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Theoretical Background for Inward Rectification

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Theoretical background has been reviewed for inward rectification due to a potassium current termed IRK. The Eyring rate theory in which the thermodynamic rate coefficient for chemical reactants (channels and ions in this case) can be described in terms of energy barriers for potassium ions can mimic not only the polarity and degree of rectification but also the voltage-dependence of the barium-induced IRK block. The model predicts that the blocking site locates 30–70% depth from the outer margin of the IRK channel.

Keywords : Potassium channel, barium, Constant-field theory, GHK-model, Arrhenius, Energy-barrier model, Eyring rate theory, Woodhull model

INTRODUCTION

Excitable membranes show nonlinear changes in their conductance with voltage, a phenomenon referred to as rectification [8]. The polarity of rectification is called inward when ions get into the cell through channels more easily than they get out, and outward rectification describes the reversed phenomenon.

The functional significance of rectification depends critically on the polarity and degree of rectification. For example, strong inward rectification due to a potassium current termed IRK is essential for the stable resting membrane potential at about −90 mV and long plateau (range, 100–300 ms) that is a feature of the action potential in cardiomyocytes; under physiological conditions, very little IRK flows outwardly at potentials positive to −40 mV, which thereby avoiding the short-circuit for the action potential and hence minimizing the ATP consumption for pumping out sodium and calcium ions entering the cells during the plateau [8]. A potassium current with electrophysiological properties identical or at least quite similar to IRK also occurs in many cell types including skeletal muscles, nerve and glial cells, endocrine cells, immune cells, and endothelial cells [14].

Regarding the pharmacology of IRK, it has been known that IRK is sensitive to the block by barium with an IC_{50} value less than 100 μM [1, 4, 6, 7–9, 14, 18, 20]. The block is characterized by its time- and voltage-dependence, and greater and faster block occurs with increased negativity of the membrane potential. Fig. 1 shows whole-cell/patch-clamp recordings from human endothelial cells, and is an example of the barium-induced IRK block [15, 17, 19].

Voltage-dependent block on ionic currents has been found in many excitable membranes and studies of these mechanisms provided quite useful information about the structure and function of ion channels [1]. In this article, we will review the theoretical background for the actions of barium on the IRK channels by focusing on two different biophysical models: first, the constant-field model and second, the energy-barrier model.

Since these models basically deal with elementary properties of ions in solution, those readers who might not be familiar with thermodynamics are suggested to skip those equations with which results in Fig. 3 and Fig. 5 have been calculated.

Constant-field model

Fig. 2 illustrates electrodiffusion of ion S having valence of z_s across a hypothetical membrane in the presence of electrochemical gradients for S. According to Nernst [13] and Planck [16], the ionic current carried by S (I_s) can be expressed as the Nernst-Planck

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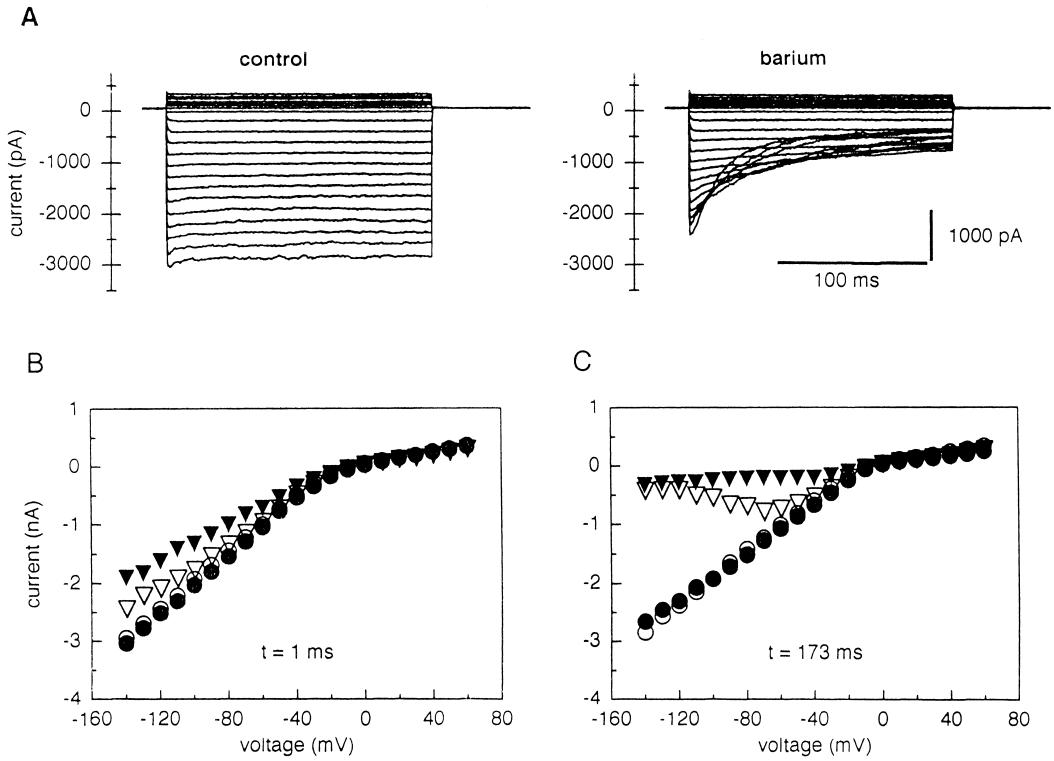

Fig. 1 The barium-induced IRK block in cultured human umbilical vein endothelial cells. *A*: shown are superimposed IRK traces in response to 21 different command pulses (not shown here for the simplicity of the illustration) from the holding potential of 0 mV. The pulses started from +30 mV with −10 mV increments. Left, control in 150 mM potassium. Right, barium (60 μM). *B* and *C*: *I-V* curves when measured at 1 and 173 ms after the onset of command pulses. Filled circles, control. Open triangles, barium (60 μM). Filled triangles, barium (300 μM, current traces not shown in *A*). Open circles, wash. METHODS: cultured endothelial cells were obtained with methods described by Jaffe *et al.* [11]. The bath solution was composed of (mM) 150 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 12 dextrose (pH 7.3 with KOH). The pipette solution was composed of (mM): 120 K-aspartate, 40 NaCl, 5 HEPES, 11 EGTA (pH 7.3 with KOH). Liquid junction potential of 10 mV has been corrected for all the data points. Experiments were carried out at 22–24 ℃. Results presented here are unpublished observations by T Tokimasa, A Surprenant and RA North.

equation,

$$I_s = -z_s F D_s (dc_s/dx + (F z_s c_s/RT)(d\Psi/dx)) \quad \cdots\cdots (1)$$

where D_s, dc_s/dx and $d\Psi/dx$ denote diffusion coefficient of Fick, the concentration profile for S and the electrical potential profile within the membrane, respectively. F, R and T are Faraday's constant, gas constant and the absolute temperature, respectively.

The most commonly used theory for describing ionic current of excitable membrane is based on Goldman [5] and Hodgkin and Katz [10], and hence the theory is called the Goldman-Hodgkin-Katz (GHK) constant-field model. There are basic assumptions for this model. First, the movement of ions within the membrane follows the Nernst-Planck equation; second, ions move across the membrane without interacting with each other; and finally, $d\Psi/dx$ in Eq. 1 is constant defined as $-d\Psi/dx = E$. The integration of Eq.1 across the membrane having the thickness of l gives,

$$I_s = P_s z_s^2 EF^2/RT \cdot ([S]_i - [S]_o \exp(-z_s FE/RT))/(1-\exp(-z_s FE/RT)) \quad \cdots\cdots (2)$$

where P_s represents permeability of S which can be defined by multiple of D_s and water-membrane partition coefficient (β_s) divided

by the thickness of the membrane or $P_s = D_s \beta_s / l$. Fig. 3 illustrates current-voltage (*I-V*) curves based on Eq. 2 for four different $[S]_o/[S]_i$ values assuming that ion S is a monovalent cation having the P_s value at 1.0 cm/s. It is clear from Fig. 3 that GHK model can only describe outward rectification with negative reversal potentials, and inward rectification with positive reversal potentials. This is not realistic for IRK however.

Energy-barrier model (Eyring rate theory)

An alternative formulation is called the energy-barrier model or the Eyring rate theory [3] in which the thermodynamic rate coefficient for chemical reactants can be described in terms of energy barriers which must be hopped over by reactants (Fig. 4). This theory can be applied for ionic currents if we assume that each ion crossing the membrane from one side to the other must hop over an energy barrier.

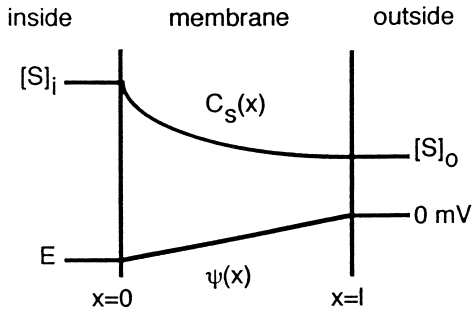

Fig. 2 Electrodiffusion in hypothetical membrane. Cs(x) and ψ(x), respectively denote the concentration profile of ion S, and the electrical potential profile within the membrane having the thickness of l.

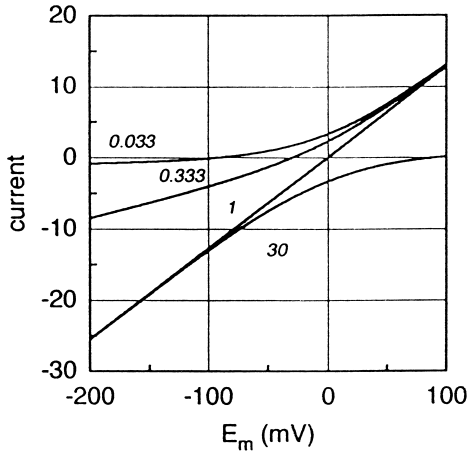

Fig. 3 *I–V* curves predicted with the GHK-equation. Curves are drawn from Eq. 2 with $[S]_o/[S]_i$ value at 0.033, 0.333, 1 and 30 as indicated. Ordinate is in an arbitrary unit.

Based on the law of mass reaction, the flux of a reactant is proportional to the concentration of the reactant, and the proportionality constant is named rate coefficient *k*. Hence, for ion flux through a single energy barrier in Fig. 4, the influx (J_i) and efflux (J_o) can be written as,

$$J_i = k_1 \beta_s [S]_o \quad \cdots\cdots (3)$$
$$J_o = k_2 \beta_s [S]_i \quad \cdots\cdots (4)$$

where K_1 and K_2 are rate coefficient, $[S]_o$ and $[S]_i$, β_s have the same meaning as that for the constant-field model. According to Arrhenius [2], the rate coefficients at thermodynamic equilibrium can be expressed as,

$$K_1 = A \cdot \exp(-U/RT) \quad \cdots\cdots (5)$$
$$K_2 = A \cdot \exp(-U/RT) \quad \cdots\cdots (6)$$

where U represents the standard free energy of activation and A is a constant which is characteristic for hopping over the barrier. When an electric field E is applied to the membrane, the free energy of activation is no longer symmetrical since the energy barrier is influenced by a factor of $\delta z_s FE$, where δ represents the location of the barrier. Thus,

$$K_1 = A \cdot \exp(-(U + (1-\delta) z_s FE/RT)) = k(0) \cdot \exp((1-\delta) z_s FE/RT) \quad \cdots\cdots (7)$$

$$K_2 = A \cdot \exp(-(U - \delta z_s FE/RT)) = k(0) \cdot \exp(\delta z_s FE/RT) \quad \cdots\cdots (8)$$

where, $K(0) = A \cdot \exp(-U/RT)$. The net current flow across the membrane is,

$$I_s = z_s F (J_o - J_i) \quad \cdots\cdots (9)$$

Hence,

$$I_s = z_s F \beta_s k_o([S]_i \cdot \exp(\delta z_s FE/RT) - [S]_o \cdot \exp(-(1-\delta) z_s FE/RT)) \quad \cdots\cdots (10)$$

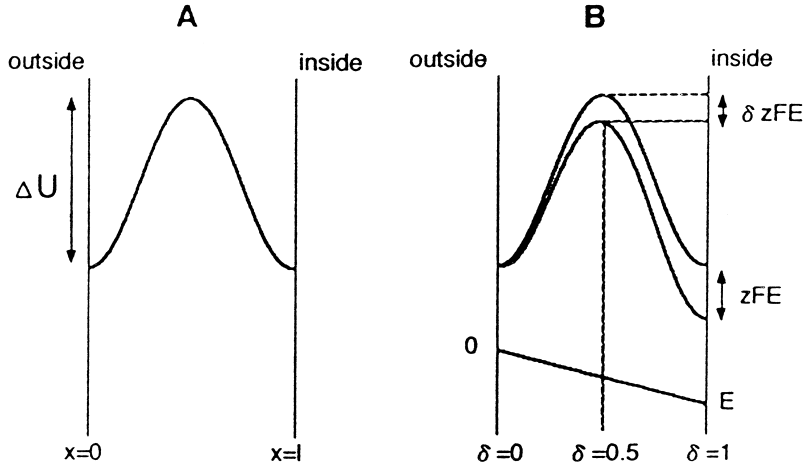

Fig. 4 The energy-barrier model. Symmetrical free energy profile such as that shown in *A* becomes asymmetrical when the membrane potential of E is applied to the membrane. In *B*, the profile is drawn on an assumption that δ equals 0.5.

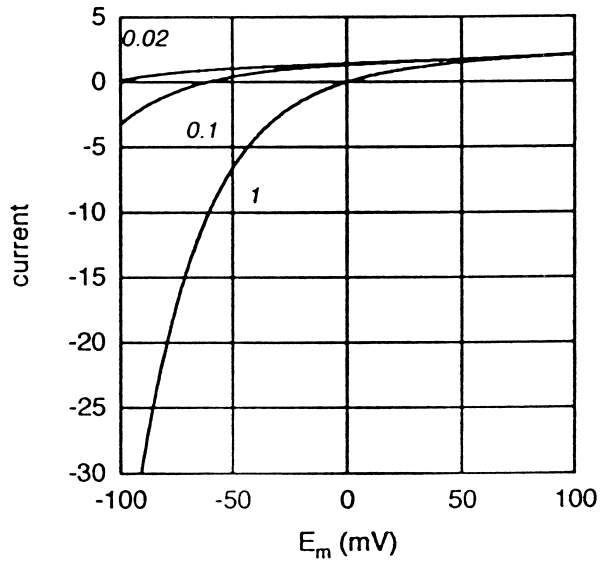

Fig. 5 *I–V* curves predicted with the energy-barrier model. Curves are drawn from Eq. 10 with $[S]_o/[S]_i$ value at 0.02, 0.1 and 1 as indicated. Like in Fig. 4, ordinate is in an arbitrary unit.

Fig. 5 shows *I–V* curves calculated with Eq. 10. Unlike the constant field model, the Eyring rate theory can predict either inward or outward rectification with positive or negative reversal potentials. As can be seen in Fig. 5, the degree of inward rectification depends on the ratio $[S]_o/[S]_i$ for any fixed value of δ.

Woodhull model for channel block

In this section, we will consider some extension of the Eyring rate theory developed by Woodhull [21]. This model (now called Woodhull model) is based on the following assumptions (Fig. 6). First, there is a barium binding site (X) inside the channel flanked by energy barriers, and the binding of barium to the site X follows first-order kinetics with a one-to-one ratio. Given that barium can reach the site X from either side of the membrane,

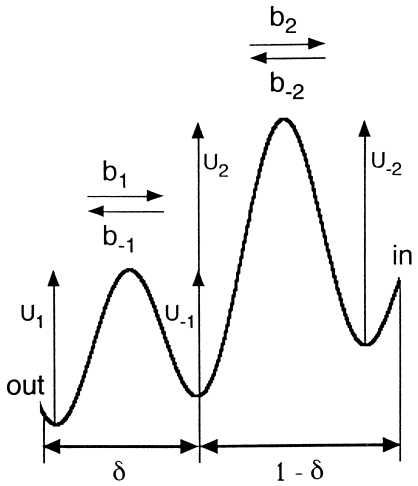

Fig. 6 The Woodhull model illustrating the Free energy profile of the channel and the blocking site. U_i and K_i. represent the heights of the energy barriers and the rate constants, respectively.

$$Ba_o \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} BaX \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} Ba_{in}$$

where K_i (s^{-1}) denotes the rate constant for the direction specified; K_1 and K_{-2} are pseudo first-order rate constants since they include the factors for the concentration of barium. Second, the K_i varies as exponential function of the membrane potential as in the Eyring rate theory [2]. Third, potassium cannot pass through the channel when barium occupies the site X, and a current carried by barium is negligible compared to IRK. Fourth, potassium does not interfere with the binding of barium to the site X. Fifth, all channels are open but the Woodhull model does not deal with how they open. Finally, the system as a whole is in a steady-state of blockage.

The probability (p) that the site X is not occupied by barium is constant at the steady-state and hence the p value can be described by a ratio of the sum of rates for leaving the site X to the sum of all rates or $p=(k_{-1}+k_2)/(k_1+k_2+k_{-1}+k_{-2})$. Given that a voltage ($E$) is applied to the channel and the site X locates at δ ($0\leq\delta\leq1$, $\delta=0$ at the outer margin of the membrane), the rates can be expressed by,

$$k_1=[Ba]_o\ a_1\ \exp((-U_1/RT)-(-z\,\delta\,FE/2RT)) \quad \cdots\cdots (11)$$

$$k_{-1}=a_{-1}\ \exp((-U_{-1}/RT)+(z\,\delta\,FE/2RT)) \quad \cdots\cdots (12)$$

$$k_2=a_2\ \exp((-U_2/RT)-((-z\ (1-\delta)\,\delta\,FE/2RT))) \quad \cdots\cdots (13)$$

$$k_{-2}=[Ba]_i\ a_{-2}\ \exp((-U_{-2}/RT)+((z\ (1-\delta)\,\delta\,FE/2RT))) \quad \cdots\cdots (14)$$

where $[Ba]_o$ and $[Ba]_i$ denote the concentration of barium in the bulky solution surrounding the channel, and a_i denotes the proportionality constant in case $[Ba]_o$ and $[Ba]_i$ are different from the real concentration of barium within the channel. Let,

$$b_i=a_i\exp(-U_i/RT) \quad \cdots\cdots (15)$$

then,

$$p=\frac{\frac{b_{-1}}{b_1}\exp\left(\frac{z\,\delta\,FE}{RT}\right)+\frac{b_2}{b_1}\exp\left(\frac{(2\delta-1)zFE}{a\,RT}\right)}{[Ba]_o+[Ba]_i\frac{b_{-2}}{b_1}\exp\left(\frac{zFE}{2RT}\right)+\frac{b_{-1}}{b_1}\exp\left(\frac{z\,\delta\,FE}{RT}\right)+\frac{b_2}{b_1}\exp\left(\frac{(2\delta-1)zFE}{a\,RT}\right)} \quad \cdots\cdots (16)$$

This is a general formulation for the barium-induced IRK block in which the relationship between b_1, b_{-1}, b_2 and b_{-2} is restricted to $b_{-2}/b_2=b_1/b_{-1}$, since, if $E=0$ and $[Ba]_o=[Ba]_i$, the net transport must be zero for barium. The δ value can be estimated from this equation.

There is another method to estimate the δ value by measuring the time constant of the barium-induced IRK block (see Fig. 1). The reversible binding between the channel (R) and barium (Ba) can be formulated as,

$$Ba + R \underset{K_2}{\overset{K_1}{\rightleftharpoons}} BaR$$

where BaR denotes the blocked channel, and K_1 ($s^{-1}\,M^{-1}$) and K_2 (s^{-1}) represent the forward and reverse rate constants. The quotient K_2/K_1 equals the equilibrium dissociation constant K_d. In equilibrium,

$$k_1[Ba][R]=k_2[BaR] \quad \cdots\cdots (17)$$

The extent of the block expressed as the ratio of currents with and without barium (defined as y) can be written as,

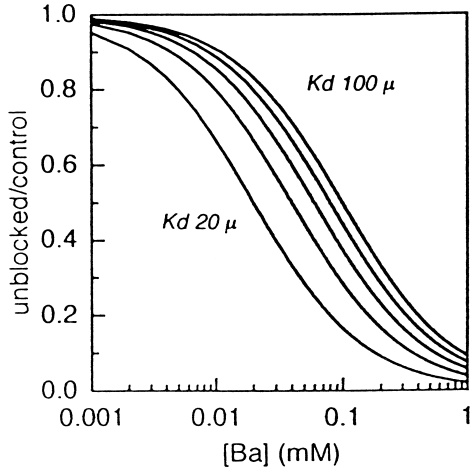

Fig. 7 Actions of barium on IRK at the steady-state. Theoretical curves are drawn from Eq. 18 with the K_d value at from left to right 20, 40, 60, 80 and 100 μM. Tha quotient "unblocked/control" in ordinate denotes the amplitude of the unblocked IRK relative to its respective control at the steady-state. The K_d value would be about 20 μM in human endothelial cells as judged by the degree of the IRK block shown in Fig. 1.

$$y = [R]/([R] + [BaR]) = K_d/(K_d + [Ba]) \quad \cdots\cdots (18)$$

This is a modified form of the Langmuir bimolecular absorption isotherm. Fig. 7 shows a series of theoretical curves drawn from Eq. 18 with 5 different K_d values between 20 and 100 μM, implying that the K_d value would be close to 20 μM in human endothelial cells (see Fig. 1). Hagiwara *et al* [7] have demonstrated that the y value linearly related to the time constant (τ) of the blocking action of barium which was quite easy to be measured. Hence, the τ value can be written as a function of [Ba] and membrane potential (V) in a formula,

$$\tau = \alpha\ K(V)/(K(V) + [Ba]) \quad \cdots\cdots (19)$$
$$K(V) = K(0)\ \exp(2\ \delta\ FV/RT) \quad \cdots\cdots (20)$$

where α is a scaling factor in $\alpha = \tau/y$, $K(0)$ represents the K_d value at 0 mV. Fig. 8 shows an example for the voltage-dependence of τ in human endothelial cells. The δ value obtained from the curve fitting to the data points is about 0.35, indicating that the blocking site locates at about one third from the outside margin of the channel. Comparable though somewhat larger values (0.5–0.7) have been reported in other cell types such as the guinea-pig cardiomyocytes [9], the starfish egg cells [8] and the frog skeletal muscles [18], indicating the validity of the Woodhull model for IRK.

OVERVIEW

The Woodhull model based on the rate theory can provide useful information about the properties of potassium channels responsible for IRK. The amino acid sequence of the pore region of many potassium channels have recently been elucidated by molecular cloning [14]. Kavanaugh *et al* [12] identified a tyrosine at the C-terminal end of the pore region of the *Shaker* channel (now called *Kv1. 1*) that determines sensitivity of this potassium channel to tetraethylammonium (TEA); point mutation of this tyrosine in *Kv1. 1* to valine (the equivalent residue in *Kv1. 2* which is insensitive to TEA) made *Kv1. 1* insensitive to TEA, and the reverse mutation in *Kv1. 2* made the channel sentitive to TEA. Hence, a motif responsible for the energy-barrier as well as the barium binding site could be identified in a near future so as to reveal the structure-function-relationship for the IRK channels.

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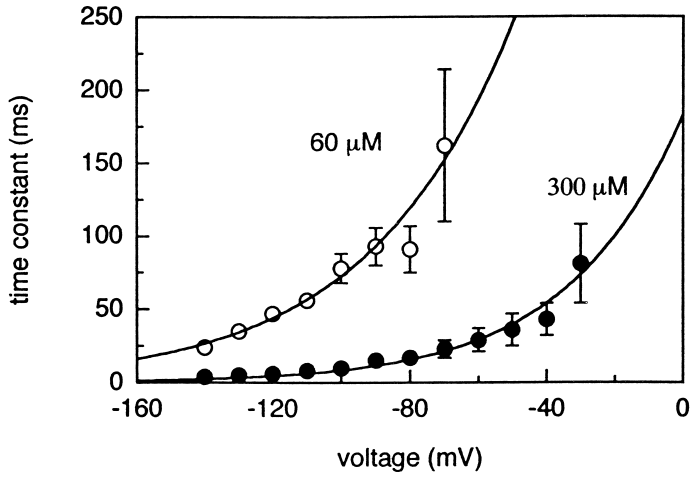

Fig. 8 Voltage-dependence of the time constant for the barium-induced IRK block. Shown are data points (means ± SEM) obtained from 5 cells with 60 μM (open circles) and 300 μM (filled circles). The mean values were fitted (least squares method) to Eq. 19 and $a = 4098$, $K(0) = 14$ μM and $\beta = 0.39$ gave the best fit for results with 300 μM. Respective values were 3160, 18 μM and 0.32 for the results with 60 μM. Continuous lines are drawn from Eq. 19. Results presented here are unpublished observations by T Tokimasa, A Surprenant and RA North.

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