J. Membrane Biol. 185, 237–247 (2002)
DOI: 10.1007/s00232-001-0126-x

The Journal of
Membrane
Biology

Properties of a Sodium Channel (Na_x) Activated by Strong Depolarization of *Xenopus* Oocytes

A. Vasilyev, E. Indyk, R.F. Rakowski*
Department of Physiology and Biophysics, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064, USA

Received: 5 June 2001/Revised: 4 October 2001

Abstract. Short (< 1 sec) duration depolarization of *Xenopus laevis* oocytes to voltages greater than +40 mV activates a sodium-selective channel (Na_x) with sodium permeability five to six times greater than the permeability of other monovalent cations examined, including K^+, Rb^+, Cs^+, TMA^+, and $Choline^+$. The permeability to Li^+ is about equal to that of Na^+. This channel was present in all oocytes examined. The kinetics, voltage dependence and pharmacology of Na_x distinguish it from TTX-sensitive or epithelial sodium channels. It is also different from the sodium channel of *Xenopus* oocytes activated by prolonged depolarization, which is more highly selective for Na^+, requires prolonged depolarization to be activated, and is blocked by Li^+. Intracellular Mg^{2+} reversibly inhibits Na_x, whereas extracellular Mg^{2+} does not have an inhibitory effect. Intracellular Mg^{2+} inhibition of Na_x, is voltage dependent, suggesting that Mg^{2+} binding occurs within the membrane field. Eosin is also a reversible voltage-dependent intracellular inhibitor of Na_x, suggesting that a P-type ATPase may mediate the current. An additional cytoplasmic factor is involved in maintaining Na_x since the current runs down in internally perfused oocytes and excised membrane patches. The rundown is reversible by reintroduction of the membrane patch into oocyte cytoplasm. The cytoplasmic factor is not ATP, because ATP has no effect on Na_x current magnitude in either cut-open or inside-out patch preparations. Extracellular Gd^{3+} is also an inhibitor of Na_x. Na_x activation follows a sigmoid time course. Its half-maximal activation potential is +100 mV and the effective valence estimated from the steepness of conductance activation is 1.0. Na_x deactivates monoexponentially upon return to the holding potential (−40 mV). The deactivation rate is voltage dependent, increasing at more negative membrane potentials.

Key words: *Xenopus* — Oocyte — Sodium — Channel — Endogenous — Depolarization

Introduction

Oocytes of the South African clawed toad *Xenopus laevis* are widely used as a system for expression and characterization of exogenous membrane channels [24]. Such studies require an understanding of the endogenous channel activity present in the oocyte membrane. A variety of endogenous *Xenopus* oocyte channels have been described. These include various chloride channels having different voltage dependence and pharmacology [1, 3, 11, 17, 27, 29, 30], at least one kind of calcium channel [14, 18], potassium channels [7, 8, 12, 15], hemi-gap-junctional channels [2, 10] and stretch activated cation channels [16, 26, 31, 32]. In addition, at least three different passive sodium currents are known to be present in *Xenopus* oocytes. One of them is mediated by an epithelial-like amiloride-sensitive sodium channel [23, 28]. The whole-cell current produced by this channel is small (tens of nanoamperes per oocyte) and is inhibited by 10 μM extracellular amiloride. Another sodium channel found in *Xenopus* oocytes is TTX-sensitive [19]. It is inhibited by nanomolar concentrations of TTX, activated by depolarization positive to −40 mV and is inactivated within a few milliseconds. A third type of sodium current is activated by prolonged depolarization [4, 5, 6, 20, 22]. It is characterized by its slow induction rate [6], is strongly selective for sodium and is inhibited by Li^+.

* *Present address:* Department of Biological Sciences, Irvine Hall, Ohio University, Athens, OH 45701

Correspondence to: R.F. Rakowski; e-mail: rakowski@ohio.edu

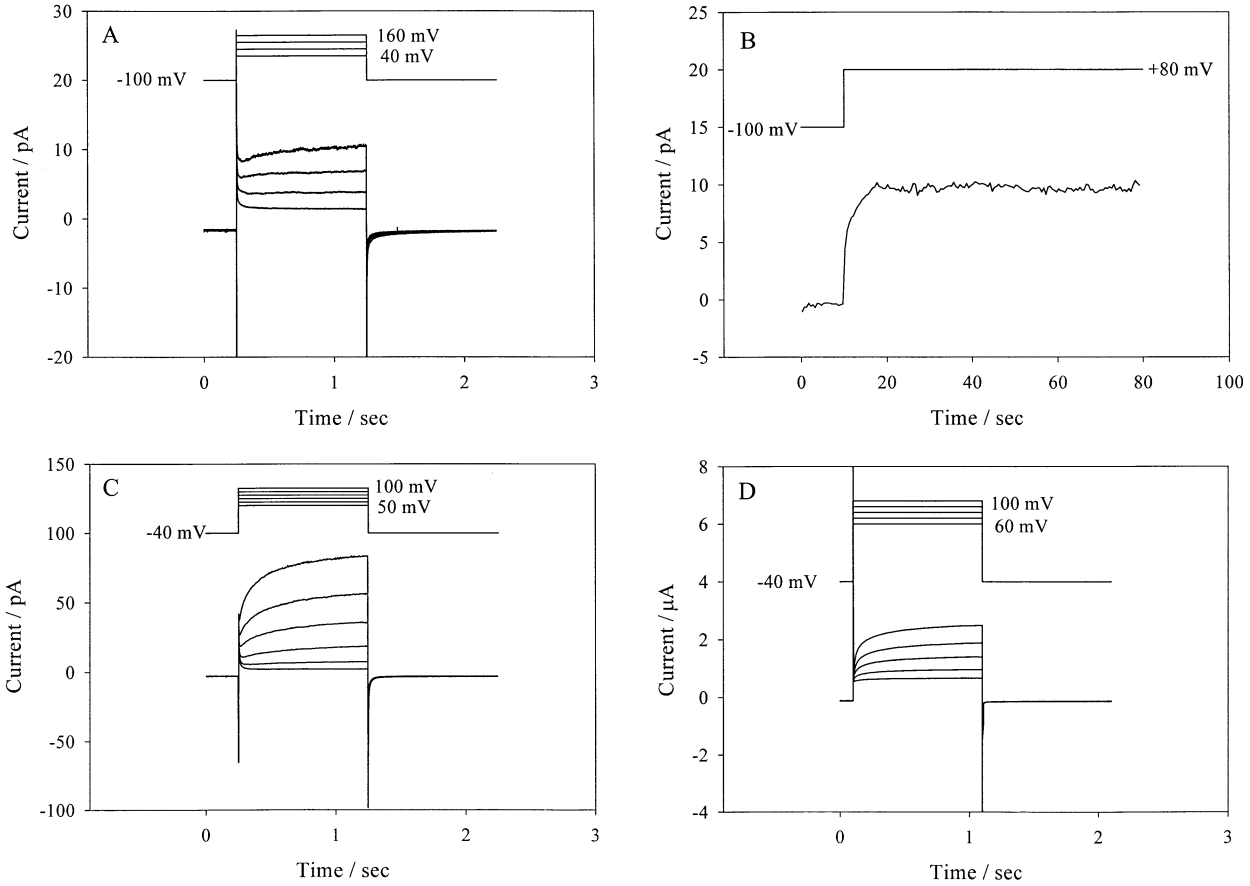


Fig. 1. Examples of the time course of current activation when the membrane voltage is stepped to positive potentials. The record in (*A*) was obtained from an on-cell patch of *Xenopus* oocyte membrane using a small (~2 MΩ patch electrode. Panel (*B*) shows that the current remains steady for long times after activation. (Inside-out patch configuration). In (*C*) the size of the patch pipette was increased (200 kΩ). In (*D*) the record was obtained using a two-microelectrode voltage clamp. Pipette and bath solutions were (in mM): 100 NaCl, 20 TEA Sulfamate, 10 $MgSO_4$, 20 HEPES, 2 EGTA-TMA, pH = 7.5 (Similar results were obtained without TEA^+ in the solutions).

In this paper we report the characteristics of an endogenous oocyte sodium channel (Na_x) with novel biophysical properties and pharmacology. Na_x differs from the amiloride-sensitive sodium channel in that it is not inhibited by 100 µM amiloride and requires depolarization to voltages greater than +40 mV to be activated. It is also different from the TTX-sensitive Na^+ channel in that it is not inhibited by high concentrations of STX and does not inactivate. In our tests of excised patches the current remained stable for over a minute. It also can be distinguished from the sodium channel activated by prolonged depolarization in that it has a fast time course of activation and is not inhibited by Li^+.

Materials and Methods

Stage V–VI oocytes were obtained from adult female African clawed toads, *Xenopus laevis* (Nasco, LaCrosse, Wisconsin) [9]. Oocytes were treated for two hours with 2 mg/ml type IA (Sigma, St. Louis, MO) collagenase dissolved in Ca^{2+}-free oocyte Ringer solution (in mm): 87.5 NaCl, 2.5 KC1, 1.0 $MgCl_2$, 5.0 TRIS/HEPES, pH = 7.6. The collagenase treated oocytes were manually defolliculated and kept at 4°C in normal Barth saline solution of the following composition (in mM): 87 NaCl, 3 KC1, 1 $MgCl_2$, 5 TRIS/HEPES, titrated to pH 7.6 with NaOH; 50 units/ml penicillin and 50 mg/ml streptomycin (Sigma). Oocytes were used within 4 days after defolliculation.

For patch-clamp experiments, oocytes were prepared by manual removal of the vitelline membrane after 5 to 15 minutes of incubation in devitelinizing solution (mM): 100 KCl, 109 NaCl, 5 HEPES, 1 $MgCl_2$, 2 $CaCl_2$, pH = 7.4. Glass pipettes (100 µl micropipettes, VWR Scientific, West Chester, PA) were pulled to a 1–20 µm diameter tip, depending on the intended experiment, and coated with silicon elastomer (Sylgard, Dow Corning, Midland, MI). The pipette tips were fire-polished against an electrically heated 3-mm wide heating filament (Sutter Instruments, Novato, CA) covered with melted glass. The data from the clamp amplifier (BC-525C, Warner Instruments, Hamden, CT) were digitally sampled by using an ITC-16 AD converter (Instrutech Corporation, Port Washington, NY) using HEKA Pulse data acquisition software (HEKA elektronik, Lambrecht/Pfalz, Germany). Sigma Plot 5.0 software (Jandel Scientific, Corte Madera, CA) was used to plot and analyze the data.

Cut-open oocyte clamp experiments were performed according to Taglialatela et al. [25]. In our modification of this method a quartz canula was connected via PE10 tubing to a small chamber positioned ~5 cm above the level of the oocyte. The intracellular solution flow rate was controlled by changing the height of this chamber. The data from the CA-1 High Performance Oocyte

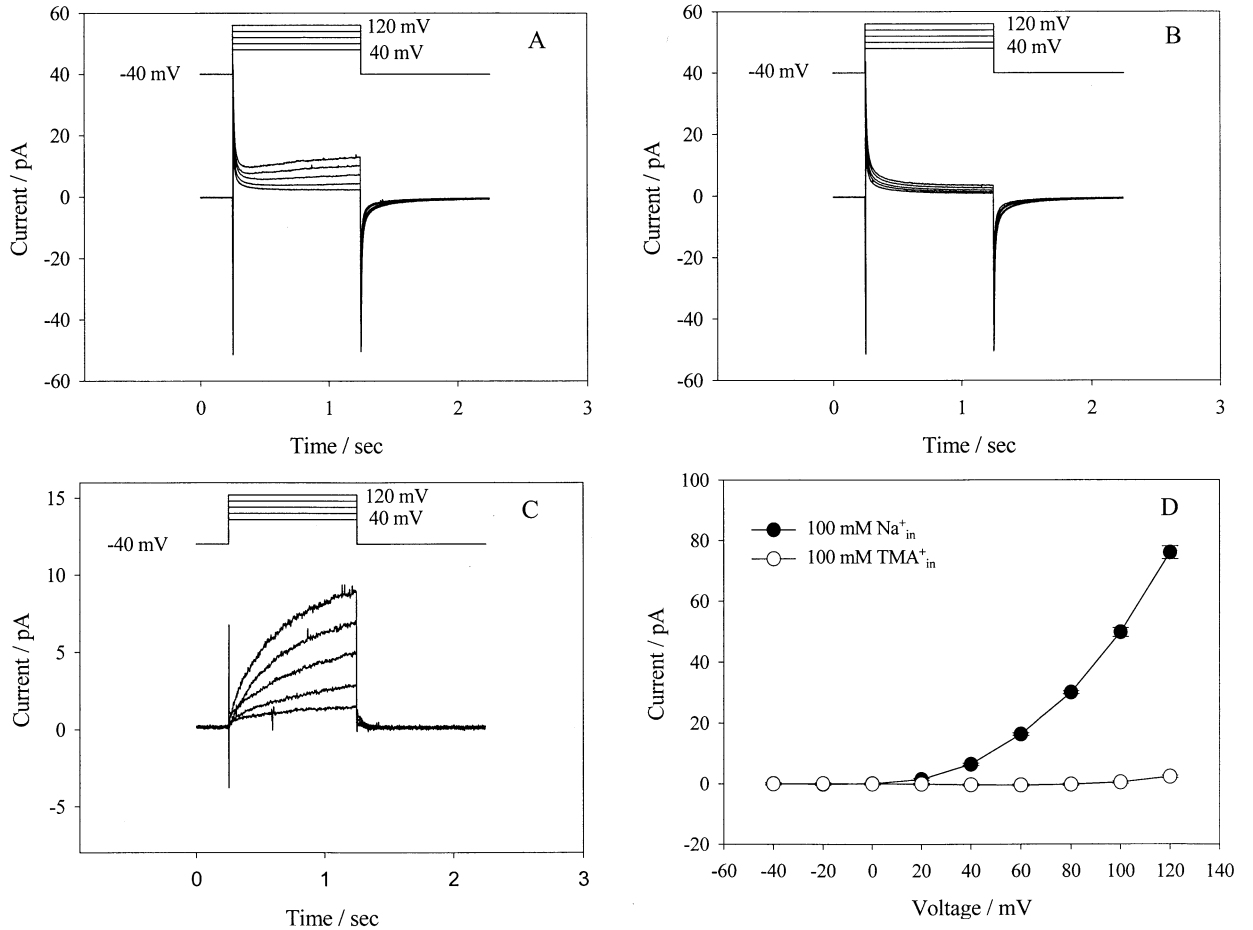


Fig. 2. Na_x is sensitive to removal of intracellular sodium. (*A*) Inside-out patch current in the presence of symmetrical 100 mM Na^+_o and 100 mM Na^+_i solutions. (*B*) Membrane current in the same patch after intracellular Na^+ was replaced with TMA^+. (*C*) The difference current (*A-B*). (*D*) Current-voltage relationship at the end of 1-sec depolarizing pulses in 100 mm $[Na^+]_i$ (filled circles) or 100 mM $[TMA^+]i$ 0 mM $[Na^+]_i$ (empty circles). These current-voltage data were obtained by subtracting a linear component of the total current and are presented here as the algebraic sum of four independent patches. Error bars in *D* represent square roots of the sums of variances of the individual records. The total subtracted linear conductance was (sum of four patches) 225 ± 8 pS and reversal potential −1.8 ± 1.8 mV reflecting passive patch currents. Pipette solution in (*A*) and (*B*) contained (in mM) 100 NaCl, 20 TEA Sulfamate, 10 $MgSO_4$, 20 HEPES, 2 EGTA-TMA, pH = 7.5. The bath solution in (*A*) had the same composition as the pipette solution, whereas in (*B*) 100 mM NaCl was replaced with 100 mM TMA Cl.

Clamp (Dagan Corporation, Minneapolis, MN) were processed in the same way and with the same hardware as in patch-clamp experiments.

For two-microelectrode experiments, we used an OC-725 voltage clamp amplifier (Warner Instruments). The current and voltage signals were sampled using a TLl-100 kHz AD converter (Axon Instruments, Burlingame, CA). Pclamp6 software was used to collect data and to perform the initial analysis of the results. Data were further analyzed using Sigma Plot 5.0 software. With the exception of Figs. 1 and 2, the data reported in this study were obtained by removing capacitance and leak currents. In order to do that, a series of five 20-mV prepulse traces were scaled and subtracted from the test-pulse record.

Results

As shown in Fig. 1, depolarization of *Xenopus* oocyte membrane to potentials positive to +40 mV activates a current with the following characteristics: the current does not activate until the membrane potential is brought above ≈+40 mV, does not inactivate (for durations up to 1 min) and deactivates rapidly upon repolarization. Since various anion and cation channels previously described in *Xenopus* oocytes have been shown to have a similar voltage dependence, we examined the ion selectivity of this depolarization-activated current in order to characterize it further.

Using the inside-out patch-clamp technique we found that substituting K^+ or TMA^+ for intracellular Na^+ virtually abolished the outward current activated by membrane depolarization (Fig. 2). This result suggested that Na^+ carries the current activated by strong depolarization (hereafter we will refer to it as Na_x—*sodium*-dependent endogenous *Xenopus* current).

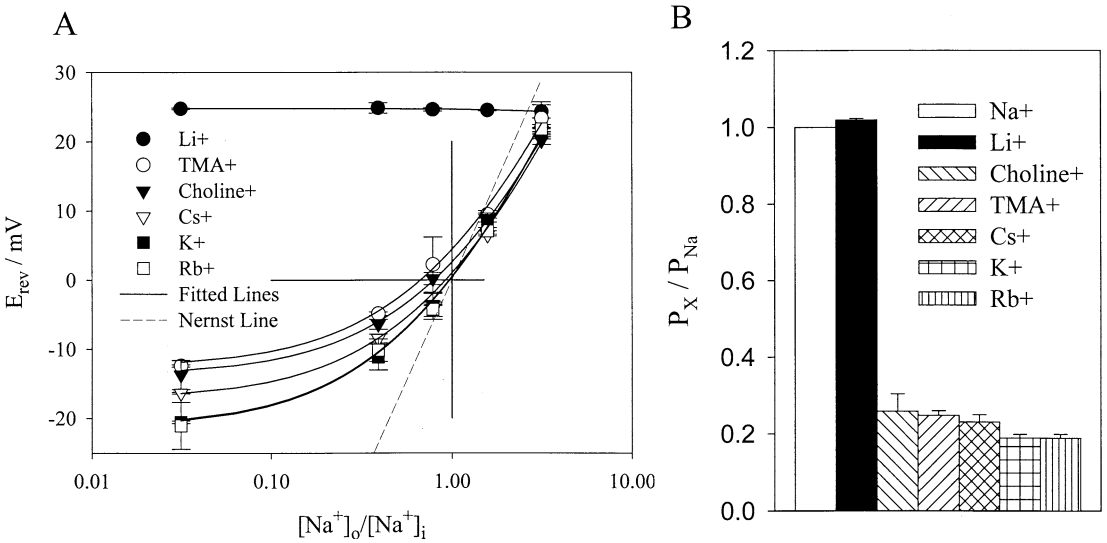


Fig. 3. Relative permeabilities of different monovalent cations determined by using the cut-open oocyte technique. In panel (*A*) the reversal potential was determined after lowering of the extracellular sodium concentration from 110 mM to 0 mM in three intermediate steps: 55 mM, 22.5 mM and 11.25 mM. The regression lines were obtained by fitting the constant-field equation (Eq. 3) to the data with P_x/P_{Na} being a free parameter and where "X" is a substitute ion. Panel (*B*) shows the permeability ratio P_x/P_{Na}. Internal solution contained (in mM): 70 KCl, 30 NaCl, 5 Na_2EDTA, 5 HEPES, pH = 7.5. External solution contained (in mM): 1 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, pH = 7.5 and various concentrations of NaCl — 110, 55, 22.5, 11.25 and 0 mM. Sodium was replaced by different monovalent cations. The Na_o^+-free data are shown in panel (*A*) but plotted at a value $[Na^+]_o/[Na^+]_i = 0.03$ for convenience of presentation on this logarithmic scale. The reversal potential value of 0 was used to obtain the fit shown.

To further investigate the ion selectivity of Na_x, we studied the effect of extracellular ion substitutions on the reversal potential of the current. The cut-open oocyte technique was used because of its good temporal resolution and the ability to control both intracellular and extracellular ion composition. Current records were obtained after depolarization to +120 mV for 100 msec and then stepping to potentials varying from −60 mV to +60 mV in 20-mV increments. The deactivating-current traces (similar to those shown below in Fig. 5*D*) were fit with a single exponential function (Equation 1) to obtain time-zero values of Na_x at each membrane potential.

$$I_t = I_\infty + I_0 e^{-a(t-t_0)} \tag{1}$$

where t_0 is the time at the start of repolarization. These instantaneous *I-V* relationships $I_v(V) = I_0(V) + I_\infty(V)$ were further fit by a polynomial function (Equation 2)

$$I_v = \sum A_i (V - V_0)^i \quad i = 1, \ldots, n \tag{2}$$

where n is the number of voltage levels in the data set and A_i is a free parameter. This function estimates the reversal potential V_0 more accurately than a linear function because of the slight outward rectification of the instantaneous *I-V* relationship.

Six different monovalent cations were used as Na^+ substitutes: Li^+, TMA^+, $Choline^+$, K^+, Rb^+, and Cs^+. The intracellular solution contained 70 mM K^+ not only to simulate the normal intracellular ion composition, but also to enable us to resolve inwardly-directed deactivating currents as the extracellular Na^+ concentration was lowered. The constant field equation (Equation 3) was fit to the data.

$$E_{\text{rev}} = \frac{RT}{F} \ln \frac{[\text{Na}]_o + \alpha[X]_o}{[\text{Na}]_i + \beta[\text{K}]_i} \tag{3}$$

The only free parameters in this equation (α and β) represent the relative permeabilities of the ion of interest (X) or of potassium respectively ($\alpha = P_x/P_{Na}$ and $\beta = P_K/P_{Na}$) When potassium was used to substitute for Na^+ externally, the best fit yielded equal values of α and β as expected from their definition. Figure 3 shows that Na_x is sodium-selective. Similar to the TTX-sensitive Na^+ channel, Li^+ permeates as well as Na^+, but Na_x is insensitive to a high concentration of STX (Figs. 4 A–C). Na_x also shows no sensitivity to extracellular amiloride (100 μM), an inhibitor of epithelial sodium channels (Fig. 4).

To examine the voltage dependence of steady-state Na_x conductance, we performed double-exponential fits to the activation traces (with the leak component subtracted as described in Methods) as shown in Fig. 5A. The values of G_∞ from Equation 4 were then normalized to the extrapolated value at +200 mV.

$$I(t) = \left(G_\infty - Ae^{-a(t-t_0)} - Be^{-a(t-t_0)}\right) \Big/ (V - E_{\text{rev}}) \tag{4}$$

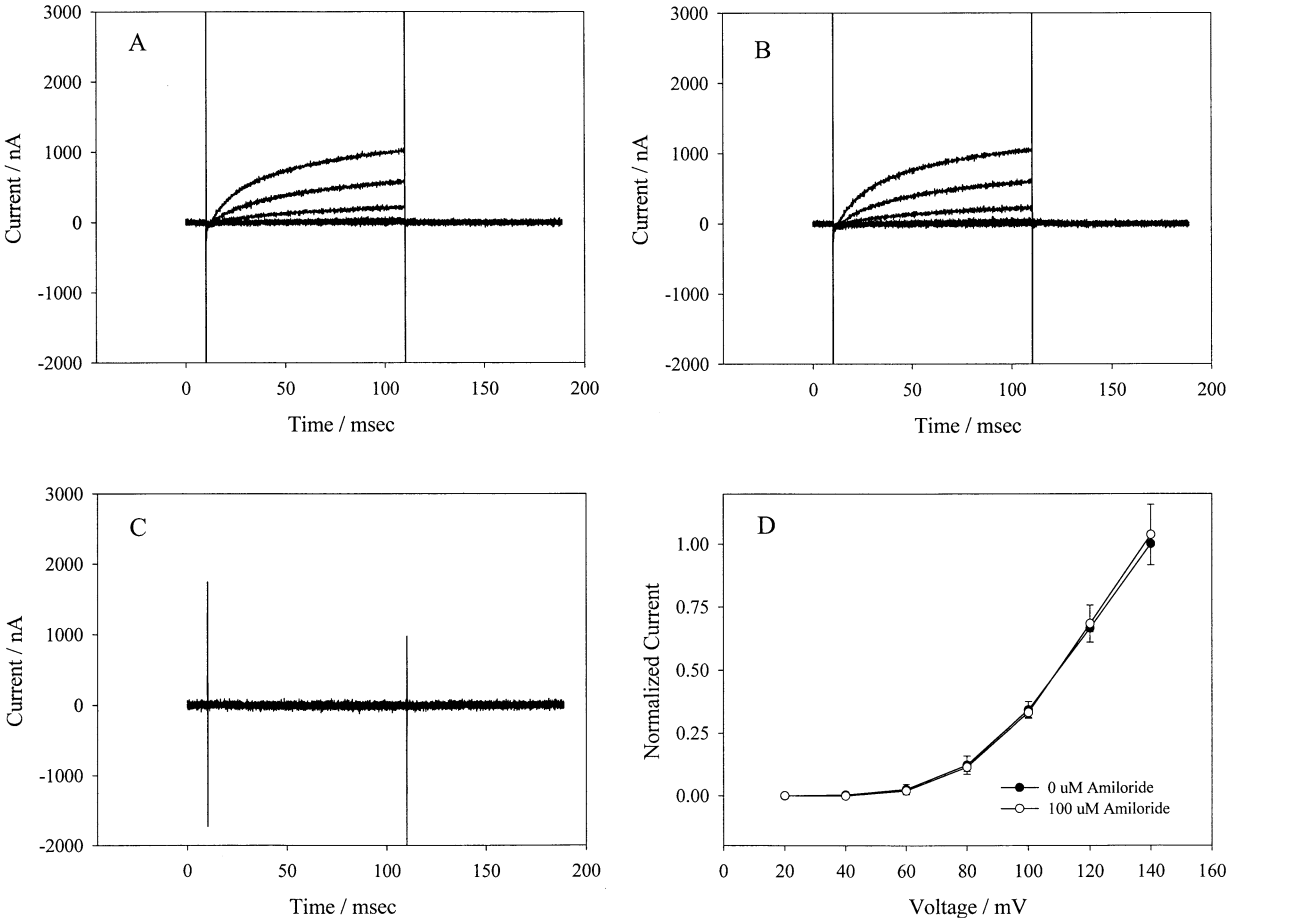


Fig. 4. STX and Amiloride have no effect on Na_x. The cut-open oocyte preparation was used for this experiment. In panel (*A*) Na_x current is shown before the addition of saxitoxin. Panel (*B*) shows Na_x current after 300 μM STX was added to the extracellular medium. Panel (*C*) shows the difference (*A*)-(*B*). In panel (*D*) filled circles indicate currents averaged over the last 5 msec of 100-msec depolarizing pulses at various voltages normalized to the value obtained at +140 mV. Open circles show the same currents after addition of 100 μM external amiloride. They are also normalized to the value obtained at +140 mV before the addition of amiloride ($n = 4$). Internal solution (in mM): 70 KCl, 30 NaCl, 5 Na_2EDTA, 5 HEPES, pH = 7.5. External solution: 110 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, pH = 7.5.

The results are plotted in Fig. 5*B* as filled circles. Alternatively, the conductance values were measured as instantaneous-current (time-zero) values by fitting deactivating traces with a single-exponential function upon return to the holding potential (−40 mV). These values were directly proportional to the conductance at the corresponding depolarizing voltage since all deactivating currents were measured at single (−40 mV) voltage. The time-zero values of $I(V)$ normalized to the extrapolated value at +200 mV are shown in Fig. 5*B* as filled circles. Equation 5 was fit to the normalized time-zero current data.

$$G(V) = G_{\max} \Big/ \left(1 + e^{-\delta F(V - V_{1/2})/RT}\right) \qquad (5)$$

The values of δ (dielectric coefficient) estimated by these two methods were 1.04 ± 0.08 and 0.99 ± 0.07, respectively, consistent with channel gating being governed by a single charge crossing the entire membrane field. The corresponding values of $V_{1/2}$ were 98.3 ± 2.3 mV and 99.4 ± 2.4 mV.

Modifiers of Na_x

Na_x is relatively insensitive to extracellular Mg^{2+}. In contrast, intracellular Mg^{2+} is a potent inhibitor of Na_x, as shown in Figs. 6*A* and *B*. We investigated the dose dependence of Mg^{2+} inhibition. The results are shown in Figs. 6*C* and *D*. The data are well described by Equation 6 that assumes a single binding site for Mg^{2+} inhibition of Na_x:

$$i = (K_m + a[\mathrm{Mg_i}])/([\mathrm{Mg_i}] + K_m) \qquad (6)$$

(i is the normalized steady-state current plotted in Fig. 6*C*, K_m is the Mg^{2+} binding constant, a is the ratio of Na_x conductance with Mg^{2+} bound to the inhibitory site to Na_x conductance in Mg^{2+}-free

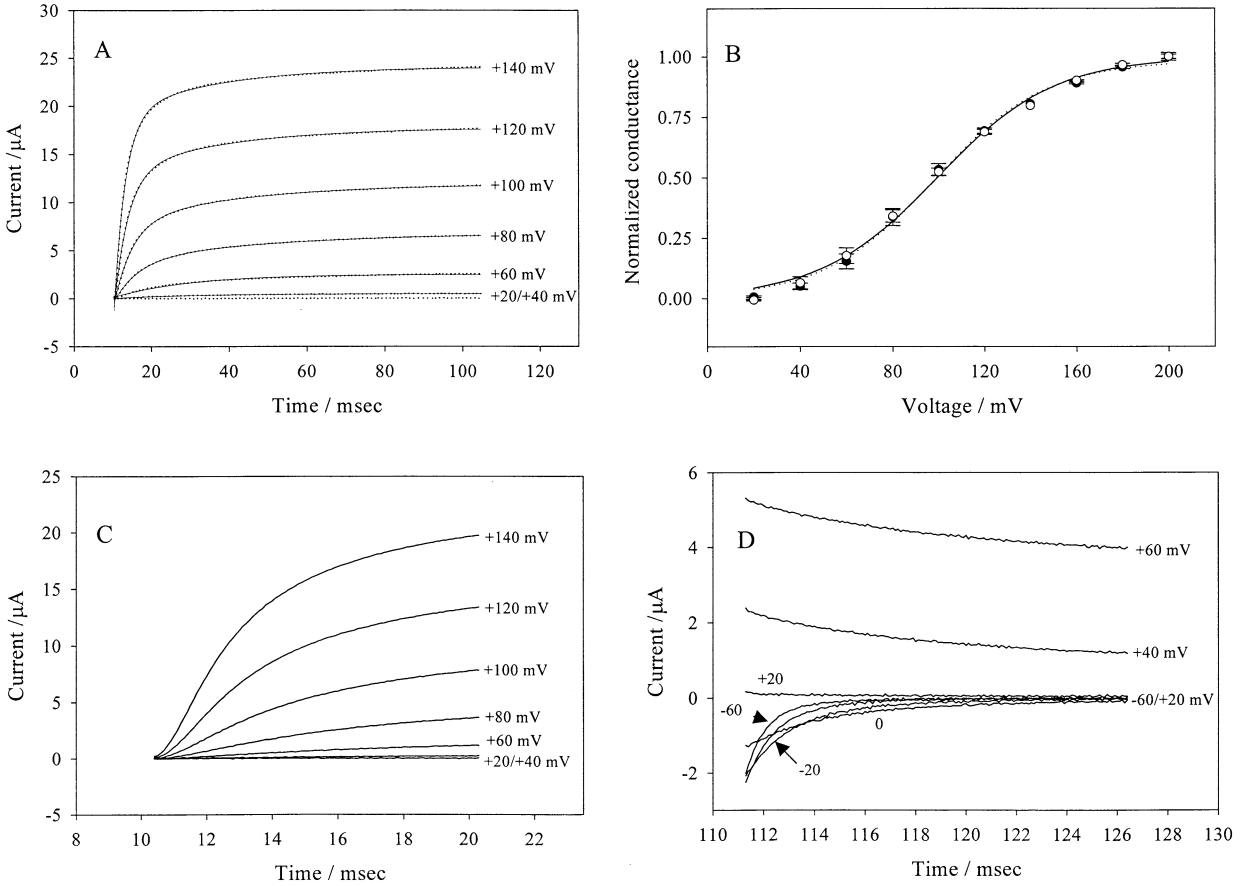


Fig. 5. Time course and voltage dependence of Na_x current. In panel (*A*) the dotted lines represent Na_x current; the solid lines, exponential fits to these data $I = I_\infty - Ae^{-a(t-t_0)} - Be^{-a(t-t_0)}$. Pulses were made to a series of voltages ranging from +20 mV to +140 mV in 20-mV increments from a holding potential of −40 mV. Panel (*B*) shows the estimates of the voltage dependence of steady-state total conductance of Na_x. Filled circles—predicted steady-state *I-V* data were used to calculate the *G-V* relationship. Open circles, the *G-V* relationship was determined directly by measuring the instantaneous *I-V* after return to a fixed voltage of −40 mV from various membrane potentials. The solid and the dotted lines represent the least-square fits of a Boltzmann function to the corresponding data. In panel (*C*) the Na_x activation traces are shown on an expanded time scale to show that activation of Na_x follows a sigmoidal time course. Panel (*D*) shows that the deactivation rate of Na_x is voltage dependent. A prepulse was made to +120 mV and subsequent test pulses were from −60 mV to +60 mV in 20-mV increments. Internal solutions (in mM): 70 KCl, 30 NaCl, 5 Na_2EDTA, 5 HEPES, pH = 7.5. External solutions: 110 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, pH = 7.5.

conditions). The values of K_m can be plotted as a function of membrane voltage and fit using Equation 7.

$$K_m = k_m e^{\delta FV/RT} \tag{7}$$

The values of δ (dielectric coefficient) and k_m obtained from the fit were $\delta = -0.33 \pm 0.02$ and $k_m = 3.0 \pm 0.2$ mM.

Note that intracellular Mg^{2+} not only affects the magnitude of the steady-state current but also changes the kinetics of current activation as shown in Figs. 6*E* and *F*. When no internal Mg^{2+} is present, Na_x activates with a sigmoid time course with ~1 msec delay before activation. On the other hand, when intracellular Mg^{2+} is present, the time course is a double-exponential function of time without a sigmoid foot.

Na_x is inhibited by intracellular eosin (Fig. 7). The normalized data at various membrane voltages can be fit assuming a single intracellular binding site for eosin inhibition. Remarkably, at all membrane potentials, the maximum amount of eosin inhibition of Na_x is one half, indicating that eosin is a partial inhibitor of the current. If Equation 6 is fit to the data at various voltages (eosin in place of Mg^{2+}), the values of *a* fall within the range 0.43 to 0.55. Here we used a single value of *a* to fit the entire data set. K_m values appear to be dependent on voltage, suggesting that the eosin-binding site is located within the membrane field. These values are plotted in panel 7*B*. The curve represents a least-squares fit of Equation 7 to the data and yields values of $\delta = 0.47 \pm 0.03$ and $k_m = 2.9 \pm 0.4$ μM.

Extracellular Gd^{2+} also inhibits Na_x. But despite the presence of a high-affinity component of Gd^{3+}

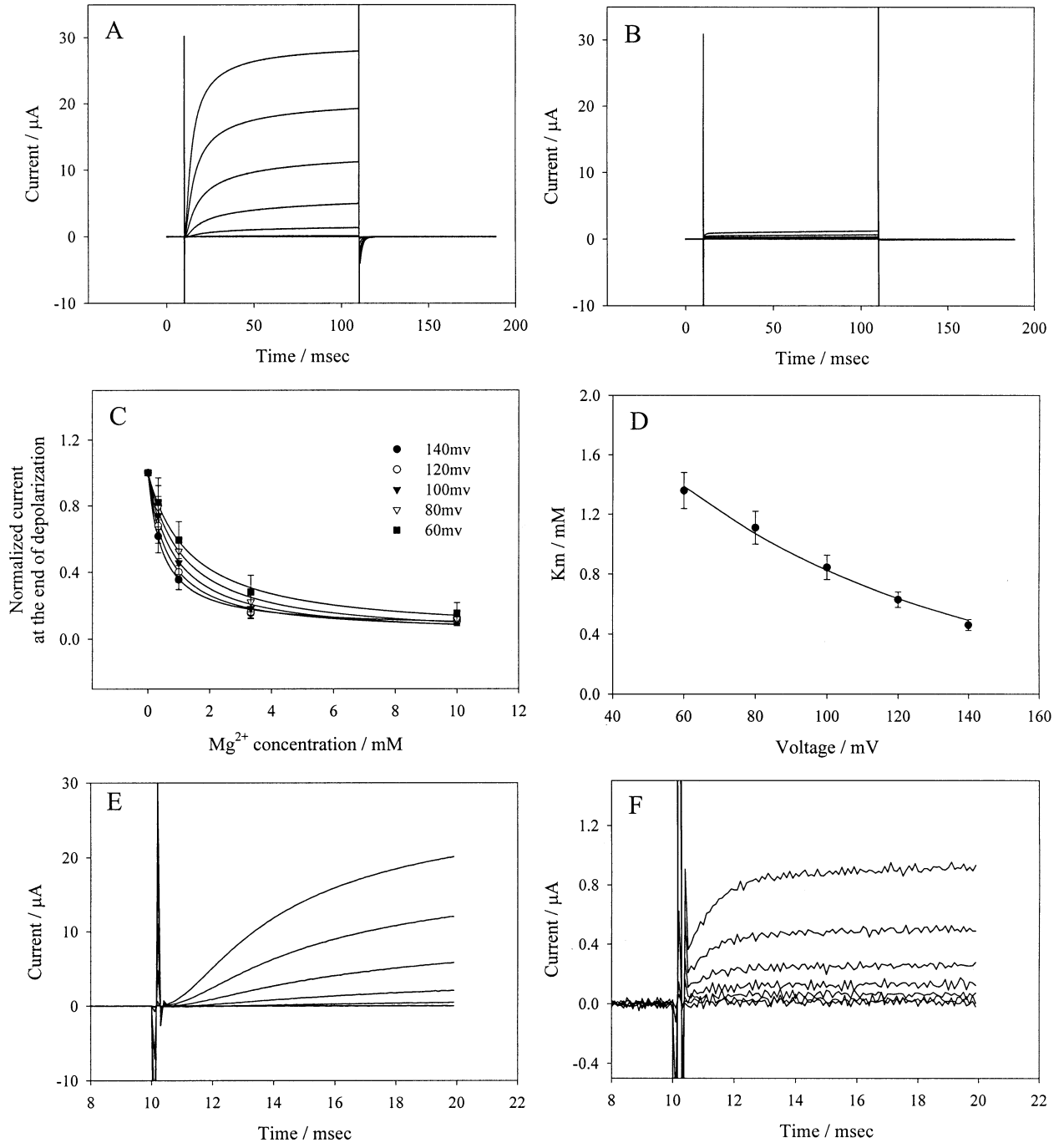
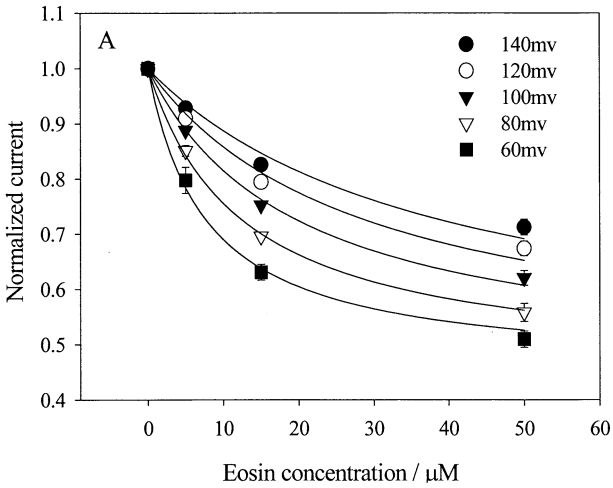


Fig. 6. The effect of intracellular Mg^{2+} on the magnitude and kinetics of Na_x. This experiment was done using the cut-open oocyte technique. Voltage pulses were made from the −40 mV holding potential to a series of voltages +20/+140 mV in 20-mV increments. (*A*) Na_x before the addition of 10 mM intracellularMg^{2+}. (*B*) Na_x current was measured 6 min after 10 mM Mg^{2+} was added to the intracellular medium. (*C*) The magnitude of Na_x curret is plotted as a function of intracellular [Mg^{2+}]. The values are normalized to the current magnitude when no intracellular Mg^{2+} was present. This procedure was repeated at various membrane voltages (shown using different symbols). Smooth curves represent the least-square fits to the data using equation (6). Panel (*D*) shows the estimated values K_m as a function of membrane voltage. The smooth line is the least-square fit of equation 7 to the data. The estimated parameter values are $k_m = 3.0 \pm 0.2$ mM and $\delta = -0.33 \pm 0.02$. (*E*) The time course of activation of Na_x has a sigmoidal shape in 0 mM $[Mg^{2+}]_i$. (*F*) The activation time course is faster, and the curve shape is exponential in 10 mM $[Mg^{2+}]_i$. Intracellular solution (in mM): 70 KCl, 30 NaCl, 5 BAPTA, 5 HEPES, pH = 7.5 with up to 10 mM $MgCl_2$ added. The external solution was the same as in Figure 5.

inhibition (Fig. 8*A*,*C*), the amount of Gd^{3+} required to block most of Na_x is relatively high (K_m = 246 ± 20 μM). This value is much larger than, for example, the amount of Gd^{3+} needed to half-inhibit the stretch-activated channels in *Xenopus* oocytes.



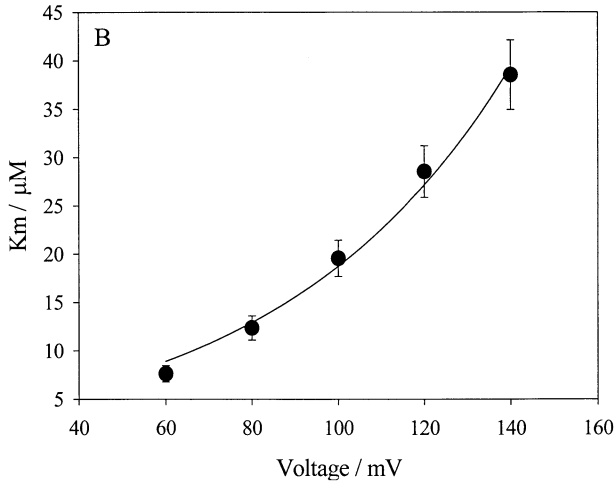


Fig. 7. Inhibitory action of intracellular eosin on Na_x. (*A*) The normalized magnitude of Na_x current is plotted as a function of intracellular eosin concentration. The values are normalized to the current with no intracellular eosin present. This procedure was repeated at various membrane voltages. The smooth curves represent the least-square fits to the data using equation 6 ($[eosin]_i$ in place of $[Mg^{2+}]_i$). The estimated values of K_m are plotted as a function of membrane voltage in panel (*B*). The smooth lines represent the least square fit of equation 7 to the data. The estimated values are $k_m = 2.9 \pm 0.4$ mM and $\delta = 0.47 \pm 0.03$. Internal solutions (in mM): 70 KCl, 30 NaCl, 5 Na_2EDTA, 5 HEPES, pH = 7.5. Various amounts of eosin were added to give the indicated concentration. External solution: 110 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, pH = 7.5.

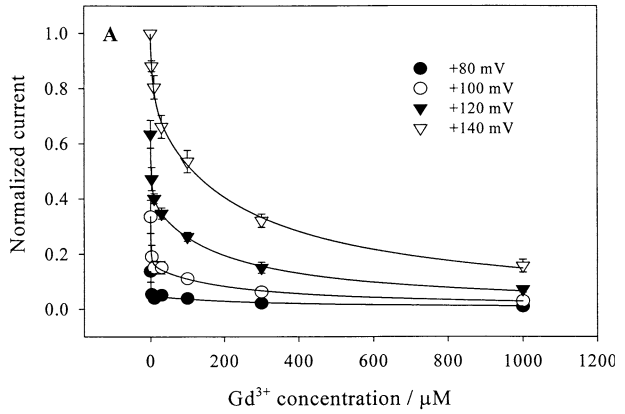
In cut-open oocyte experiments of long duration (more than 1 hour) a slow rundown of Na_x was observed that was independent of the presence of intracellular Mg^{2+}. This slow rundown suggested that a cytoplasmic factor was required to maintain Na_x. This hypothesis was tested by using the excised inside-out patch technique as shown in Fig. 9. A large-diameter patch pipette was used (20 μm). Upon patch excision (Fig. 9*A* (*I–IV*)), the magnitude of Na_x decreased within a few minutes. When the membrane patch was moved back into the intracellular medium (*V*), a rapid recovery of Na_x took place. This cytoplasmic factor does not appear to be ATP or its Mg^{2+}complex, as explained below.

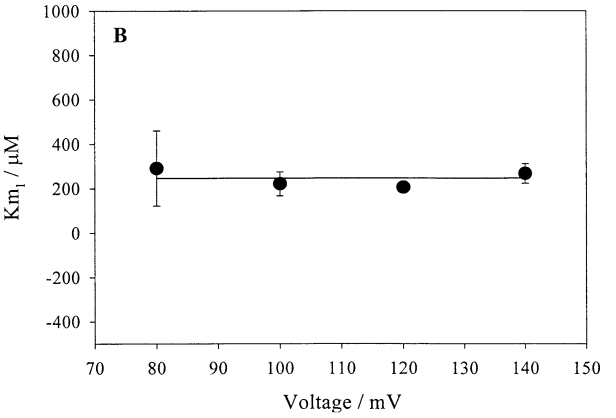
In the experiment shown in Fig. 9*B* the cut-open oocyte was perfused for 4 hours with a solution containing no Mg^{2+} or ATP. Rundown of Na_x was observed. The shape and the magnitude of Na_x were then examined under a variety of conditions. First, 1 mM $MgCl_2$ was applied intracellularly and inhibition of Na_x was observed (*I*). Na_x recovered after Mg^{2+} was washed out (*II*). Second, the addition of 5 mM Na_2ATP had no effect on Na_x (*III*). After Na_2ATP was replaced with 5 mM MgATP, a decrease in Na_x current magnitude occurred, consistent with the effect of Mg^{2+} alone (*IV*). Na_x current recovered to its initial level when 0 mM ATP, 0 mM Mg^{2+}, 5 mM EDTA solution was used to remove free Mg^{2+} from the intracellular medium (*V*). The results of this experiment indicate that it is unlikely that either ATP or its magnesium complex is the cytoplasmic factor that prevents Na_x rundown.

Discussion

The pharmacology, voltage dependence, and ion selectivity of Na_x indicate that it is a novel form of Na^+ conductance that is endogenous in *Xenopus* oocytes. There are certain similarities between Na_x and the Na^+ current activated by prolonged depolarization of oocyte membrane [4, 5, 6, 20, 22]. First, both currents require depolarization above +40 mV to become activated. Second, they are both non-inactivating currents. Third, they are inhibited by intracellular Mg^{2+}. On the other hand, there are marked differences that set these two currents apart. The sodium current described by Baud et al. [6] requires prolonged depolarization to become activated, which is its signature feature [22]. Na_x, on the other hand, is activated within a few milliseconds from its fully deactivated state at −40 mV holding potential. The slowly activated sodium current described by Baud, et al. [6] closely follows the Nernst equation with respect to sodium concentration. Na_x is less selective for sodium. The slowly activated sodium current is blocked by Li^+, whereas Na_x is permeable to both Na^+ and Li^+. In addition to these qualitative differences, there are significant differences in the measured values for voltage dependence and gating charge [22]. Therefore it is likely that Na_x and the slowly activated Na^{2+} current are mediated by different channel proteins.

Assuming a single-channel conductance on the order of 10 pS, the Na_x channel has to be present in the membrane at a very high density, comparable to that of membrane pumps, to yield whole-cell currents





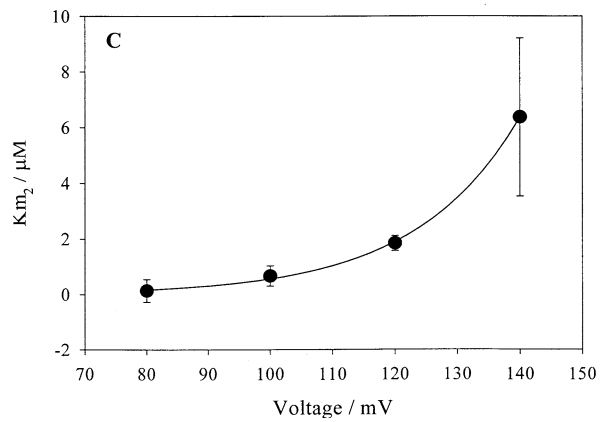


Fig. 8. The effect of extracellular gadolinium on Na_x. (*A*) Na_x was measured at the end of 100-msec depolarizing pulses to various potentials at various concentrations of external Gd^{3+}. The current values were normalized to the value at 140 mV and in 0 Gd^{3+} and plotted as a function of Gd^{3+} concentration. Solid lines represent least-square fits to the data at +80, +100, +120, and +140-mV pulses using equation: $I = a/(1 + [Gd^{3+}]/K_{m1}) + b/(1 + [Gd^{3+}]/K_{m2})$. (*B*) shows that K_{m1} is independent of membrane voltage. The value of K_{m1} = 246 ± 20 mM. (*C*) shows that K_{m2} is voltage dependent. Theoretical curve was obtained by fitting equation: $K_{m2} = k_{m2}\, e^{\delta\, FV/RT}$ to the data. The estimated parameters were: k_{m2} = 1.3 ± 0.4 nM, δ = 1.52 ± 0.05.

of the magnitude observed in our experiments. The fact that Na_x is inhibited by micromolar concentrations of intracellular eosin suggests that Na_x is mediated by a P-type ATPase. We propose that an endogenous P-type ATPase assumes a channel-like conformation upon membrane depolarization to voltages greater than +40 mV, producing currents several orders of magnitude larger than normal pump currents [13, 21]. The time course of Na_x activation varies, depending on the composition of the intracellular medium. This may reflect the tight interaction between cytoplasmic composition (cytoplasmic factor, intracellular Mg^{2+}) and channel gating.

Supported by NIH grant NS-22979.

References

1. Ackerman, M.J., Wickman, K.D., Clapham, D.E. 1994. Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J. Gen. Physiol.* **103:**153–179
2. Arellano, R.O., Woodward, R.M., Miledi, R. 1995. A monovalent cationic conductance that is blocked by extracellular divalent cations in *Xenopus* oocytes. *J. Physiol.* **484:**593–604
3. Barish, M. 1983. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol.* **342:**309–325
4. Baud, C. 1983. Developmental change of a depolarization-induced sodium permeability in the oocyte of *Xenopus laevis*. *Developm. Biology* **99:**524–528
5. Baud, C., Kado, R.T. 1984. Induction and disappearance of excitability in the oocyte of *Xenopus laevis*: A voltage-clamp study. *J. Physiol.* **356:**275–289
6. Baud, C., Kado, R.T., Marcher, K. 1982. Sodium channels induced by depolarization of the *Xenopus laevis* oocytes. *Proc. Natl Acad. Sci.* **79:**3188–3192
7. Bauer, C.K., Falk, T., Schwarz, J.R. 1996. An endogenous inactivating inward-rectifying potassium current in oocytes of *Xenopus laevis*. *Pfluegers Arch.* **432:**812–820
8. Dryer, S.E. 1994. Na^+-activated K^+ channels: A new family of large-conductance ion channels. *Trends Neurosci.* **17:**155–160
9. Dumont, J. 1972. Oogenesis in *Xenopus laevis* (Daudin). *Morph.* **136:**153–180
10. Ebihara, L. 1996. *Xenopus* connexin 38 forms hemi-gap-junctional channels in the nonjunctional plasma membrane of *Xenopus* oocytes. *Biophys. J.* **71:**742–748
11. Kowdley, G.C., Ackerman, S.J., John, III, I.E., Jones, L.R., Moorman, J.R. 1994. Hyperpolarization-activated chloride currents in *Xenopus* oocytes. *J. GenPhysiol.* **103:**217–230
12. Krause, J.D., Foster, C.D., Reinhart, P.H. 1996. *Xenopus laevis* oocytes contain endogenous large conductance Ca^{2+}-activated K^+ channels. *Neuropharmacology* **35:**1017–1022
13. Lafaire, A.V., Schwarz, W. 1986. Voltage dependence of the rheogenic Na^+/K^+ ATPase in the membrane of oocytes of *Xenopus laevis*. *J. Membrane Biol.* **91:**43–51
14. Lory, P., Rassendren, F.A., Richard, S., Tiaho, F., Nargeot, J. 1990. Characterization of voltage-dependent calcium channels

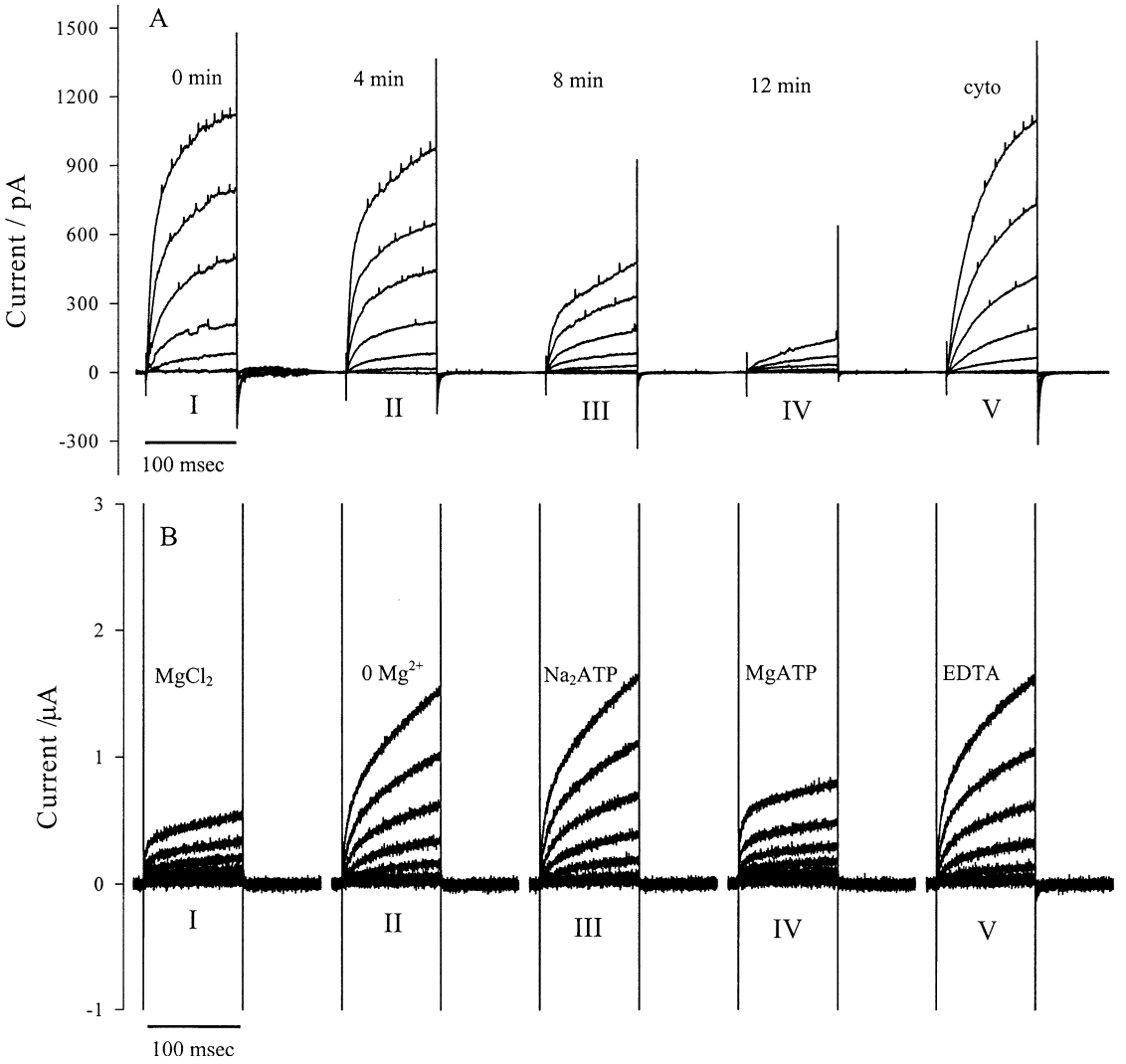


Fig. 9. Experiment showing that the presence of a cytoplasmic factor is essential for maintaining Na_x. In panel (*A*) Trace *I* shows the Na_x current in a giant patch shortly after excision to form the inside-out configuration. Bath solution contained (in mM): 70 KCl, 30 NaCl, 5 Na_2EDTA, 5 HEPES, pH = 7.5. Pipette solution was 110 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, pH = 7.5. Traces *II*, *III*, and *IV* show the Na_x current in the same patch at 4, 8, and 12 minutes after excision. Trace *V* shows the Na_x current after the membrane patch was reintroduced into the oocyte. In panel (*B*) the cut-open oocyte was internally perfused for 4 hours with solution containing (in mM) 100 NaCl, 2 BAPTA, 5 HEPES, pH = 7.5 prior to the experiment. Then 1 mM $MgCl_2$ was added (*I*) followed by a washout (*II*).After that, 5 mM Na_2ATP was added to the internal solution (*III*) followed by the addition of 5 mM MgATP (*IV*). The oocyte was perfused with 5 mm EDTA to ensure the removal of Mg^{2+}. The external solution was the same as the pipette solution in (*A*).

expressed in *Xenopus* oocytes injected with mRNA from rat heart. *J. Physiol.* **429:**95–112

15. Lu, L., Montrose-Rafizadeh, C., Hwang, T.C., Guggino, W.B. 1990. A delayed rectifier potassium current in *Xenopus* oocytes. *Biophys. J.* **57:**1117–1123
16. Methfessel, C., Witzemann, V., Takahashi, T., Mishina, M., Numa, S., Sakmann, B. 1986. Patch clamp measurements on *Xenopus laevis* oocytes: Currents through endogenous channels and implanted acetylcholine receptor and sodium channels. *Pfluegers Arch.* **407:**577–588
17. Miledi, R., Parker, I. 1984. Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol.* **357:**173–183
18. Parker, I., Miledi, R. 1987. Dependent calcium channels expressed in *Xenopus* oocytes injected with inositol trisphosphate activate a voltage-dependent calcium influx in *Xenopus* oocytes. *Proc. R. Soc. Lond.* **231:**27–36
19. Parker, I., Miledi, R. 1987. Tetrodotoxin-sensitive sodium current in native *Xenopus* oocytes. *Proc. R. Soc. Lond.* **232:**289–296
20. Peres, A., Mancinelli, E. 1985. Sodium conductance and the activation potential in *Xenopus laevis* eggs. *Pfluegers Arch.* **405:**29–36
21. Rakowski, R.F., Vasilets, L.A., Latona, J., Schwarz, W. 1991. A negative slope in the current-voltage relationship of the Na^+/K^+ pump in *Xenopus* oocytes produced by reduction of external $[K^+]$. *J. Membrane Biol.* **121:**177–187
22. Rettinger, J. 1999. Novel properties of the depolarization-induced endogenous sodium conductance in the *Xenopus laevis* oocyte. *Pfluegers Arch.* **437:**917–924

23. Staub O., Verrey, F., Kleyman, T.R., Benos, D.J., Rossier, B.C., Kraehenbuhl, J.P. 1992. Primary structure of an apical protein from *Xenopus laevis* that participates in amiloride-sensitive sodium channel activity. *J. Cell Biol.* **119:**1497–1506
24. Stühmer, W., Parekh, A. 1995. Electrophysiological recordings from *Xenopus* oocytes. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 341–356. Plenum Press, New York
25. Taglialatela, M., Toro, L., Stefani, E. 1992. Novel voltage clamp to record small, fast currents from ion channels expressed in *Xenopus* oocytes. *Biophys. J.* **61:**78–82
26. Taglietti, V., Toselli, M. 1988. A study of stretch-activated channels in the membrane of frog oocytes: Interactions with Ca^{2+} ions. *J. Physiol.* **407:**311–328
27. Takahashi, T., Neher, E., Sakmann, B. 1987. Rat brain serotonin receptors in *Xenopus* oocytes are coupled by intracellular calcium to endogenous channels. *Proc. Natl. Acad. Set.* **84:**5063–5067
28. Weber, W.-M., Liebold, K., Clauss, W. 1995. Amiloride-sensitive Na^+ conductance in native *Xenopus* oocytes. *Biochim. Biophys. Acta* **1239:**201–206
29. Weber, W.-M., Liebold, K.M., Reifarth, F.W., Clauss, W. 1995. The Ca^{2+} induced leak current in *Xenopus* oocytes is indeed mediated through a Cl^- channel. *J. Membrane Biol.* **148:**263–275
30. White, M.M., Aylwin, M. 1990. Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. *Mol. Pharmacol.* **37:**720–724
31. Yang, X.-C., Sachs, F. 1989. Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and calcium ions. *Science* **243:**1068–1071
32. Yang, X.-C., Sachs, F. 1990. Characterization of stretch-activated ion channels in *Xenopus* oocytes. *J. Physiol.* **431:**103–122