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Determining K^+ channel activation curves from K^+ channel currents

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Abstract Potassium ion channels are generally believed to have current-voltage (*IV*) relations which are linearly related to driving force ($V - E_K$), where V is membrane potential and E_K is the potassium ion equilibrium potential. Consequently, activation curves for K^+ channels have often been measured by normalizing voltage-clamp families of macroscopic K^+ currents with ($V - E_K$), where V is the potential of each successive step in the voltage clamp sequence. However, the *IV* relation for many types of K^+ channels actually has a non-linear dependence upon driving force which is well described by the Goldman-Hodgkin-Katz relation. When the GHK dependence on ($V - E_K$) is used in the normalization procedure, a very different voltage dependence of the activation curve is obtained which may more accurately reflect this feature of channel gating. Novel insights into the voltage dependence of the rapidly inactivating I_A channels Kv1.4 and Kv4.2 have been obtained when this procedure was applied to recently published results.

Key words Potassium channels · Current-voltage relations · *Xenopus* oocytes · HEK-293 cells

Discussion

One of the distinguishing features of a voltage-gated ion channel is its steady-state activation curve, sometimes referred to as its g-V curve, where g represents conductance and V membrane potential. The g-V curve is often measured from a voltage clamp family of macroscopic currents, with the current corresponding to each voltage step being normalized relative to the driving force, $V - E$, where E is the equilibrium potential for the ion in question. For a delayed rectifier-type K^+ channel, the currents for this procedure are measured at the end of voltage steps which are relatively long compared to the activation time constant of the channel conductance for any given step. In other words, the assumption is made that $I_K = g_K(V,\ t)(V - E_K)$, and $g_K(V,\ t \to \infty)$ is obtained by the procedure just described. For rapidly inactivating K^+ channels (I_A type) the peak outward current is typically used for this analysis because I_A activation kinetics are much more rapid than I_A inactivation kinetics. The assumption is made that the degree of inactivation at the time of occurrence of the peak current is approximately the same at each potential. Consequently, the voltage dependence of channel activation can be reasonably approximated by normalizing the peak current by $V - E_K$. This procedure, which has been widely used, is illustrated by the original results obtained from Kv4.2 channels heterologously expressed in *Xenopus* oocytes by Baldwin et al. (1991) which are reproduced in Fig. 1.

The above analysis assumes that the current-voltage relation (*IV*) for K^+ channels is ohmic, i.e., linearly proportional to ($V - E_K$) for physiological saline, which has a much lower K^+ concentration relative to that of cytoplasm. This conclusion is based at least in part on the original results from Hodgkin and Huxley (1952) on the delayed rectifier I_K from squid giant axons. Other work from squid axons and various other preparations has shown that the voltage dependence of K^+ channel *IV* relations is well described by the Goldman-Hodgkin-Katz equation (Goldman 1943; Hodgkin and Katz 1949), i.e., $I_K \approx qV/kT\{\exp(q(V - E_K)/kT) - 1\}/\{\exp(qV/kT) - 1\}$, where q is the unit electronic charge, k is the Boltzmann constant, and T is absolute temperature (Frankenhauser 1961; Binstock and Goldman 1971; Siegelbaum et al. 1982; Spruce et al. 1987; Clay 1991) (at room temperature, $kT/q \approx 25$ mV). Consequently, the voltage and time-dependent properties of

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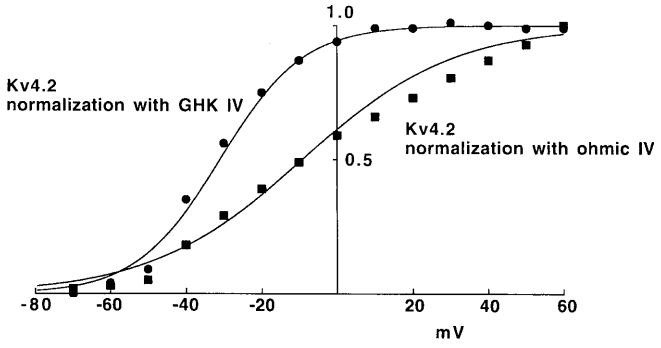

Fig. 1 Activation curve for Kv4.2 K^+ channels expressed in *Xenopus* oocytes. The data represented by (■) were taken directly from Fig. 4B of Baldwin et al. (1991). These results were obtained by normalizing peak outward currents by $(V - E_K)$ with $E_K = -100$ mV. The results represented by (●) were obtained by first removing the ohmic normalization. That is, the original data points (■) were multiplied by $(V - E_K)$. They were then renormalized by $\alpha(V/25)\{\exp((V - E_K)/25)\}/\{\exp(V/25) - 1\}$, where α is a normalization factor (same for all points) chosen so that the average of the results at +10, +20, ... +60 mV was unity. The curves represent $\{1 + \exp(-(V - V_{1/2})/V_s)\}^{-1}$, where $V_{1/2} = -9.2$ mV and $V_s = 20$ mV for the original data, and $V_{1/2} = -31$ mV and $V_s = 11$ mV for the renormalized results

the K^+ current for many K^+ channel types is more accurately represented by:

$$I_K \approx g_K(V,t)V\{\exp(qV - E_K)/kT) - 1\}/\{\exp(qV/KT) - 1\} \quad (1)$$

than by $I_K \approx g(V,t)(V - E_K)$. Voltage-clamp families of K^+ currents should therefore be normalized by the GHK dependence on $(V - E_K)$, rather than by $(V - E_K)$ itself when determining K^+ channel activation curves. This procedure has been used by Taglialatela and Stefani (1993) to obtain the g-V curve for Kv2.1 channels expressed in *Xenopus* oocytes. The Kv4.2 *Xenopus* oocyte results of Baldwin et al. (1991) have been reanalyzed this way in Fig. 1. Two notable features of the analysis are a clear saturation of the conductance with membrane depolarization and a significantly steeper voltage dependence of activation than originally reported. Both sets of results in Fig. 1 are described by the Boltzmann relation $\{1 + \exp(-(V - V_{1/2})/V_s)\}^{-1}$, where $V_{1/2}$ is the potential where half-activation of the conductance occurs and V_s is the relative steepness of activation at the midpoint (Fig. 1, legend). The reanalyzed Kv4.2 activation curve from *Xenopus* oocytes is also shown in Fig. 2A, along with a recent result obtained from expression of Kv4.2 channels in HEK-293 cells by Petersen and Nerbonne (1999). The latter activation curve was also renormalized using the GHK equation. Similarly, the renormalized activation curve for Kv1.4 channels expressed in HEK-293 cells (Petersen and Nerbonne 1999) is shown in Fig. 2B along with the activation curve for Kv1.4 channels expressed in *Xenopus* oocytes (Bertoli et al. 1994). The latter results were obtained from tail current amplitudes, a procedure which avoids the problem of normalizing currents from different potentials, since tail currents are measured at a single potential, usually the holding potential.

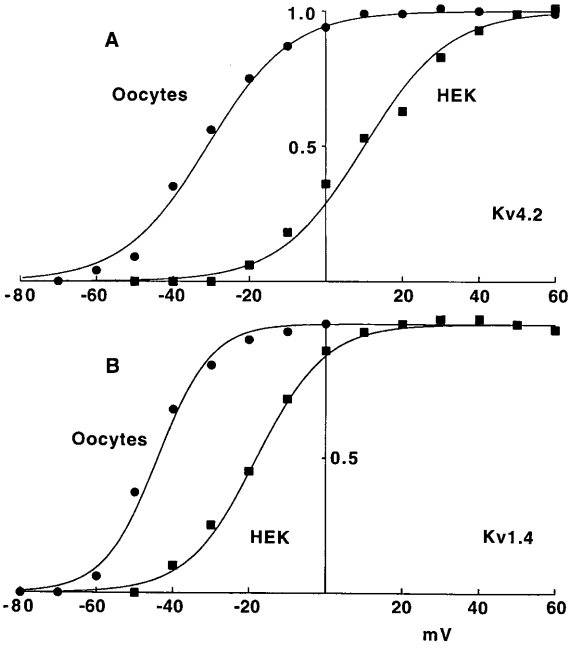

Fig. 2A, B Activation curves for Kv4.2 and Kv1.4 K^+ channels heterologously expressed in *Xenopus* oocytes and HEK-293 cells. **A** Kv4.2 channel activation curve in oocytes (●) and HEK cells (■). The oocyte result is the same as the renormalized curve in Fig. 1. The HEK-293 result was taken from Fig. 6 of Petersen and Nerbonne (1999) and renormalized as described in Fig. 1 legend, with the normalization factor chosen so that the average of the results at +50 and +60 mV was unity. The curves represent the Boltzmann equation with $V_{1/2} = -31$ and 10 mV for the oocyte and HEK results, respectively, and $V_s = 11$ mV for both curves. **B** Kv1.4 channel activation curve in oocytes (●) and HEK cells (■). The former was taken directly from fig. 3 of Bertoli et al. (1994). The HEK result was taken from fig. 2 of Petersen and Nerbonne (1999) and renormalized as described in Fig. 1 legend, with the normalization factor chosen so that the average of the results at +20, +30, ... +60 mV was unity. The parameters for the Boltzmann curves are $V_{1/2} = -44$ and −18 mV and $V_s = 7$ and 9 mV for the oocyte and HEK cell results, respectively

The analysis in Fig. 2 reveals a novel finding, namely the activation curves for Kv1.4 and Kv4.2 in both *Xenopus* oocytes and HEK cells are shifted along the voltage axis relative to each other by 30–40 mV in both expression systems, with the Kv1.4 curve lying negative to the respective Kv4.2 curve in each case. Moreover, the Kv1.4 activation curve appears to have a steeper voltage dependence relative to that of Kv4.2. The relative positions of these curves on the voltage axis reinforce the point made by Petersen and Nerbonne (1999) concerning the influence of expression environment on channel gating. The voltage shift could be attributable to endogenous, auxiliary K^+ channel subunits either in HEK cells, or *Xenopus* oocytes, or perhaps both.

As noted above, Eq. (1) may serve as a general description of K^+ channels. One possible exception may be the inwardly rectifying K^+ channel (Katz 1949). However, the factors responsible for the rectification [intracellular Mg^{2+} (Matsuda 1988); cytoplasmic spermine and spermidine (Ficker et al. 1994)] obscure the voltage dependence of the current which would pass

through these channels in the absence of those factors. A similar situation occurs for Na^+ channels. For example, the classic, rapidly activating, tetrodotoxin-sensitive Na^+ channel in nerve axons has an *IV* relation which is well described by the GHK equation in Ca^{2+}-free external medium (Vandenberg and Bezanilla 1991). That is, the current inwardly rectifies, as predicted by GHK in this case, because the concentration of sodium ions outside of the cell is substantially larger than it is intracellularly. However, extracellular calcium ions block inward current through this channel in a voltage-dependent, instantaneous manner (Yamamoto et al. 1984), so that the *IV* relation appears to be approximately ohmic for physiological conditions over the range of membrane potentials spanned by the action potential. In other words, the GHK nature of the *IV* relation is obscured by a normally occurring factor, in this case extracellular Ca^{2+}. The applicability of the GHK equation is also not readily apparent for channels which are not perfectly selective, or at least nearly so, under physiological conditions, for the major ion in question over a range of membrane potentials which is sufficiently large to see the nonlinearity of the GHK relation. Calcium ion channels appear to fall in this category (Hess et al. 1986). Potassium ion channels in general are selective solely to potassium ions (under physiological conditions) over a broad range of membrane potentials. For example, the classic delayed rectifier K^+ channel in squid giant axons does not allow extracellular sodium ions to permeate the channel for potentials even as negative as −200 mV (J.R. Clay, unpublished observation).

For the reasons just given, the analysis in this report is most directly relevant to K^+ channels. An accurate determination of K^+ channel activation curves is significant both for modeling of K^+ channel gating and neuronal firing (Clay 1998). The procedure outlined above should be of general use for obtaining data sets for both purposes.

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