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Expression of an inwardly rectifying K^+ channel from rat basophilic leukemia cell mRNA in *Xenopus* oocytes

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Rat basophilic leukemia cells (RBL-2H3) have previously been shown to contain a single type of voltage-activated channel, namely an inwardly rectifying K^+ channel, under normal recording conditions. Thus, RBL-2H3 cells seemed like a logical source of mRNA for the expression cloning of inwardly rectifying K^+ channels. Injection of mRNA isolated from RBL-2H3 cells into *Xenopus* oocytes resulted in the expression of an inward current which (1) activated at potentials negative to the K^+ equilibrium potential (E_K), (2) decreased in slope conductance near E_K, (3) was dependent on $[K^+]_o$ and (4) was blocked by external Ba^{2+} and Cs^+. These properties were similar to those of the inwardly rectifying K^+ current recorded from RBL-2H3 cells using whole-cell voltage clamp. Injection of size-fractionated mRNA into *Xenopus* oocytes revealed that the current was most strongly expressed from the fraction containing mRNA of approximately 4–5 kb. Expression of this channel represents a starting point for the expression cloning of a novel class of K^+ channels.

Inward rectifier; Potassium channel; *Xenopus* oocyte; RNA expression

1. INTRODUCTION

Inwardly rectifying K^+ channels permit a greater influx of K^+ ions at potentials negative to the K^+ equilibrium potential, E_K, than efflux of K^+ ions at depolarized potentials. At potentials negative to E_K, the inwardly rectifying K^+ channels conduct an inward current which decreases near E_K and shows rectification. At potentials up to about 30 mV positive to E_K a small conductance of K^+ in the outward direction is seen. The inwardly rectifying properties of a resting K^+ conductance in skeletal muscle fibers were first described by Katz [1], and the biophysical characteristics of this channel in starfish eggs were worked out by Hagiwara and Takahashi [2]. Inwardly rectifying K^+ channels (I_{K1}) have been found in many cell types, including cardiac myocytes, and are thought to play a role in determining the resting membrane potential.

Another type of inwardly rectifying K^+ channel is activated by neurotransmitters coupled to G proteins. For example, acetylcholine activates an inwardly rectifying K^+ channel ($I_{K.ACh}$) in cardiac atrial myocytes [3]. Activation of this channel contributes to the control of heart rate. These two types of hyperpolarization-activated inwardly rectifying K^+ channels, the resting inwardly rectifying K^+ channel (I_{K1}) and the G protein-activated inwardly rectifying K^+ channel ($I_{K.ACh}$), have not previously been cloned or expressed from exogenous mRNA.

Rat basophilic leukemia cells (RBL-2H3) have been shown to have an inwardly rectifying K^+ channel. This is the only voltage-dependent channel found in these cells under normal recording conditions [4,5]. Thus, RBL-2H3 cells seemed like a logical source of mRNA for the expression and eventual cloning of inwardly rectifying K^+ channels. We report that injection of *Xenopus* oocytes with mRNA isolated from RBL-2H3 cells resulted in the expression of an inwardly rectifying K^+ channel.

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2. MATERIALS AND METHODS

2.1. *Isolation of RNA*

RBL-2H3 cells were a generous gift from Dr Henry Metzger (NIH). RBL-2H3 cells were maintained in Minimum Essential Media (Gibco) supplemented with 10% fetal calf serum (Gibco) and grown at 37°C in 5% CO_2. RBL-2H3 RNA was prepared using the guanidinium isothiocyanate method [6]. Poly(A)$^+$ RNA (0.35 mg) was fractionated according to size on a 10–30% sucrose gradient [7].

2.2. *Electrophysiological recording*

Oocytes were removed from female *Xenopus laevis* (Nasco, Fort Atkinson, WI) under tricaine anesthesia (1.5 g/l) and defoliculated manually after treatment with 5 mg/ml collagenase (Sigma type 1A) in OR-2 solution (see below) for 2 h at room temperature in a shaking water bath. Oocytes were injected 19–26 h later with RNA (67–184 ng of total RNA in 0.1 M KCl) and incubated at 19°C for 3–6 days in OR-2 solution containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$ and 10 mM HEPES, pH 7.4 (NaOH), 230 mOsm/kg supplemented with gentamicin (50 μg/ml).

The two-electrode voltage-clamp technique was used to record K^+ currents with an AxoClamp (Axon Instruments, Foster City, CA)

using the virtual ground circuit. Electrodes were pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) and filled with 3 M KCl (resistance < 1 MΩ). Currents were initially recorded in OR-2 without gentamicin from a holding potential of −80 mV. Currents were elicited with voltage steps of 400 ms to potentials between −160 and +80 mV every 3 s. The solution was then changed to a high K^+, Ca-free solution containing 90.2 mM KCl, 20 mM $MgCl_2$ and 10 mM HEPES, pH 7.4 (KOH), 230 mOsm/kg. The Ca^{2+}-free solution was used to avoid conductance through the endogenous Ca^{2+} channel and activation of the Ca^{2+}-dependent Cl^- channel. The holding potential in the high K^+ solution was −10 mV (the zero current potential). All experiments were done at room temperature, 22–24°C. Currents were filtered at 300 Hz and sampled at 1 ms/point. A Macintosh IIcx computer (Apple) and a MacAdios II data acquisition board (GW Instruments, Somerville, MA) were used to stimulate and record using software designed by Dr Stephen Ikeda. Data analysis was performed using Igor (WaveMetrics, Lake Oswego, OR) and software designed by Dr Stephen Ikeda.

The whole-cell patch-clamp technique [8] was used to record inwardly rectifying K^+ currents from RBL-2H3 cells. The external solution contained 140 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES and 15 mM glucose, pH 7.4 (NaOH), 307 mOsm/kg. The internal solution contained 120 mM K isethionate, 20 mM KCl, 10 mM HEPES, 1.1 mM EGTA, 4 mM MgATP, 0.1 mM NaGTP and 0.1 mM $MgCl_2$, pH 7.2 (KOH), 288 mOsm/kg. Cells were voltage-clamped with an Axopatch 1-D (Axon Instruments) to a holding potential of −10 mV and stepped to potentials between −160 and +80 mV every 3 s. Currents were filtered at a frequency of 5 kHz and sampled at 250 μs/point.

3. RESULTS

The primary conductance in RBL-2H3 cells is carried by inwardly rectifying K^+ channels [4,5]. Fig. 1 (inset) shows whole-cell currents from an RBL-2H3 cell elicited by voltage steps from a holding potential of −10 mV to membrane potentials between −160 and −20 mV. The current–voltage (*I/V*) relationship of the peak inward current for potentials between −160 and +80 mV displays inward rectification (Fig. 1). The inwardly rectifying current was blocked by 1 mM $BaCl_2$ added to the external solution (Fig. 1). The current shows partial inactivation during voltage steps to potentials between −160 and −120 mV. This inactivation has been shown to be due to a voltage-dependent block by external Na^+ [5]. No other conductances are present under these recording conditions.

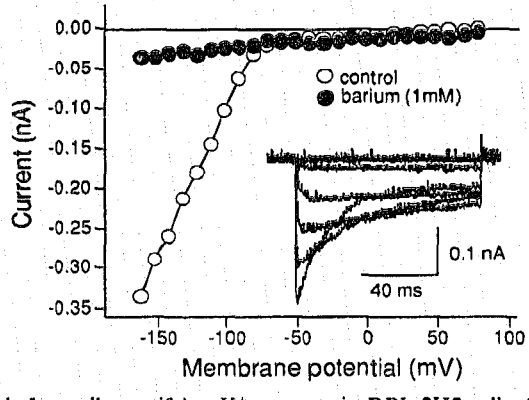

Fig. 1. Inwardly rectifying K^+ currents in RBL-2H3 cells. Currents were recorded in the whole-cell voltage-clamp mode from a holding potential of −10 mV to voltage steps from −160 to +80 mV in 10 mV increments. Inset: current traces shown are for every other voltage step from −20 to −160 mV. The *I/V* curve is plotted for peak currents in the absence (○) and presence of 1 mM $BaCl_2$ (●) in the external solution.

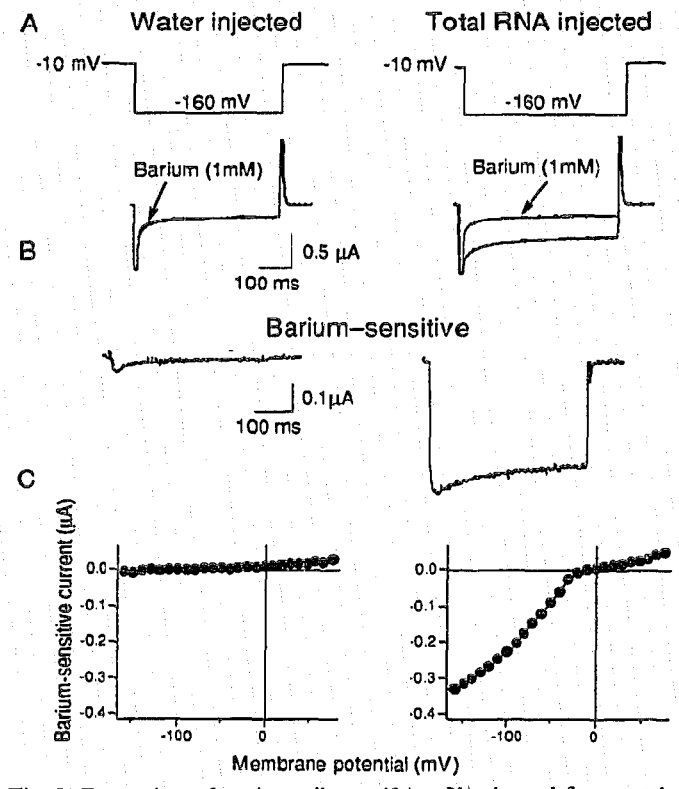

Fig. 2. Expression of an inwardly rectifying K^+ channel from total RNA isolated from RBL-2H3 cells in *Xenopus* oocytes. A. Currents were recorded in a high K^+ (90 mM) external solution using the two electrode voltage clamp technique. The voltage paradigm shows that the oocytes were voltage clamped to a holding potential of −10 mV and stepped to −160 mV for 400 ms. The current traces under the voltage paradigm show raw records from a water-injected oocyte and an oocyte injected with 184 ng of total RNA after 4 days of incubation. The small inward current in the water-injected oocyte was little affected by superfusion with 1 mM $BaCl_2$ (left). The larger inward current expressed in the oocyte injected with total RNA was inhibited by 1 mM $BaCl_2$ (right). B. The current in the presence of barium was subtracted from the current in the absence of barium and is shown as the barium-sensitive current. C. The *I/V* plots of the barium-sensitive inward currents (measured at the end of the voltage step) are shown for both water and total RNA injected oocytes.

Oocytes injected with total RNA isolated from RBL-2H3 cells expressed small inward currents when recorded in OR-2 containing 2.5 mM $[K^+]_o$. To enhance detection of an inwardly rectifying K^+ channel, currents were recorded in high external K^+ solution (90 mM). Since inwardly rectifying K^+ channels are blocked by external $BaCl_2$, the sensitivity of the currents to external $BaCl_2$ was used to determine expression of this channel in oocytes. Fig. 2 shows that oocytes injected with total RNA isolated from RBL-2H3 cells express an inwardly

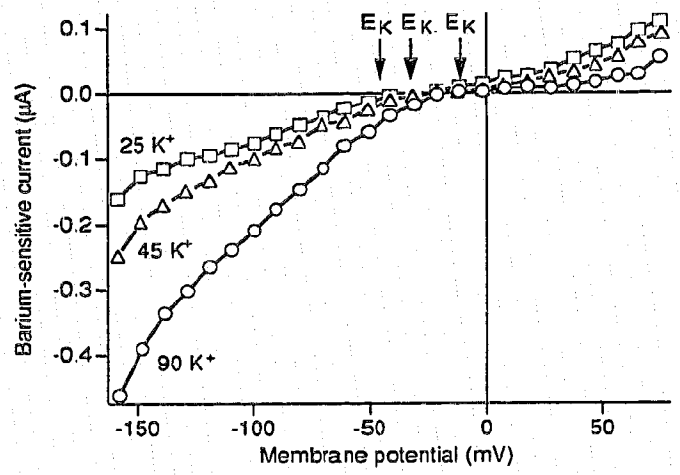

Fig. 3. Dependence of the barium-sensitive inwardly rectifying current on the external K^+ concentration. The *I/V* curve shows the barium-sensitive current recorded in three different external K^+ concentrations, 25 K^+ (□), 45 K^+ (△) and 90 K^+ (○) for an oocyte injected with 67 ng total RNA isolated from RBL-2H3 cells. The arrows indicate the depolarizing shift of the K^+ equilibrium potential, E_K, with increasing $[K^+]_o$.

rectifying channel. Currents were recorded from both water and total RBL-2H3 RNA (184 ng) injected oocytes after 4 days of incubation. The oocytes were voltage-clamped to a holding potential of −10 mV (the zero holding current potential in 90 mM K^+) and stepped to a membrane potential of −160 mV (Fig. 2A). In the water-injected oocyte a voltage step to −160 mV elicited a small endogenous inward current which was little affected by superfusion with 1 mM $BaCl_2$. The oocyte injected with total RNA displayed a larger inward current which was blocked by superfusion with 1 mM $BaCl_2$ (Fig. 2A) and reduced by 1 mM CsCl (not shown). To determine the barium-sensitive current, the current recorded in 1 mM $BaCl_2$ was digitally subtracted from the current in the absence of $BaCl_2$ (Fig. 2B). *I/V* plots of the barium-sensitive inward current, measured at the end of 400 ms voltage steps to membrane potentials between −160 and +80 mV, show that oocytes injected with total RNA isolated from RBL-2H3 cells expressed a barium-sensitive, inwardly rectifying K^+ channel (Fig. 2C). The amplitude of the barium-sensitive inward current measured at the end of a step to −160 mV was −0.224 ± 0.04 μA (mean ± SEM, n = 4) for oocytes injected with total RNA and −0.015 ± 0.01 μA (n = 4) for control oocytes injected with water. The endogenous inward current seen in water-injected oocytes appeared to be seasonally expressed. Oocytes from the same *Xenopus* displayed this current in the winter, but not during the fall.

The depencence of the amplitude of the barium-sensitive inward current expressed from total RNA isolated from RBL-2H3 cells on the external potassium concentration, $[K^+]_o$ is shown in Fig. 3. The amplitude of the barium-sensitive current increased with increasing $[K^+]_o$ (KCl substituted for NaCl to maintain osmolarity). The slope conductance decreased near E_K for each change in $[K^+]_o$. In 25 mM $[K^+]_o$ the slope conductance decreased near E_K, but in 90 mM $[K^+]_o$ the slope conductance decreased at a potential negative to E_K.

Sucrose gradient fractions of poly(A)$^+$ mRNA isolated from RBL-2H3 cells were injected into *Xenopus* oocytes. Fig. 4A shows a bar graph of the amplitudes of the barium-sensitive current from individual oocytes injected with sucrose gradient fractions A–J (two oocytes per fraction). Current amplitudes were measured at the end of a 400 ms voltage step to −160 mV from a holding potential of −10 mV in 90 mM $[K^+]_o$. Injection of fraction D which contained mRNA of approximately 4–5 kb resulted in the expression of the largest barium-sensitive inward current. Fig. 4B compares the barium-sensitive currents from fractions C and D. Injection of

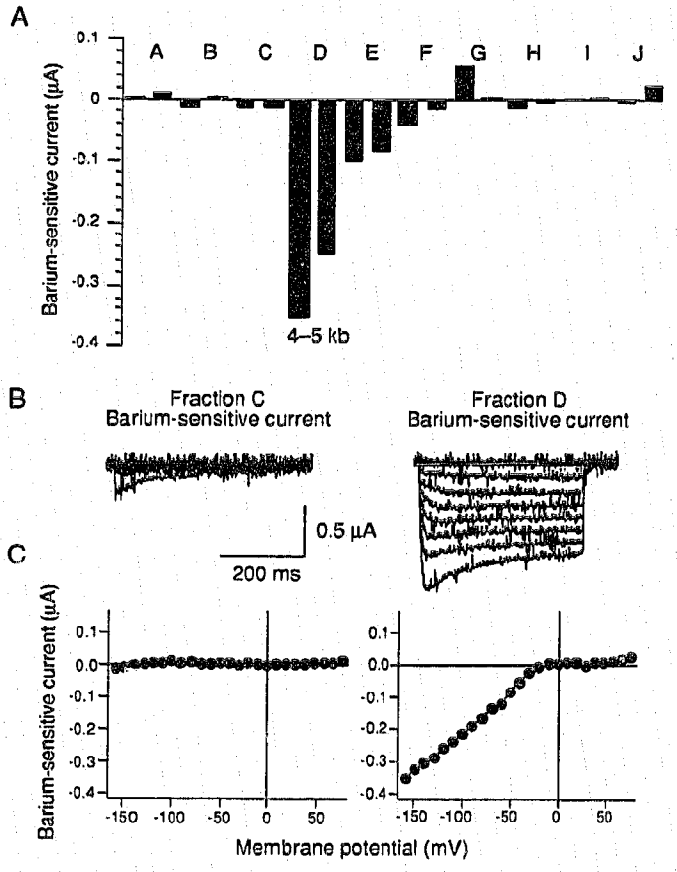

Fig. 4. Expression of an inwardly rectifying K^+ channel from size fractionated RNA. A. This histogram shows the amplitude of the barium-sensitive current from single oocytes 4 days after injection of RNA fractions A–J from a sucrose gradient (2 oocytes per fraction). Fraction D (4–5 kb) gave the largest inward current. B. Traces of the barium-sensitive currents recorded in a high K^+ (90 mM) external solution and are shown for voltage steps from −160 to 0 mV in 20 mV increments for oocytes injected with fractions C and D. C. *I/V* plots of the current amplitude (measured at the end of the voltage step) show that fraction D expressed an inwardly rectifying channel while fraction C did not show expression.

fraction C resulted in the expression of a small barium-sensitive inward current similar to that seen in control oocytes injected with water (see Fig. 2B). Injection of fraction D resulted in the expression of larger barium-sensitive inward currents. The *I/V* curve of barium-sensitive currents expressed from fraction D showed inward rectification (Fig. 4C).

The inwardly rectifying currents expressed in the oocyte show an apparent inactivation at potentials more negative than −100 mV. Inactivation of the inwardly rectifying current during voltage steps to potentials more negative than −100 mV has been shown to be due to a block by external Na^+ [5,9]. While there is no extracellular Na^+ present, 20 mM Mg^{2+} is present and probably contributes to the inactivation of this current.

4. DISCUSSION

Under normal recording conditions, rat basophilic leukemia cells (RBL-2H3) have a single type of voltage-activated channel, namely an inwardly rectifying K^+ channel [4,5]. Consequently, we chose RBL-2H3 cells as a source of mRNA for the expression of inwardly rectifying K^+ channels in *Xenopus* oocytes. The single channel conductance of the inwardly rectifying K^+ channel in RBL-2H3 cells (26 pS in 130 K^+) [5] is identical to the inwardly rectifying K^+ channel, I_{K1}, in cardiac ventricular myocytes (27 pS in 145 K^+) [10]. Expression of this channel from mRNA isolated from RBL-2H3 cells may lead to the eventual cloning of the heart inward rectifier, I_{K1}.

RNA from RBL-2H3 cells, when injected into *Xenopus* oocytes, resulted in the expression of an inwardly rectifying channel which was blocked by external $BaCl_2$ (1 mM) and CsCl (1 mM). An endogenous inward current found in oocytes appeared to be insensitive to $BaCl_2$. The inwardly rectifying K^+ channel has been shown to be blocked by Ba^{2+} and Cs^+ in many cell types including cardiac ventricular myocytes, mouse macrophage tumor cells, skeletal muscle, starfish and tunicate eggs, and RBL-2H3 rat basophilic leukemia cells [4,5,10–17]. We used this characteristic of barium-sensitivity to detect expression of this channel from exogenous mRNA injected into *Xenopus* oocytes. The inward current expressed from the injection of mRNA isolated from RBL-2H3 cells activated at potentials negative to the K^+ equilibrium potential (E_K), decreased in slope conductance near E_K and was dependent on $[K^+]_o$. These properties were similar to those of the inwardly rectifying K^+ current recorded from RBL-2H3 cells using whole-cell voltage clamp. Injection of size-fractionated mRNA into *Xenopus* oocytes revealed that the current was most strongly expressed from the fraction containing mRNA of approximately 4–5 kb.

Inwardly rectifying K^+ currents show rectification, or a decrease in slope conductance near E_K. The mechanism of inward rectification in cardiac ventricular myocytes has been shown to be due to an open channel block by intracellular Mg^{2+} [18,19]. Hille and Schwarz [20] modelled channel rectification produced by an internal monovalent blocker and predicted that the channel conductance would rectify near E_K with changes in $[K^+]_o$. A divalent internal blocker, such as Mg^{2+}, would also produce rectification near E_K, except in high $[K^+]_o$. In high $[K^+]_o$, the membrane potential where rectification begins to occur would be negative to E_K because Mg^{2+} has a higher valence and thus a greater voltage dependency for entry into the channel pore than the monovalent permeant ion [20].

Modulation of the inwardly rectifying K^+ current has been shown in a variety of cell types. The inwardly rectifying K^+ channels in RBL-2H3 cells, mouse renal juxtaglomerular cells and human dermal fibroblasts are inhibited by intracellular GTPγS [21–23]. Angiotensin II, substance P and thyrotropin-releasing hormone have been shown to inhibit the inwardly rectifying K^+ channels in mouse renal juxtaglomerular cells, rat neurons and GH_3 pituitary cells, respectively [22,24,25]. The inhibitory effects of GTPγS in RBL-2H3 cells and of substance P in neurons are mediated through a pertussis toxin-insensitive G protein [21,26].

In cardiac atrial myocytes a different inwardly rectifying K^+ channel, $I_{K,ACh}$, is activated by acetylcholine, adenosine and somatostatin through pertussis toxin-sensitive G proteins [3,27–30]. Somatostatin and enkephalin activate a similar channel through pertussis toxin-sensitive G proteins in both neurons and clonal pituitary cells [31–35]. Thus, G proteins may modulate both activation and inhibition of different inwardly rectifying K^+ channels. We anticipate that these channels may be members of a diverse family of inwardly rectifying K^+ channels.

We have expressed an inwardly rectifying K^+ channel in *Xenopus* oocytes from exogenous mRNA isolated from RBL-2H3 rat basophilic leukemia cells. To our knowledge this is the first reported expression in *Xenopus* oocytes of an inwardly rectifying K^+ channel.

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