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Inward Rectification of the MinK Potassium Channel

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Abstract. The minK protein induces a slowly activating voltage-dependent potassium current when expressed in *Xenopus* oocytes. We have used macroscopic minK currents to determine the open channel current-voltage relationship for the channel, and have found that the minK current is inwardly rectifying. The channel passes inward current at least 20-fold more readily than outward current. Both rat and human minK exhibit this property. The rectification of minK is similar to that reported for a slow component of the cardiac delayed rectifier, strengthening the hypothesis that minK is responsible for that current.

Key words: MinK — Potassium current — Rectification — *Xenopus* oocytes — Cardiac delayed rectifier

Introduction

Potassium channels perform a number of important cellular functions, including maintenance of resting potential, repolarization of action potentials, and potassium transport. By cloning the genes encoding potassium channels and studying these proteins in relative isolation, it is possible to gain insight into the regulation of these functions. Of the potassium channels cloned thus far, all but one have multiple membrane-spanning regions and share a conserved pore region (Kaczmarek, 1991; Ho et al., 1993; Kubo et al., 1993). The one exception is minK (also called I_{sk}), a small protein that induces a slowly activating voltage-dependent potassium current when expressed in the oocytes of the frog *Xenopus laevis*.

Because the minK protein contains only 129–130 amino acids and one proposed transmembrane helix, it differs from all other known potassium channels (Fig. 1) (Takumi, Ohkubo & Nakanishi, 1988). The minK current was first seen in *Xenopus* oocytes that had been injected with rat uterine RNA (Boyle et al., 1987); the protein is now known to be expressed in heart (Folander et al., 1990), uterus (Pragnell et al., 1990), T-lymphocytes (Attali et al., 1992), and secretory epithelia (Sugimoto et al., 1990). The function of minK in these tissues is unclear. On the basis of the kinetics of the minK current in oocytes, however, some researchers have suggested that it carries the slow component of the cardiac delayed rectifier current (Folander et al., 1990), but this has not been proven. MinK blockers can interfere with lymphocyte activation (Attali et al., 1992), but no slow potassium current has been reported in these cells. In secretory epithelia, the minK protein has been localized to the apical membranes of cells in kidney proximal tubule, submandibular gland (Sugimoto et al., 1990), and the stria vascularis of the cochlear duct (Sakagami et al. 1991),where it is proposed to play a role in potassium secretion.

To understand more fully the physiological role of the minK protein, we have studied the characteristics of the current expressed in *Xenopus* oocytes. We have found that the minK current is strongly inwardly rectifying. This property should be considered in future attempts to define a physiological role for minK.

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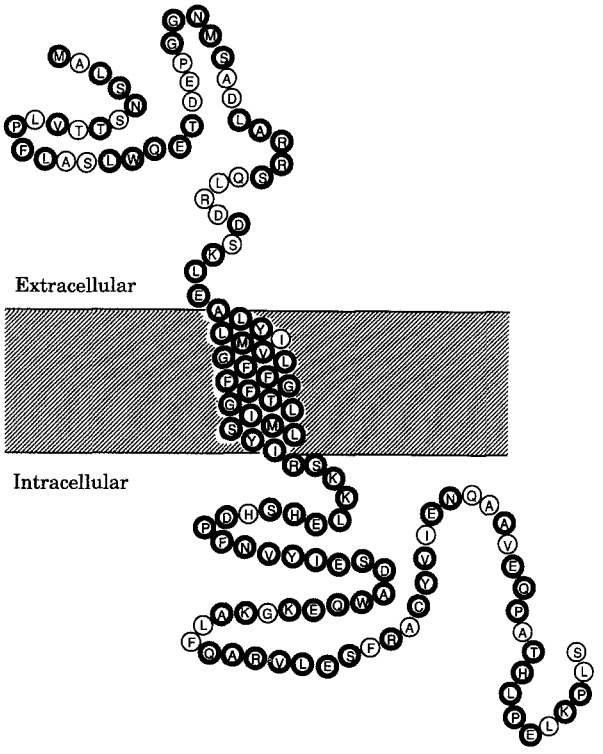

Fig. 1. Sequence and proposed transmembrane topology of the rat minK protein (Takumi et al., 1988). Residues conserved in the human minK protein are shaded (Murai et al., 1989). The evidence for this orientation is that a residue in the carboxyl-terminal domain is a substrate for protein kinase C (Busch et al., 1992), and an epitope inserted into the amino-terminal domain is recognized by extracellular antibodies (E.M. Blumenthal and L.K. Kaczmarek, *manuscript submitted*).

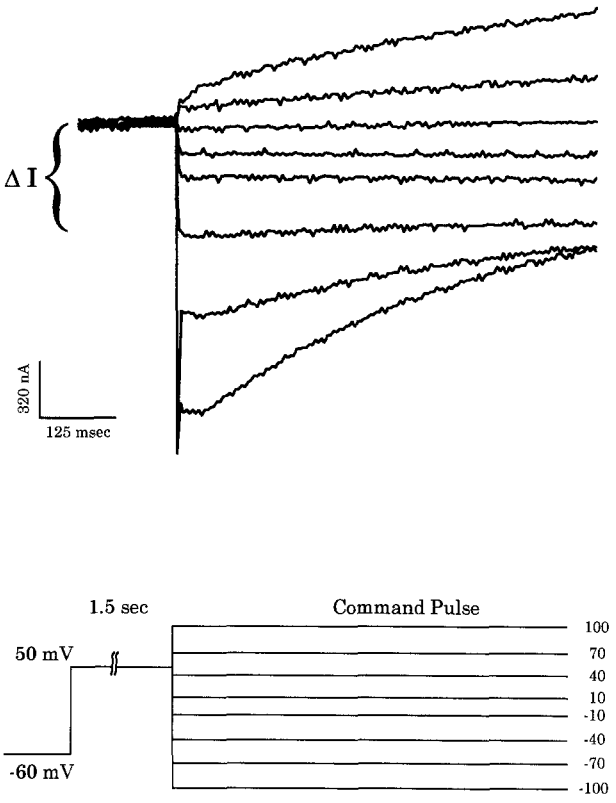

Fig. 2. Currents from an oocyte expressing rat minK. The voltage protocol is shown at the bottom. The instantaneous current step, ΔI, was measured as the difference in current between the end of the prepulse and the very beginning of the command pulse (disregarding the capacitive transient), as shown for the pulse to -40 mV. The slow increase in current at 100 mV is due to an endogenous sodium current (Baud, Kado & Marcher, 1982), and the small, brief increases in inward current at -70 and -100 mV are due to an endogenous chloride current (Peres & Bernardini, 1983).

Materials and Methods

In Vitro Transcription and Oocyte Injection

The constructs used were an artificial rat minK gene (Hausdorff et al., 1991) with a $K_v1.5$ 3′ untranslated region (E.M. Blumenthal & L.K. Kaczmarek, *manuscript submitted*), rat $K_v1.6$ (Swanson et al., 1990), and a human minK construct (a gift of Dr. R. Swanson; Merck, Sharp and Dohme). Capped run-off transcripts were prepared and purified, and stage V and VI *X. laevis* oocytes (Dumont, 1972) were isolated and injected as described previously (Blumenthal & Kaczmarek, 1992). Oocytes were injected with 50 nl of RNA at 0.2 mg/ml (rat minK), 0.05 mg/ml (human minK), or 0.02 mg/ml ($K_v1.6$). Oocytes were stored at 17–19°C in OR3 medium (50% L-15 (GIBCO, without phenol red), 15 mM HEPES pH 7.6, 50 μg/ml gentamycin (Sigma)), and currents were recorded 2–7 days post-injection.

For EGTA injections, oocytes were injected with 50 nl of 20 mM EGTA pH 7.0 (KOH) and stored at room temperature in OR3 for 57–84 min before currents were recorded.

Electrophysiology

Oocyte membrane currents were recorded using standard two-electrode voltage clamp procedures with a WPI S-7100 voltage clamp controlled by a PDP-11/24 computer (Indec). The recording and analysis software was a modified version of BClamp (Indec). Electrodes were filled with 3 M KCl and had resistances of 0.5–2 MΩ. Currents were filtered at 1,000 Hz, digitized, and stored on disk. The recording solution contained 98 mM K-aspartate, 0.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM HEPES pH 7.6 (KOH). The voltage protocol is shown in Fig. 2 and described in the Results.

Data Analysis

For construction of current-voltage relationships, the magnitudes of the instantaneous current steps resulting from a series of voltage steps were recorded. For minK, this current step could be measured directly from the records (*see* Fig. 2). For $K_v1.6$, "instantaneous" currents were measured after the capacitive transient (*see* Fig. 4). This was only possible for steps to potentials

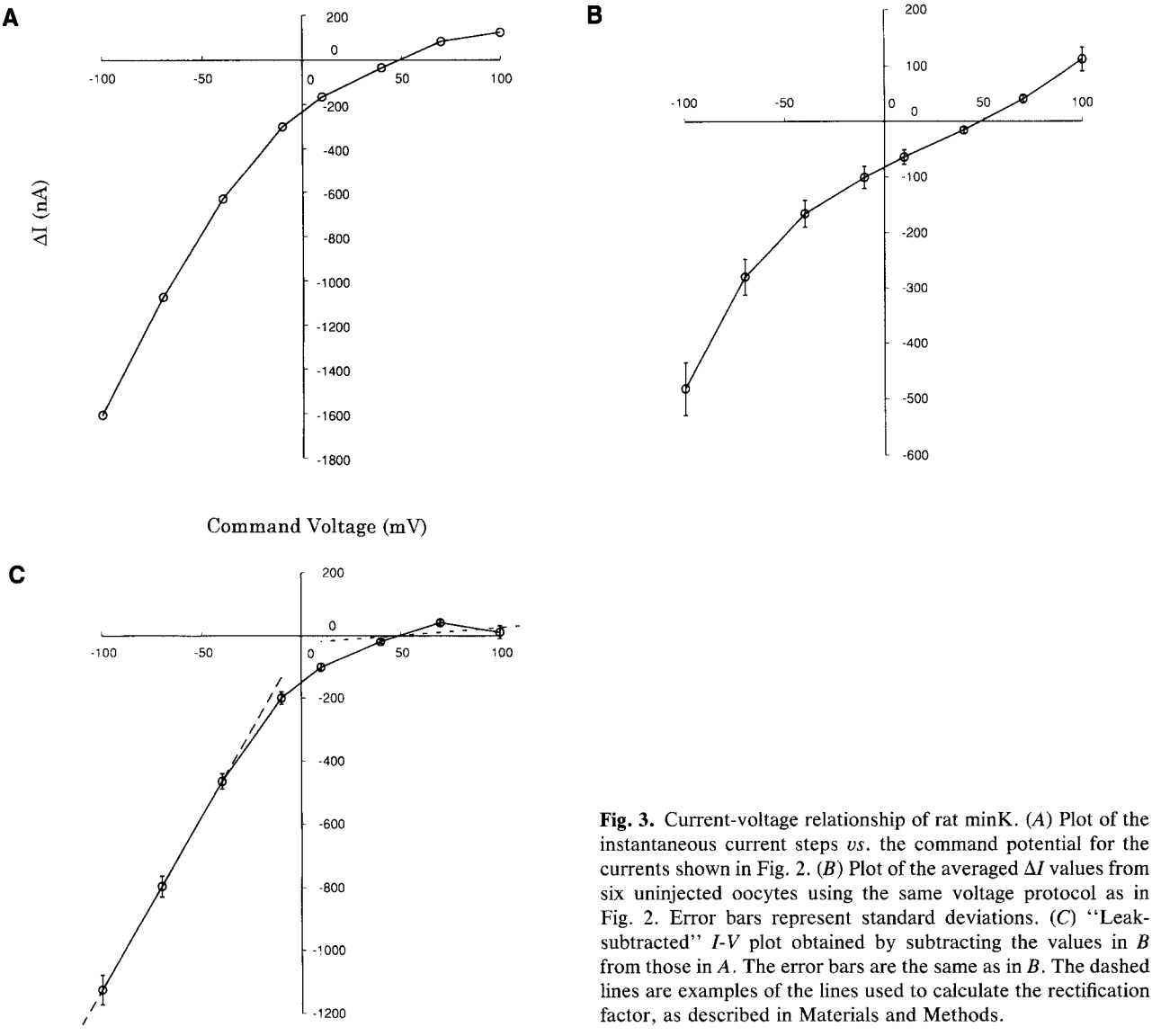

Fig. 3. Current-voltage relationship of rat minK. (*A*) Plot of the instantaneous current steps *vs.* the command potential for the currents shown in Fig. 2. (*B*) Plot of the averaged ΔI values from six uninjected oocytes using the same voltage protocol as in Fig. 2. Error bars represent standard deviations. (*C*) "Leak-subtracted" *I-V* plot obtained by subtracting the values in *B* from those in *A*. The error bars are the same as in *B*. The dashed lines are examples of the lines used to calculate the rectification factor, as described in Materials and Methods.

of −40 mV or more positive and did not take into account any rapid deactivation of the current.

To assess the contribution of leak and endogenous currents to the *I-V* curve, parallel experiments were conducted on uninjected oocytes from the same donor frogs. The current values for six oocytes from each donor were averaged and the average *I-V* curves were subtracted from the *I-V* curves of minK and $K_v1.6$-expressing oocytes to create "leak-subtracted" current-voltage relationships for each oocyte. The rectification factor was calculated from the resulting curves as follows: for each oocyte, best fit lines were calculated by linear regression for the currents at 40, 70, and 100 mV (line A) and either at −40, −70, and −100 mV for minK or at −10 and −40 mV for $K_v1.6$ (line B). The rectification factor is the ratio of the slope of line A over the slope of line B. Examples of these lines are shown in Fig. 3*C*.

Results

We have attempted to determine the open channel current-voltage relationship for the rat minK potassium current expressed in *Xenopus* oocytes. Because attempts in this and other laboratories to measure single minK channels have been unsuccessful (S. Goldstein and F. Sigworth, *personal communication*), we measured the instantaneous change in macroscopic minK current in response to voltage steps. While the oocytes were perfused with a high (100 mM) potassium solution, the membrane potential was held at −60 mV, stepped to 50 mV for 1.5 seconds to activate minK, and then stepped to a series of potentials between 100 and −100 mV (Fig. 2). After subtracting leak and endogenous currents, the instantaneous current should represent current flowing through the open minK channels. For a channel behaving as an ohmic conducting pore, the current step should be a linear function of voltage (Hille, 1984). As shown in Fig. 3, this was not the case for minK. The inward currents flowing through

Table. The rectification factor is the ratio of inward to outward slope conductances, calculated as described in Materials and Methods

mRNA injected	Oocytes	Rectification factor
Rat minK	10	0.04 ± 0.01
Human minK	5	0.07 ± 0.01
Rat $K_v1.6$	6	0.62 ± 0.02*

Values are avg ± SEM.
* Significantly different from rat minK by Student's *t*-test, $P < 0.001$.

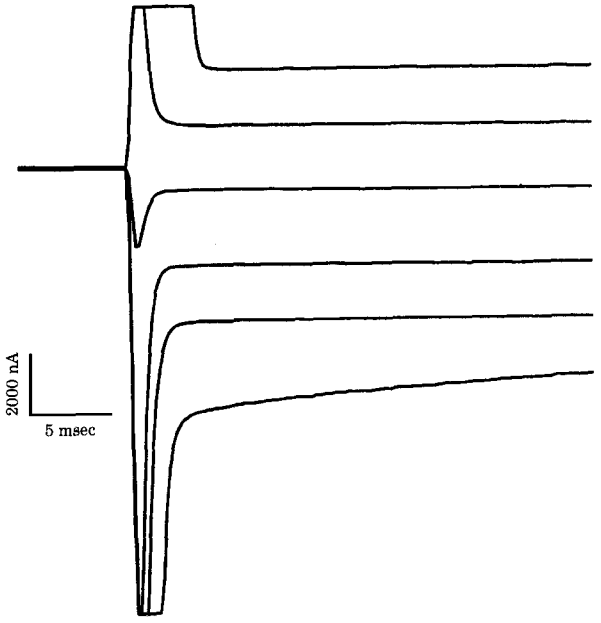

Fig. 4. Currents from an oocyte expressing rat $K_v1.6$. The voltage protocol is similar to that shown in Fig. 2, except that the holding potential was −80 mV, the pulse to 50 mV lasted 50 msec, and the command pulses were to −40, −10, 10, 40, 70, and 100 mV.

minK channels in response to steps to negative potentials were much larger than the corresponding outward currents, suggesting that the open channel current-voltage relationship is strongly inwardly rectifying. In fact, in seven out of ten oocytes, the slope conductance at 100 mV was negative.

To quantify the degree of rectification, we estimated the slope conductances of the current at 70 and −70 mV (*see* Fig. 3*C*). The ratio of these two slopes was defined as the rectification factor. Although this procedure underestimates the degree of rectification, the rectification factor of minK was calculated to be 0.04 (Table), indicating that minK passes inward current at least 20-fold more readily than outward current.

We next examined oocytes expressing the human minK, which shows 76% identity to the rat protein (Murai et al., 1989; Fig. 1), to determine whether rectification is conserved between the two species. The current-voltage relationship of the human channel indicated that its inward rectification was indistinguishable from that of the rat channel (Table).

To ensure that the rectification is specific to minK and is not a property of all potassium channels expressed in *Xenopus* oocytes, we conducted the same experiments on the Shaker-like delayed rectifier $K_v1.6$. The pulse protocol was modified to account for the different kinetics and voltage dependence of this channel; oocytes were held at −80 mV, pulsed to 50 mV for 50 msec to activate the current, and the membrane potential was then stepped to a series of potentials between 100 and −40 mV. As shown in Figs. 4 and 5, the behavior of $K_v1.6$ is close to that of an ideal potassium-selective ohmic pore under these conditions.

The inward rectification of many channels has been shown to be due to a voltage-dependent block by internal divalent cations. We therefore examined the effects of intracellular divalent cations on minK. As it was not possible to change the intracellular solution directly, we injected oocytes with several agents that alter the ionic environment. Injecting oocytes with the calcium chelator EGTA did not affect rectification (*data not shown*). The concentration of EGTA used (50 nl of 20 mM EGTA, for a final concentration of at least 1 mM) was sufficient to block activation of the endogenous calcium-activated chloride current (*data not shown*). Thus, internal calcium does not appear to be responsible for the rectification of minK.

To determine the role of magnesium ions, we injected oocytes with the chelators EDTA or Na_2ATP (Sands & Barish, 1992). Both of these procedures induced large, variable, time-independent leak currents. We were unable to isolate the currents flowing through minK channels in these oocytes and therefore could not determine whether the rectification of minK is due to block by intracellular magnesium ions.

Discussion

We have shown that the minK potassium channel is inwardly rectifying, passing inward current at least 20-fold more readily than outward current. We must stress, however, that although minK rectifies, it is not a "classical" inward rectifier. Such currents are typically activated by hyperpolarization and their voltage dependence varies with the external potassium concentration (Hagiwara, Miyazaki & Rosenthal, 1976). MinK does not share

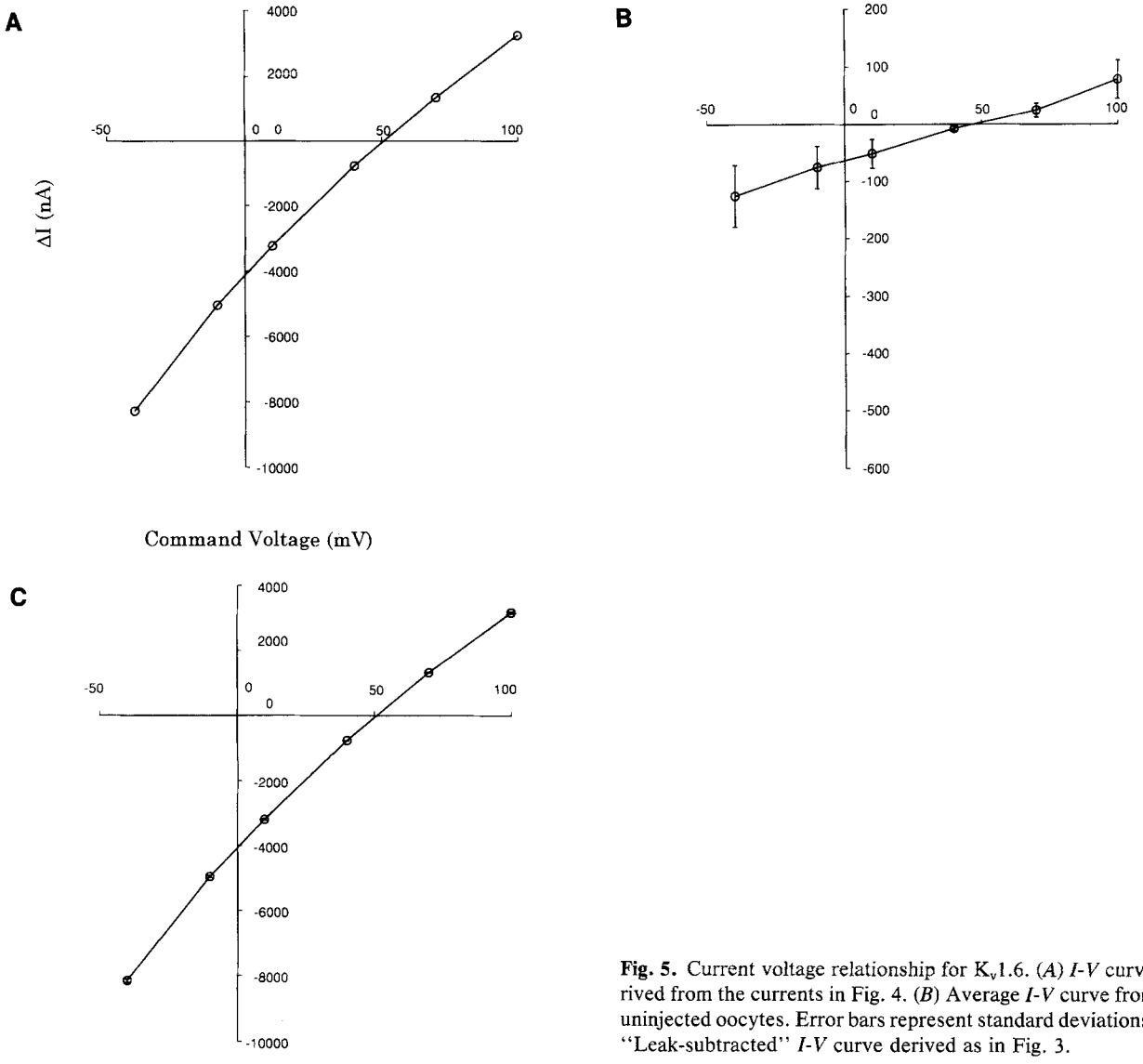

Fig. 5. Current voltage relationship for $K_v1.6$. (*A*) *I-V* curve derived from the currents in Fig. 4. (*B*) Average *I-V* curve from six uninjected oocytes. Error bars represent standard deviations. (*C*) "Leak-subtracted" *I-V* curve derived as in Fig. 3.

these properties (E.M. Blumenthal and L.K. Kaczmarek, *unpublished data*). Recently, a number of potassium channels have been cloned that give rise to inwardly rectifying currents when expressed in oocytes (Schachtman et al., 1992; Ho et al., 1993; Kubo et al., 1993). These channels show varying degrees of homology with the superfamily of voltage-dependent potassium channels, and share a putative pore domain, but none has any similarity to minK.

We used macroscopic currents in this study to estimate the open channel current-voltage relationship of minK; this approach was necessary due to the inability of any laboratory to record single minK channels. A recent paper has reported single channels following the incorporation of the putative transmembrane domain of minK into lipid bilayers (Ben-Efraim, Bach & Shai, 1993). Because these channels have kinetics, selectivity, and voltage dependence different from the macroscopic minK current, their physiological relevance is still unclear.

There are three mechanisms by which rectification could occur (Matsuda, 1991): first, the rectification could be due to channel gating; second, the outward currents could be impeded by voltage-dependent block of the channel; third, the channel pore could be inherently rectifying due to asymmetric energy barriers. While we cannot eliminate the first possibility, we believe it is unlikely; the current traces do not show inactivation of the current at depolarized potentials or activation at hyperpolarizing potentials, as would be necessary for this explanation. While our voltage clamp was too slow to resolve fast (<5 msec) events, such transitions are

unlikely because they would be opposite to the known voltage dependence of minK, which opens at depolarized potentials, and they would be orders of magnitude more rapid than any other known minK transitions.

Voltage-dependent channel block is another well-studied mechanism of rectification. In many cases, this block can be attributed to intracellular magnesium ions. This accounts for at least some of the rectification of potassium channels (Matsuda, Saigusa & Irisawa, 1987; Vandenberg, 1987), sodium channels (Pusch, 1990), and nicotinic acetylcholine receptors (Sands & Barish, 1992). We were unable to determine the role of intracellular magnesium in minK rectification due to the adverse effects on the oocytes of injecting magnesium chelators. We have shown, however, that chelating intracellular calcium had no effect on rectification.

Both human and rat minK channels are inwardly rectifying. These proteins are 76% identical in amino acid sequence (Murai et al., 1989); conserved residues are shown in Fig. 1. The small size of the minK protein makes this an attractive system for identifying the residues involved in rectification and localizing the channel pore. In recent work, however, we have shown that other, endogenous oocyte factors may be involved in the formation of minK channels (E.M. Blumenthal & L.K. Kaczmarek, *manuscript submitted*). It is also possible, therefore, that the rectification may be due to some other protein complexed with minK.

The possible physiological role of minK rectification is unknown. It has been suggested that minK could be involved in potassium secretion by endothelial cells (Sugimoto et al., 1990; Sakagami et al., 1991). Since minK is activated by depolarization, potassium secretion would be voltage dependent. If, however, rectification were due to block of the channel by some intracellular ion, regulation of the levels of that ion could allow fine-tuning of potassium secretion that is independent of membrane potential.

Some researchers have suggested that minK functions as the slow component of the delayed rectifier in cardiac cells (Folander et al., 1990). This current shows kinetics and voltage dependence similar to minK. In addition, the estimated open channel current-voltage relationship of the current in myocytes from bullfrog atrium (Duchatelle-Gourdan, Hartzell & Lagrutta, 1989) and guinea pig ventricle (Matsuura, Ehara & Imoto, 1987) appears to rectify in a manner similar to that reported here. Thus, our results offer further evidence that minK may be responsible for part of the cardiac delayed rectifier currents.

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