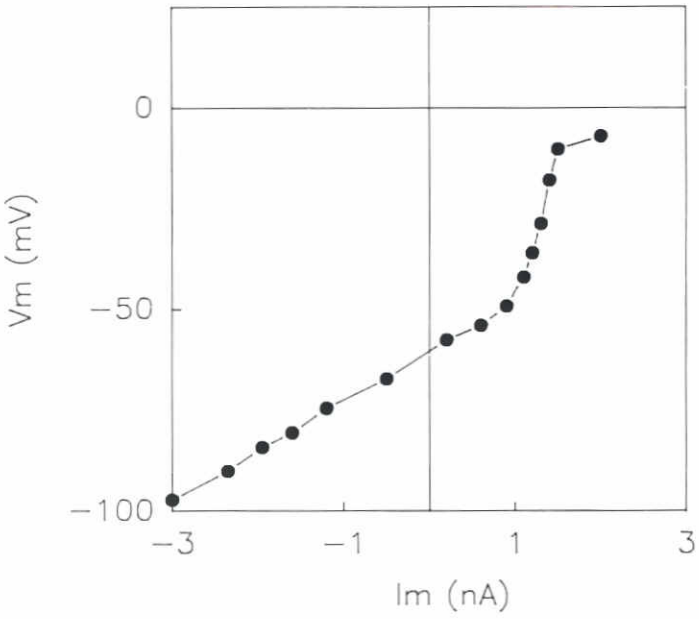

Fig. 4. Current voltage plot, *recorded in current-clamp of immature* Patella *oocyte.*

Generally, peak depolarization was reached within 100 ms from the initiation of stimulus, depending upon stimulus intensity. The action potential showed no slope change during the rising phase. The repolarizing phase never presented a plateau, but exhibited an exponential decay, as illustrated in the semi-log plot in Fig. 3, inset. After spiking, hyperpolarization was rarely observed in the immature oocyte. When present, it never exceeded 4 mV. The maximum rate of rise was 0.55 mV/s.

A total membrane capacity of 1.78 nF on average was obtained from the time constant of the membrane response measured in the linear portion of the I/V curve. Assuming the oocyte as a sphere of 220 μm diameter (mean ± SD = 220 μm ± 12, n= 40) the specific membrane capacity was 1.17 $\mu F/cm^2$ ± 0.2,(n= 11), which is comparable with values found in other kinds of unfertilized eggs. 1.1 $\mu F/cm^2$ in the starfish oocyte of *Asterina pectinifera* (Miyazaki, *et al.*, 1975a), 1 $\mu F/cm^2$ in the tunicate (Miyazaki, *et al.*, 1974a, b) 1μF/cm2 in the amphibian (Jaffe *et al.*, 1978).

Ionic dependence of the action potential.

Action potentials could be elicited in the complete absence of Na^+ ion in the external medium (Fig. 5B). The threshold for initiation of such an action potential was 8 mV less negative in 0-Na-ASW than in ASW. The substitution of NaCl by N-methyl glucamine resulted in a reduction of 10 mV in the peak amplitude of the action potential (Fig. 5B). The total duration of the action potential was shortened to 150 ms. Complete recovery of the action potential was observed following washing with normal ASW.

A tenfold decrease in the external Ca^{2+} concentration affected the parameters of the action potential in the same way as observed following Na^+ subsitution. The peak amplitude was reduced by 7 to 10 mV and the duration to 120 ms (Fig. 5C). Return in ASW allowed a complete recovery .

Medium deprived of Na^+ and Ca^{2+} and supplemented with 20 mM Co^{2+} completely abolished the action potential (data not shown).

These experiments indicate that the rising phase of action potential of *Patella* oocyte membrane is Ca^{2+} and Na^+ dependant.

Analysis of the current voltage relation

As shown in Fig. 2, relatively long current pulses (500 ms) were used to measure membrane resistance. Fig. 4 is a plot of the applied current *versus* the peak voltage response of an immature oocyte (I/V curve). Membrane potential was measured at the end of the current pulse. At the termination of the pulse, there was an overshoot beyond the resting potential (Fig. 2). The slope resistance of the current-voltage curve was 3-4 Mohms at -90 mV; at the resting potential, the value of the slope resistance was 17.2 Mohms. Thus, an inward going rectification exists in the hyperpolarizing region of the current-voltage curve. At the critical potential of about -40 mV, the slope resistance of the current-voltage curve became almost infinite, corresponding to the action potential. The current-voltage curve is markedly S-shaped. These features of the current-voltage

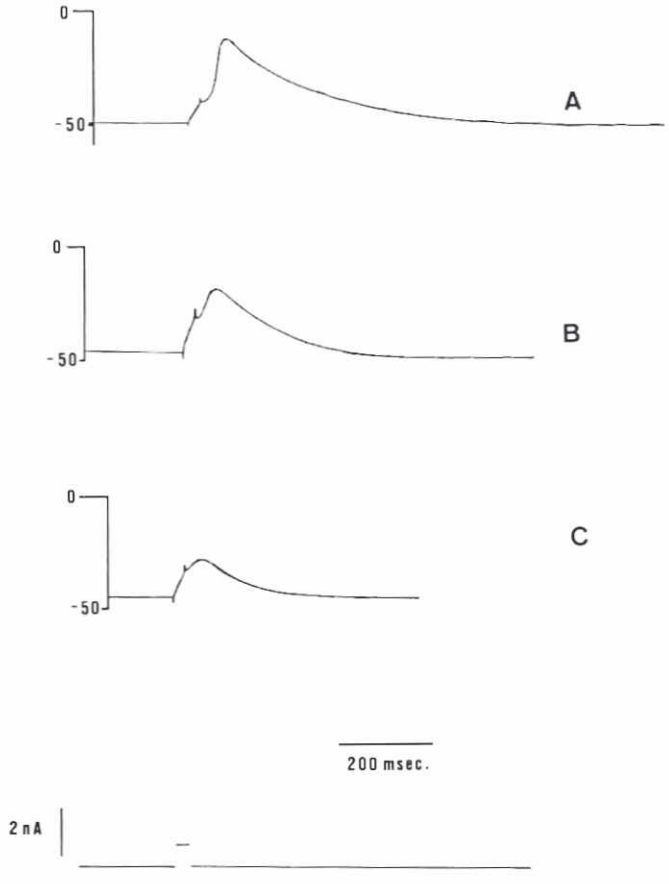

Fig. 5. Effects of decreasing extracellular Na^+ or Ca^{2+} on time course of action potential of immature *Patella* oocyte. (A) *control in ASW,* **(B)** *in 0-Na-ASW,* **(C)** *in 0-Ca-ASW.*

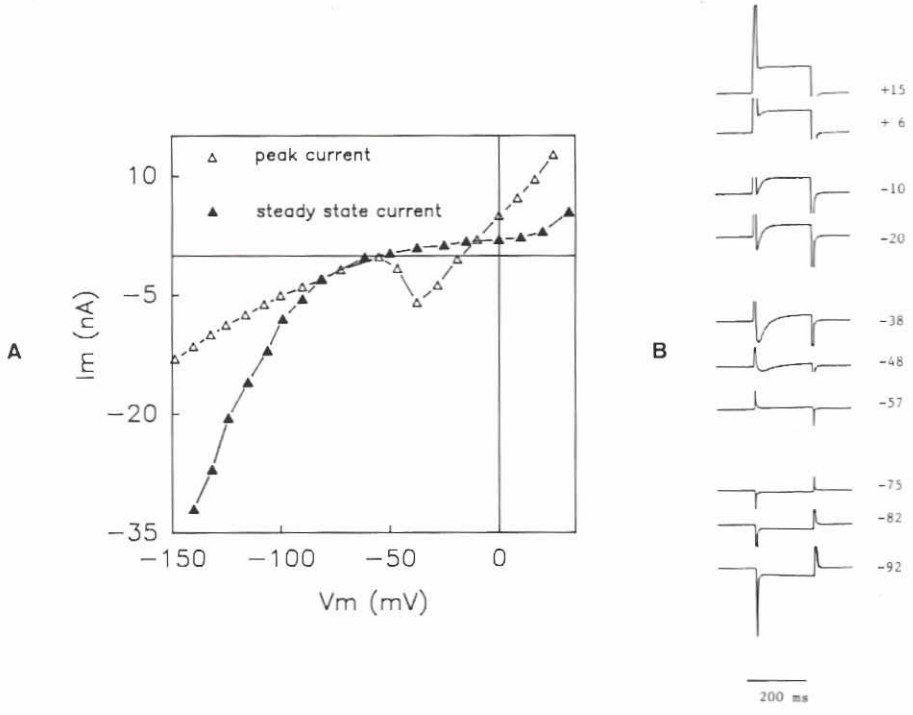

Fig. 7. Current voltage plot recorded in voltage clamp on an immature *Patella* oocyte, for peak and steady state current. (A) *Peak and steady-state currents.* **(B)** *Original recording used to plot the I/V curve. The values reported in (B) correspond to the clamped voltage (holding potential -70 mV).*

curve are similar to those observed in many invertebrate oocytes such as starfish, ascidians, sea urchins (see Introduction).

Voltage clamp analysis of the current-voltage relation

Membrane currents recorded under voltage clamp in the immature oocyte are shown in Fig. 6. Three principal voltage dependent currents were found:

1) Inwardly rectifying K^+ currents were recorded during hyperpolarizing voltage steps between -50 and -150 mV (see traces in Fig. 6B). These currents showed no inactivation during a 800 ms voltage step and were blocked by 10 μM barium.
2) Between -10 and +15 mV, an outward current can be recorded corresponding to the delayed outward rectification.
3) A transient inward current recorded at potential between -50 and -15 mV (see current traces of the inset, Fig. 6B). This current was identified as a Ca^{2+} current because its amplitude increased with increasing calcium concentration, and could be blocked by addition of 25 mM cobalt or 10 mM gadolinium to the external solution (data not shown).

The current voltage relation for the immature oocyte in 0-Na-ASW is shown in Fig. 7. For potential more negative than -60 mV, where inwardly rectifying K^+ conductance was prominent, steady state currents were plotted. For potential more positive than -60 mV, peak currents were taken as well.

The inward current was divided by about 3 in amplitude when Na^+ was removed from the external solution as for Ca^{2+} currents in starfish oocytes (Moody and Lansman, 1983) or in *Dentalium* eggs (Baud *et al.*, 1987).

The Na^+ dependence of this inward current could arise either from a binding effect of external Na^+ on the Ca^{2+} channel, or from the activation of a non-specific cation channel by Ca^{2+} entering the cell by the Ca^{2+} channel (Colquhoun *et al.*, 1981).

Meiotic maturation

For convenience, the maturation process was divided into an early phase and a late phase (Fig. 8C).

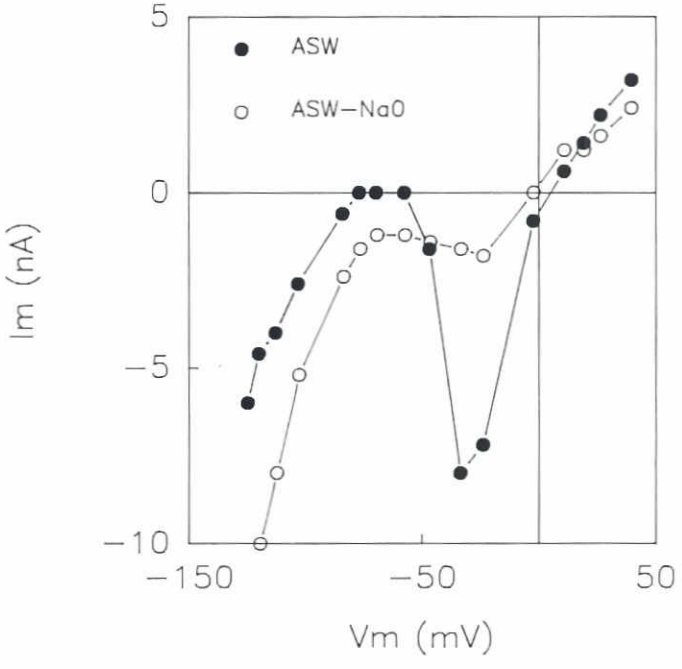

Fig. 7. Effect of Na deprivation on I/V plot recorded fom a voltage-clamped immature *Patella* oocyte. *The values plotted represent the amplitude of the peak current.*

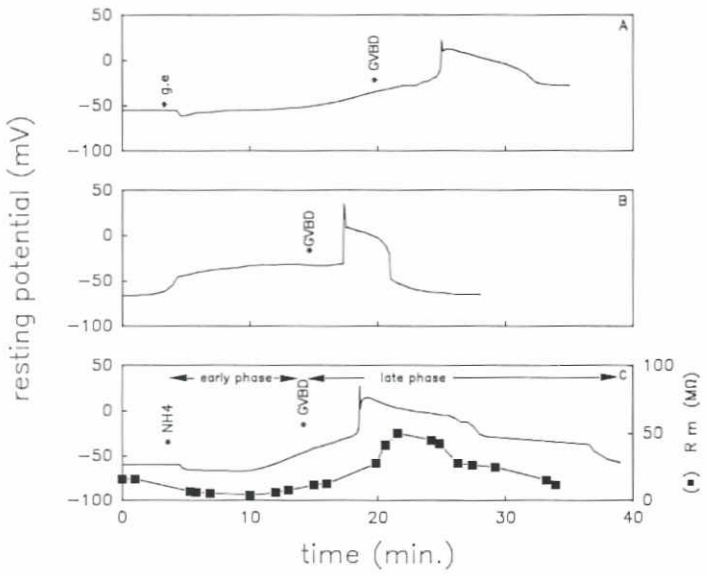

Fig. 8. Evolution of the membrane potential of *Patella* oocyte *following meiosis induction by* **(A)** *ganglia extracts,* **(B)** *spontaneous maturation,* **(C)** *NH_4Cl, 10 mM. (C) also shows membrane resistance as recorded by injecting a hyperpolarizing current.*

The early phase consists of the first 10 min after the application of the agonist up to germinal vesicle breakdown (GVBD).

The late phase began at GVBD and ended 45 min after agonist application.

Resting potential and I/V curve during maturation

Fig. 8A,B,C illustrates the changes in membrane potential observed when meiosis reinitiation (A) was induced by ganglion extracts, (B) occured spontaneouly, and (C) was triggered by NH_4Cl. As can be seen, the shapes of these recording are similar for ganglia extracts and NH_4Cl, although the time course of the early phase appears to be shorter with ammonia.

Fig. 8C superimposes membrane resistance and membrane potential. Two to three minutes after NH_4^+ addition, the response to hyperpolarizing current pulses of constant intensity gradually decreased and the membrane potential hyperpolarized from its original value of -60 mV to -72 mV. After 8 to 10 min, the membrane repolarized spontaneously until GVBD and abruptly depolarized up to +28 mV ± 6 (n=12) (action potential). During this period, membrane resistance increased by 5-fold and this value was maintained during the 10 min of the plateau phase. Then, the membrane repolarized and resistance decreased to its original value.

During the early phase, with our monoelectrode technique, it was difficult to obtain action potential (too low resistance), but when obtained, repolarization was longer.

During the late phase (plateau), only a gradual depolarization up to -20 mV occured when the oocyte was stimulated. No action potential could be obtained.

After an ammonia-induced GVBD was observed in ASW, the P_{Na}/P_K ratio was 0.045, instead of 0.039 as observed in the immature oocyte. The estimated internal K^+ concentration decreased to 174 mM in the mature oocyte instead of 287 mM as found in the immature oocyte.

In order to better analyze the changes in ionic conductance that occur during meiotic maturation, we used substituted external media as well as ionic channel permeability inhibitors to block ionic conductance (Fig. 9A,B).

The maturation process, as monitored by the occurence of GVBD, was the same in ASW, in 0-Na-ASW and in 0-K-ASW and the timing of GVBD was not affected.

As illustrated in Fig. 9A, 0-K-ASW hyperpolarized the membrane by 4-5 mV. When ammonia-induced maturation was performed in that medium, the membrane potential followed a time course similar to that observed in normal medium, but the amplitude of the hyperpolarization increased significantly (10 mV instead of 6 mV, n= 25) ; the «overshoot» after GVBD is normal.

In order to examine the actual contribution of K^+ conductance in maintaining the resting potential, we used TEA as an inhibitor of potassium permeability. Unexpectedly, TEA chloride (150 mM) was found to induce GVBD in nearly 50% of the treated oocytes. In contrast, TEA bromide (150 mM) did not trigger GVBD. All experiments were therefore performed with TEA bromide.

As shown in Fig. 9B, 3 min after TEA addition, the membrane began to depolarize, and after 5 min the membrane potential attained its threshold value of -35 mV. At this time, an overshoot is triggered (+ 20 mV), similar to that observed with NH_4Cl (see Fig. 9A). After a sustained plateau at + 3.5 mV, a slow spontaneous repolarization takes place. The changes in potential obtained with TEA bromide are thus similar to those obtained with NH_4Cl (except

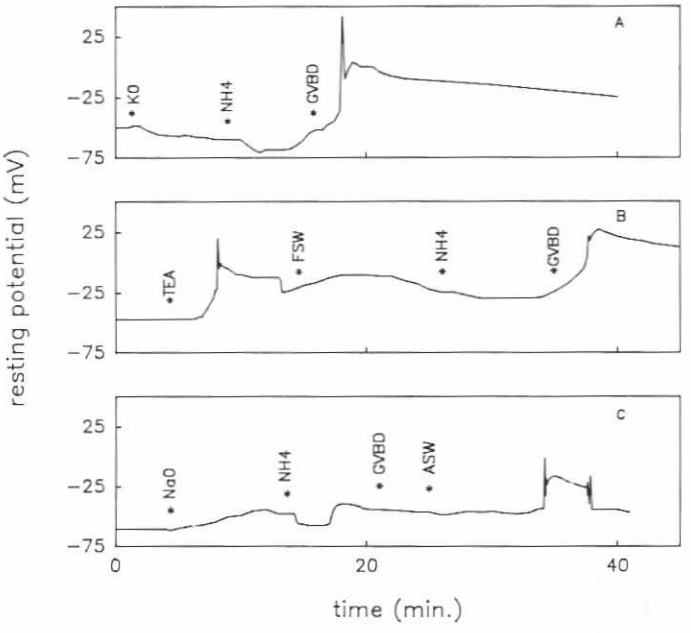

Fig. 9. Evolution of the membrane potential of *Patella* oocytes during meiosis reinitiation *triggered in 0-K-ASW* **(A)**, *ASW supplemented with 150 mM TEA bromide* **(B)** *or 0-Na-ASW* **(C)**. *In (B), the addition of 10 mM NH_4Cl after TEA has been washed resulted in GVBD.*

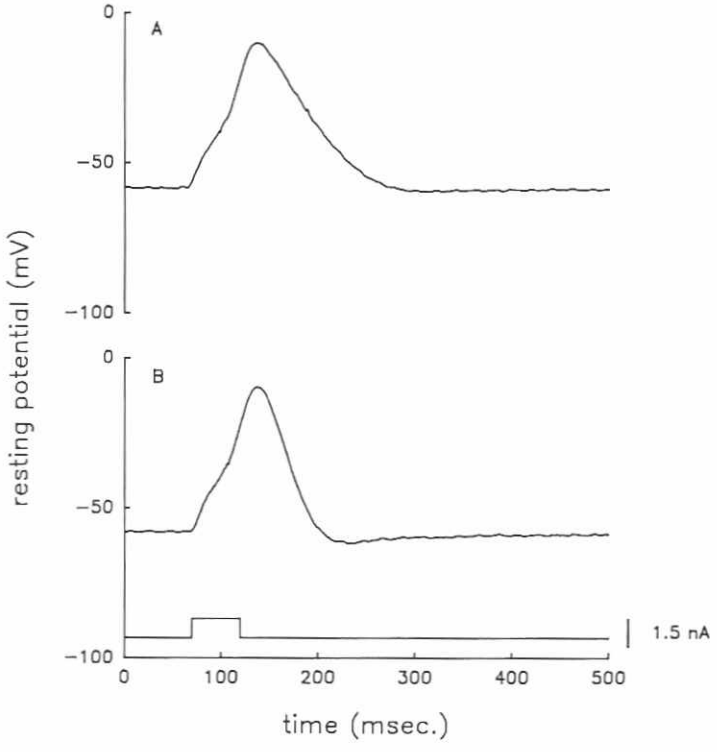

Fig. 10. Changes in action potentials observed for the same depolarizing current *applied to an immature oocyte* **(A)** *or to a mature oocyte taken after repolarization of the membrane potential has occurred (late phase) 35 min after NH_4Cl 10 mM treatment* **(B)**.

for the original hyperpolarization phase), although no GVBD occurs under these conditions. These phenomena are reversible and a second application of 150 mM TEA, after washing the oocytes produces the same evolution (data not shown). After such a wash with filtered SW or ASW, a further addition of NH_4Cl produces a normal hyperpolarization followed by GVBD and the usual overshoot (Fig. 9B).

In 0-Na-ASW, (Fig. 9C) the membrane depolarizes by 5mV. After the addition of 10 mM NH_4Cl, a hyperpolarization of about 5 mV precedes a depolarization of about 10 mV, but the typical «overshoot» is not observed, even after the application of strong depolarizing stimulations (10 to 20 nA). The membrane potential stabilizes at -40 to -35 mV. Although no spontaneous action potential was recorded, GVBD occured, 9 minutes after the addition of NH_4Cl in the 0-Na-ASW medium.

Replacing 0-Na-ASW by normal ASW allows the oocyte to depolarize and to produce a typical «overshoot», although the peak amplitude is now slightly attenuated (+1 mV).

Changes in cell excitability

At the end of the late phase of maturation, the excitability of the oocyte is modified (Fig. 10) and the amplitude of the action potential increases up to 50.5 mV ± 4 (n=7). The threshold is slightly negative -42.2 ± 6 mV (n=7) and the speed of depolarization increases from 0.55 V/s to 0.67 V/s (n=7). However total duration of the action potential (post-potential included) is not modified 371.6 ± 19 ms (n=7). There is always a post-potential corresponding to a hyperpolarization of 4 mV (Fig. 10). This hyperpolarization never exceeds 1 mV in immature oocytes when it exists.

During the plateau phase of the action potential (late phase) it was impossible to trigger any action potential but, when the oocyte was artificially repolarized to -60 mV by negative current injection (Fig. 11), a depolarizing stimulus of 1.5 nA did trigger a normal action potential. The inset of Fig. 11 demonstrates that the action potential is similar to the one obtained at the end of the late phase.

Evolution of the current-voltage relation (current clamp) during maturation

Fig. 12 gives the I/V curves obtained at different times during ammonia-induced maturation.

During the early phase of maturation the slopes of the inward and outward going rectifications decrease about 2-fold, indicating an increase in membrane resistance. During the late phase of maturation, the slope near resting potential also decreases (Fig. 11).

When immature oocytes are treated with TEA bromide (50 or 150 mM), the inward and outward-delayed rectifications disappear (Fig. 13). The effect of TEA is reversible. These I/V curves are plotted during the depolarizing phase before the action potential.

Evolution of ionic currents during maturation (voltage clamp)

Measures performed in voltage clamp conditions presented in Fig. 14 confirm the evolution observed in current clamp (see Fig. 12). Particularly, we noticed an increase in the ionic currents corresponding to the inward and outward going rectifications following ammonia stimulation (Fig. 14).

We also observed that the transient inward current did not disappear (Fig. 14), but the total current became positive. It seems that an outward inactivating current is added. After GVBD the outward current disappears, and the transient inward current alone remains: the oocyte can produce an action potential (Fig. 13).

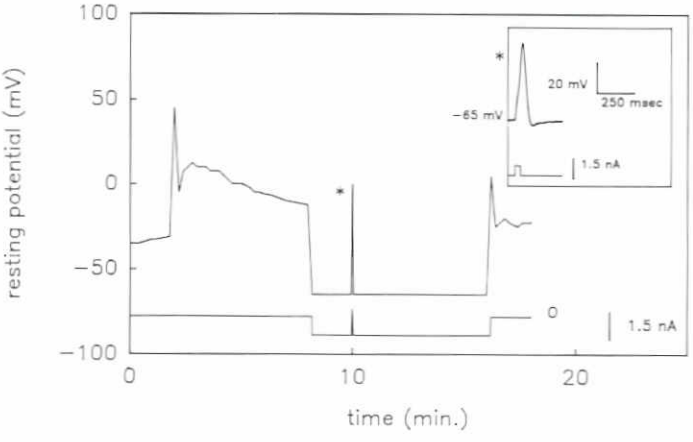

Fig. 11. Evidence for the persistence of excitability *Patella* oocyte. *During the overshoot, it is impossible to trigger an action potential, but repolarizing the membrane potential by injection of a constant current restores the ability to trigger an action potential using a depolarizing pulse (*). Inset represents the action potential (*) with an enlarged time-scale.*

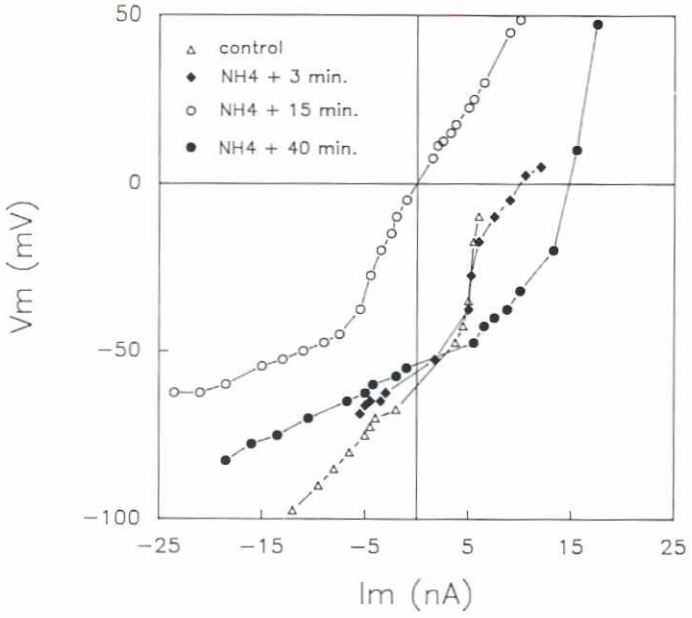

Fig. 12. I/V plot recorded in current clamp during the maturation of *a Patella* oocyte *taken 3, 15 and 40 min following the addition of 10 mM NH_4Cl.*

Discussion

The oocyte of *Patella vulgata* is an excitable cell with a resting potential of about -61 mV, mainly dependant on potassium. Sodium and calcium ions have little effect in maintaining the resting potential. The major role of K^+ in maintaining the resting potential of the oocytes of tunicates, echinoderms, amphibians and mammalians is well established (for review, see Hagiwara and Jaffe, 1979). However the oocyte of the mollusk *Illyanasa* seems to behave differently, exhibiting a low resting potential independent of K^+ ions (Conrad *et al.*, 1977; Moreau and Guerrier, 1981).

In the oocyte of *Patella* an all-or-none action potential could be evoked from a resting potential of -70 mV. The action potential is relatively simple and the depolarizing phase is Na^+ and Ca^{2+} dependant. Similar data were obtained for starfish oocytes (Miyazaki *et al.*, 1975a; Shen and Steinhardt, 1976; Moody and Lansman, 1983), tunicates (Miyazaki *et al.*, 1974a, b; Okamoto *et al.*, 1976; Thompson and Knier, 1983), *Ctenophora* (Barish, 1984) and for the mollusk *Dentalium* (Baud *et al.*, 1987; Moreau *et al.*, 1989). The exponential repolarizing phase seems to be the result of the inactivation of only one class of channel, *i.e.* the Na-Ca channel.

The electrophysiological properties of the oocyte are modified during meiosis reinitiation. In the ovary *Patella* oocytes are arrested during prophase of the first maturation (germinal vesicle stage). Release from the prophase block involves a change in intracellular pH (Guerrier *et al.* 1986a, b); the second block (metaphase 1) depends on a rise in intracellular free calcium. These factors can modulate ionic permeability.

Two main events characterize the evolution of the resting potential: a hyperpolarization (early phase) and a depolarization followed by an overshoot (late phase). The electrical properties evolve in a similar way whether maturation is induced by a ganglia extract or by NH_4Cl, 10 mM. These data suggest that the variation of pH induced by NH_4Cl acts on a physiological chain of events. It has been shown that such agonists induce an alkalinization of the internal medium necessary for triggering the maturation process (Azzi, unpublished results).

During the early phase of maturation, the observed hyperpolarization is linked to an increase of the inward going rectification. The analysis of the I/V plot obtained in voltage-clamp conditions points to an increase in membrane conductance for hyperpolarizing potentials. This hyperpolarization could be blocked by the addition of TEA. This strongly suggests that potassium channels do open at that time and are responsible both for the hyperpolarization of the membrane potential and for the simultaneous observed decrease in the membrane resistance.

Different mechanisms may work to control the opening of the K^+ channels during the early maturation phase:

- Ca^{2+} may play a role in the activation of potassium channels (Meech, 1974), but in *Patella*, an increase in intracellular calcium seems to be excluded at the germinal vesicle stage since it has been shown that raising intracellular calcium ions is only effective for releasing the oocyte from the second block which occurs in metaphase 1 (Guerrier *et al.*, 1986a, b).

- A more probable mechanism would involve the activation of pH dependant potassium channels. These channels have been studied by Strickholm (1961) who showed that raising the intracellular pH increased potassium conductance. During maturation of the *Patella* intracellular pH increases (Guerrier *et al.*, 1986). This phenomenon may well explain the hyperpolarization observed during the early phase of the maturation process. Such a transient hyperpolarization suggests that NH_4Cl and the ganglia extracts do not exert a permanent effect, but that the regulatory mechanism acts through a compensation for the initial pH effect. In *Xenopus* oocyte maturation,

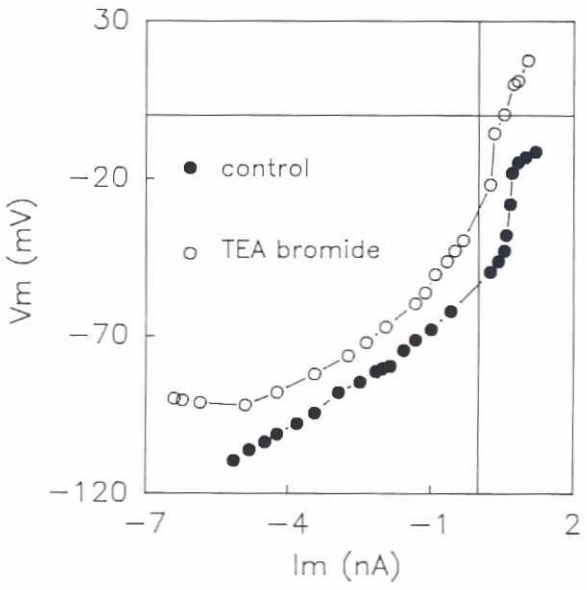

Fig. 13. Effect of 50 mM TEA bromide on the I/V curve recorded in current clamp.

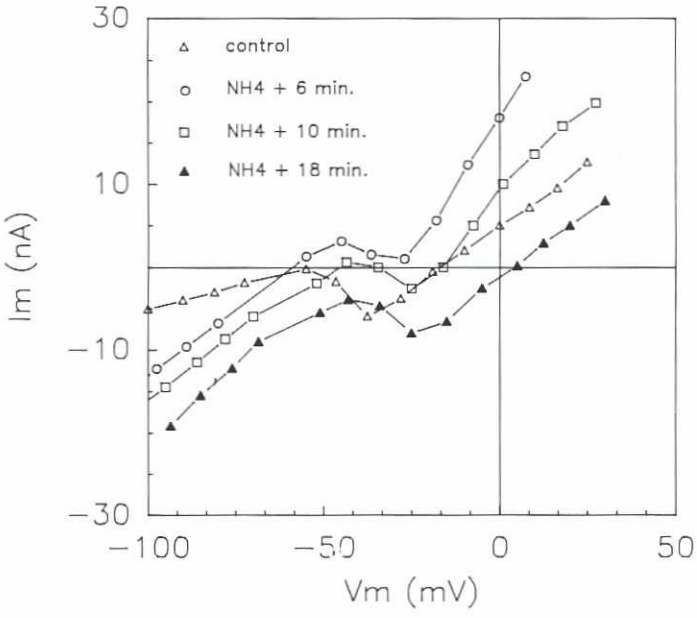

Fig. 14. Time course evolution of the membrane currents measured in voltage clamped *Patella* oocyte during ammonia-induced maturation.

hyperpolarization also follows progesterone treatment (Kado *et al.*, 1981; Vilain *et al.*, 1989). The process of membrane depolarization that follows seems also to involve a change in K^+ ion permeability. In the presence of TEA the variation in resting potential is similar to that observed during the late phase of meiosis. Such an overshoot has also been reported to occur after GVBD in oocytes of the starfish *Astropecten irregularis* or *Astropecten aurantiacus* (Dale *et al.*, 1979; Guerrier *et al.*, 1984) as well as in oocytes of the amphibian *Ambystoma* (Baud and Barish, 1984). These rapid depolarizations were attributed to a loss in the selectivity of the membrane for K^+ ions. It is this loss of selectivity that induces the progressive and sustained depolarization.

The evolution of the resting potential as observed during the late phase of maturation does not appear to be in direct relation to maturation, although it should be a consequence of this event. The observed phenomenon occurs a long time after GVBD and can also be obtained in the absence of GVBD as observed in the presence of TEA. This phenomenon is reversible and can be elicited several times.

It do not exclude the possibility that there are physical events involved that may partly or totally explain this last step in the evolution of membrane potential. We have seen that during *Patella* oocyte maturation, the diameter of the oocyte increases from 210 up to 240 µm, possibly due to the absorption of water. This phenomenon correlates with an important decrease in the intracellular K^+ concentration, which falls from 247 to 187 mM (see results). This dilution fits with the 1.5-fold volume variation. The positive potential shift corresponding to this dilution should be of about 15 mV and the effect of such a depolarization would be to limit the leakage of potassium by reducing the driving force. Blocking the membrane potential at positive values triggers a closure of the potassium channels. The effect of this depolarization may be reinforced by the activation of hypothetical stretch-channels (Taglietti and Toselli, 1988), which may accelerate the overshoot. Stretch-channels are sensitive to changes in volume of the cell and are involved in osmoregulation. Their permeability is mainly K^+dependant. Maintaining internal ionic concentrations may depend on the evolution of the potential and on activity of stretch-channels.

In conclusion the variations in permeability recorded during maturation seem mainly to depend on modifications resulting from transduction of the meiotic signal, which involves a change in the intracellular pH. It is unlikely, that by acting on membrane permeability one could induce or inhibit maturation in *Patella* oocytes.

Materials and Methods

Solutions

Artificial sea water (ASW) was made up as follows: NaCl, 452 mM; KCl, 10 mM; $MgCl_2$, 25 mM; $MgSO_4$, 17.2 mM; $CaCl_2$, 10 mM, adjusted to the different pH values following the addition of Tris (hydroxymethyl)aminoethane (TRIS), 6 to 25 mM. Sodium-free sea water (0-Na-ASW) was prepared by replacing the 452 mM of NaCl by equimolar concentrations of N-methyl glucamine neutralized by HCl. Chloride-free sea water (0-Cl-ASW) was prepared by Na-isethionate replacement for NaCl, K_2SO_4, for KCl, $CaSO_4$ for $CaCl_2$, $MgSO_4$ for $MgCl_2$.

NH_4Cl was made as a 2M stock solution in distilled water containing 10 mM TRIS, pH 8.5, and diluted to the required final concentration (10-20 mM), pH being adjusted with TRIS, before treatment.

Handling of gametes

Adult limpets were collected regularly from late September to early April along the coast near Roscoff. The external appearance of *Patella vulgata* is presented in Fig. 1A.

Prophase-blocked oocytes (Fig. 1B) were obtained by dissecting the gonad in filtered sea water (FSW) or ASW. Oocytes were used either with remaining follicular cells or after removing them. To remove follicular cells, the oocytes were washed at first in a solution containing 0.32 M sucrose, 2 mM $CaCl_2$, 50 mM TRIS pH 8, and afterwards, in calcium and sodium-free sea water (0-Na-0-Ca-ASW)

Germinal vesicle breakdown (GVBD) was either induced in the natural way, using paleopedial ganglia, prepared according to the procedure described by Shirai *et al.* (1987) or by adding a weak base to the medium, essentially NH_4Cl, 10 to 20 mM pH 8.5. GVBD was scored as the disappearance of germinal vesicle by gently squeezing the oocyte under a coverslip.

Electrical measurements

All current clamp measurements were done with an intracellular microelectrode. The electrodes were filled with 3 M KCl and had a resistance of 7 to 20 megohms. Measurements were made with an M 707 WPI amplifier (Hamden, Connecticut). The bath was directly connected to ground via an agar bridge and an Ag-AgCl electrode. The single electrode was used both for passing current and for recording potential, with a compensation to adjust the potential drop within the electrode.

Voltage clamp experiments were done with a conventional two-electrode voltage clamp amplifier (RK-300, Biologic, Grenoble, France). The resistance in series with the voltage electrode, calculated from the time constant of the capacity transient, was less than 1.5 Kohms.

Membrane parameters (R_m and C_m) were determined by computer reading or direct measurement using a Gould-Brush chart recorder.

I/V curves are defined as the result of the plot of membrane current *versus* membrane voltage in conditions of voltage clamp recording and membrane voltage, *versus* membrane current in current-clamp.

The bath was continuously perfused by a peristaltic pump and maintained at 17-19 °C.

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