Mg^{2+}-dependent inward rectification of ROMK1 potassium channels expressed in *Xenopus* oocytes

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1. ROMK1 potassium channel currents were examined in *Xenopus* oocytes by two-microelectrode voltage-clamp and patch-clamp techniques following injection of oocytes with *in vitro* transcribed ROMK1 cRNA. Macroscopic currents recorded from intact cells rectified inwardly at positive potentials.

2. In inside-out membrane patches rectification is manifested as an apparent reduction of single channel current (at 500 Hz) in the presence of 0·1–10 mM Mg^{2+}, without a decrease in the channel open probability. No inward rectification is observed when membrane patches are isolated into solutions containing potassium as the only internal inorganic cation.

3. Mg^{2+} block can be described by a simple one-site model for Mg^{2+} binding with K_0 ([Mg^{2+}] causing half-maximal block at 0 mV) of 16·7 mM and δ (the fraction of the membrane field sensed by the blocking Mg^{2+}) of 0·35.

4. The voltage dependence of channel block by cytoplasmic Mg^{2+} was shifted approximately −50 mV by a reduction in extracellular [K^+] from 140 to 0 mM, corresponding to a decrease of K_0 to 4·4 mM.

5. At negative membrane potentials, ROMK1 channels exhibit a single subconducting state that is approximately 4/10 of the full conductance. The incidence of subconductance states is not appreciably enhanced in the presence of Mg^{2+}.

Potassium channels have been classified into two broad groups (Hille, 1992): (1) delayed, or outward, rectifying channels that are activated by membrane depolarization; and (2) inward rectifying channels, which include muscarinic-activated and ATP-sensitive channels, that conduct current more readily in the inward direction than the outward direction. With the notable exception of the minK channel (Takumi, Ohkobi & Nakanishi, 1988), the delayed rectifier channels are all members of the *Shaker* superfamily of polypeptides (Wei, Covarrubias, Butler, Baker, Pak & Salkoff, 1990). Each of these channels are characterized by cytoplasmic *N*- and *C*-terminal domains and six membrane-spanning hydrophobic domains, one of which (S4) contains repeated positive charges and is believed to form the voltage sensor responsible for voltage-dependent activation (Papazian, Timpe, Jan & Jan, 1991), and hence functional outward rectification. Inward rectification in the second class of channels results from two mechanisms: voltage-dependent block by cytoplasmic cations and an intrinsic voltage dependence of the open probability (Matsuda, Saigusa & Irisawa, 1987; Vandenberg, 1987; Matsuda, Matsuura, & Noma, 1989; Oliva, Cohen & Pennefather, 1990; Kelly, Dixon & Sims, 1992). In the well-studied inward rectifiers from cardiac myocytes, both mechanisms are partly responsible for rectification. Internal block by Mg^{2+} appears as a rapid flickering block in cardiac inward rectifiers (Matsuda, 1988) and conductance sublevels that are 2/3 or 1/3 of the full conductance have been observed. It has been proposed that this behaviour, which is also observed with blockade by Cs^+ and Rb^+ ions (Matsuda *et al.* 1989), provides evidence that inwardly rectifying K^+ channels are formed from three parallel conducting pores (triple barrelled), possibly by the assembly of three single-barrelled subunits.

Recently, two inwardly rectifying potassium channels have been cloned and expressed in *Xenopus* oocytes (Ho *et al.* 1993; Kubo, Baldwin, Jan & Jan, 1993). These two channels share a similar molecular architecture that is different from that of *Shaker* family channels. The IRK1 channel, isolated from a macrophage cell line (Kubo *et al.* 1993), demonstrates many of the properties of the 'classic' inwardly rectifying channels found in the heart:

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rectification is very pronounced above the reversal potential for potassium (E_K). ROMK1, a MgATP-activated potassium channel cloned from kidney medulla (Ho *et al.* 1993), shows significantly less rectification, which is typical of 'mild' inward rectifiers such as ATP-sensitive and muscarinic-activated potassium channels (Hille, 1992). ROMK1 and IRK1, which exhibit ~40% amino acid identity to one another, belong to a new superfamily of potassium channels. Both appear to have only two membrane-spanning domains in addition to an H5 pore region. Similarities between the H5 regions in these channels and those in *Shaker* family members provide a distant ancestral link between the two superfamilies.

We have characterized the inward rectification in ROMK1 channels. A voltage-dependent block of the channel by cytoplasmic Mg^{2+} causes inward rectification that is partly relieved by increased external K^+. There is no contribution of channel gating to inward rectification. A preliminary presentation of some of these results has been made to the Physiological Society (Nichols, Ho & Hebert, 1993).

METHODS

Oocyte expression of ROMK1 channels

ROMK1 cDNA (Ho *et al.* 1993) was propagated in the transcription-competent vector pSPORT1 in *E. coli.* cRNA was transcribed *in vitro* from linearized template cDNA using T7 RNA polymerase and a cap analogue diguanosine triphosphate. Stage V–VI *Xenopus* oocytes were isolated by partial ovariectomy under tricaine anaesthesia. Oocytes were then defolliculated by treatment with 1 mg ml^{-1} collagenase (Sigma Type IA; Sigma, St Louis, MO, USA) in zero Ca^{2+} ND96 (see Solutions below) for 1 h. Between 2 and 24 h after defolliculation, oocytes were pressure injected with ~50 nl of 1–100 ng μl^{-1} cRNA. Oocytes were maintained in 2 mM Ca^{2+} ND96 supplemented with penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}) for 2–7 days prior to experimentation.

Electrophysiology

Two-microelectrode voltage clamp. Whole oocytes were voltage clamped using a two-microelectrode voltage clamp (Warner Instruments, Inc., Hamden, CT, USA) in a 200 μl chamber mounted on the stage of an SMZ-1 microscope (Nikon Inc., Garden City, NY, USA). Experiments were performed in zero Ca^{2+} ND96 with modifications as described in Results. Electrodes were filled with 3 M KCl and had tip resistances of 1–5 MΩ. Experiments were performed at room temperature. pCLAMP software and a Labmaster TL125 D/A converter (Axon Inc., Foster City, CA, USA) were used to generate voltage pulses. Data were normally filtered at 1 kHz, signals were digitized at 22 kHz (Neurocorder, Neurodata, NY, USA) and stored on videotape. Experiments were replayed onto a chart recorder, or digitized into a microcomputer using Axotape software (Axon Inc.). Alternatively, signals were digitized on-line using pCLAMP and stored on disk for off-line analysis.

Inside-out membrane patch experiments. Oocytes were placed in a hypertonic solution (mM: 60 KCl, 10 EGTA, 40 Hepes, 250 sucrose, 8 $MgCl_2$, pH 7·0) for 5–30 min in order to allow the separation of the oocyte membrane from the vitelline membrane; the latter was then removed using Dumont no. 5 forceps. Oocytes were patch clamped using fire-polished micropipettes pulled from thin-walled glass (WPI Inc.) on a horizontal puller (Sutter Instrument Co., Novato, CA, USA). Electrode resistance was typically 1–2 MΩ when electrodes were filled with 145 mM KCl or NaCl and had tip diameters of 1–10 μm. Membrane patches were voltage clamped using an Axopatch 1B patch clamp (Axon Inc.) in a chamber that allowed rapid change of solution (< 2 s), mounted on the stage of an inverting microscope (Nikon Diaphot, Nikon Inc.). Voltage pulse generation and recording was as described above. Currents were generally filtered at 0·5–3 kHz and then digitized (Neurocorder, Neurodata Inc.) and stored on videotape. Experiments were then re-digitized into a microcomputer (at 3–10 kHz). Single channel current amplitudes were generally determined by fitting amplitude histograms. Open channel probability was high (> 0·95) at positive potentials, and the number of channels in a patch was readily determined from the maximum number of overlapping single channels. Open probability was determined from idealized records containing one or a few channels using Fetchex software (Axon Inc.).

Solutions

For two-microelectrode experiments, the standard extracellular solution (ND96) contained (mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 5 Na-Hepes, pH 7·5. For external $[K^+]$ replacement experiments, mixtures of ND96 and KD98 (which contained (mM): 98 KCl, 1 $MgCl_2$, 5 K-Hepes, pH 7·5) were used. Variations from the standard solutions are described in the Results section. For inside-out patch experiments, both patch pipette and bath solutions were KINT, containing (mM): 140 KCl, 10 K-Hepes, 1 K-EGTA, pH 7·3; or NaINT, containing (mM): 140 NaCl, 10 Na-Hepes, 1 Na-EGTA, pH 7·3; or a mixture of these two solutions. All experiments were performed at room temperature.

RESULTS

ROMK1 rectification results from internal cation block, not gating

Figure 1*A* shows typical currents in a two-microelectrode voltage-clamp experiment, recorded from an oocyte injected with ROMK1 mRNA (10–100 ng μl^{-1}) in response to steps from a holding potential of −40 mV to voltages between −120 and +120 mV and to slow voltage ramps from −120 to +120 mV, in 2 mM external $[K^+]$ (K_o^+). Two significant points are apparent. Firstly, the currents show little or no time dependence over this voltage range. Secondly, there is a significant inward rectification of currents positive to the reversal potential (E_K). Figure 1*B* shows currents in response to ramp changes in voltage, in the presence of different external $[K^+]$. At positive potentials, outward currents became larger as external $[K^+]$ was increased, even though the driving force for outward flow of K^+ ions was reduced, resulting in a 'cross-over' of the current–voltage relationships. At voltages positive to +100 mV, or negative to −100 mV, both injected and non-injected oocytes showed the development of time-dependent currents. These were probably chloride currents, or non-selective cation currents. Since the

ROMK1 current is essentially time independent in this voltage range, and rectifies inwardly at positive potentials, it was easily distinguishable from the native currents. In non-injected oocytes, native currents were considerably smaller than ROMK1 currents in injected oocytes, typically less than 300 nA at +100 mV and −300 nA at −100 mV. Any injected oocytes showing significant time-dependent currents at large potentials were rejected from the analysis. In injected oocytes, the degree of rectification appeared to be essentially unaffected by the presence or absence of external Ca^{2+}, Mg^{2+} or Ba^{2+} at 2 mM or less, although external Ba^{2+} itself caused voltage-dependent block at negative potentials (Ho *et al.* 1993).

The rectification was further examined using excised, inside-out membrane patches. Contaminating stretch-activated channels were a more significant problem with single channel studies, since they could be activated during the application of the suction that is necessary to form on-cell patches. However, these channels were distinguishable from ROMK1 channels by virtue of their non-selectivity between cations, variable open channel current and high intrinsic open channel noise. Patches containing any channels other than ROMK1 channels were rejected from analysis. Figure 2 shows records of single channel ROMK1 currents in inside-out membrane patches exposed to 0, 1 and 10 mM internal Mg^{2+}. Internal Mg^{2+} reduces outward ROMK1 currents by reducing the apparent amplitude of single channel currents at positive potentials, consistent with a voltage-dependent block. There is little increase in the noise associated with the open channel, suggesting that Mg^{2+} block of ROMK1 is very rapid.

Figure 3*A* shows the apparent single channel current–voltage relationship in different internal [Mg^{2+}]. As internal [Mg^{2+}] is increased, the degree of rectification

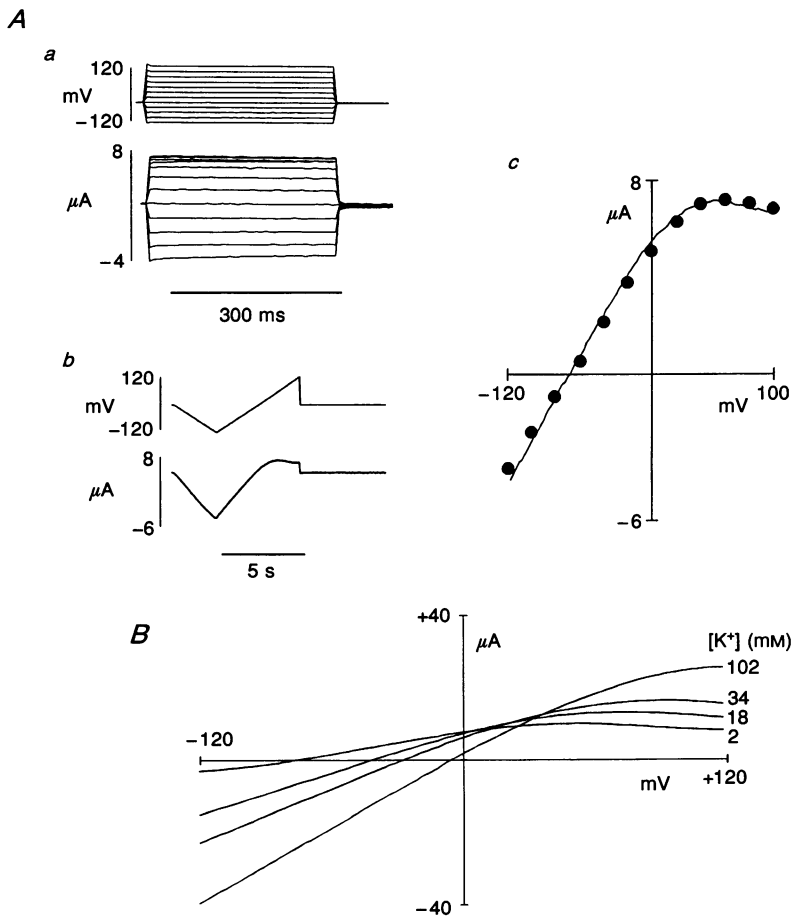

Figure 1. Whole-cell ROMK1 currents measured with two-microelectrode voltage clamp
A a, currents (below) in response to voltage steps (above) from −40 mV to voltages between −120 and +120 mV; *b*, currents (below) in response to voltage ramps (above) from 0 mV to −120 and +120 mV; *c*, steady-state (200 ms) current following a voltage step (●) superimposed on currents obtained during ramp from −120 mV to +120 mV (continuous line). All records in *A* were obtained from the same cell in 2 mM K^+. *B*, currents in response to 500 ms linear ramps from −120 to +120 mV are plotted *versus* membrane potential. Currents were measured in the external [K^+] indicated. All records in *B* were obtained from the same cell.

becomes more pronounced. Rectification is effectively abolished in the absence of Mg^{2+}. ROMK1 channels spontaneously 'run down' following patch excision (Ho *et al.* 1993), and this effect can be reversed by application of MgATP. At positive potentials, the Mg^{2+}-dependent rectification was independent of the extent of channel run-down. Thus there appears to be no overlap between the Mg^{2+} binding site responsible for rectification, and the MgATP binding site responsible for channel activation. Figure 3*B* shows the relative current–voltage (membrane

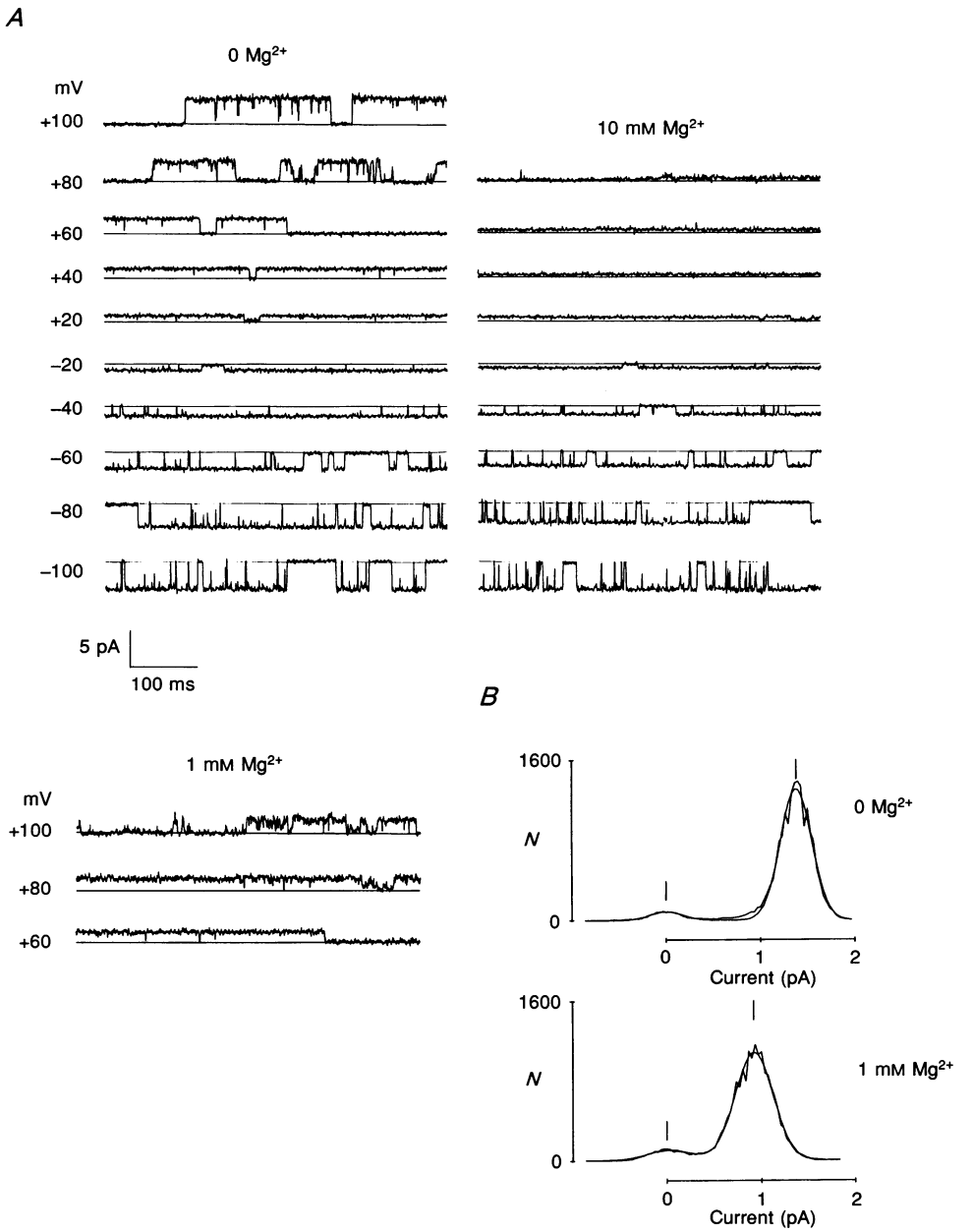

Figure 2. Effect of internal Mg^{2+} on ROMK1 single channel currents
A, currents are shown from one patch in the presence of 0, 1 and 10 mM internal Mg^{2+}, at the voltages indicated. Only currents in the outward direction are shown for 1 mM Mg^{2+}. All currents were recorded with 140 mM KINT solutions present on both sides of the membrane, with $MgCl_2$ added to internal solutions; $f_c = 500$ Hz. *B*, amplitude histogram calculated for currents recorded at +60 mV in zero (above) or 1 mM Mg^{2+} (below), and fitted with the sum of two Gaussian curves. Number of observations of a given amplitude (N) are plotted against current amplitude. Current records were filtered at 500 Hz and digitized at 1·5 kHz. Filtering at 3 kHz did not increase the apparent open channel current (not shown) in the presence of Mg^{2+}.

potential) relationship in different [Mg^{2+}]. The relationship can be described by a simple one-site model for Mg^{2+} binding (Woodhull, 1973):

$$K_{V_m} = K_0 \exp(-\delta Z V_m F/RT), \quad (1)$$

where Z is the valency of the blocking ion, δ is the fraction of the membrane field sensed by the blocking ion, K_{V_m} and K_0 are the Mg^{2+} concentrations causing half-maximal block at V_m and 0 mV, F, R and T have their usual meaning, and V_m is the membrane potential. Relative current (I_{rel}) is then given by:

$$I_{rel} = K_{V_m}/(K_{V_m} + M), \quad (2)$$

where $M = [Mg^{2+}]$. Simultaneous fitting of the three curves in Fig. 3*B* by linear regression gives a value for δ of 0·35, $K_0 = 16{\cdot}7$ mM, and $K_{+100} = 1{\cdot}0$ mM.

In order to investigate the possibility that rectification might arise in part from an intrinsic voltage dependence of gating, channel open probability was examined as a function of voltage, in the presence of different [Mg^{2+}] (Fig. 4). It is apparent from Fig. 4 that open probability is largely independent of internal [Mg^{2+}] and does not decrease at positive potentials, nor increase at negative potentials. On the contrary, open probability decreases below 0 mV. Thus the intrinsic voltage dependence of gating will tend to minimize the apparent rectification of the macroscopic currents, rather than contribute to rectification. Most patches contained multiple channels, and the low frequency with which single channel patches were obtained and the long open times observed at positive

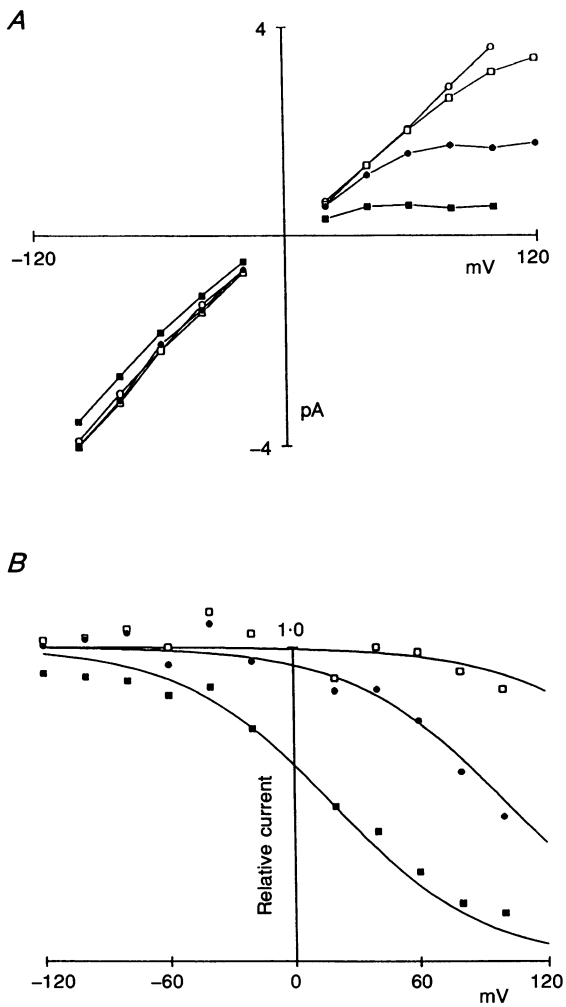

Figure 3. Effect of internal Mg^{2+} on ROMK1 single channel current–voltage relationship
A, plot of mean single channel current ($n = 2$–7 patches) *versus* membrane potential in 0 (○), 0·1 (□), 1 (●) and 10 mM internal Mg^{2+} (■). Standard errors of the mean in this and Figs 4 and 5 were generally smaller than the symbols and are not shown. *B*, plot of mean single channel current (from *A*) relative to the current in zero internal Mg^{2+} *versus* membrane potential. The fitted lines are described in the text.

potentials precluded a detailed analysis of Mg^{2+} effects on channel lifetimes.

Mg^{2+} block is relieved by extracellular K^+

Internal cation block of potassium channels has been shown in many cases to be relieved by the flow of potassium ions through the channel, a so-called 'knock-off' effect (Hille & Schwartz, 1978; Marty, 1983; Yellen, 1984; Matsuda, 1991). In Fig. 1, the increase in outward current associated with increasing K_o^+ from 0 to 140 mM is consistent with such an effect. Figure 5*A* shows the voltage dependence of single channel current in the presence and absence of Mg^{2+}, at 0 and 140 mM K_o^+. The single channel current–voltage relationships in the presence of 1 mM internal Mg^{2+} cross over one another at ~+50 mV. The normalized current–voltage relationship in 1 mM Mg^{2+} (relative to zero Mg^{2+}) (Fig. 5*B*) is shifted by approximately −50 mV in the absence of external potassium, with a corresponding reduction of K_0 to 4·4 mM.

ROMK1 shows a characteristic subconductance at negative potentials

Studies of Mg^{2+} block of inward rectifier channels in cardiac cells have shown that at intermediate inhibitory concentrations of Mg^{2+}, subconductances that are one-third and two-thirds of the full conductance become apparent (Matsuda, 1988). In the present study, no well-resolved subconductances were induced by Mg^{2+}. Many patches showed a single subconductance at negative membrane potentials (Fig. 6), in the presence and absence of Mg^{2+}. The incidence of subconductance states was variable from patch to patch, although the amount of time spent at the subconducting level was always much less than 1 % of the total time spent at the full open level. Observation of individual records suggested that the current in the subconductance state was slightly less than one-half of the full conductance, and Gaussian fits to amplitude histograms (Fig. 6*B*) revealed a mean amplitude for the subconducting level of 44 ± 3 % of the full current (S.D., $n = 30$ paired observations) at voltages between −100 and −40 mV. The amplitude variabilities within both subconducting and fully conducting states were similar, suggesting that the subconducting amplitude is not systematically over- or underestimated. The incidence, and amplitude (relative to the fully conducting state), of the subconducting state was similar in the presence or absence of Mg^{2+} (Fig. 6*B*). At positive membrane potentials, various additional very brief-duration subconductance levels were also observed in the presence or absence of Mg^{2+} (see Fig. 2*A*), although the stability and frequency of such events was too low to permit an analysis.

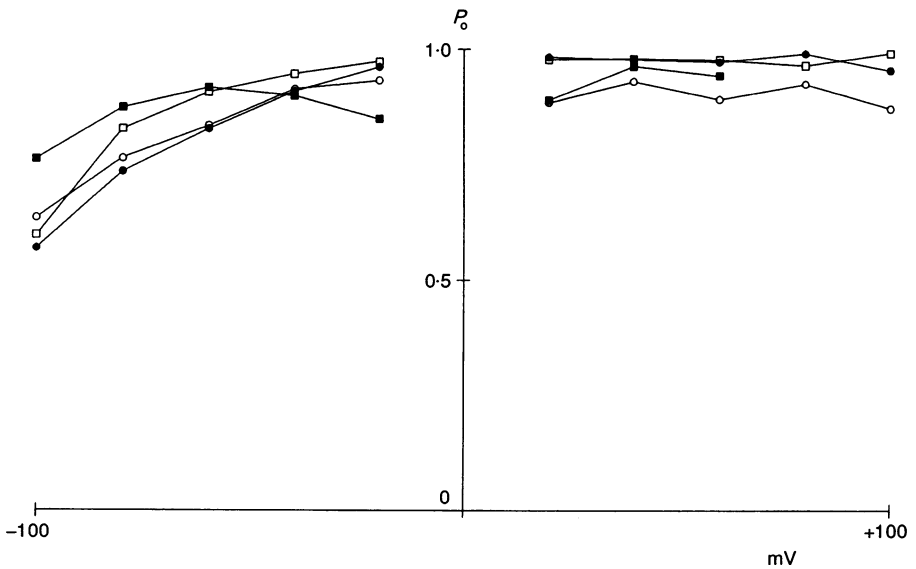

Figure 4. Voltage dependence of mean open probability (P_o) of ROMK1 channels in different internal [Mg^{2+}]

The data points show mean values of P_o ($n = 2$–7 patches). Open probability was estimated from patches containing one to five channels. ROMK1 channel activity runs down following patch isolation (Ho *et al.* 1993) and so only data from patches which retained > 90 % activity at a test potential (between −40 and −80 mV) following exposure to test solutions and voltages were included in the analysis. Symbols: ○, 0; □, 0·1; ●, 1; ■, 10 mM Mg^{2+}.

DISCUSSION

ROMK1 channel inward rectification is due to cation block, not channel gating

Inward, or anomalous rectification (Katz, 1949) is a property of a wide range of non-voltage-gated potassium channels, and these channels can be divided broadly into two groups. Rectification in the first group of 'classical' inward rectifiers and the second group of 'mild' inward rectifiers (Hille, 1992) has been well characterized in cardiac and skeletal muscle. In all inward rectifiers studied to date, rectification results from a combination of voltage-dependent gating, and internal cation block of the open channel. In ROMK1 channels, there is no evidence for a reduction of channel open probability as the membrane is depolarized (Fig. 4). Instead, inward rectification results from a block of the single channel current by internal Mg^{2+} (Fig. 2). With respect to other cations, Na^+ is the most likely physiologically relevant cation that could cause open channel block. However, preliminary experiments (not shown) suggest that Na^+ block of ROMK1 is very weak, K_0 being $\gg$ 50 mM, and it therefore seems unlikely that internal Na^+ contributes significantly to rectification *in vivo*.

The voltage dependence of rectification in 'classical' inwardly rectifying channels is very steep; an e-fold decrease of conductance occurs for a potential change of 5–10 mV, equivalent to the movement of 2·5–5 elementary charges across the membrane field (Kurachi, 1985; Matsuda *et al.* 1987; Burton & Hutter, 1990), and the half-maximal

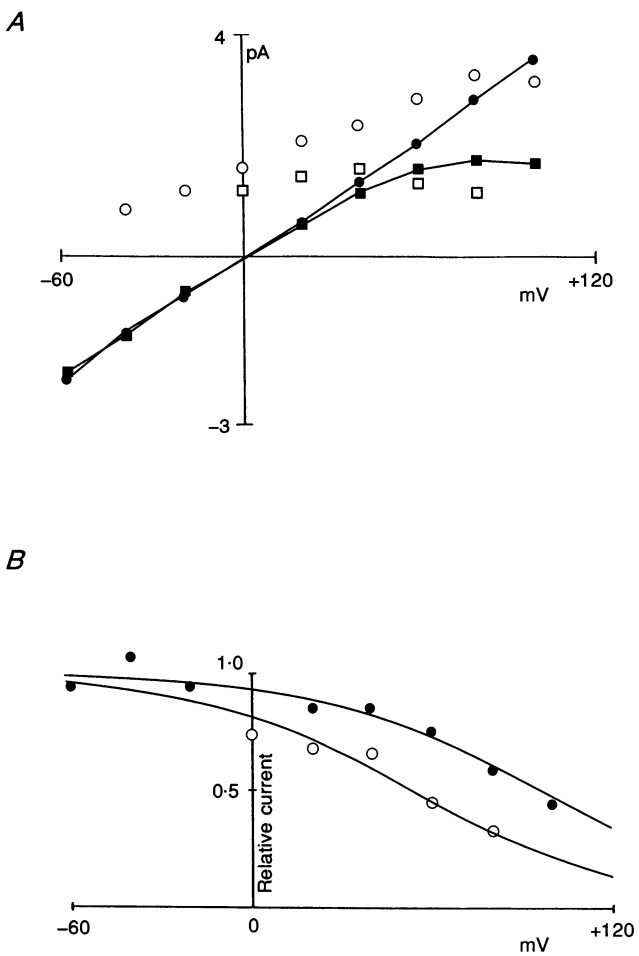

Figure 5. External $[K^+]$ dependence of internal Mg^{2+} block of ROMK1 single channel currents
A, plot of mean single channel current ($n = 2$–6 patches) *versus* membrane potential in 0 (circles) and 1 mM (squares) internal Mg^{2+}, with zero (open symbols) or 140 mM (filled symbols) K_o^+. External $[K^+]$ was varied by equimolar substitution of Na^+ for K^+ in the patch pipette. *B*, plot of voltage dependence of mean single channel current in 1 mM Mg^{2+}, relative to the current in zero Mg^{2+}, for data shown in *A* (filled symbols, 140 mM K_o^+; open symbols, zero K_o^+). The fitted lines are described in the text.

blocking concentration of Mg^{2+} is typically in the micromolar range (Matsuda, 1988). For the second group of 'mild' inward rectifiers, which includes the K^+_{ATP} channels of pancreatic, cardiac, skeletal and neuronal tissues, and acetylcholine-activated K^+ channels in the heart, rectification is typically equivalent to the movement of 0·5–1 elementary particles across the membrane field (reviewed in Hille, 1992), and the half-maximal blocking concentration of Mg^{2+} is typically in the millimolar range (e.g. Horie, Irisawa & Noma, 1987). IRK1 has the properties of a 'classical' inward rectifier (Kubo *et al.* 1993), whereas the Mg^{2+}-dependent rectification of ROMK1 clearly characterizes this channel as a 'mild' inward rectifier.

Internal block of ROMK1 by Mg^{2+} is rapid and weak, resembling Mg^{2+} block of K^+_{ATP} channels

In ROMK1, the half-maximal blocking concentration of Mg^{2+} at 0 mV (K_0) is 16·7 mM; eqn (1) predicts K_{+40} is 4·2 mM, and K_{+70} is 2·4 mM. This latter value is three orders of magnitude higher than the blocking concentration required for cardiac iK1 channels ($K_{+70} = 1{\cdot}7\ \mu M$; Matsuda, 1988). However, the Mg^{2+} block is very similar to that observed in ATP-sensitive K^+ channels in heart and pancreas. In the heart, K_{+40} was ~5 mM in 140 mM K^+_o (Horie *et al.* 1987, their Fig. 4*B*),

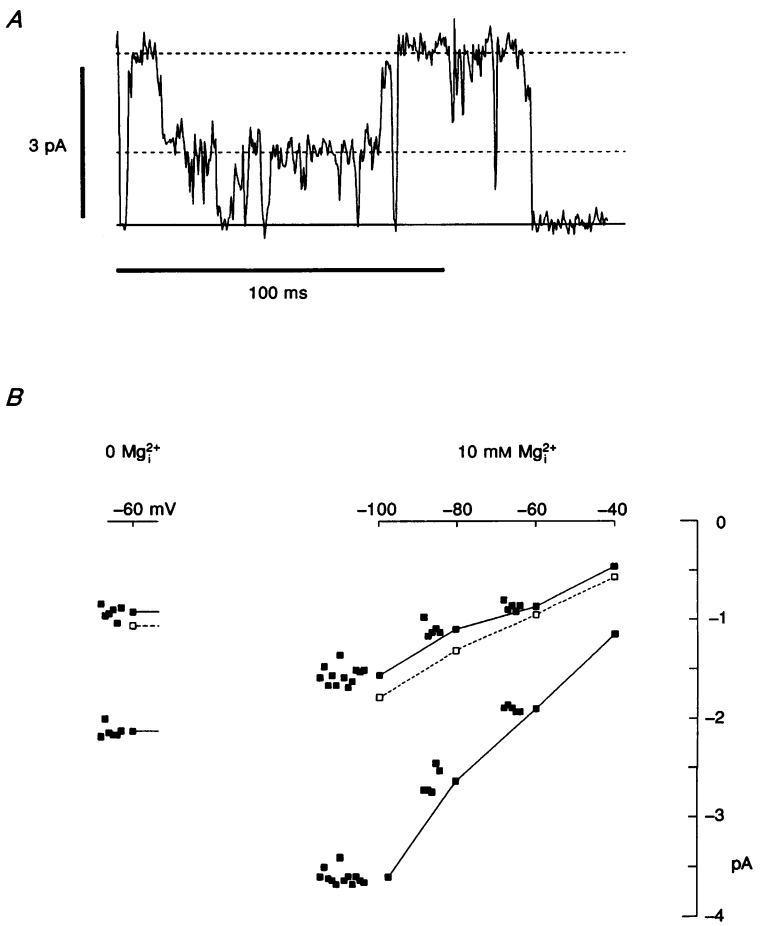

Figure 6. Subconductance behaviour of ROMK1 channels
A, record of membrane current at −100 mV, showing typically observed subconductance that is slightly less than one-half of the full conductance. *B*, amplitude histograms of full and subconducting currents were calculated, and individual estimates of full and subconductance currents at negative voltages ($n = 1$–12 at each voltage), together with mean values in the presence of 0 and 10 mM internal Mg^{2+}, are plotted. The dashed line and open symbols indicate values corresponding to exactly one-half of the full conductance.

with the Mg^{2+} blocking site being situated 35 % of the electrical distance from the inner mouth of the channel; decreasing K_o^+ from 140 mM to zero decreased the K_{+40} ~10-fold from ~5 mM to ~0·5 mM. The data in Fig. 5 predict that K_{+40} for ROMK1 in zero potassium should be 1·46 mM, suggesting that the extent of the relief of block by external K^+ is similar in the two channels. In pancreatic β-cells, K_0 for Mg^{2+} was 34 mM with 140 mM K_o^+, and again a binding site situated 35 % of the electrical distance from the inner mouth of the pore was predicted (Ciani & Ribalet, 1988). We presume that the apparent reduction of single channel current by Mg^{2+} results from an unresolved block of the open channel, rather than from an allosteric action decreasing the single channel current. Horie *et al.* (1987) estimated the kinetics of Mg^{2+} block of cardiac K_{ATP}^+ channels from analysis of noise associated with open channels. Their analysis predicts that blocking and unblocking rates are both about 15000 s^{-1} in 1 mM Mg^{2+} at 0 mV. At 500 Hz filtering, the increase in open channel noise in the presence of internal Mg^{2+} was not marked in the present study (Fig. 2), consistent with similar rapid kinetics in ROMK1. Detailed studies have not been performed for renal K_{ATP}^+ channels, to which ROMK1 may be related, although the rectification observed in the experiments of Wang and colleagues (Wang, Schwab & Giebisch, 1990*a*; Wang, White, Geibel & Giebisch, 1990*b*), obtained in the presence of 1·5–2 mM Mg^{2+} would suggest that the Mg^{2+} block of ROMK1 is similar to that of the K_{ATP}^+ channels found in the collecting duct and loop of Henle.

Relief of Mg^{2+} block of ROMK1 by external K^+

The apparent reduction of unitary current by Mg^{2+} presumably results from a rapid, unresolvable block of the open channel. It has been suggested that the repulsive effect of K^+ ions in neighbouring binding sites within the pore should decrease the affinity for binding of Mg^{2+}, and hence relieve the block (Hille & Schwartz, 1978; Marty, 1983; Yellen, 1984; Matsuda, 1991). As shown in Fig. 5*A*, some relief of Mg^{2+} block of channel current is observed as external K^+ is increased from 0 to 140 mM. The relief, equivalent to a shift in the voltage dependence of approximately +50 mV (Fig. 5*B*), is not as marked as that observed for external K^+ relief of internal Na^+ block of Ca^{2+}-activated K^+ channels (Yellen, 1984), but as noted above, is similar to that observed with internal Mg^{2+} block of the cardiac inward rectifier channels (Horie *et al.* 1987).

Mg^{2+} block of ROMK1 channels compared to cation block of other cloned K^+ channels

The present demonstration of rectification in a potassium channel clone due to internal magnesium block now makes it possible to address the structural requirements for such block. If the steep rectification of IRK1 (Kubo *et al.* 1993) is also due to Mg^{2+} block, then the Mg^{2+} sensitivity must be quite different from ROMK1, suggesting a structural difference at the Mg^{2+}-binding site. Much recent work has localized external and internal cation binding sites to the H5 loop in voltage-activated K^+ channels (MacKinnon & Miller, 1989; MacKinnon, Heginbotham & Abramson, 1990; MacKinnon & Yellen, 1990; Hartmann, Kirsch, Drewe, Taglialatela, Joho & Brown, 1991; Yellen, Jurman, Abramson & MacKinnon, 1991; Heginbotham, Abramson & MacKinnon, 1992; Heginbotham & MacKinnon, 1992). Within the H5 region, there is almost complete identity between ROMK1 and IRK1, the only difference being a threonine in IRK1 and a valine in ROMK1 at position 140 (V140). Internal Mg^{2+} blocking of NMDA channels has recently been ascribed to an asparagine residue at position 598 within the putative pore, which, when mutated to a glutamine permits Mg^{2+} permeability and lessens the Mg^{2+} block of the channel (Burnashev *et al.* 1992; Mori, Masaki, Yamakura & Mishina, 1992). In addition, replacement of an arginine residue by glutamine at a homologous site in α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor channels alters the cation permeability of these channels (Boulter *et al.* 1990; Sakimura *et al.* 1990). A glutamine residue (Q139) immediately precedes a valine (V140) within ROMK1 H5. This (Q139, V140) site is adjacent to the tyrosine–glycine (YG) sequence that appears to be a major requirement for K^+ selectivity in voltage-dependent potassium channels (Heginbotham *et al.* 1992) and is thus a reasonable candidate site for internal cation block. On the other hand, recent work (Isacoff, Lopez, Jan & Jan, 1993; Lopez, Jan & Jan, 1993; Slesinger, Jan & Jan, 1993) suggests an involvement of both the S4–S5 linker and the S6 region, which potentially contribute to the inner vestibule of the channel pore, in allowing Ba^{2+} access to internal binding sites in *Shaker* channels, suggesting that other regions may contribute to cation blocking sites in ROMK1 and other inwardly rectifying potassium channels.

ROMK1 subconductance states are not altered, or induced, by Mg^{2+}

Subconductance states have been well characterized for the cardiac inward rectifier potassium channel by Matsuda and colleagues (Matsuda, 1988; Matsuda *et al.* 1989). In these studies, current fluctuations between the closed state and three evenly spaced open levels were readily observed in the presence of internal Mg^{2+}, or external Cs^+ and Rb^+. The even spacing of the sublevels has been taken as evidence that the cardiac inward rectifier K^+ channel is composed of three pore-forming units that normally gate simultaneously. Blocking of one of the pore-forming units by non-permeant Mg^{2+} from the inside, or Rb^+ or Cs^+ from the outside reduces the conductance of the channel by one-third, and simultaneously increases the closing rate of the other pore-forming units. The subconductance levels are observed at potentials both positive and negative to the reversal

potential. Similar sublevels have not been observed in the present results which argue, therefore, against such a triple-barrelled structure for ROMK1. Although various subconductances were apparent at positive membrane potentials, only one clearly resolvable subconductance state of ROMK1 channels was evident. This was only well resolved at negative potentials, and was 0·44 times the full conductance (Fig. 6). Because this is not a simple fraction of the full conductance, and since ROMK1 channels are presumably only forming as homomultimers (the oocyte is injected with a single cRNA species), it is difficult to reconcile these results with a model in which the subconductance arises because of a breakdown in the simultaneous gating of an integral number of identical pore-forming units, unless the closing of one unit could affect the conductance of a second unit (e.g. Berry & Edmonds, 1993). The subconductance was proportionately the same reduction (i.e. 0·44 × full conductance) in the presence and absence of Mg^{2+}. This result argues that Mg^{2+} can block the full and subconducting states equally well. Subconductance states of K^+_{ATP} channels appear infrequently and have not been studied in detail. However, it is intriguing to note that in an early paper characterizing these channels in cardiac cells (Kakei, Noma & Shibasaki, 1985) observed a similar subconductance (~0·45; their Fig. 2*B*), again consistent with a similar pore structure for K^+_{ATP} channels and ROMK1 channels.

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