Internal Na\(^+\) and Mg\(^{2+}\) Blockade of DRK1 (Kv2.1) Potassium Channels Expressed in X. Oocytes

**Inward Rectification of a Delayed Rectifier**

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**ABSTRACT** Delayed rectifier potassium channels were expressed in the membrane of X. oocytes by injection of rat brain DRK1 (Kv2.1) cRNA, and currents were measured in cell-attached and inside-out patch configurations. In intact cells the current-voltage relationship displayed inward-going rectification at potentials > +100 mV. Rectification was abolished by excision of membrane patches into solutions containing no Mg\(^{2+}\) or Na\(^+\) ions, but was restored by introducing Mg\(^{2+}\) or Na\(^+\) ions into the bath solution. At +50 mV, half-maximum blocking concentrations for Mg\(^{2+}\) and Na\(^+\) were 4.8 ± 2.5 mM (n = 6) and 26 ± 4 mM (n = 3) respectively. Increasing extracellular potassium concentration reduced the degree of rectification of intact cells. It is concluded that inward-going rectification resulting from voltage-dependent block by internal cations can be observed with normally outwardly rectifying DRK1 channels.

**INTRODUCTION**

DRK1 is a potassium channel cloned from rat brain (Frech, VanDongen, Schuster, Brown, and Joho, 1989), and is a member of the voltage-gated Shaker superfamily of channels that do not conduct at negative potentials (hyperpolarization) and which are activated by depolarization (Hille, 1992; Hodgkin, Huxley, and Katz, 1949; Lu, Montrose-Rafizadeh, Hwang, and Guggino, 1990; Walsh, Arena, Kwok, Freeman, and Kass, 1991). These channels thus show outward (normal) rectification and are primarily responsible for action potential repolarization. A number of clones of delayed rectifier potassium channels in the Shaker superfamily have been isolated and characterized in recent years (Covarrubias, Wei, and Salkoff, 1991; Swanson, Marshall, Smith, Williams, Boyle, Luneau, Antanavage, Oliva, Buhrow et al., 1990).

The phenomenon of inward or anomalous rectification, as first described by Katz...
(1949), means that membrane conductance increases with hyperpolarization and decreases with depolarization. Inward rectifying potassium channels are primarily responsible for the resting potential in nerve and muscle cells, and have been found and described in many tissues (Kelly, Dixon, and Sims, 1992; Matsuda and Stanfield, 1989; Matsuda, Saigusa, and Irisawa, 1987; Oliva, Cohen, and Pennefather, 1990; Vandenberg, 1987). Two of them were recently cloned by expression in oocytes (Ho, Nichols, Lederer, Lyton, Vassilev, Kanazirska, and Hebert, 1993; Kubo, Baldwin, Jan, and Jan, 1993). The mechanisms underlying inward rectification are different for different channels, but are of two major types: (a) single channel kinetics may be changed in a way causing channel closure (Silver and DeCoursey, 1990), or opening (Latorre, Coronado, and Vergara, 1984) when the membrane potential becomes more positive; (b) internal particles, such as ions, may block the channel in a voltage-dependent manner (Horie, Irisawa, and Noma, 1987). In some channels, both processes take place simultaneously (Matsuda, 1988). In this paper we demonstrate that classic outward delayed rectifier potassium channels expressed in oocytes (DRK1, Frech et al., 1989) display inward rectification at positive potentials due to cation block, in intact cells. This property closely resembles that of "mild" inwardly rectifying potassium channels (Hille, 1992). Sequence analysis of predicted pore regions of DRK1 and cloned inwardly rectifying potassium channels (ROMK1, IRK1) reveals homology which may help to explain the similarity in rectifying properties of such different channels.

MATERIALS AND METHODS

Oocyte Expression of DRK1 Channels

DRK1 cDNA was propagated in the transcription-competent vector pBluescript SK(−) in Escherichia coli TG1 (Frech et al., 1989). cRNA was transcribed in vitro using T7 RNA polymerase and capping from linearized cDNA. Stage V-VI Xenopus oocytes were isolated by partial ovariectomy under tricaine anesthesia, and then defolliculated by treatment with 1 mg/ml collagenase (Sigma Type 1A, Sigma Chemical Co., St. Louis, MO) in zero Ca^{2+} ND96 (below) for 1 h. 2–24 h after defolliculation, oocytes were pressure-injected with ~50 nl of 100–200 ng/μl cRNA. Oocytes were kept in 2 mM Ca^{2+} ND96 (below), supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) for 1–7 d before experimentation.

Electrophysiology

Oocytes were placed in hypertonic solution (ND96 plus additional 100 mM NaCl) for 5–30 min to shrink the oocyte membrane from the vitelline membrane. The vitelline membrane was removed from the oocyte using Dumont No. 5 forceps. Oocytes were patch-clamped using fire-polished micropipettes pulled from thin-walled glass (WPI Inc., New Haven, CT) on a horizontal puller (Sutter Instrument, Co., Novato, CA). Electrode resistance was typically 0.5–2 MΩ when filled with 100 mM KCl or NaCl, with tip diameters of 2–20 μm. Experiments were performed at room temperature in a chamber mounted on the stage of an inverted microscope (Nikon Diaphot, Nikon Inc., Garden City, NY). PClamp software and a Labmaster TL125 D/A converter were used to generate voltage pulses. Data were normally filtered at 1 kHz, signals were digitized at 22 kHz (Neurocorder, Neurodata, NY) and stored on video tape. Experiments could then be digitized into a microcomputer using Axotape (Axon Instruments, Inc., Foster City, CA). Alternatively, signals were digitized on-line using PClamp, and stored on disk for
off-line analysis. Currents from giant patches were not subtracted for artifacts except when leakage current at holding potential −100 mV was more than 5% of peak current amplitude at zero mV.

In most experiments with repetitive pulsing, the interpulse duration was 3 s with a pulse duration of 100 ms. Use-dependent inactivation of DRK1 currents did not exceed 10% with this voltage protocol.

**Solutions**

The standard high 'sodium' extracellular solution (ND96) contained (mM): NaCl 96, KCl 2, MgCl₂ 1, HEPES 5, pH 7.5 (with NaOH) and standard high potassium extracellular solution (KD98) contained (mM): KCl 98, MgCl₂ 1, HEPES 5, pH 7.5 (with KOH). The bath (internal) solution (KD140) for measurements with inside-out patches contained (mM): KCl 140, HEPES 5, K₂EGTA 1, pH 7.2 (with KOH). Mg²⁺ and Na⁺ ions were introduced into internal KD140 solution as MgCl₂ and NaCl salts without adjusting total molarity. Corrections should be made for true Mg²⁺ concentration because of the chelating properties of EGTA. For the composition of KD140 solution true values of free Mg²⁺ were 9.6, 4.75, 1.88, 0.926, and 0.488 mM for 10, 5, 2, 1, and 0.5 mM of added MgCl₂ respectively. All concentrations mentioned in this paper (text or figures) are referred to as added concentrations.

**Analysis**

Wherever possible, data are presented as mean ± SD. Microsoft Solver was used to fit data by a least-square algorithm. Relative currents (R) for cell-attached patches were obtained as follows: inside-out currents were measured with different concentrations of K⁺ in the pipette and peak current-voltage (I/V) relations at potentials positive to +40 mV were fitted using a modified GHK equation:

$$I = A \cdot \left( V \cdot \frac{[S]_i}{1 - \exp(-\nu_i)} \right)^n + A \cdot \left( V \cdot \frac{[S]_0}{1 - \exp(\nu_0)} \right)^n,$$

where $[S]_i$ and $[S]_0$ are potassium concentration in the bath and in the pipette, respectively, $A$ is a scale factor, $\nu_i$ is an abbreviation for ZVF/RT (Z is the effective valency of the gating particle (see Results); $F$, $R$, and $T$ have their usual meaning) and $n$ stands for deviation of I/V relations from independence principle (GHK equation, Hille, 1992). Peak I/V relations at potentials positive to +40 mV are well described by Eq. 1 because open channel probability reaches steady state values in this voltage range (unpublished observation). I/V relations were best fitted with $n = 1.25$, and arbitrarily scaled relative currents ($A$ is a free parameter) were then calculated, dividing the theoretical I/V relation by the experimental one, using $[S]_i$ and $[S]_0$ which give a true reversal potential. The fitting procedure is described in Results.

**RESULTS**

**Currents in Cell-attached Patches**

Oocytes were injected with relatively high concentrations (100–200 ng/μl) of DRK1 cRNA. In 2–5 d, the density of delayed rectifier potassium channels was sufficient to obtain cell-attached patch currents of up to hundreds of picoamps with giant patches. Cell-attached currents in response to step depolarizations from −100 mV, and the peak current-voltage relationship, for a representative patch are illustrated in Fig. 1. Slowly activating chloride currents have been reported in *Xenopus* oocytes (Miledi and Parker, 1984) and, in some of our experiments, these currents contributed signifi-
cantly to the total current at positive potentials. All patches which displayed significant Cl⁻ currents were discarded from further analysis. We did not observe any significant endogenous delayed rectifier potassium currents (Lu et al., 1990). The major point to be derived from Fig. 1 is that DRK1 current declines at very positive voltages. The average membrane potential at which the current-voltage relation reached a maximum was +82 ± 12 mV (n = 17, from 10 oocytes, with ND96 - 2 mM K⁺ solution in the pipette).

**Figure 1.** DRK1 currents in cell-attached patches. (A) Records of current in cell-attached patch elicited by 100 ms voltage steps from holding potential -100 mV to potentials indicated. (B) Peak current-voltage relation for the patch presented in A. 100-ms voltage steps from holding potential -100 mV are indicated at each current trace. All currents were leak subtracted and filtered at 1 kHz. Membrane potential for cell-attached measurements (A, B) was zeroed by bathing the oocyte in KD98 potassium solution (see Materials and Methods).

**Currents in Inside-out Membrane Patches**

Similar anomalous rectification at positive potentials has been described for ATP-sensitive and mACh-activated potassium channels that have been classified as mild inward rectifiers (Hille, 1992). In such channels, the inward-going rectification results from voltage-dependent block by internal cations. To verify the suggestion that inward-going rectification of DRK1 currents is due to blockage by internal cations, currents from excised inside-out patches were measured in a bath solution containing K⁺ as the only significant cation (Fig. 2). As shown in Fig. 2 B the peak current-voltage relationship did not rectify up to +150 mV in these conditions. DRK1 channels run down within a few minutes after patch excision, complicating, and limiting, time consuming measurements, such as dose-response, or current-voltage, relations. Nevertheless, a reasonable number of patches showed only 10–30% rundown over tens of minutes—only these patches were used for analysis. In most cases, channel rundown occurred as an approximately linear function of time (rather than exponential) and when necessary, data were corrected assuming this approximation.
Inside-out Patch Currents. Block by Internal Sodium Ions

When sodium ions were added to the intracellular (bath) solution, current amplitude was reduced at positive potentials, with little or no change in the activation kinetics. The peak current-voltage relationship showed inward rectification at positive potentials (Fig. 3 B), resembling the current-voltage relation obtained in cell-attached configuration (Fig. 1 B). The relative amplitude of peak current in the presence of 10 mM Na⁺ is shown in Fig. 4 A using a logarithmic ordinate (Ln[1/R-1], where R is relative current). Data points can be fitted by linear regression analysis indicating that the dissociation constant for sodium block decreases exponentially with increasing

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**FIGURE 2.** DRK1 currents in inside-out patches. (A) Records of current in an inside-out patch in bath solution containing no Mg²⁺ or Na⁺ ions (KD140, see Methods). 100 ms voltage steps from holding potential −100 mV are indicated at each current trace. (B) Peak current-voltage relation for the patch presented in A. All currents were leak subtracted and filtered at 1 kHz.

**FIGURE 3.** Voltage-dependent block by Na⁺ ions in inside-out patch. (A) Records of current elicited by a voltage step to +50 mV in zero Na⁺ (pre), during exposure to 10 mM Na⁺ (10 Na) and after return to zero Na⁺ (post). (B) Peak current-voltage relations with (○) and without (■) Na⁺ ions (10 mM) in the bath solution. Holding potential −100 mV indicated by arrow.
membrane potential. Assuming that the dissociation constant may be described by (Woodhull, 1973):

$$K_{Na}(V) = K_{Na}(0) \exp \left(-\frac{ZVF}{RT}\right)$$  \hspace{1cm} (2)

where $Z$ is the effective valency of the blocking ion, $K_{Na}(0)$ is the dissociation constant at zero potential, $F$, $R$, and $T$ have their usual meaning, and $V$ is the membrane potential. Linear regression gives a value for $Z$ of 0.53. Effective valency is defined as $Z = z\delta$, where $z$ is the true valency of the blocking ion and $\delta$ describes the location of the ion binding site in the electrical field. Thus, the Na$^+$ binding site ($\delta$) is located 53% of the electrical distance from the internal edge of the pore.

Fig. 4B shows the DRK1 current ($I/I_0$) at two different potentials (+50 mV and +100 mV) with different concentrations of internal sodium ions, relative to the current in the absence of Na$^+$. The data are well fit by a Hill equation:

$$\frac{I}{I_0} = \frac{1}{1 + \left[\frac{[Na^+]_i}{K_d(V)}\right]^n}$$  \hspace{1cm} (3)

with $K_d(+50) = 27.5$ mM (Hill coefficient $n = 1.1$) and $K_d(+100) = 10.8$ mM (Hill coefficient $n = 0.95$) suggesting one-to-one binding of sodium ions within the pore of the channel. The averaged value for $K_d(+50)$ was 26.2 mM ($n = 3$).

**Inside-out Patch Currents. Block by Internal Magnesium Ions**

As with sodium, a similar analysis was carried out with Mg$^{2+}$ ions applied to the internal surface of the membrane. The data are shown in Figs. 5 and 6. Again, Mg$^{2+}$ ions reduced peak DRK1 current. Relative block of DRK1 currents versus potential, at 0.5, 2, and 5 mM added internal Mg$^{2+}$ was exponentially voltage-dependent (Fig. 6A), giving the electrical distance for Mg$^{2+}$ binding site $\delta = 0.32$ ($Z = 0.65$) for this particular experiment. Averaged electrical distance obtained from four different patches with different concentrations of Mg$^{2+}$ ions was $\delta = 0.3 \pm 0.05$. 
Figure 5. Voltage-dependent block by Mg$^{2+}$ ions in inside-out patch. (A) Records of current elicited by a voltage step to +50 mV in zero Mg$^{2+}$ (pre), during exposure to 2 mM Mg$^{2+}$ (2 Mg) and after return to zero Mg$^{2+}$ (post). (B) Peak current-voltage relations measured with different concentrations of added internal Mg$^{2+}$. Holding potential is indicated by arrow.

Figure 6. (A) Relative current (plotted as Ln(1/[R-1])) measured with 2 mM Mg$^{2+}$ versus membrane potential. Straight line is linear regression approximation with effective valency $Z = 0.65$ (Results, Eq. 2). (B) Dose-response relations for Mg$^{2+}$ block of DRKI current at +100 mV (○) and +50 mV (■). Continuous lines are least square fit by Hill equation (see Results).
Representative dose-response curves measured at +50 and +100 mV (Fig. 6 B) give $K_d(+50) = 3.16$ mM (Hill coefficient $n = 1.21$) and $K_d(+100) = 1.52$ mM (Hill coefficient $n = 1.16$), assuming a one-to-one binding of Mg$^{2+}$ ions, as for internal Na$^+$. Dose-response curves for magnesium block (as well as for sodium) showed fairly wide variability in inside-out patches giving large scatter for estimated parameters: averaged value for $K_d(+50)$ and Hill coefficient were $4.8 \pm 2.5$ mM and $1.01 \pm 0.20$ ($n = 6$), respectively. The time course of current activation was essentially unchanged by either Mg$^{2+}$ or Na$^+$ (Figs. 3 A, 5 A).

Inward Rectification Depends on External K$^+$ Concentration

In other channels showing inward rectification due to internal cation block, the block is relieved by elevation of external potassium. Results presented in Fig. 7 A show that the same is true for delayed rectifier DRK1 channels. Cell-attached patch currents were measured in different patches from the same cell with pipettes containing different concentrations of K$^+$. Current-voltage relations were then normalized to the peak values in each case to show the effect of external [K$^+$]. Because currents were recorded from different patches (but in each series of experiments on the same cell) the only informative data are the peak values for current-voltage relations. Averaged
values for peak potentials with 0, 11, and 98 mM [K+]o were 77 ± 8.6 mV (n = 10), 104 ± 3.7 mV (n = 10) and 116 ± 6.5 mV (n = 12), respectively. More information can be extracted from graphs presented in Fig. 7A by the use of current-voltage relations obtained in inside-out patches. For this purpose current-voltage relations from inside-out patches were measured with different concentrations of potassium ions in the pipette (0, 11, and 98 mM), and without magnesium or sodium ions in the bath solution, and fitted by the modified GHK equation (see Experimental procedures). Relative currents R (in arbitrary scale) were then obtained by dividing cell-attached current-voltage relations by theoretical inside-out relations.

The major assumption of the theory of ionic blockage is that the dissociation constant depends exponentially on voltage (Eq. 2). Therefore, relative current $R = I/I_o$ (equation 3) should be a linear function of voltage after ordinate transformation $\ln(1/R - 1)$. Thus effective valency can be estimated, even with arbitrarily scaled relative currents. Data were plotted using $\ln(A/R - 1)$ (where R is the relative current and A is the scaling factor) as ordinate and fit by straight line varying both Z and A (Fig. 7B). All currents were well fit by a straight line with effective valency Z ranging from 0.65 to 0.95 (mean value 0.76, n = 6).

**Discussion**

The results demonstrate that currents through a delayed rectifier potassium channel (DRK1, Kv2.1) expressed in oocytes display inward going rectification at strong positive membrane potentials (Fig. 1), defining a property of mild inward rectifier potassium channels and not normally associated with voltage-dependent outward delayed rectifiers. To reveal the basis for this phenomenon cell-attached currents were compared with currents from inside-out patches in internal solutions of different ionic compositions. Only two possible mechanisms have been shown to underlie inward rectification of potassium currents: (a) intrinsic channel kinetics depending on voltage in such a way as to cause open channel probability to decrease at positive potentials; (b) internal ions or particles blocking the channel in a voltage-dependent manner. Excision of patches from oocytes into solutions containing K+ as the only small cation abolishes inward rectification, suggesting a cation blocking mechanism for the inward rectification of DRK1 channels. Addition of either sodium or magnesium ions to the internal solution caused inward rectification of patch currents at strong positive potentials, resembling, in general, the currents observed in cell-attached configuration, and strongly arguing that inward going rectification in the cell-attached mode is due to voltage-dependent blockage by internal cations.

This type of inward rectification has been well described for many voltage-independent potassium channels from a variety of tissues. Many mono- and divalent cations, such as Na+, Li+, Rb+, Ba2+ and Mg2+, applied to the inner surface of the membrane can block outward currents in a voltage-dependent manner (Hille, 1992; Matsuda et al., 1987; Matsuda, Matsuura, and Noma, 1989; Matsuda, 1991). It has been shown, in particular, that internal Na+ blocks ATP-sensitive K+ channels of guinea pig ventricular cells (Horie et al., 1987) and of frog skeletal muscle (Quayle and Stanfield, 1989), the Ca2+-activated K+ channel of bovine chromaffin cells (Marty, 1983). A weak internal Na+ block of the outward delayed rectifier potassium
current of giant axons has been reported (French and Wells, 1977). Forsythe, Linsdell and Stanfield (1992) have recently described a Mg\(^{2+}\) and Na\(^{+}\) dependent rectification in fast transient A-type channels of the rat locus coeruleus neurones, although rectification was not observed in A-type currents from dorsal root ganglion neurones (Kasai et al., 1986). In these cases, the molecular nature of the channel type underlying the current is unknown. Rettig, Wunder, Stocker, Lichtinghagen, Mastiaux, Beckh, Kues, Pedarzani, Schroter, Ruppersberg, Veh, and Pongs (1992) demonstrated a Mg\(^{2+}\) block in the rapidly activating Raw3 (Kv3) member of the Shaw-like group of channels. Although not studied in detail, the degree of block appears to be similar to that described in the present paper for block of DRK1, a member of the Shab-like group of channels. Rectification has not been described for cloned Shaker-like channels.

The potency of internal Na\(^{+}\) to block outward currents is different for different channels. At +50 mV 20 mM Na\(^{+}\) reduces currents through inwardly rectifying potassium channels by \(~50\%\) in guinea-pig ventricular cells (Matsuda, 1991), by \(~50\%\) in Ca\(^{2+}\)-activated K\(^{+}\) channels of bovine chromaffin cells (Marty, 1983), by \(~60\%\) in ATP-sensitive potassium channels in guinea-pig ventricular cells (Horie et al., 1987) and very weakly (<10\%) delayed outward current in squid axon (French et al., 1977; Bezanilla and Armstrong, 1972). DRK1 channels belong to the Shab family of voltage-dependent delayed rectifier, and little or no Na\(^{+}\) block was expected. However, in contrast to delayed rectifiers of squid axon, \(~50\%\) block of DRK1 channels was observed at +50 mV with 20 mM Na\(^{+}\), which is in the range of that observed for mild inward rectifier potassium channels (Hille, 1992). However, the 'electrical distance' (δ) of the Na\(^{+}\) binding site within the pore, is different from that of ATP-sensitive potassium channels in guinea-pig ventricular cells (0.53 for DRK1; 0.35 for K\(_{ATP}\) channels, Horie et al., 1987), and in contrast to K\(_{ATP}\) channels, dose-response curves give Hill coefficient ~1 for DRK1 channels rather than 2 for K\(_{ATP}\) channels (Horie et al., 1987).

As with block by Na\(^{+}\), internally applied Mg\(^{2+}\) ions effectively block inward rectifier K\(^{+}\) currents in a variety of tissues (Oliva et al., 1990; Horie et al., 1987; Vandenberg, 1987; Matsuda et al., 1987). As shown (Fig. 5), sensitivity of DRK1 to internal Mg\(^{2+}\) is in the millimolar range and this is presumably one of the underlying mechanisms for inward-going rectification seen with DRK1 potassium channels in cell-attached configuration. With low potassium in the pipette (2 mM, extracellular side) half maximum Mg\(^{2+}\) concentration measured at +50 mV was about 4.8 mM for DRK1. It is interesting to note that while the electrical distance δ for the Mg\(^{2+}\) binding site in DRK1 channels is very close to that of K\(_{ATP}\) channels in guinea-pig ventricular cells (Horie et al., 1987), ~0.3 and 0.32, respectively, half-inhibiting concentrations measured at +50 mV are 10-fold different (4.8 mM in DRK1 vs 0.5 mM in K\(_{ATP}\) channels). Nevertheless, the Mg\(^{2+}\) sensitivity of DRK1 channels is fairly close to that of other mild inward rectifiers—half-inhibition occurs in the millimolar range in K\(_{ATP}\) channels of RINm5F cells (Findlay, 1987).

Voltage-dependent block by internal cations is normally reduced by raising the external permeant K\(^{+}\) ion concentration (Yellen, 1984; Hille and Schwarz, 1978; Marty, 1983; Matsuda, 1991) and this is major evidence for the blocking site residing...
in the channel pore itself. Bezanilla and Armstrong (1972) first showed that internal \( \text{Na}^+ \) and \( \text{Cs}^+ \) block of delayed rectifier potassium channels in squid can be relieved by raising external \( \text{K}^+ \) concentration and this appears to be true also for inward rectification of DRK1 channels. Peak current-voltage relations were shifted to more positive potentials by about 40 mV (relief) by increasing extracellular potassium from 0–2 mM to \( \sim 100 \text{mM} \). The effective valency \( Z \) of internal blocking particles is about 0.76 when measuring currents in cell-attached configuration. This is higher than the effective valency for pure internal \( \text{Mg}^{2+} \) or \( \text{Na}^+ \) block in inside-out patches (0.65 and 0.53, respectively). Probably both cations (i.e. \( \text{Mg}^{2+} \) and \( \text{Na}^+ \)) are important for inward rectification in cell-attached configuration. For example, the estimated concentration of free \( \text{Na}^+ \) in oocytes is \( \sim 20 \text{mM} \) (De Laat, Buwalda, and Habets, 1974; no data available for \([\text{Mg}^{2+}]\)), and a contribution of \( \text{Na}^+ \) ions to the inward rectification in intact cells would be expected.

By expression in oocytes, two inwardly rectifying potassium channels have recently been cloned, from rat kidney (ROMK1, Ho et al., 1993) and from a mouse macrophage cell line (IRK1, Kubo et al., 1993), permitting a comparison of their molecular structures with that of DRK1. In striking contrast to DRK1 and other voltage-gated potassium channels, ROMK1 and IRK1 have only two putative transmembrane domains (rather than six) and there is almost no overall homology. However, sequence homology of the predicted pore forming region (H5 linker) is high: sequence comparison of ROMK1 and DRK1 or other voltage-activated potassium channels H5 regions reveals similarity of 44–60%, depending on the segment length (Ho et al., 1993). Both DRK1 and ROMK1 (Nichols, Ho, and Hebert, 1994) rectify by cation block and millimolar quantities of \( \text{Mg}^{2+} \) are necessary for half inhibition at +50 mV, putting them both in a class of mild inward rectifiers. In some cases single amino acid substitutions in the H5, pore forming, region of voltage-dependent \( \text{K}^+ \) channels results in dramatic changes in selectivity and external cation blocking characteristics (Heginbotham, Abramson, and MacKinnon, 1992), permeability (Kirsch, Drew, Hartmann, Tagialalatale, De Biasi, Brown, and Joho, 1992), or sensitivity to internal or external TEA (Heginbotham and MacKinnon, 1992; MacKinnon and Yellen, 1990; Yellen, Jurman, Abramson, and MacKinnon, 1991), suggesting that particular amino acids may be responsible for some properties. Further examination of the H5 region of DRK1 and inwardly rectifying channels may well suggest a role for specific amino acids in this region in internal cation blocking and rectification. The present results further demonstrate that \( \text{Mg}^{2+} \) and \( \text{Na}^+ \) dependent inward rectification is not a unique property of classic and mild inward rectifiers, (Hille, 1992) and even delayed outward rectifier \( \text{K}^+ \) channels can display it under normal physiological conditions.

DRK1 was a gift from R. Joho (University of Texas Southwestern Medical School, Dallas, TX) and A. Brown (Baylor College of Medicine, Houston, TX). We are grateful to Dr. E. Makhina for preparation of cRNA.

This work was supported by Grant HL45742 to C. G. Nichols from the NIH.

Original version received 5 May 1993 and accepted version received 5 October 1993.
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