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Rapid report

Ion permeation through a G-protein activated (GIRK1/GIRK5) inwardly rectifying potassium channel

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Abstract

In order to further investigate a G-protein activated inwardly rectifying potassium channel subunit, GIRK1 was expressed in *Xenopus* oocytes (where it coassembles with the endogenous GIRK5). The mechanism underlying ion permeation and rectification were measured in isolated inside-out patches. Single channel current amplitudes under symmetrical K^+ concentrations at different holding potentials were evaluated. Inward-rectification of K^+-currents through open GIRK1/GIRK5 channels was removed by washing out polyamines and Mg^{2+} ions. We developed a simple 'two-sites-three-barrier' (2S3B) Eyring rate theory model of K^+ ion permeation for GIRK1/GIRK5 channels. The resulting optimized parameter-set will be used as a working model for subsequent investigation regarding K^+ permeation process through the GIRK1/GIRK5 channel. © 1998 Elsevier Science B.V.

Keywords: GIRK; Inward rectifier; Potassium channel; Eyring rate theory; G-Protein; Ion permeation

The inwardly rectifying K^+ channels belonging to the GIRK family are known to play a crucial role in controlling excitability in heart and brain. In heart tissue, GIRK channels are opened by interaction with activated G-proteins, mediated by muscarinic m2 receptors. In brain, the activation process of the GIRK channels follows a similar course, except that the neurotransmitters and receptors involved fall in the class of serotonin (5HT1A), opioid, $GABA_B$ and other ones (see [1], for review). Inward-rectification results from a voltage-dependent block of channels by internal Mg^{2+} [2] and cationic polyamines (spermine, spermidine, putresceine; see [1], for review). Despite the existent body of structural and functional data, little is known about the molecular mechanism of gating of the GIRK1/GIRK5 channel [1,3], its rectification and open-pore properties. Moreover, recent work [4,5] involving non G-protein gated inwardly rectifying channels describe rectifying properties even in the absence of Mg^{2+} and polyamimes, calling attention on the existence of an intrinsic voltage-dependent mechanism which might be enhanced by the above-mentioned ions, generating inward-rectification.We report here that in the case of GIRK1/GIRK5 channels, Mg^{2+} and polyamine ions are seemingly mainly responsible elements for the rectification mechanism. A 'two-sites-three-barrier' (2S3B) Eyring model for K^+ permeation through the open channel was adapted and its parameters evaluated by fitting it to the experimental I/V data. This

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analysis of energy barriers, wells and electrical distances will be regarded as a ground for future studies of GIRK open-channel properties.

Removal of *Xenopus laevis* oocytes, defolliculation, mRNA synthesis and injection were carried out as described [6]. Oocytes injected with 1 ng GIRK1 cRNA were kept in an incubator at 19°C and used 3–7 days after injection for electrophysiological recordings. Patch-clamp experiments were performed on devitellinized oocytes, which were placed into a 500 μl volume recording chamber filled with bathing-solution (BS). After patch formation, inside-out patches were achieved by briefly air-exposing the pipette tip. GIRK1/GIRK5 channels were activated by bath application of GTP-γ-S (100 μM). Currents were recorded at different holding potentials using an Axopatch 1D amplifier, equipped with the IHS integrating headstage (Axon Instruments; Burlingame; USA) and connected via a TL-125 A/D converter to an IBM compatible computer. Mg^{2+} and polyamine free experiments were carried out on the same membrane-patch as used for control experiments, by replacing BS with BS0Mg by means of gravitational superfusion. In order to ensure complete wash-out of polyamines, BS0Mg was superfused through the recording chamber for at least 4 min before measurements were performed. Holding potentials ranged from −120 to +50 mV. Current traces were low-pass

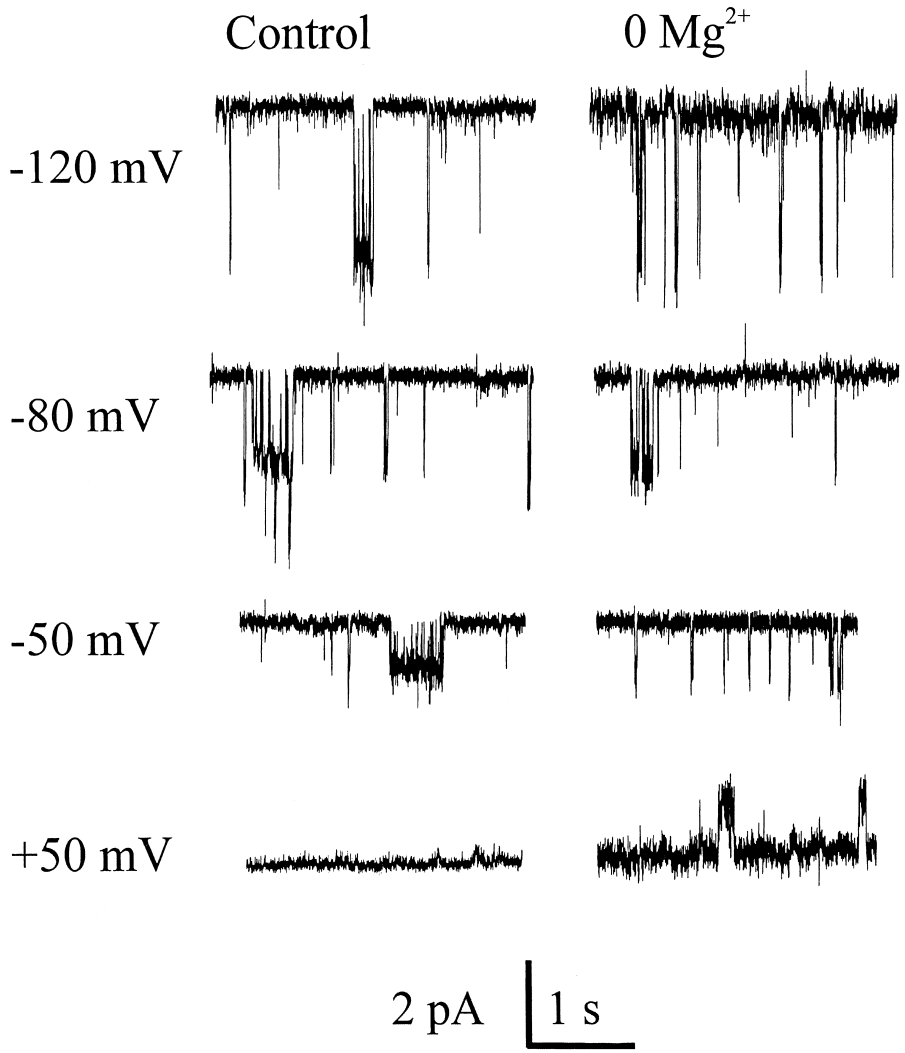

Fig. 1. Voltage dependence of the current elicited by K^+ ions movement through GIRK1/GIRK5 channels in presence of Mg^{2+} and polyamine ions (left panel) and after their wash-out (right panel).

filtered at 1 kHz, digitized at 5 kHz and stored on hard-disk for subsequent analysis. Single-channel current amplitudes were measured by computer-aided inspection of the original traces. Current amplitude values, statistics and I/V curve of the channel were estimated with the use of Origin 4.1 software (Micro-Cal Software, Sunnyvale, CA). Mathematica 3.0 (Wolfram Research, IL) was employed for implementation of the 2S3B model according to Begenisich and Cahalan [7].

BS composition was (in mM): 140 KCl, 10 NaCl, 1 EGTA, 4 $MgCl_2$, 1 Na^+-ATP, 10 HEPES (titrated with KOH at pH 7.5). BS0Mg (in mM): 140 KCl, 10 NaCl, 1 EDTA, 1 Na^+-ATP, 10 HEPES (titrated with KOH at pH 7.5). The composition of pipette solution was (in mM): 150 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 0.05 $GdCl_3$, 10 HEPES (titrated with KOH at pH 7.5). In the presence of Mg^{2+} and polyamines, the current mediated by GIRK1/GIRK5 heteromultimers showed a quite strong rectification behavior (see Fig. 1, left panel). After extensive dialysis of BS and replacing it with BS0Mg, the current flux through the channel is restored in the range of positive potentials (Fig. 1, right panel). The main conclusion inferred from these data is that, for the particular case of the GIRK1/GIRK5 channel, inward-rectification is produced mainly by extrinsic cations and little arguments would entail the quest for additional mechanisms dominating occurrence of the rectification property. Fig. 2 (top panel) shows I/V curves of the single-channel currents before and after washout of Mg^{2+} and polyamines. As judged by the I/V curve, removal of Mg^{2+} and polyamines turns the channel into a weakly inward rectifying K^+-selective pore with single channel slope conductances of 32.9 ± 0.5 and 22.3 ± 1.3 (mean value $\pm$ SEM, $n = 3$, $p < 0.01$), measured in the range from -50 to -30 mV and from $+30$ to $+50$ mV, respectively. The single channel slope conductance, measured in the range from -120 to -80 mV was 28.9 ± 0.6 pS and 31.9 ± 0.6 pS before and after wash-out, respectively (mean value $\pm$ SEM, $n = 3$, mean values are not statistically significant different at the $p < 0.05$ level). Since removal of inward-rectification allows to evaluate single channel currents over a wider range of potential values, we developed a description of ion permeation on the frame of Eyring rate theory. The corresponding Eyring parameters (energies, electrical distances, interaction energy), as derived from a non-linear optimization Levenberg-Marquardt algorithm applied to our experimental data, are presented in Fig. 2 (lower panel). Even this preliminary stage of the modeling enabled us to correctly predict two important characteristic properties of single-file models. Namely, the single-channel conductance in the range of low concentrations of the permeating ion was predicted to vary proportional to the square root of $[K^+_{out}]$ ($\sigma \approx [K^+_{out}]^{0.43}$) and at high $[K^+]$, conductance was seen to reach a saturating value and further decline (data not shown). Nevertheless, further experiments (e.g. evaluation of the single-channel conductance at various potassium concentrations) will be carried out in order to refine our permeation model for the particular case of the GIRK1/GIRK5 channel and narrow the potential freedom of its set of parameters.

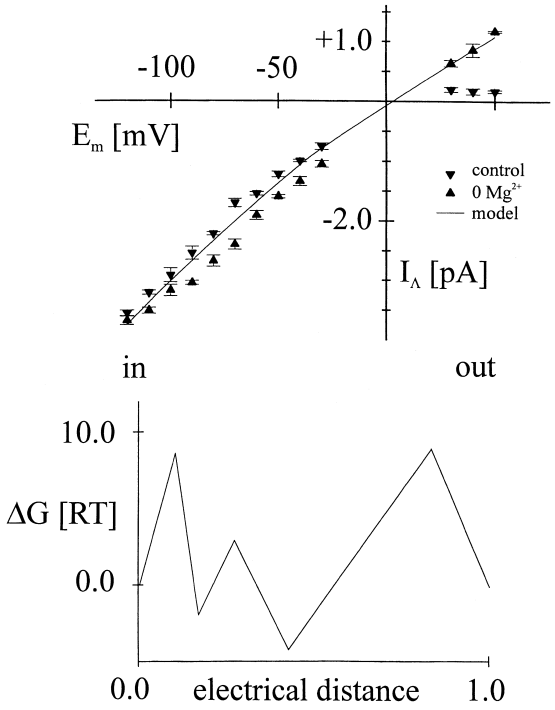

Fig. 2. I/V curves for K^+ permeation through GIRK1/GIRK5 channels in presence and absence of Mg^{2+} and polyamines (bars represent SEM). The continuous line represents the best prediction given by a 2S3B ('two-sites-three-barriers') model for K^+ permeation through the ion channel at different holding potentials having removed the inward-rectification property of the channel. The schematic energy diagram of the 2S3B model for K^+ permeation through GIRK1/GIRK5 channels in absence of Mg^{2+} and polyamines is shown at the lower panel.

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