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A Voltage-dependent and pH-sensitive Proton Current in *Rana esculenta* Oocytes

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Abstract. Voltage clamp technique was used to study macroscopic ionic currents in *Rana esculenta* oocytes. Depolarization steps led to the activation of a single type of outward current (I_{out}) when contaminant potassium and calcium-dependent chloride currents were pharmacologically inhibited. The voltage threshold of I_{out} activation was 10 mV and this current, which did not inactivate, presented a deactivation the time constant of 73 ± 21 msec (n = 26) corresponding to a membrane voltage of −60 mV. Its reversal potential (E_{rev}) was dependent on the magnitude of the depolarization and also on pulse duration. These changes in E_{rev} were thought to reflect intracellular ion depletion occurring during activation of the remaining outward current. Furthermore, the activation threshold of I_{out} was clearly affected by modifications in extracellular and intracellular H^+ concentrations. Indeed, intracellular alkalinization (evoked by external application of ammonium chloride) or extracellular acidification induced a rightward shift in the activation threshold while intracellular acidification (evoked by external application of sodium acetate) or extracellular alkalinization shifted this threshold toward a more negative value. Lastly, I_{out} was dramatically reduced by divalent cations such as Cd^{2+}, Ni^{2+} or Zn^{2+} and was strongly decreased by 4 Aminopyridine (4-AP), well-known H^+ current antagonists already described in many cell types. Therefore, it was suggested that the outward current was prominently carried by H^+ ions, which may play a key role in the regulation of intracellular pH and subsequent pH dependent processes in *Rana* oocyte.

Key words: *Rana esculenta* oocytes — H^+ current — pH — Voltage clamp

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Introduction

Amphibian oocytes display a wide variety of ion channels, namely: calcium (Ca^{2+}), potassium (K^+), chloride (Cl^-), sodium (Na^+) and proton (H^+) channels. The distribution of these channels depends on the type of oocyte and each type displays individual characteristics. For example, the Na^+ channel described in *Xenopus* oocytes (Baud, Kado & Marcher, 1982) is not found in any other amphibian oocytes. Ca^{2+} channels that were first analysed in *Xenopus* oocyte have also been reported in *Pleurodeles* oocytes but their electrical and pharmacological characteristics are quite different. *Pleurodeles* oocyte possesses a dihydropyridine-sensitive Ca^{2+} channel (Ouadid et al., 1994) whereas *Xenopus* oocyte exhibits a Ca^{2+} channel insensitive to L or N Ca^{2+} channel blockers (Bourinet et al., 1992). Although the Ca^{2+} signaling features have been extensively studied in the past years, it is surprising to find that only few studies have been carried out on the means by which these oocytes regulate their internal pH. It has recently been reported that immature *Xenopus* oocyte can regulate its internal pH by a working Na/H exchange (Burckhardt, Kroll & Fromter, 1992). However this pathway is not ubiquitous and other mechanisms such as the activation of H^+ channels have been reported as a means of regulating the internal pH in *Ambystoma* oocytes (Barish & Baud, 1984) and even in other cell types (*see* for review DeCoursey & Cherny, 1994).

Here, we report the probable existence of a voltage dependent H^+ pathway in immature fully grown ovarian oocytes of *Rana esculenta.* The corresponding H^+ current is subjoined to the other currents already described in *Rana esculenta:* potassium and calcium-dependent chloride currents (Peres et al., 1985; Taglietti et al., 1984; Toselli et al., 1989). This H^+ current is similar to those described in *Ambystoma* oocyte and in other cells, for example, *Lymnaea stagnalis* and *helix aspersa* neu-

rones (Thomas & Meech, 1982; Byerly, Meech & Moody, 1984; Meech & Thomas, 1987), macrophages (Kapus et al., 1993), granulocytes (Demaurex et al., 1993) and skeletal muscle (Bernheim et al., 1993).

Materials and Methods

Oocyte Preparation

Rana esculenta were purchased from Arthus Frères (85690 Notre Dame de Mont, France). Pieces of the ovary were surgically removed and individual oocytes were dissected in a ND96 physiological medium (in mM): NaCl, 96; KCl, 2; $MgCl_2$, 2; $CaCl_2$, 1.8 and N-2-hydroxyethyl piperazine-N′-2-ethanesulfonic acid (HEPES), 5; pH 7,45 NaOH. Oocytes with the largest diameter (1 mm) corresponding to stage VI (Dumont 1972) were selected. Without further treatment, single oocytes could be maintained for 2–6 days at 19° C in ND96 supplemented with 50 μg/ml gentamicin. This medium was renewed daily.

Electrophysiological Measurements

In a 0.3 ml perfusion chamber, a single fully grown ovarian oocyte was impaled with two standard glass microelectrodes (0.5–2.0 MΩ resistance) filled with 3M KCl and maintained under voltage clamp conditions by using a Dagan 8500 amplifier (Minneapolis, MN). Stimulation of the preparation, data acquisition and analysis were performed using the pClamp5.5 software (Axon Instrument, Burlingame, CA). In addition, membrane currents were continuously displayed by means of the BIO 1000 software (Nortek, Villeneuve d'Ascq, France). Drugs used were applied externally by direct addition to the superfusate (flow rate: 3 ml · min^{-1}).

To determine reversal potential (E_{rev}), the leak subtracted current amplitude at the end of the depolarizing pulse was measured for each membrane potential (1st value). The corresponding instantaneous deactivation current (at the –60 mV holding potential) was determined by a digital fitting and extrapolation to time zero of the relaxation current (2nd value). These two values were plotted with their corresponding voltages. The potential for which the instantaneous current is zero corresponds to E_{rev}. This quick way of measuring E_{rev} was valid since the instantaneous current-voltage relationship was linear between –60 and +60 mV. This relation, presented in Fig. 1, was established in the customary way, which consists in stepping back to membrane potentials above and below the E_{rev}.

All the experiments were performed using media made by adding 10 mM tetraethylammonium chloride (TEACl) and 10 mM caffeine to the perfusate. TEA was used to prevent activation of the potassium current (IK^+) described in *Rana esculenta* oocyte (Peres et al., 1985; Taglietti et al., 1984). Caffeine was used to prevent an oscillatory calcium-dependent chloride current (previously described by Toselli et al., 1989), activated by calcium release from the inositol 1,4,5 trisphosphate sensitive store (*unpublished data*).

Media were made as follows: (i) chloride-free medium, in mM: NaOH, 96; KOH, 2; $MgOH_2$, 2; $CaOH_2$, 1.8; TEAOH, 10; caffeine, 10 (pH 7.45 titrated with methane sulfonic acid) and (ii) sodium free medium, in mM: Choline Cl, 96; KCl, 2; $MgCl_2$, 2; $CaCl_2$, 1,8; TEACl, 10; caffeine, 10 (pH 7,45 with TEAOH).

Buffers used to obtain different pH values were used at 5mM concentration as follows: pH 8.95 and pH 8.05, EPPS (n(2-Hydroxyethyl)-piperazine-N′-(3-propanesulfonic acid) pKa = 8.0); pH 7.45, HEPES (1,8; N-2-hydroxyethyl piperazine-N′-2-ethanesulfonic acid; pKa = 7.5); pH 6.95, PIPES (Piperazine-n,n′-bis-(2-ethanesulfonic acid) pKa = 6.8).

The variability of the results was expressed as the standard error of the mean, with n indicating the number of oocytes contributing to the mean. Student's t-test was used for statistical analysis. The figures presented show current traces and curves which are representative of the mean. Electrical measurements were conducted on fully grown immature oocytes routinely voltage clamped at a holding potential (HP) of –60 mV.

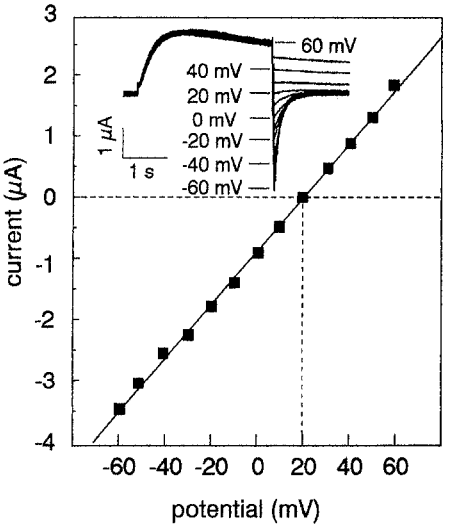

Fig. 1. Instantaneous remaining current as a function of the membrane potential in ND96 supplemented with TEACl (10 mM) and caffeine (10 mM). The outward current was activated by 120 mV depolarizing pulse from a holding potential of –60 mV. At the end of the pulse, the membrane potential was repolarized to various levels and the tails of current were recorded (inset). The instantaneous currents were extrapolated to time zero by measuring the deactivation time constant and the current 50 msec after the repolarizing pulse by using pClamp5.5 software. To facilitate extrapolations of the current tails to time zero (end of the depolarizing pulse), both the leakage and capacity current were automatically subtracted (pClamp5.5 software, P/N protocol).

Results

As illustrated in Fig. 2*A*, a depolarization step to 40 mV from –60 mV during 20 sec evoked an outward oscillatory current in ND96 medium supplemented with TEACl. TEA (10 mM) was used to block the outward K^+ current already described in *Rana* oocytes (Peres et al., 1985). The oscillatory current has been described as a calcium-dependent chloride current (Toselli et al., 1989). Activation of this current is produced by calcium release from the $InsP_3$ sensitive stores (*unpublished data*). Caffeine (10 mM concentration), a well-known inhibitor of $InsP_3$-induced Ca release, blocked the chloride current. Under these conditions (ND96- TEA- caffeine) a large remaining outward current (I_{out}) was observed during

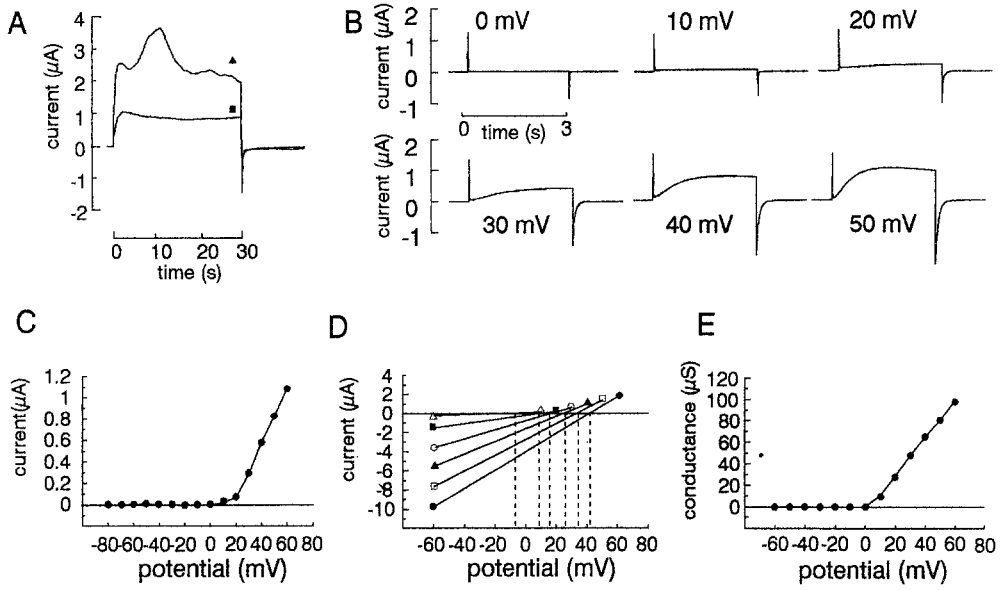

Fig. 2. Membrane currents elicited by depolarizations in immature *Rana esculenta* oocyte. (*A*) Currents recorded in ND96 medium supplemented by TEACl (10 mM) (▲). Depolarization to +40 mV from the −60 mV holding potential during 30 sec elicited an oscillatory calcium-dependent chloride current; it is blocked by application of caffeine (10 mM) (■). (*B*) Currents recorded after shifting membrane voltage from −60 mV to the potentials indicated during 3 sec in ND96 supplemented with TEACl (10 mM) and caffeine (10 mM). (*C*) Current-voltage relationship for the time-dependent currents shown in *B*, currents were measured at the end of the depolarizing pulses. (*D*) Determination of E_{rev} for each depolarizing pulse: amplitude of each dynamic current, measured at the end of the pulse and the corresponding amplitude of the instantaneous deactivation current were plotted with the potential. The potential at which the current is zero is E_{rev} value. (*E*) Conductance-voltage relationship obtained from the current amplitudes shown in *C* and corresponding reversal potentials determined in *D*.

adequate stimulation (Fig. 2*A*). The purpose of this study was to examine the characteristics of this current.

Characterization of the Remaining I_{out}

Incremented 30-sec depolarizations of an oocyte bathed in modified ND96 medium from 0 to 60 mV elicited a time-dependent outward current. This current progressively increased for positive values >0 mV (Fig. 2*B*). Currents recorded for a membrane potential between 10 to 40 mV did not exhibit any apparent inactivation. However in this oocyte, at a 50 mV membrane potential, I_{out} reached a peak and then slowly declined. Such a decrease in current amplitude is clearly visible in other cases even for applied potentials more negative than 50 mV (Fig. 3*A* for example). This slight decrease was always partial and relatively slow as a function of time. After depolarizations strong enough to activate I_{out}, an inward deactivation current was recorded when the membrane potential returned to HP (−60 mV). Each deactivation current decayed with the same time constant (73 ± 21 msec; n = 26) and the instantaneous current-voltage relationship was linear (Fig. 1). These results taken together suggest that a single current could be activated during depolarization. Figure 2*C* shows the current-voltage relationship obtained from currents measured at the end of the 3-sec pulse from −80 to +60 mV. To determine E_{rev} of the activated I_{out}, current amplitude measured at the end of each depolarizing pulse and the corresponding instantaneous deactivation current were plotted *vs.* the membrane voltage (for more details, *see* Materials and Methods, Fig. 1). The potential value corresponding to zero current represents E_{rev}. The value of E_{rev} was determined for every voltage step-elicited current (Fig. 2*D*) and as illustrated, the slope of each linear relation increased with depolarization. This slope, which corresponds to the conductance, never reached a steady value for any of the potentials tested (from 10 to 60 mV). Furthermore, the instantaneous deactivation current linearly increased with potential. Figure 2*D* also shows that E_{rev} increased with depolarization magnitude. For a 3-sec depolarization at 40 mV, E_{rev} was 8.56 ± 6 mV (n = 15) and only −4.2 ± 5.7 mV (n = 12) for 1 sec; this difference was highly significant ($P < 0.001$) whatever the level or duration of the depolarization. Moreover, the deactivation time constant remained unchanged with the level of depolarization and with pulse duration. The change in E_{rev}, associated with the fact that a single current seems to be activated, led us to propose the existence of a phenomenon of accumulation and/or depletion subsequent to a voltage dependent activation of the dynamic conductance. This conductance was calculated for each depolarizing pulse by using the corresponding

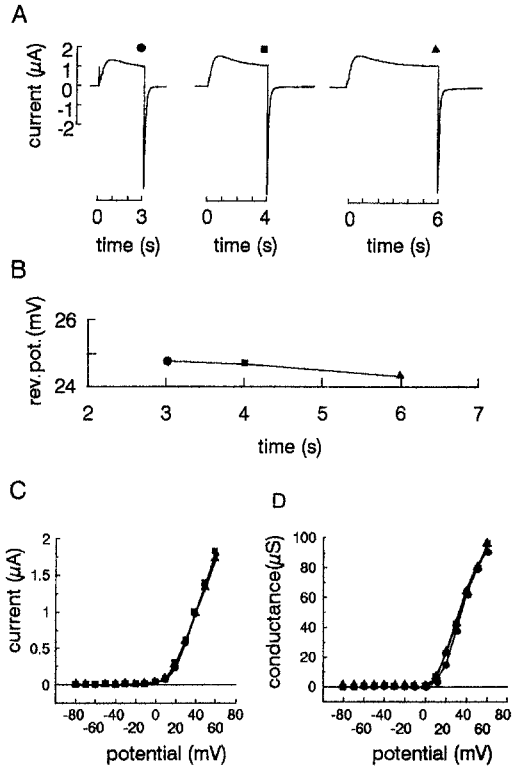

Fig. 3. Effect of pulse duration on current decay in ND96 supplemented with TEACl (10 mM) and caffeine (10 mM). (*A*) Successive depolarizations of 3, 4 and 6 sec to a membrane potential of +40 mV from a −60 mV holding potential. (*B*) Reversal potential values according to pulse duration (determined from the current traces shown in part *A*). Reversal potentials were determined as above (Fig. 2*D*). (*C*) Current-voltage relationship for 3- (●), 4- (■) and 6-sec (▲) pulse durations. (*D*) Conductance-voltage relationships obtained from the current amplitude presented in *C* (the corresponding reversal potential value, to calculate the conductance, was determined for each current recorded).

reversal potential. The likely sigmoidal conductance-voltage relationship, presented in Fig. 2*E*, corroborates the fact that the conductance was not maximal even for a 60 mV membrane potential. After fitting the conductance-voltage relationship by using the Boltzman equation, the deduced maximal conductance was estimated at 135 μS and the potential of half activation at 39 mV (Fig. 2*E*).

The decrease in current amplitude with pulse duration and the change in E_{rev} with the rate of activation have been interpreted as a consequence of an intracellular phenomenon of ionic depletion or accumulation. The decrease in current amplitude was not due to an inactivation process since the current amplitude, E_{rev} and the amplitude of the tail current were unchanged when the step duration was increased from 3 sec to 6 sec (Fig. 3*A* and *B*). Moreover, for all activation potentials, the current-voltage relationship (Fig. 3*C*) and the conductance-voltage relationship (Fig. 3*D*) were not modified. Thus,

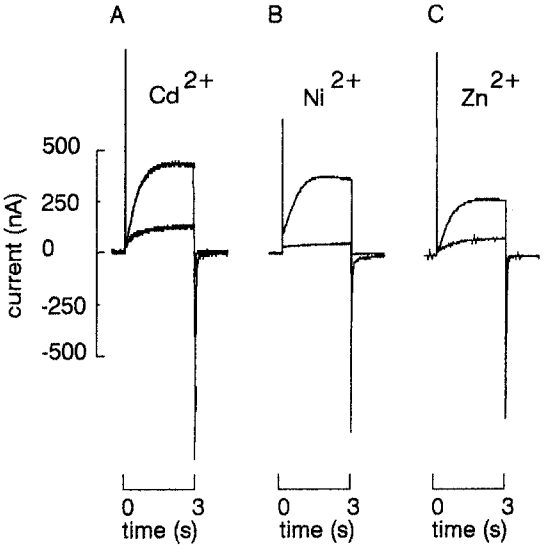

Fig. 4. Effect of heavy metals of ND96 supplemented with TEACl (10 mM) and caffeine (10 mM) on the current activated by a 3 sec depolarization to +40 mV from the −60 mV holding potential. (Cd^{2+}, Ni^{2+} or Zn^{2+} was used at a 1 mM concentration.)

Fig. 3 illustrates: (i) the absence of inactivation; (ii) saturation of ion accumulation/or ion depletion phenomenon over time.

Blockade of I_{out} by Heavy Metals

The effects on I_{out} of various divalent cations such as cadmium (Cd^{2+}), nickel (Ni^{2+}) and zinc (Zn^{2+}) used at 1mM concentration are described in Fig. 4. In our study, these divalent cations led to a significant inhibition of I_{out} (on average 85 + 5%, n = 7; Fig. 4, *A–C*). The reduction in current amplitude induced by heavy metals operated by shifting the current-voltage relation in a rightward direction and consequently decreased the outward current for all potentials (*data not shown*). As previously reported, Cd^{2+}, Ni^{2+} or Zn^{2+} correspond to putative blockers of H^+ conductance (Barish & Baud, 1984; Byerly et al., 1984; Meech & Thomas, 1987; Byerly & Suen, 1989; Mahaut-Smith, 1989; DeCoursey, 1991; Demaurex et al., 1993; Bernheim et al., 1993; Kapus et al., 1993). Moreover, 4-AP, also known as a H^+ channel blockers (Meech & Thomas, 1987) strongly affected I_{out} (5 mM, n = 5, *data not shown*). As (i) the deactivation time constant of I_{out} was fitted by a monoexponential, (ii) the instantaneous current-voltage relationship was linear and (iii) the divalent cations led to a dramatic inhibition of I_{out}, this current is thought to be due to activation of a H^+ conductance. Taking into account all the results mentioned above, we carried out an investigation to determine the ionic nature of I_{out} elicited in *Rana* oocytes with the idea that it could be related to a H^+ current.

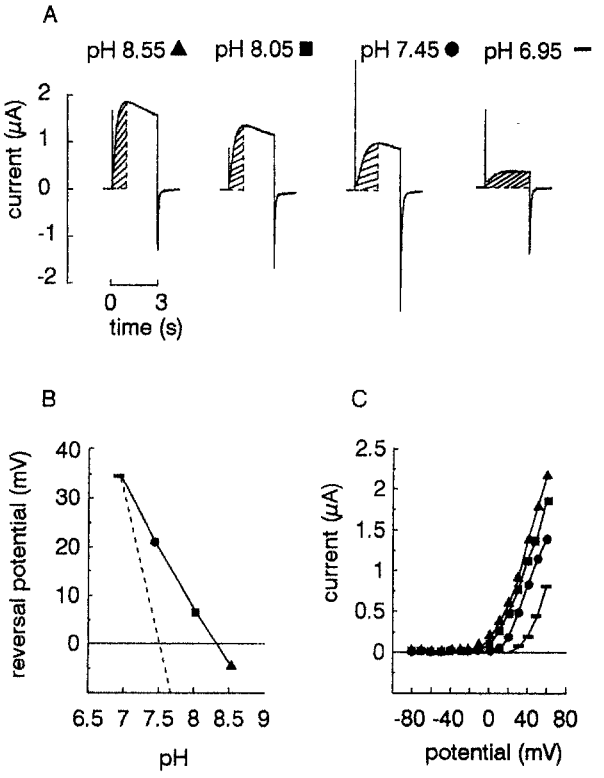

Fig. 5. Effect of changing external pH in ND96 supplemented with TEACl (10 mM) and caffeine (10 mM). (*A*) Effect of different external pHs on the current activated by a 3-sec depolarization to +40 mV from a −60 mV holding potential. External pH was changed around the 7.45 control value (●) to 8.05 (■), 8.55 (▲) and 6.95 (—). (*B*) Reversal potential, according to pH, determined as above for currents activated by a 40 mV imposed voltage shown in *A*, at pHs of 6.95 (—), 7.45 (●), 8.05 (■), 8.55 (▲). Slope of the relation linking reversal potential to pH is 30 mV; broken line represents the linear relation, in function of pH, expected by the Nernst equation. (*C*) Current-voltage relationships for different external pHs of 6.95 (—), 7.45 (●), 8.05 (■), 8.55 (▲).

Effect of External pH on I_{out}

External pH was modified around the control value of 7.45 to 8.05, 8.55 and 6.95 to produce an increase or decrease in the H^+ chemical gradient. Currents recorded under these conditions are presented in Fig. 5*A*. The outward current amplitude was clearly increased when external pH increased. Inversely, I_{out} was reduced when pH was lower than the control value of 7.45. During the depolarizing pulse the progressive decrease of the current amplitude over time was more prominent and started earlier when pH was augmented (Fig. 5*A*). Graphical integrations from the beginning to the I_{out} peak (measured at pH 8.55, 8.05, 7.45, 6.95) are represented for each current by the hatched area in Fig. 5*A*. These integral values were nearly identical, indicating that the beginning of the decrease in current amplitude depended on the amount of charges flowing through the oocyte membrane. Interestingly, the deactivation time constant decreased when external pH was increased (83 msec for a 7.45 pH, 58 msec for a 8.05 pH and 54 msec for a 8.55 pH). Inversely, the deactivation time constant increased to 92 msec for a 6.95 pH. Variations in the current amplitude produced following external pH modifications are correlated to a modification of E_{rev}. Figure 5*B* illustrates the effects of external pH variations on E_{rev}. E_{rev} was shifted toward more negative values when pH was more alkaline (+6 mV for a 8.05 pH, −5 mV for 8.55 and +21 mV for the normal pH of 7.45). By contrast, E_{rev} was shifted toward a more positive potential value when pH was lower than 7.45 (it was 35 mV for a 6.95 pH). If I_{out} was carried by H^+ ions, the shift in E_{rev} value expected by the Nernst equation would be of 58 mV for a change of one pH unit. In our experiments, the shift was only 30 mV (Fig. 5*B*). But despite this discrepancy, I_{out} was thought to be mainly carried by H^+ ions because of the probable existence of an intracellular accumulation-depletion phenomenon. Indeed, such a situation could lead to a significant variation in E_{rev} associated with an important modification of I_{out} amplitude. In particular, depletion of intracellular H^+ ions could explain the shift lower than 58 mV even if the current was very largely or exclusively carried by H^+ ions. The fact that E_{rev} shifted with the rate of I_{out} activation (Fig. 2*D*) or with the pulse duration (<3 sec) argues strongly for such a depletion process. When external pH was alkaline, the H^+ chemical gradient increased, leading to an increase in the current amplitude and to a significant H^+ ion depletion during the depolarizing pulse. Thus, H^+ depletion cannot bring about a shift of 58 mV. From these points, we postulate that H^+ ions play a very significant role in the remaining outward current recorded in our conditions (ND96 supplemented with TEACl and caffeine). Finally, external pH alterations were also able to cause a change in the activation potential threshold (Fig. 5*C*), indicating that the biophysical properties of the molecular H^+ carrier entity are also modified by the external H^+ surrounding.

Effect of Internal pH on I_{out}

To assess the involvement of H^+ ions in I_{out}, the internal pH (pH_i) dependence of the current was analyzed at a clamped extracellular pH (7.45). Internal alkalinization was performed by adding ammonium chloride (NH_4Cl, 20 mM concentration) to the control medium since such a 20 mM NH_4Cl concentration has been recently reported to produce an intracellular alkalinization of about 0.4 pH unit in *Rana* oocyte (Keicher & Meech, 1994). On the other hand, and as already described by Bode et al. (1994), we used extracellular sodium acetate ($NaCH_3COOH$, 20 mM) to produce internal acidification. The extent of the internal pH variation brought about by $NaCH_3COOH$ has not been yet determined in *Rana es-*

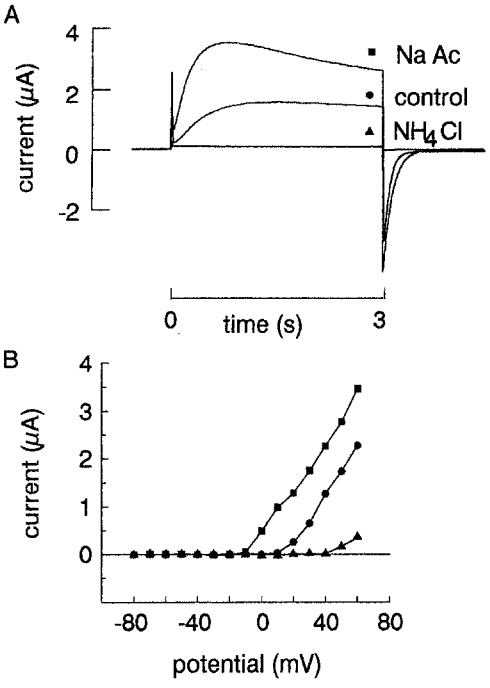

Fig. 6. Effect of internal pH modifications. (*A*) Effect of intracellular acidification by adding 20 mM of sodium acetate (■) and effect of intracellular alkalinization by adding 20 mM of ammonium chloride (▲) on the current recorded in control conditions in ND96 supplemented with TEACl (10 mM) and caffeine (10 mM) (●). Currents were activated by a 3-sec depolarization to +40 mV from a −60 mV holding potential. (*B*) Corresponding current-voltage relationships at normal pH (●), intracellular acidification (■) and intracellular alkalinization (▲). Currents were measured at the end of the depolarizing pulses.

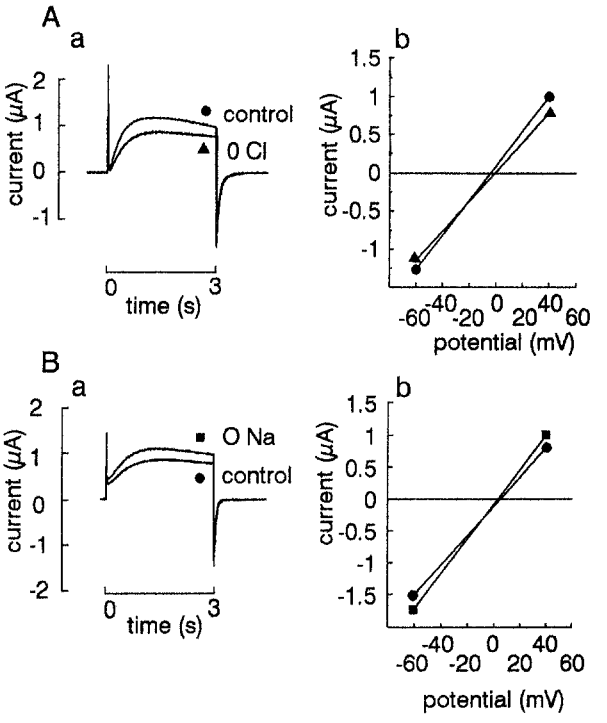

Fig. 7. Effect of Na^+ removal or Cl^- removal on the high voltage activated current in ND96 supplemented with TEACl (10 mM) and caffeine (10 mM). (*A*) Effect of Cl^- removal (methane sulfonate ions instead of external Cl^- ions) on the activated current (*a*) and E_{rev} (*b*). (*B*) Effect of Na^+ removal (choline ions instead of Na^+ ions) on the activated current (*a*) and E_{rev} (*b*). Currents were activated by 3-sec depolarization to +40 mV from a −60 holding potential. Currents were measured at the end of the depolarizing pulses. Reversal potentials were determined as above.

culenta oocyte. However intracellular acidification obtained by adding 20 mM sodium acetate led to an increase in current amplitude (Fig. 6*A*). When the membrane potential returned to its initial value, the current deactivated more slowly. Indeed, the time constant in the presence of external sodium acetate was 124 ± 22 msec (n = 7) as compared to a control value of 73 ± 27 msec (n = 26). Furthermore, E_{rev} was modified, becoming more negative when the oocyte was challenged with 20 mM sodium acetate (*data not shown*). On the other hand, when the intracellular medium was alkalinized by addition of NH_4Cl to the extracellular side, the current vanished (Fig. 6*A*). Therefore its time constant of deactivation and E_{rev} cannot be determined. These results corroborate the hypothesis that the H^+ ions did carry the outward current. The beginning of the decrease in current magnitude occurring during the test pulse was observed earlier when the intracellular medium was acidified. These results are in agreement with the occurrence of an intracellular H^+ ion depletion which can play a key role in the development and kinetics of I_{out}. The current-voltage relationship, presented in Fig. 6*B*, shows that the change in current amplitude was associated with a change in the activation potential threshold. This threshold evolved from 10 mV in the control medium to −10 mV in the presence of external sodium acetate and to 50 mV in the presence of NH_4Cl.

Influence of External Cl^-, Na^+ or K^+ Concentrations on I_{out}

Lastly, we investigated the possible participation of other ions species in the development of I_{out}. The substitution of external chloride ions by methane sulfonate led to a moderate decrease in the current amplitude and a slight modification of E_{rev} (Fig. 7*A*). ΔE_{rev} was +3.6 ± 3.1 mV (n = 20). This slight shift was not significant and led us to rule out major participation of Cl^- ions in the depolarization-induced I_{out}. Moreover, substitution of sodium by choline resulted in an increase in the current amplitude associated with a discrete change in E_{rev} (ΔE_{rev}: −4.1 ± 2.2 mV, n = 18; *see also* Fig. 7*B*). As for the Cl^- ion situation, the shift in E_{rev} was not large enough to conclude in favor of sodium permeability. Substitution in the external medium of K^+, Na^+, and Cl^- by TEA^+ and methane sulfonate did not significantly

affect either current amplitude or E_{rev} (*data not shown*), indicating that H^+ ions carried the remaining current.

Discussion

Our results clearly demonstrate that depolarizations of the oocyte of *Rana esculenta* elicited a large outward dynamic current activated for positive membrane voltages, under conditions where K^+ and Ca^{2+}-dependent Cl^- currents are eliminated. The outward current presented only one component and did not display real inactivation. This current was significantly reduced by Cd^{2+}, Ni^{2+} or Zn^{2+} and 4-AP, substances which are known to inhibit the H^+ conductance. All the results, taken together, demonstrate that the current described was very close to the H^+ currents described in other preparations (Barish & Baud, 1984; Byerly et al., 1984; Meech & Thomas, 1987; Byerly & Suen, 1989; Mahaut-Smith, 1989; DeCoursey, 1991; Demaurex et al., 1993; Bernheim et al., 1993; Kapus et al., 1993).

Ionic Selectivity of I_{out} Elicited by Depolarization

In a classical way, to conclude in favor of ionic selectivity, for example H^+ conductance, a change in the extracellular or intracellular pH of one unit must produce a 58 mV shift in E_{rev}. Our results indicate that if such a modification in the H^+ external concentration was made, the shift in E_{rev} was only 30 mV. Furthermore in our conditions, it was not possible to demonstrate that other ions carried the outward current to any significant extent. Modification of the Na^+ gradient had almost no effect on the current amplitude or on E_{rev}. Similar results have been described in the *Ambystoma* oocytes (Barish & Baud, 1984). In these two oocyte models, such a slight effect of Na^+ withdrawal could be attributed to: (i) a weak contamination of the outward H^+ current by a small membrane Na^+ current; (ii) a small permeability to Na^+ through the putative H^+ channels. In this latter case, the Na^+ permeability in *Rana* oocyte does not appear to be significant because of the discrete shift in E_{rev} obtained by Na^+ removal. Moreover, it was not possible to obtain any deactivation characteristics corresponding to a Na^+ current despite the fact that such a current has been described in the immature *Rana* oocyte (Taglietti et al., 1984). We have no explanation for this discrepancy, unless the stages of maturity of the oocytes can be somewhat different. Moreover, it must be noted that E_{Na} calculated from results of Keicher & Meech (1994) obtained on the fully grown ovarian oocytes of *Rana pipiens* is relatively high (80 mV). A similar value was found in the *Xenopus* oocyte (Baud et al., 1982). Under these conditions and contrary to the previous study carried out on *Rana esculenta* oocytes (Taglietti et al., 1984), INa^+ activated for moderate depolarizations (to a 40 mV membrane potential) must undergo the inward direction. So in our opinion, no additional outward current carried by Na^+ can overlap the H^+ component.

Several papers have concluded in favor of the existence of a H^+-selective conductance whose properties are similar to those described here, despite the fact that an E_{rev} shift lower than 58 mV was observed for one unit pH variation (*see* for review, DeCoursey & Cherny, 1994). In granulocytes for example, the reported shift was only 40 mV (Demaurex et al., 1993) and 30 mV in muscle myotube (Bernheim et al., 1993). In these two preparations, the intracellular H^+ concentration was clamped by using high H^+ buffer concentration. Nevertheless, in spite of the presence of internal H^+ buffer, the observed shift inferior to 58 mV was attributed to a local change in H^+ concentration underneath the membrane. Furthermore in myotubes and granulocytes, it was well demonstrated, when intracellular medium was not buffered, that activation of the H^+ current led to a H^+ intracellular depletion that caused an intracellular alkalinization. This alkalinization was correlated with a decrease in the current amplitude and to a shift in E_{rev}. On the other hand, a variation in E_{rev} to a value brought closer to 58 mV was determined in *Ambystoma* oocytes (Barish & Baud, 1984) and in macrophages (Kapus *et al.*, 1994), two sets of experiments where no variation in internal H^+ concentration was observed. Indeed, the pulse duration for the determination of E_{rev} in *Ambystoma* oocytes was short enough to exclude any H^+ depletion and, in macrophage, internal pH was strongly buffered. However, in *Ambystoma* oocytes, H^+ ion depletion was observable when applying large and/or long lasting pulses (Barish & Baud, 1984). Thus, one may postulate that in the *Rana esculenta* oocyte, the current activated by strong depolarizations is mainly carried by H^+ ions and from now, this current will be called H^+ current (IH^+).

Intracellular H^+ Ion Depletion in *Rana* Oocyte

In *Rana esculenta* oocyte, E_{rev} varied with the depolarizing level and with pulse duration. In addition, the decrease in current amplitude observed during pulse application, which could not be attributed to a real inactivation, was a function of extracellular and intracellular pH. These results taken together argue for efficient H^+ depletion which led to intracellular alkalinization in this kind of oocyte. In fact, the more H^+ current was activated, the more H^+ ions flowed out of the oocyte and thus, the greater was the rise in the intracellular alkalinization. Such a phenomenon of H^+ depletion during IH^+ activation is well documented (*see* for review, DeCoursey & Cherny, 1994). For example, in macrophages (Kapus et al., 1994), activation of the H^+ current for 7 sec at an imposed voltage of +60 mV led to an alkalinization near 1 pH unit when the intracellular medium is not

artificially buffered. For *Ambystoma* oocyte, H^+ depletion is not observed for a 2-sec pulse at +60 mV (Barish & Baud, 1984). By contrast, for the same stimulation, H^+ depletion was clearly seen in *Rana esculenta* oocyte. This discrepancy can be explained by the fact that the two oocyte models exhibited currents of comparable amplitude whereas their respective diameters are obviously different (1 mm for *Rana* and 2 mm for *Ambystoma,* giving a volume 8-fold higher for *Ambystoma*). Consequently, in view of these considerations, *Rana esculenta* oocyte might be more sensitive to intracellular H^+ depletion than *Ambystoma* oocyte when they undergo the same experimental voltage clamp protocol. However, additional experiments with a direct evaluation of intracellular pH using fluorometric methods are needed to quantify the H^+ depletion during depolarization.

Physiological Relevance of the H^+ Current

The role of the H^+ pathway is not known but the voltage-dependent H^+ current can produce substantial changes in pH_i (Thomas & Meech, 1982; Demaurex et al., 1993; Kapus et al., 1993; DeCoursey & Cherny, 1994). It seems possible that H^+ fluxes through these channels might contribute to some of the physiologically generated modifications in pH_i that have been reported in photoreceptors, skeletal muscle, fertilized eggs and during oocyte maturation (Brown, Meech & Thomas, 1976; Brown & Meech, 1979; Westerblad & Allen, 1992; Shen & Steinhardt, 1978; Webb & Nuccitelli, 1981; Lee & Steinhardt, 1981; Cicirelli, Robinson & Dennis Smith, 1983). In *Ambystoma* oocytes, during the first hours of progesterone-induced maturation, a depolarization associated with intracellular alkalinization was reported (Baud & Barish, 1984). During the first 5 hr, the H^+ current is clearly detectable. In this context, it may be suggested that the H^+ current could play a pivotal role in the occurrence of intracellular alkalinization. In *Rana esculenta* oocytes, the working H^+ current described in this paper could be involved in a similar alkalinization process during maturation. During cellular activation, a local intracellular accumulation of H^+, decreasing internal pH, could shift the current-voltage characteristics of the H^+ current to negative voltages in such a way that protons are extruded even at the resting potential. Another possibility is that H^+ conductance can operate when the oocyte is depolarized: during its activation, the H^+ current can create a relative proton sink below the membrane. This could generate a pulsing driving force facilitating H^+ diffusion from the core toward the membrane. Further experiments would be necessary to approach the role of the H^+ current during oocyte maturation and more generally to determine the regulation of intracellular pH in relation to the presence of the voltage-activated H^+ current.

References

Barish, M.E., Baud, C. 1984. A voltage-gated hydrogen ion current in the oocyte membrane of the Axolotl, *Ambystoma. J. Physiol.* **352:**243–263

Baud, C., Barish, M.E. 1984. Change in membrane hydrogen and sodium conductances during progesterone-induced maturation of *Ambystoma* oocytes. *Dev. Biol.* **105:**423–434

Baud, C., Kado, R.T., Marcher, K. 1982. Sodium channels induced by depolarization of the *Xenopus leavis* oocyte. *Proc. Natl. Sci. USA* **79:**3188–3192

Bernheim, L., Krause, R.M., Baroffio, A., Hamann, M., Kaelin, A., Bader, C.R. 1993. A voltage-dependent proton current in cultured human skeletal muscle myotubes. *J. Physiol.* **470:**313–333

Bode, H.P., Eder, B., Trautmann, M. 1994. An investigation on the role of vacuolar-type proton pumps and luminal acidity in calcium sequestration by nonmitochondrial and inositol-1,4,5-triphosphate-sensitive intracellular calcium stores in clonal insulin-secreting cells. *Eur. J. Biochem.* **222:**869–877

Bourinet, E., Fournier, F., Nargeot, J., Charnet, P. 1992. Endogenous *Xenopus*-oocyte Ca-channels are regulated by protein kinases A and C. *FEBS. Lett.* **299:**5–9

Brown, H.M., Meech, R.W. 1979. Light induced changes of internal pH in a barnacle photoreceptor and the effect of internal pH on the receptor potential. *J. Physiol.* **297:**73–93

Brown, H.M., Meech, R.W., Thomas, R.C. 1976. pH changes induced by light in a large *Balanus* photoreceptors. *Biophys. J.* **16:**33*a*

Burckhardt, B.C., Kroll, B., Fromter, E. 1992. Proton transport mechanism in the cell membrane of *Xenopus leavis* oocytes. *Pfluegers Arch.* **420:**78–82

Byerly, L., Hagiwara, S. 1982. Calcium currents in internally perfused nerve cell bodies of *Limnea stagnalis. J. Physiol.* **322:**503–528

Byerly, L., Meech, R., Moody, W. 1984. Rapidly activating hydrogen ion currents in perfused neurones of the snail, *Lymnaea stagnalis. J. Physiol.* **351:**199–216

Byerly, L., Suen, Y. 1989. Characterization of proton currents in neurone of the snail *Lymnaea stagnalis. J. Physiol.* **413:**75–89

Cicirelli, M.F., Robinson, K.R., Dennis Smith, L. 1983. Internal pH of *Xenopus* oocytes: a study of the mechanism and role of pH changes during meiotic maturation. *Dev. Biol.* **100:**133–146

DeCoursey, T.E. 1991. Hydrogen ion current in alveolar epithelial cells. *Biophys. J.* **60:**1243–1253

DeCoursey, T.E., Cherny, V.V. 1994. Voltage-activated hydrogen ion currents. *J. Membrane Biol.* **141:**203–223

Demaurex, N., Grinstein, S., Jaconi, M., Schegel, W., Lew, D.P., Krause, K.H. 1993. Proton currents in human granulocytes: regulation by membrane potential and intracellular pH. *J. Physiol.* **466:**329–344

Dumont, J.N. 1972. Oogenesis in *Xenopus leavis* (Daudin) I. Stages of the oocyte development in laboratory maintained animals. *J. Morphol.* **136:**153–180

Kapus, A., Romarek, R., Yi Qu, A., Rotstein, O.D., Grinstein, S. 1993. A pH sensitive and voltage dependent proton conductance in the plasma membrane of macrophages. *J. Gen. Physiol.* **102:**729–760

Keicher, E., Meech, R. 1994. Endogenous Na^+-K^+ (or $NH4^+$)-$2Cl^-$ cotransport in *Rana* oocytes; anomalous effect of NH_4^+ on pH_i. *J. Physiol.* **475.1:**45–57

Kostyuk, P.G., Krishtal, O.A. 1977. Separation of sodium and calcium currents in the somatic membrane of mollusc neurones. *J. Physiol.* **270:**545–568

Lee, S., Steinhardt, R.A. 1981. pH changes associated with meiotic maturation in oocytes of *Xenopus leavis. Dev. Biol.* **85:**358–369

Mahaut-Smith, M.P. 1989. The effect of zinc on calcium and hydrogen ion currents in intact snail neurons. *J. Exp. Biol.* **145:**455–464

Meech, R.W., Thomas, R.C. 1987. Voltage dependent intracellular pH in *Helix aspersa* neurones. *J. Physiol.* **390:**433–452

Ouadid, H., Browaeys-Poly, E., Vilain, J.P., Guilbault, P. 1994. Endogenous DHP-sensitive Ca^{2+} channels in *Pleurodeles* oocytes. *FEBS Lett.* **351:**58–62

Peres, A., Bernardini, G., Mancinelli, E., Ferroni, A. 1985. A voltage-dependent K^+ channel controlling the membrane potential in frog oocytes. *Pfluegers Arch.* **403:**41–46

Shen, S.S., Steinhardt, R.A. 1978. Direct measurement of intracellular pH during metabolic depression of the sea urchin egg. *Nature* **272:**253–254

Taglietti, V., Tanzi, F., Romero, R., Simoncini, L. 1984. Maturation involves suppression of voltage-gated currents in the frog oocyte. *J. Cell. Physiol.* **121:**576–588

Thomas, R.C., Meech, R.W. 1982. Hydrogen ion currents and intracellular pH in depolarized voltage-clamped snail neurones. *Nature* **299:**826–828

Toselli, M., Taglietti, V., Tanzi, F., D'angelo, E. 1989. Calcium-dependent chloride currents in the immature oocyte of the frog, *Rana esculenta. Arch. Ital. Biol.* **127:**69–80

Webb, D.J., Nuccitelli, R. 1981. Direct measurement of intracellular pH changes in *Xenopus* eggs at fertilization and cleavage. *J. Cell. Biol.* **91:**562–567

Westerblad, H., Allen, D. 1992. Changes of intracellular pH due to repetitive stimulation of single fibres from mouse skeletal muscle. *J. Physiol.* **449:**49–71