A delayed rectifier potassium current in *Xenopus* oocytes

Luo Lu, Chahrzad Montrose-Rafizadeh, Tzyh-Chang Hwang, and William B. Guggino
Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA

ABSTRACT A delayed voltage-dependent K^+ current endogenous to *Xenopus* oocytes has been investigated by the voltage-clamp technique. Both activation and inactivation of the K^+ current are voltage-dependent processes. The K^+ currents were activated when membrane potential was depolarized from a holding potential of −90 to −50 mV. The peak current was reached within 150 ms at membrane potential of +30 mV. Voltage-dependent inactivation of the current was observed by depolarizing the membrane potential from −50 to 0 mV at 10-mV increments. Voltage-dependent inactivation was a slow process with a time constant of 16.5 s at −10 mV. Removal of Ca^{2+} from the bath has no effect on current amplitudes, which indicates that the current is Ca^{2+}-insensitive. Tail current analysis showed that reversal potentials were shifted by changing external K^+ concentration, as would be expected for a K^+-selective channel. The current was sensitive to quinine, a K^+ channel blocker, with a K_i of 35 μM. The blockade of quinine is voltage-independent in the range of −20 to +66 mV. Whereas oocytes from the same animal have a relatively homogeneous current distribution, average amplitude of the K^+ current varied among oocytes from different animals from 30 to 400 nA at membrane potential of +30 mV. Our results indicate the presence of the endogenous K^+ current in *Xenopus* oocytes with characteristics of the delayed rectifier found in some nerve and muscle cells.

INTRODUCTION

Xenopus oocytes have been used as a very powerful tool to study the molecular mechanism of a variety of ion channels because they translate and process exogenous messenger RNA efficiently and are suitable to voltage- and patch-clamp techniques (see reference 7 for review). However, a number of endogenous currents have been observed in *Xenopus* oocyte membrane. These include: a Ca^{2+}-dependent Cl^- current (17) that can be induced by various Ca^{2+}-mobilizing neurotransmitters (8, 11, 20), by injection of Ca^{2+}-mobilizing second messenger inositol-1,4,5-triphosphate (21, 23), or by entry of Ca^{2+} through a voltage-dependent Ca^{2+} channel (4, 7); a voltage-dependent Ca^{2+} current, which can be blocked by Cd^{2+}, but not by organic Ca^{2+} channel blockers, such as nifedipine (9, 16, 19); a Na^+ current induced by depolarization of the membrane potential (22); a fast transient Ca^{2+}-independent K^+ current observed in follicle-enclosed oocytes (24); and a preliminary observation of the Ca^{2+}-insensitive K^+ current with the peak currents < 30–40 nA at the membrane potential of +30 mV (17, 25).

The present study demonstrates an endogenous Ca^{2+}-independent and quinine-sensitive K^+ current in *Xenopus* oocytes. Activation of the K^+ current is a voltage-dependent process with some properties of the delayed rectifier similar to K^+ currents in nerve membranes (6). Inactivation of the K^+ current is also a voltage-dependent process with a very slow time course. It has been observed that the average amplitude of the K^+ current varied from 30 to 400 nA, depending upon the individual animal, but the oocytes from the same animal had a homogeneous current size. The characterization of this endogenous K^+ current is quite important for the future studies on expressed exogenous K^+ channels in *Xenopus* oocytes.

Address correspondence to William B. Guggino, Dept. of Physiology, The Johns Hopkins University, School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205.

MATERIALS AND METHODS

Oocyte preparation

Adult female *Xenopus laevis* frogs (*Xenopus* I, Michigan) were anesthetized by immersion in a 0.15% tricaine methanesulfonate (Ayerst) solution for 30 min. A small incision was made on either side of the abdomen to remove several ovarian lobes. The lobes were torn apart and immersed in a Ca^{2+}-free OR-2 solution (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM Hepes-Tris, pH 7.5). Oocytes were defolliculated by incubation with the Ca^{2+}-free OR-2 solution containing 2 mg/ml collagenase (Sigma, Chemical Co., St. Louis, MO, type 1A) at room temperature (22–24°C) for 2–3 h. After digestion, oocytes were washed five times with OR-2 solution and five times with a modified Barth's solution (MBS, 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 15 mM Tris-HCl, pH 7.6, and penicillin 100 μg/ml, streptomycin 100 μg/ml). Stage 5–6 oocytes were selected and stored at 18°C in modified Barth's solution.

Voltage clamp

Two microelectrode voltage clamp experiments were performed in a continuously perfused bath (10 ml/min) at room temperature. The microelectrodes with resistances of 1.5 to 2.0 MΩ, filled with 3 M KCl, were used for delivering current pulses or for voltage clamping. The bath was connected through an Ag-AgCl-Agar-3 M-KCl bridge to voltage-recording amplifier (World Precision Instruments, Inc., New Haven, CT). The data were filtered with a eight-pole Bessel filter (Frequency Devices, Haverhill, MA) at 200 Hz. Voltage pulse protocols and data acquisition were performed by a pCLAMP program (Axon Instruments, Inc., Burlingame, CA). Leakages were eliminated by digital subtractions. Membrane currents were measured in two different Cl^--free and Ca^{2+}-free solutions which contained: 96 mM Na-gluconate, 2 mM K-gluconate, 12 mM Mg-gluconate, 5 mM EGTA, and 5 mM Hepes-NaOH, pH 7.4; or 96 mM NaOH, 2 mM KOH, 12 mM Mg gluconate, 5 mM EGTA, 5 mM Hepes, titrated with methanesulfonic acid (Fluka, AG, Buchs, Switzerland), pH 7.4. Inward membrane currents carried by intracellular Cl^- ion were completely abolished by addition of 500 μM 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, Fluka) or 500 μM anthracene-9-carboxylic acid (9-AC, Fluka) in the bath solution. It has been reported that internal perfusion of DIDs modified K^+ conductance in giant squid axon. We examined that epithelial cell Cl^- channel blockers, such as DIDS, 9-AC and *N*-phenylanthranilic acid (DPC), have no effect on the endogenous K^+ currents in *Xenopus* oocytes at concentration of 500 μM (14). All Cl^- channel blockers (DIDS or 9-AC) were freshly added to bath solution to a final concentration of 500 μM before each experiment.

RESULTS

Outward membrane currents in oocytes from an adult female *Xenopus* were elicited by depolarizing voltage steps from a resting potential of −90 to +66 mV for 1,200 ms (Fig. 1 *A*). When membrane potential was clamped at +30 mV, the outward current rose to a peak (285 ± 11 nA, $n = 34$, from the same donor) within 150 ms and then declined very slowly to a nonzero steady-state current. Replacement of bath solution with a Cl^--free and high-concentration Ca^{2+} solution (11.8 mM Ca^{2+} and 1 mM Mg^{2+} instead of 12 mM Mg^{2+} and 5 mM EGTA) had no effect on the peak or on the steady-state current. Because gluconate has been reported to affect the intracellular pH (26), in some experiments we replaced the Cl^- with methanesulfonate instead of gluconate. This substitution of anion did not change the characteristics of the outward current. In a long-duration recording, the peak current decreased by 27 ± 3% ($n = 17$) after 5 s of depolarization to +30 mV (Fig. 1 *B*). The decay phase at +30 mV was fitted by a single exponential ($e^{-t/\tau}$) with a time constant of 11.7 s. The voltage-current relationship showed that the currents were activated by depolarization of the membrane potential to a threshold potential of −50 mV, and outwardly increased at −15 mV similar to that of delayed rectifier K^+ current (Fig. 1 *C*).

The reversal potential (E_R) of the current was determined by the tail current analysis. Tail current experiments were done by depolarizing membrane potential from −90 to +30 mV for 150 ms and then repolarizing membrane potential to −165 mV at 15-mV intervals. Tail currents were recorded at various membrane potentials. Peak tail currents reversed the polarity from −90 to −105 mV with an external K^+ concentration of 2 mM (Fig. 2 *A*), as would be expected for a K^+-selective channel. Reversal potentials were plotted as a function of external K^+ concentration with a slope of 46 mV (Fig. 2 *B*) where K^+ concentration was increased by isotonic replacement of Na^+ with K^+.

Inactivation of the K^+ current appeared to be a voltage-dependent process and was studied by changing prepulse amplitude (Fig. 3 *A*). Depolarization prepulses were varied over the range of −90 to 0 mV for 6 s, and then a standard command pulse (+30 mV) was applied for 2.5 s. The ratio of current amplitudes was measured in the presence of depolarization prepulse (I) or in the absence of the prepulse (for I_{max}, the membrane potential was depolarized from −90 to +30 mV without a prepulse

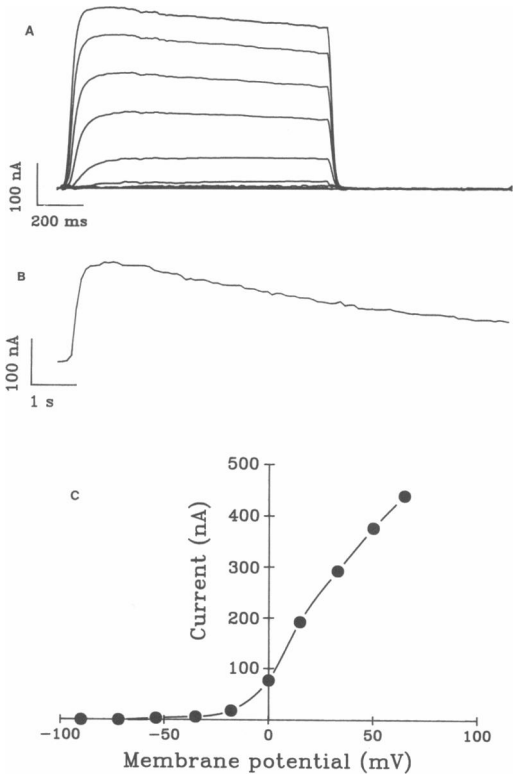

FIGURE 1 Membrane currents recorded from *Xenopus* oocytes in Cl^--free and Ca^{2+}-free solution with 500 μM 9-AC. (*A*) Outward currents were evoked by depolarizing the membrane potential from −90 to +68 mV in 16-mV increments. (*B*) Current during a prolonged depolarization of membrane potential to +30 mV for 10 s from a holding potential of −90 mV. (*C*) Outward currents measured at the end of 200 ms were plotted as a function of membrane potential after leakage subtraction.

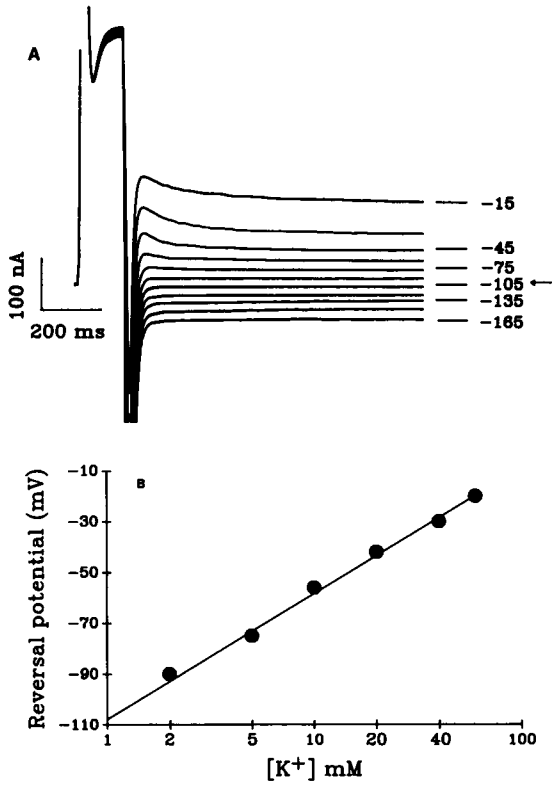

FIGURE 2 Current relaxation recording of reversal potential. (*A*) Tail current experiments were done by depolarizing membrane potential to +30 mV for 150 ms from a holding potential of −90 mV and then repolarizing membrane potential to voltages indicated at the end of each trace. The reversal potential (V_R) is indicated by arrow ([K^+]out = 2 mM). (*B*) Effect of external K^+ concentrations on the reversal potential of the tail currents. Points shown are the means of three independent experiments, where the standard error bars lie within the dots.

step). The ratio, I/I_{max}, was plotted as a function of the depolarization prepulse voltage (Fig. 3 *B*). I/I_{max} decreased as the depolarization prepulse increased from −50 to 0 mV, which indicates that inactivation of the K^+ current is a voltage-dependent process. In another series of experiments, the time course of the voltage-dependent inactivation processes was determined by changing the prepulse durations (Fig. 3 *C*). In this case, the depolarization prepulse was fixed at −10 mV and prepulse durations were increased by 600 ms. The logarithm of the ratio I/I_{max} was plotted as a function of prepulse duration (Fig. 3 *D*). A calculated time constant (τ = 16.5 s) was obtained from the slope.

The outward current was also characterized with K^+ channel blockers. External application of 10 to 500 μM quinine (QN) reduced the outward current by 25–98%, respectively, at membrane potential of +30 mV (Fig. 4 *A*). A concentration-response curve was obtained from the mean values of the fractional block ($1 - I/I_o$, where I was measured with the presence of blocker and I_o was obtained from the experiments with the absence of blocker) and plotted as a function of blocker concentrations (Fig. 4 *B*). All values were measured from current amplitude at the end of a 200-ms step to a membrane potential of 30 mV. Data were fitted to a theoretical curve calculated from a single-site Michaelis-Menten type saturation $I = I_o \times [QN]/(K_i + [QN])$, where K_i is the concentration of quinine causing a half-blocking of the maximal current. The value of K_i calculated from the theoretical curve was 35 μM. The blocking effect of quinine was reversible and was not dependent upon the change of membrane potential from −20 to +66 mV at concentration of 50 μM (Fig. 4 *C*). Conductance-voltage relationships of the current measured in control and in the presence of external application of 50 μM quinine show a parallel pattern, which also suggests that the block is voltage-independent (Fig. 4 *D*). The effects of other K^+ channel blockers were also examined by application to the external solution (Table 1). The outward current was reduced 12–15% by 10 mM tetraethylammonium (TEA) or 4-aminopyridine (4-AP, n = 4). Reduction of the peak currents were not observed by external applications of 10 mM barium, 1 μM apamin, or 10 nM charybdotoxin (n = 2).

Average current size varied greatly from animal to animal. The size of the average current in our experiments varied from 30 to 400 nA at membrane potential of +30 mV (Fig. 5 *A*). Two batches of oocytes were chosen from the average current distribution histogram, one from frog A with average current amplitude of 46 nA, and the other from frog B with average amplitude of 285 nA (Fig. 5 *B*). The current of frog A oocytes (histogram on left) distributed in the range of 26 to 75 nA, whereas the oocytes from frog B (histogram on right) presented much larger currents with a range of 200 to 400 nA.

DISCUSSION

This study characterizes a delayed rectifier K^+ current in *Xenopus* oocytes based on its functional and pharmacological properties. Because the current was recorded in Cl^--free and Ca^{2+}-free solution in the presence of Cl^- channel blockers, the Ca^{2+}-dependent Cl^- current and the inward Ca^{2+} current were completely eliminated. These conditions allow us to carefully characterize the K^+ current in *Xenopus* oocyte membrane.

Our results show that activation of the endogenous K^+ current is voltage-dependent. The voltage-dependent activation process is similar to that of delayed rectifier K^+

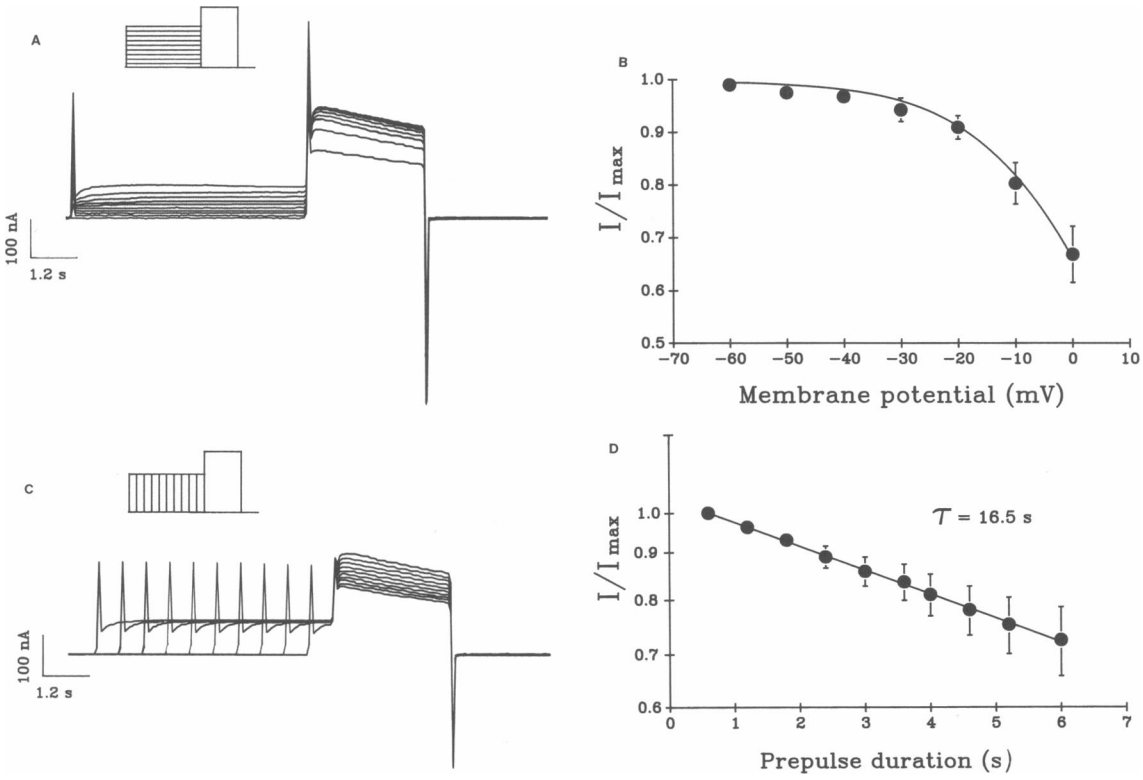

FIGURE 3 Voltage-dependent inactivation of the outward current determined by prepulse method. (*A*) Effect of prepulse depolarization on inactivation. Prepulse was stepped from −90 to 0 mV at 10-mV increments and maintained for 6 s. Then a command pulse to +30 mV was applied for 2.5 s. (*B*) The ratio (I/I_{max}) of the outward current during the prepulse (I) and in the absence of prepulse (I_{max}) is plotted as a function of prepulse voltage (n = 7). The solid line is the best fit of the Boltzman distribution with an e-fold change per 12 mV. The voltage for half-inactivation of the current is 8 mV. (*C*) Effect of changing prepulse duration on the onset of inactivation. Duration of the −10-mV prepulse was increased every 600 ms, and then a command pulse to +30 mV was applied. (*D*) Time course of voltage-dependent inactivation was studied by plotting the log of the ratio I/I_{max} (n = 6) as a function of prepulse duration (r^2 = 0.99). Time constant was calculated from the slope.

current in squid axon (6), molluscan neuron (29), or expressed K^+ channels from rat brain cDNA in *Xenopus* oocyte (5, 10, 27), but with a slower time course. From our observation (Fig. 1 *A*), the current reached a peak in 150 ms after depolarization of the membrane to +30 mV. However, the activation was faster than the K^+ channel from expressed kidney cDNA (28). Prepulse experiments (Fig. 3 *A*) showed that the K^+ currents were decreased by a prepulse to −50 mV for 6 s. This indicates that the inactivation of the K^+ current also appears to be a voltage-dependent process. The voltage-dependent inactivation process of the outward current shows a very slow time course, especially, when compared with the delayed outward current found in molluscan neuron (1, 2). The time constant of inactivation was 16.5 s (Fig. 3 *D*), which was obtained by changing prepulse durations at membrane potential of −10 mV and approximated by a single exponential function of decay. The inactivation phase of the K^+ current was also observed as single exponential decay in a long-duration recording at membrane potential of +30 mV. The time constant of inactivation at membrane potential of +30 mV was 11.7 s.

Reversal potentials measured from different concentration of external K^+ suggested that K^+ ions were responsible for the outward current. The reversal potential (E_R) value measured from 10-fold difference of external K^+ concentration was 46 mV, which is close to the slope of 57 mV change predicted by the Nernst equation for a K^+-selective channel. As reported by Frech (10), substitution of external Na^+ with *N*-methyl-*D*-glucamine$^+$ (NMDG) had a negligible effect on the expressed rat brain K^+ tail currents in *Xenopus* oocytes. From their experiment, the 10-fold change in external K^+ for the expressed rat brain K^+ channel showed a slope of 48 mV. The other report also found that the 10-fold external K^+ concentration change gives a slope of 48 mV for an expressed A-type Shaker K^+ channel in *Xenopus* oocytes (15). Therefore, a reason for the less than ideal shift in reversal potential may be that the channel is permeable to other ions. The other possibility is an

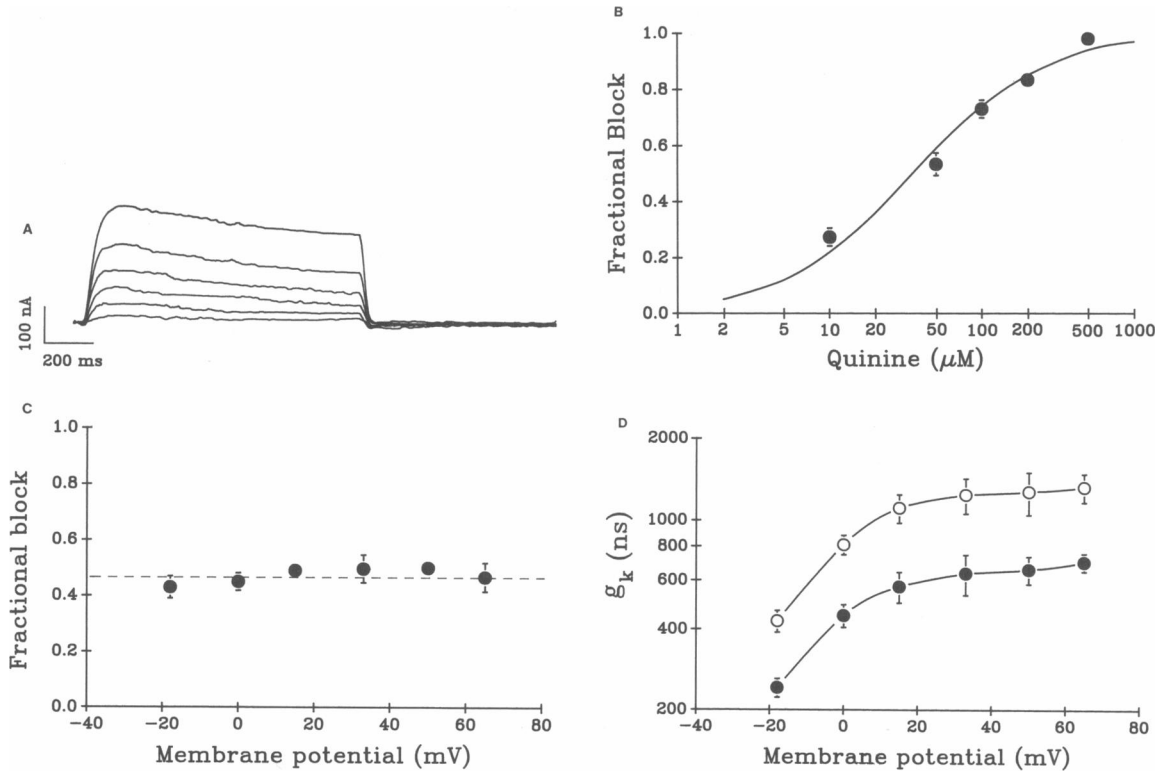

FIGURE 4 Blocking of outward K^+ current by application of quinine in the external solution. (*A*) Concentration dependence of quinine blockade of outward K^+ current. Membrane potential was stepped from a holding potential of −90 to +66 mV. The top trace was recorded in the absence of quinine. Other traces represent different concentrations (10, 50, 200, and 500 μM) of quinine applied. (*B*) Dose dependence of quinine on fractional block of the current ($1 - I/I_o$) at membrane potential of +30 mV. *(Solid line)* Theoretical line predicted from Michaelis-Menten type saturation equation with a K_i of 35 μM. Filled dots are mean values of the fractional block from three independent experiments. (*C*) Voltage-independent blocking of quinine on the outward K^+ current. Quinine concentration in external solution was 50 μM. (*D*) Conductance-voltage relationship obtained from control experiments *(open dots)* and external application of 50 μM quinine *(solid dots)*. Conductances were calculated from the ions conductance equation defined by Hodgkin and Huxley. The estimated value for the K^+ equilibrium potential (E_k) was −90 mV. Data in (*C*) and (*D*) are plotted as means with standard error bars ($n = 3$).

accumulation of K^+ ions in the noncellular fibrous layer after depolarization pulses (7). We have avoided the accumulation by extending the time interval between every voltage pulse to 30 s and by permitting > 5 min recovery period between each tail current experiment to allow the accumulated K^+ ions to diffuse away from the fibrous layer.

TABLE 1 Effect of K^+ channel blockers on the endogenous outward current in *Xenopus* oocyte

Blockers	Concentrations of the blocker	Fractional block ($1 - I/I_0$)
Quinine	35 μM	50%
4-AP	10 mM	12%
TEA	10 mM	15%
Barium	10 mM	0
Apamin	1 μM	0
CTX	10 nM	0

All blockers were applied in external bath solution at membrane potential of +30 mV. Data were measured after leakage subtraction.

Our results show that the endogenous K^+ current has distinct pharmacological properties. It is sensitive to quinine with a K_i of 35 μM at membrane potential of +30 mV. It has been reported that application of quinine and its isomer quinidine block voltage-activated delayed K^+ currents in *Myxicola* giant axon (31) and in molluscan pacemaker neurones (12). The K_i for quinidine was 28 ± 13 μM for the K^+ current in molluscan pacemaker neurones at membrane potential of +20 mV. For the molluscan channels, quinidine block was a voltage-dependent process with a calculated blocker binding site at 70–80% through the membrane electric field (12). Because quinine is positively charged at pH 7.4, we expected that quinine would block the K^+ current in a voltage-dependent fashion. However, our results showed no evidence for a voltage-dependent block in the voltage range of −20 to +66 mV (external concentration of

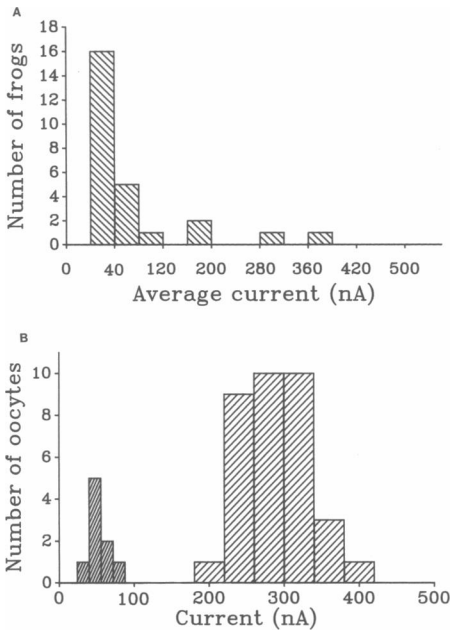

FIGURE 5 Distribution of outward currents in *Xenopus* oocyte at membrane potential of +30 mV. (*A*) Distribution of the average currents in frogs. Mean values of the K^+ current measured from 26 frogs are distributed with the increment interval of 80 nA. (*B*) Distribution of the K^+ currents in oocytes from two individual frogs. Left histogram presents the K^+ current distribution from frog A with an interval of 20 nA (mean = 47 nA, n = 9); right histogram shows the distribution of K^+ current from frog B with an interval of 50 nA (mean = 285 nA, n = 34).

quinine was 50 μM). This observation suggests that for the *Xenopus* oocyte channel, quinine does not enter the channel deep enough to sense the electric field (32).

It has been shown that K^+ channel blockers selectively block different type of K^+ channels. 4-AP externally blocks the transient K^+ currents, termed A-current, but has much less effect on some delayed outward currents (15, 29). TEA causes a reversible block in some delayed outward currents, such as K current in nerve K^+ channels (29), but has little effect on A-current or some slow outward current (C current) in neurones (29). In *Xenopus* oocyte membrane, the endogenous K^+ current was less sensitive to external 4-AP or TEA (12–15% reduction of the peak current at 10 mM 4-AP or TEA) than the expressed A-current (15, 30) or the delayed K^+ current induced by injection of rat brain cDNA (5, 27). The outward currents in oocytes were insensitive to 10 mM barium, 10 nM charybdotoxin (CTX) and 1 μM apamin. These blockers have been reported to block large conductance Ca^{2+}-activated K^+ channels (maxi K channels) or small conductance Ca^{2+}-activated K^+ channels (SK channels) in many preparations (3, 13, 15, 18).

As reported previously (4, 7), amplitude of the K^+ current in oocytes from most of frogs was in the range of 30 to 40 nA at membrane potential of +30 mV. However, we found that oocytes from some frogs had larger K^+ current. 15% of tested animals produced oocytes with average currents larger than 150 nA, and oocytes from individual frog have relatively similar current amplitude. This statistical information should be useful for investigators who attempt to express the exogenous K^+ channels in *Xenopus* oocytes. It seems to be essential that oocytes from the same frog be used for experiments and controls.

In summary, this study characterizes a delayed rectifier K^+ current in *Xenopus* oocyte. The K^+ current possesses voltage-dependent activation and inactivation properties. The time course of the voltage-dependent inactivation is very slow. The endogenous K^+ current is very sensitive to external application of quinine and less sensitive to 4-AP and TEA, or insensitive to other K^+ channel blockers. These pharmacological properties distinguish this endogenous channel from those channels recently expressed in *Xenopus* oocyte membrane (5, 27). Our data also provide the statistical information to indicate that the average current size for oocytes from frogs is distributed in a wide range, but current size from an individual frog is relatively consistent.

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