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ARTICLE

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Temperature-dependent conformational changes in a voltage-gated potassium channel

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Abstract Temperature was used as a biophysical tool to investigate the energy changes associated with conformational change during the gating of a non-inactivating voltage-gated K^+ channel present in the membrane of αT3-1 cells, a gonadotroph cell line. The time course of the current activation was described by a single exponential function at three temperatures: 15, 25 and 35 °C. The Q_{10} values were between 1.5 to 1.9 and in agreement with the activation energy determined from Arrhenius plots of the forward and backward rate constants associated with channel opening. The Gibb's free energy change associated with channel opening and closing at various membrane potentials estimated by two approaches yield similar values. The changes in Gibb's free energy ($\Delta G°$) with depolarization potential is a quadratic and more prominent at 15 than at 25 or 35 °C. The results suggest that increase in temperature favours movement of voltage sensing segments, and reduces the restraint on them brought about by other parts of the channel molecule.

Key words Thermodynamics · Hodgkin-Huxley kinetics · K^+ current

Introduction

Voltage-gated potassium channels are ubiquitous and often more than one type is present in the same cell (Rudy 1988). They control the repolarizing phase of the action potential and modulate secretion in endocrine and exocrine cells (Petersen and Maruyama 1984; Mason and Waring 1986; Marty 1989). Kinetic characterization of voltage-gated ion-channels has shown how these channels sense the change in transmembrane potential and undergo conformational change. It has been possible to estimate the energy associated with closed and open states of ion channel molecules using temperature as a biophysical tool (Tsien and Noble 1969; Cavalie et al. 1985; Pahapill and Schlichter 1990; McLarnon et al. 1993; Busch and Lang 1993; van Lunteren et al. 1993). Earlier reports have shown that the change in voltage-dependent Gibb's free energy of a voltage-gated ion-channel varies linearly with temperature (Tsien and Noble 1969; Stevens 1978; Moczydlowski 1986). Using a combination of techniques involving molecular biology, electrophysiology and fluorescence measurement to study voltage-gated ion-channels it has been demonstrated that (1) outward movement of the voltage sensor (S4 helix) (Mannuzzu et al. 1996) and (2) transduction of S4 movement to other parts of the channel molecule occurs in response to transmembrane voltage change (McCormack et al. 1991; Tomaselli et al. 1995; Shieh et al. 1997). It is logical to surmise from these studies that there should be independent energy terms associated with the movement of the voltage sensor in response to the transmembrane potential change and its interaction with other domains of the channel that influence its movement.

At the time when structural details of voltage-gated ion-channels were unavailable, Hill and Chen (1972) proposed a model in which the voltage-dependent energy change was a sum of the translation of the fixed number of charges and pull exerted on various segments of the channel protein to restore its equilibrium state. The object of this study was to apply this model to obtain thermodynamic information on conformational transitions of a non-inactivating K^+ channel present in the membrane of αT3-1, a gonadotroph cell line (Windle et al. 1990), using temperature as a variable. The non-inactivating whole cell K^+ currents were characterized by the Hodgkin-Huxley model (H-H model) (Hodgkin and Huxley 1952) at various temperatures. Since the same kinetic model was tenable at all potentials and temperatures, the rate constants determined from the kinetic model were used to estimate the thermodynamic parameters.

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Materials and methods

The αT3-1 cells were cultured as described by Tiwari et al. (1996). The electrophysiological measurements were performed at 10–35 °C with a pipette solution containing 150 mM KCl, 2 mM $MgCl_2 \cdot 6H_2O$, 5 mM HEPES and 1 mM EGTA (pH 7.35), and a bath solution containing 90 mM KCl, 50 mM choline chloride, 1 mM EGTA, 2 mM $MgCl_2 \cdot 6H_2O$, 2 mM $CoCl_2 \cdot 6H_2O$, 5 mM HEPES and 10 mM glucose (pH 7.4) (all Sigma chemicals).

Data acquisition

αT3-1 cells were plated in 35 mm culture dishes at low density and recordings performed on single isolated cells after 18 h. The bath temperature during recording was maintained by a micro incubator (PDMI-2) controlled with a bipolar temperature controller (TC-202, Medical Systems Corp.). The temperature of the bath recording solution was independently monitored by a mercury thermometer. The resistances of the patch-pipettes were between 2 and 3 MΩ and the seal resistance was >20 GΩ. K^+ currents were recorded in the presence of very low levels of free Ca^{2+} concentration. Holding the cell membrane at potentials greater than –15 mV resulted in only one type of K^+ current which activated at potentials positive to 30 mV. Whole-cell K^+ currents were recorded with a EPC-7 (List Medical) patch-clamp amplifier. Capacitance cancellation and 70% series resistance (R_s) compensation were employed to minimize artifacts in current traces. The current was filtered at 10 and 3 kHz (built-in EPC-7) and digitized at a frequency greater than 15 kHz using a CED1401 AD/DA converter connected to an IBM compatible AT-286 computer. The cell membrane was held at a desired potential (V_{HOLD}) for 4 min prior to recording. The voltage pulse protocol generation, data acquisition, leak subtraction and preliminary analysis of the acquired data were done using WCP software (J. Dempster, University of Strathclyde, Glasgow, UK). The linear leak current and capacitance transient was removed by employing the p/4 protocol where seven leak records with negative and one fourth amplitude were averaged, scaled and subtracted from an averaged set consisting of eight test records.

The "time delay" in the signal introduced by filtering was estimated by connecting a model cell to the head stage amplifier and keeping the filters and gain fixed at positions identical to the recording conditions. Voltage pulse and current outputs were captured on a digital storage oscilloscope (model 1425, L&T Gould, India). Zero time (i.e. $t = 0$) was set at the foot of the voltage pulse and the time required for the voltage pulse and current to reach 95% of the steady state was noted. The voltage pulse took 5 µs but the current trace took 170 µs. For analysis, a instrument delay of 0.20 ms was incorporated.

Data analysis

K^+ currents were analysed with a modified Hodgkin-Huxley (1952) equation:

$$I_K = a\,(1-\exp[-(t-0.2)/\tau_n])^x \quad (1)$$

where I_K is the potassium current, a is the amplitude, t is the time, τ_n is the time constant of activation and x is related to the number of independent identical transitions before the channel becomes conducting. Final analyses and curve fittings were performed on a CDC4360 computer using the IMSL subroutine RNLIN with the criterion of non-linear least squares minimization using the Marquardt-Levenberg algorithm. The analyses program developed in our laboratory allows for a fixed or variable x. τ_n and x were floated to obtain the fit of the current traces (as shown in Fig. 1 A–C). The rate constants α_n and β_n were estimated using the relation $\alpha_n = n_\infty/\tau_n$ and $\beta_n = (1-n_\infty)/\tau_n$ using the H-H model.

Estimation of thermodynamic parameters

The activation energies associated with channel opening and closing were determined by the Arrhenius equation:

$$\ln(\text{rate}) = \ln(A) - (E_a/R)/T \quad (2)$$

where "rate" can be either α_n or β_n (s^{-1}), E_a is the activation energy in kJ mol^{-1} and R is the gas constant, T is the temperature (K) and A is a pre-exponential factor.

The equilibrium constant (K_{eq}) for Scheme 1 (see results) was estimated either by $K_{eq} = \alpha_n/\beta_n$ or $K_{eq} = n_\infty/(1-n_\infty)$, because the activation kinetics of the non-inactivating K^+ channel, discussed in this paper, is a simple exponential. Thus normalized steady state activation is same as the n_∞ plot. The change in Gibb's free energy ($\Delta G°$) was determined from:

$$\ln(K_{eq}) = -\Delta G°/RT \quad (3)$$

and the change in enthalpy associated with the closed to open transition was estimated by:

$$\ln(K_{eq}) = -\Delta G°/RT = -\Delta H°/RT + \Delta S/R \quad (4)$$

For the transition state, the thermodynamic parameters $\Delta G^‡$, $\Delta H^‡$, and $\Delta S^‡$ were determined using the following equations:

$$\Delta H^‡ = E_a - RT \quad (5)$$

$$\Delta G^‡ = -RT \ln(\text{rate}) + RT \ln(kT/h) \quad (6)$$

and

$$\Delta S^‡ = (\Delta H^‡ - \Delta G^‡)/T \quad (7)$$

where k is Boltzmann's constant, h is Planck's constant and E_a, R, T have the meaning as stated above. $\Delta G^‡$ and $\Delta H^‡$ are in kJ mol^{-1} and the entropy ($\Delta S^‡$) is in J K^{-1} mol^{-1}.

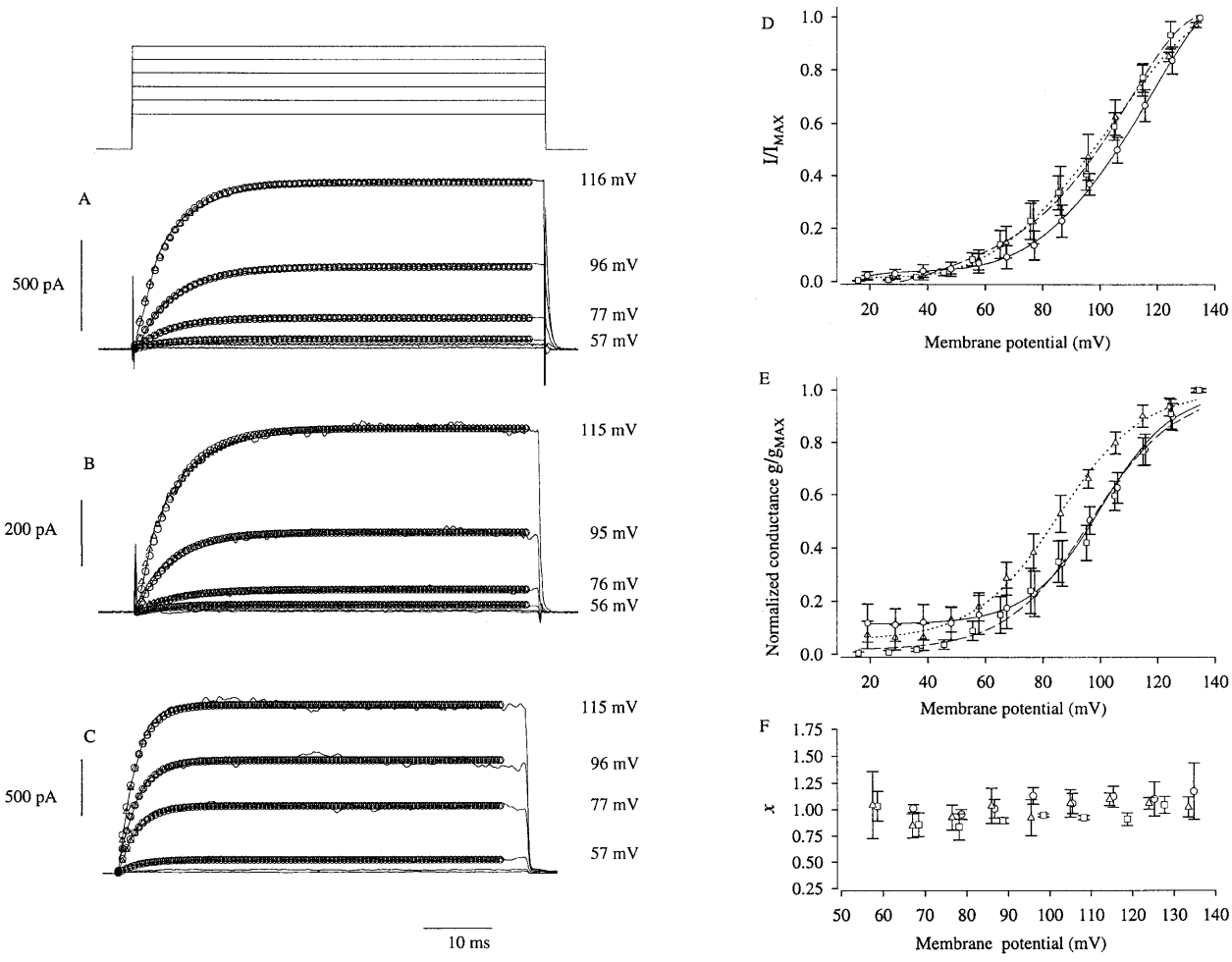

Fig. 1A–F Activation kinetics and *I-V* and *g-V* plot of non-inactivating K^+ current at different temperatures. Representative whole cell currents from V_{HOLD} = –10 mV at 15 (**A**), 25 (**B**) and 35 (**C**) °C with fit using Eq. (1). The voltage protocol is shown on top. **D** Normalized current-voltage plot at 15 (*circles*) 25 (*squares*) and 35 °C (*triangles*). **E** Conductance-voltage plot; conductance was estimated by $g = I_K/(V-V_K)$, where V_K and I_K are reversal potential and amplitude of K^+ current. V_K (–9.8 mV) was experimentally determined by reversal of tail currents. Symbols in **E** have the same meaning as in **D**. The lines through the data points in **E** are fit by the Boltzmann distribution: $g_K = (1-g_a)/(1+\exp\{(V_{b1/2}-V)\, z_{gb}\, e/kT\}) + g_a$, where *e*, *k* and *T* have their usual meaning, g_a is to account for the residual conductance of other channel(s) in the cell (Bielefeldt et al. 1992). The value of parameters z_{gb} and $V_{b1/2}$ (in mV) are: 1.97, 99.87 at 15 °C, 1.77, 96.82 at 25 °C and 1.80, 88.73 at 35 °C, respectively. Activation time course of K^+ currents at three temperatures were fit with Eq. (1) with $x = 1$ (*triangles*) and x as free parameter (*circles*). Current scale is shown on *left* and time scale is common. Recordings in **A**, **B** and **C** are from different cells. Fit has been shown with every tenth point plotted. **F** Plot of mean "*x*" versus membrane potentials at 15 (*hollow circles*), 25 (*hollow squares*) and 35 °C (*hollow triangles*) (N = 5). In all figures data with error bars are mean ± standard error of mean (sem)

Results

Current-voltage and conductance-voltage relationship and activation kinetics

The non-inactivating K^+ currents were studied by applying depolarizing pulses from +10 to +135 mV in steps of 9.7 mV from a holding potential of –10 mV. Activation of the currents increased rapidly, both with greater depolarization and on increasing the bath temperature (Fig. 1A–C). A notable feature common to both normalized currents and the conductance plot is the negligible activation at test potentials below +40 mV (Fig. 1D, 1E). The conductance plot is sigmoidal and shows saturation beyond 120 mV. Increasing the temperature in steps of 10 °C caused a mean leftward shift of about 4 mV in $V_{1/2}$ and no obvious change in the slope or gating charge (Fig. 1E).

The K^+ currents at different potentials and temperatures were analyzed with Eq. (1). When *x* was kept free, it ranged between 0.7 and 1.5 with the mean value close to 1 (Fig. 1F). The activation time course is prone to artifacts arising due to series resistance and improper R_s compensation. This, however, was not the case as no significant

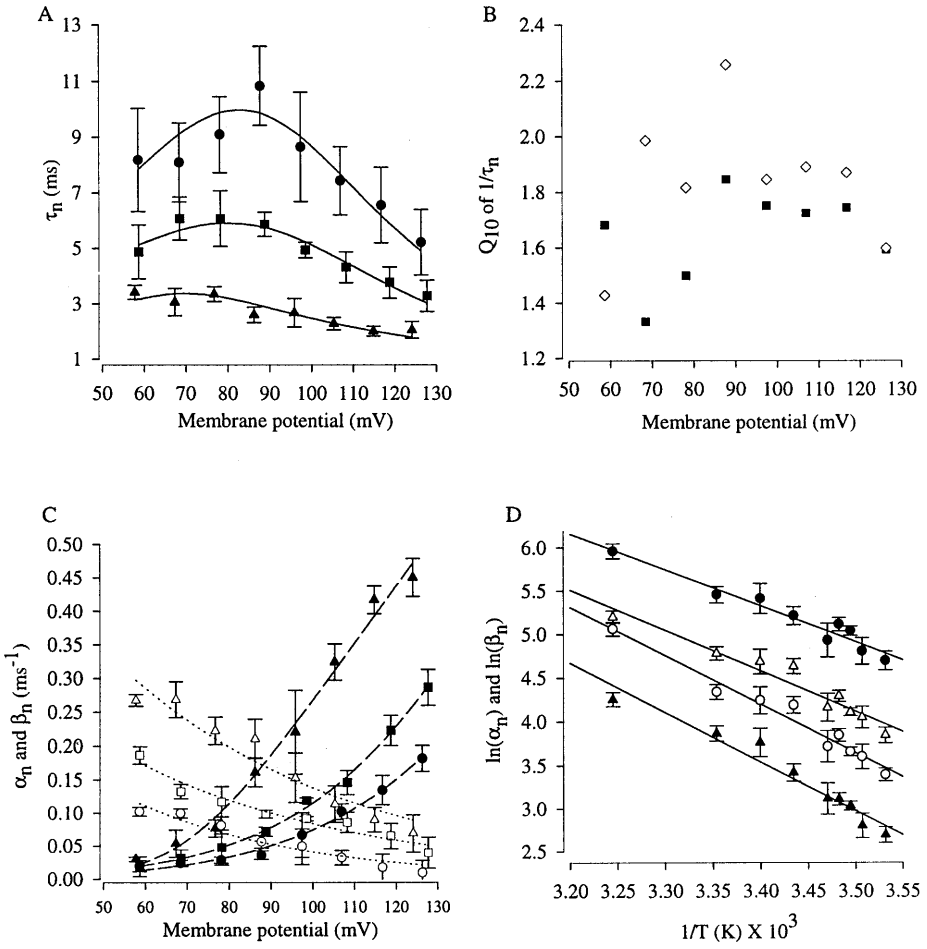

Fig. 2 A–D Effect of temperature on time constant and Arrhenius plot of α_n and β_n. **A** Plot of τ_n against membrane potential. τ_n was determined from the fit of the activation kinetics (Fig. 1). The continuous lines were drawn using $\tau_n = 1/(\alpha_n + \beta_n)$ where α_n and β_n values are from the fit in **C**. **B** Plot of Q_{10} of $1/\tau_n$ versus membrane potentials. **C** Plot of rate constants α_n and β_n versus potential. The rate constants were determined as explained in methods section. The lines through α_n and β_n are fits by: $\alpha_n = A\,(B-V)/(1-\exp[-(B-V)/C])$ (a) and $\beta_n = \beta_0 \exp(z_\beta FV/RT)$ (b) (Hodgkin and Huxley 1952). The values of the parameters A (mV^{-1} ms^{-1}), B (mV) and C (mV) obtained from the fit of α_n are: –0.0121, 130.27 and –16.97 for data at 15 °C, –0.014, 118.61 and –15.86 at 25 °C and –0.009, 69.00 and –6.32 at 35 °C, respectively. The value of the fitted parameters β_0 and z_β are: 0.49 and –0.62 at 15 °C, 0.50 and –0.46 at 25 °C and 0.85 and –0.48 at 35 °C, respectively. The fit to Eq. (a) for α_n was better than Eq. (b). **D** Plot of ln (α_n) (*circles*) and ln (β_n) (*triangles*) versus $1/T$ (K) at 88 mV (*hollow symbols*) and 116 mV (*solid symbols*). The lines are linear regressions through the data points with the slopes –5.54 and –4.16 ($r > 0.97$) for α_n, and –4.62 and –5.65 ($r > 0.96$) for β_n at 88 and 118 mV, respectively

effect was noticed on the value of x with a four-fold increase in the amplitude of the K^+ current (data not presented). Since no significant difference was observed between the fits with $x = 1$ and x as a free parameter, all analysis reported here was done with $x = 1$. The exponential kinetics of the current was observed at all potentials (above +55 mV) and temperatures and was consistent with the two state model for channel activation (Scheme 1):

$$\text{CLOSED} \underset{\beta_n}{\overset{\alpha_n}{\rightleftarrows}} \text{OPEN}$$

The plot of the activation time constant τ_n versus membrane potential was bell shaped with the maximum lying around 90 mV and showed leftward shift with increase in temperature (Fig. 2 A). The effect of temperature on τ_n was quantified by estimating the ratio at each potential for every 10 °C increase in temperature (i.e. Q_{10}) in the ranges 15–25 °C and 25–35 °C (Fig. 2 B). Mean Q_{10} was above 1.5 at all potentials and maximum around 90 mV, where kinetics was slowest. This corroborates well with the report of van Lunteren et al. (1993) on calcium current in bullfrog sympathetic neurons.

Arrhenius plot and thermodynamics of the K^+ channel

The energy barrier for the transition between the closed and open states was determined by studying the kinetics over a range of temperatures. The activation energies estimated from the Arrhenius plot for α_n were 46.16 and 34.73 kJ mol^{-1} and for β_n were 38.55 and 47.12 kJ mol^{-1} at 88 and 116 mV, respectively.

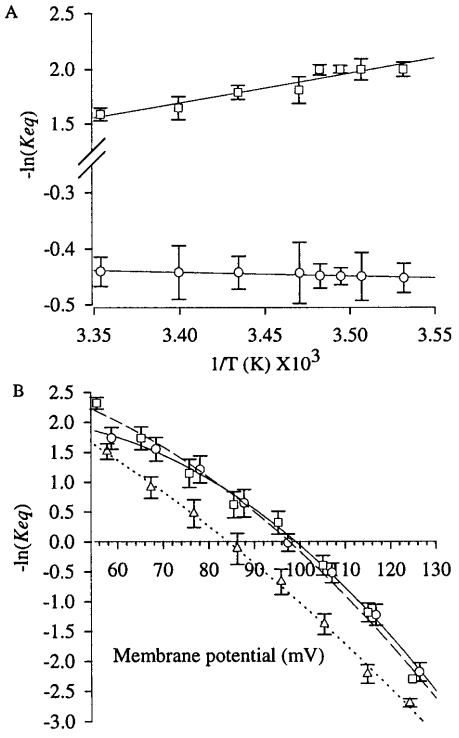

Fig. 3A, B Plot of ln (K_{eq}) versus temperature and potential. **A** Plot of –ln (K_{eq}) versus temperature. K_{eq} was estimated from α_n/β_n. The line through the data points is a linear regression with slope of –0.077 at 88 mV (*circles*) and 2.663 at 116 mV (*squares*). **B** Plot of –ln (K_{eq}) versus membrane potential at three different temperatures. K_{eq} was determined from n_∞ and data points are fit to Eq. (10) (see text). ln (K_{eq}) is zero at 99.8, 96.3 and 86.0 mV at 15 (*circles*), 25 (*squares*) and 35 °C (*triangles*), respectively (see text). The value of the fitted parameters q, δ and γ are given in Table 2

The effect of temperature on free energy, $\Delta G°$, was estimated at various temperatures at two potentials (88 and 116 mV) (Fig. 3A). The plot of ln (K_{eq}) versus $1/T$ shows that $\Delta G°$ varies linearly with $1/T$. The enthalpy of a voltage-gated ion-channel is expected to be dependent on the membrane potential. The estimated enthalpy of the channel at 88 mV is positive but at 116 mV is negative (Fig. 3A).

The effect of membrane potential on $\Delta G°$ was studied at three temperatures. $\Delta G°$ was determined at various potentials using Eq. (3). A striking feature of $\Delta G°$ is its quadratic variation with membrane potential and the curvature is most prominent at 15 °C (Fig. 3B).

A zero value of $\Delta G°$ at each temperature corresponds to the $V_{1/2}$ potentials.

Discussion

Potassium currents have been observed in a variety of gonadotrophs (Chen et al. 1989; Sikdar et al. 1989; Bosma and Hille 1992). In this paper we report the kinetics of a K^+ current in αT3-1 cells and its physico-chemical behavior. Notable features of these K^+ currents are (1) at low free intracellular Ca^{2+} concentration the K^+ current activates at high depolarizing potentials, (2) the currents do not show run down or inactivation with time and (3) the activation kinetics are well described by a single exponential at all potentials and temperatures studied. The simple exponential kinetics does not exclude the possibility of a series of transitions before the channel opens but requires a single rate-limiting voltage-dependent step between the closed and open states. The non-inactivating K^+ currents reported herein are Ca^{2+} sensitive. Increasing the free Ca^{2+} concentration (1.3 µM) in the pipette solution shifted the activation towards lower potentials and changed the activation kinetics (unpublished observation). Bosma and Hille (1992) have shown the presence of a Ca^{2+} activated K^+ current in αT3-1 cells. Our earlier study also showed presence of a large conductance Ca^{2+} activated K^+ current in αT3-1 cells (Tiwari et al. 1996). The activation kinetics and shifts in the $V_{1/2}$ of activation in response to changes in intracellular Ca^{2+} concentration are strikingly similar to those reported for the mslo channel (Cox et al. 1997a, b; Cui et al. 1997). The gating charges associated with the forward and backward rate constants ($z_{g\alpha}$ and $z_{g\beta}$) are 0.895 and 0.459, respectively, at 25 °C and do not vary significantly with increase in intracellular Ca^{2+} concentration. The extrapolated values of the rate constants α_n and β_n at zero potential are 3.48 and 498 s^{-1}, respectively, at 25 °C. These results corroborate with those of Cui et al. (1997). Moreover, the non-inactivating K^+ currents were not affected by 4-aminopyridine at 4 mM concentration but were blocked by charybdotoxin (unpublished observation). The K^+ current reported here could be from a type of mslo channel, although this requires further confirmation using molecular biology approaches, such as RT-PCR.

Effect of temperature on channel kinetics

Temperature has been used previously as a tool to understand the kinetics of a voltage-gated ion channel and a detailed thermodynamic study is possible if the model is consistent at all temperatures and potentials (Tsien and Noble 1969; Pahapill and Schlichter 1990).

An increase in temperature reduced the time constant (τ_n), alternatively, increased the transition rates. A Q_{10} value of 1.5 would be proportional to an energy barrier of 28 kJ mol^{-1} (Hille 1992). The mean value of Q_{10} was close to 1.8 at potentials greater than 80 mV. Though this value cannot be considered high, it gives an estimate of the activation energy barrier close to 42 kJ mol^{-1}. This is consistent with the activation energy determined from the Arrhenius plots for α_n and β_n at potentials of 88 and 116 mV. The earlier reports of Q_{10} for K^+ and Na^+ channels were between 1.6 and 2.5 (Collins and Rojas 1982; McLarnon et al. 1993), and our observations are consistent with these reports.

Table 1 The transition state thermodynamic parameters and effect of membrane potential

	88 mV		116 mV	
	α_n	β_n	α_n	β_n
Q_{10} (15–25 °C)	1.63	1.83	1.64	2.14
Q_{10} (25–35 °C)	2.12	1.75	1.78	1.80
$\Delta G^{\ddagger}$ (kJ mol^{-1})	61.78	60.73	58.80	63.42
(sem)	(3.54)	(2.81)	(3.74)	(4.36)
$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	–59.8	–81.6	–88.07	–62.03
E_a (kJ mol^{-1})	46.16	38.55	34.73	47.12
$\Delta H^{\ddagger}$ (kJ mol^{-1})	43.84	36.24	32.38	44.81

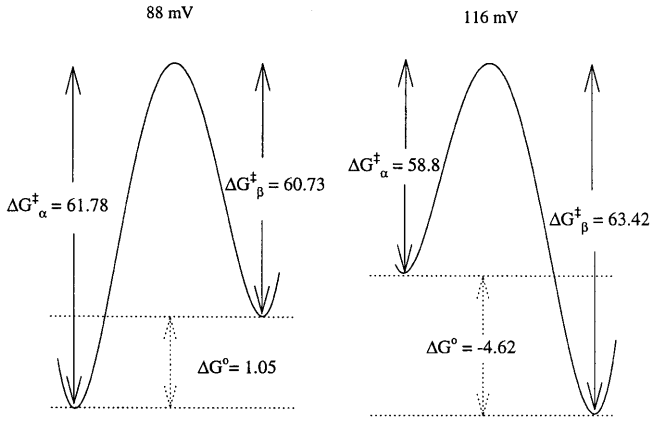

Fig. 4 Relative free energy of activation between open and closed configurations. The relative energy barrier from the two stable states (open and closed) of the channel are drawn for 88 and 116 mV. The changes in free energy of activation for α_n ($\Delta G^{\ddagger}_{\alpha}$) and β_n ($\Delta G^{\ddagger}_{\beta}$) were determined by Eq. (6). The change in free energy ($\Delta G°$) between the closed and open configurations was determined by the difference in the free energy of activation for α_n and β_n [i.e. ($\Delta G^{\ddagger}_{\alpha}$)–($\Delta G^{\ddagger}_{\beta}$) and is shown with the *dashed line* for the two potentials

Conformational change, from closed state to open state, involves an energy barrier

The activation energies (E_a) for α_n and β_n estimated from the Arrhenius plots were dependent on the depolarizing potential. With the increase in the depolarizing potential from 88 to 116 mV, the activation energy for α_n reduced from 46.16 to 34.73 kJ mol^{-1}, whereas for the same change in potential, the activation energy associated with β_n increased from 38.55 to 47.12 kJ mol^{-1}. These activation energies correlate with the Q_{10} values between 1.6 and 1.8. The entropy of the transition state is negative (Table 1) and is consistent with the observation that the "transition state" has lower entropy than the "equilibrium states".

The changes in Gibb's free energy ($\Delta G°$) determined from $\Delta G^{\ddagger}$ at 88 and 116 mV were 1.05 and –4.62 kJ mol^{-1}, respectively (Fig. 4 depicts the energy diagram for this process). $\Delta G°$ at the same potentials (T = 25 °C) estimated from steady state activation were 1.25 and –4.2 kJ mol^{-1}, respectively, indicating the consistency in the results estimated by the two methods.

Temperature and voltage dependence of equilibrium kinetics

The linearity between ln (K_{eq}) versus 1/T indicates that changes in entropy and enthalpy, associated with the channel transition, are independent of temperature between 10 and 25 °C. Figure 3B shows that the plot at 15 °C cuts the 25 °C data near 90 mV, implying that the enthalpy of the open state below 90 mV is positive, whereas at more depolarized potentials it is negative. The transition from the closed to the open state is favoured both by depolarization and increase in temperature, as evident from the leftward shift of the plot of ln (K_{eq}) versus potential with increase in temperature.

The free energy change of the voltage-gated ion-channel [$\Delta G°$ (V)] comprises two parts: (1) a voltage-independent term and (2) a voltage-dependent term. It has been suggested previously that the free energy change in a voltage-gated ion-channel varies linearly with membrane potential (Stevens 1978; Moczydlowski 1986). The result presented in Fig. 3B shows that the change in free energy, for the non-inactivating voltage-gated K^+ channel reported herein, is not linear but quadratic at three temperatures.

If movement of the voltage-sensing domain, during voltage gating, influences other parts of the voltage-gated ion-channel, then such changes should be reflected in the channel's voltage dependence. Considering a potential difference V across membrane width d, the linear voltage gradient is V/d. The charge c in the channel protein which is located at a position y from outside of the membrane surface experiences a linear electrostatic potential equal to $y\ V/d$. The domain containing the charged residue would experience two opposing forces: (1) the potential ($y\ V/d$) that exerts the pull on the charged parts and (2) the interaction of other domains present in the vicinity of the charged domain that provide a restoring force [$-a\ (y-y_0)$] where y_0 is the position of the charge "c" when the potential across membrane is zero, i.e. V = 0, and a is the coefficient of rigidity (a type of spring constant that is proportional to the magnitude of favourable interactions between the voltage sensor and other domains of the channel molecule).

Under these conditions, the net energy of the channel molecule at some potential V is given by:

$$U\ y = U_0 + (c\ y\ V/d) + (1/2)\ a\ (y-y_0)^2 \quad (8)$$

where U_0 is energy when V = 0 (Hill and Chen 1972). At equilibrium, i.e. d ($U\ y$)/dy = 0, solving and substituting the expression for y into Eq. (8) gives:

$$U\ y = U_0 + (c\ y_0\ V/d) - (1/2\ a)\ (c\ V/d)^2 \quad (9)$$

The free energy change with potential [i.e. $\Delta G\ (V)$] has the voltage-independent term ΔG_0 and voltage-dependent terms ($c\ y_0/d$) V and ($-c^2/2\ a\ d^2$) V^2. We define a function $f\ (V)$:

$$f\ (V) = \Delta G\ (V)/RT = q + \delta V + \gamma V^2 \quad (10)$$

Table 2 Coefficients of function $f(V)$ [see Eq. (10)]

	q	$\delta\times10^2$	$\gamma\times10^4$
$f(V)$ (HH)[a]	–2.33	–3.24	+2.08
$f(V)$ (DH)[a]	–3.80	–7.85	–2.34
$f(V)$ 15 °C[b]	1.38	3.68	–5.18
$f(V)$ 25 °C[b]	2.90	0.9	–3.69
$f(V)$ 35 °C[b]	3.75	–2.74	–2.03

[a] Values reported by Hill and Chen (1972)
[b] The values for the non-inactivating K^+ channel (reported here)

where $\Delta G\ (V)$ is the total free energy at a potential V, q is the voltage-independent term, δV is the linear term $[(c\ y_0/d)\ V]/RT$ and γV^2 is the quadratic term $[-(c^2/2\ a\ d^2)\ V^2]/RT$.

δV arises either due to a net difference in charge between open and closed conformations of the channel, or due to movement of a fixed set of charges in the channel molecule into a different electrostatic environment during a conformational change, or a combination of both. The quadratic term γV^2 is related to the "pull" on the charged part(s) of the molecule against its equilibrium state. Thus γV^2 is associated with the contribution by part(s) of the channel other than those involved in charge movement. The values of q, δ and γ for this channel are compared with those of squid axon in Table 2. The value of q, which is related to the free energy at zero membrane potential, is positive as expected for a channel that has $V_{1/2}$ of steady state activation at positive potentials. The order of magnitude of δ and γ values were comparable. A change in the value of δ from 3.68 at 15 °C to –2.74 at 35 °C suggests that the increase in temperature facilitates movement of the voltage sensing segment.

The curvature in the plot of $\Delta G\ (V)$ versus membrane potential was more prominent at 15 °C than at 35 °C. γ and a are inversely related; therefore a decrease in the magnitude of γ with temperature would imply an increase in the magnitude of a. This observation is consistent with the notion that at higher temperatures the channel structure is less rigid, facilitating interactions between the sensor and other domains of the channel. These favourable interactions would increase the magnitude of a, resulting in a smaller quadratic component. Earlier studies have shown that movement of S4 in response to potential change leads to conformational changes which are voltage independent (Hoshi et al. 1994; Zagotta et al. 1994) and the transduction of S4 movement to other parts of the channel molecule (McCormack et al. 1991; Tomaselli et al. 1995; Stefani 1995; Shieh et al. 1997). These observations strongly support the idea that the voltage sensor may influence the equilibrium state of other domains, which in turn may affect its own equilibrium state. A strict relation between the δV term to movement of S4 and the γV^2 term to other parts of channel is not possible at this stage. This would require experiments like those of Mannuzzu et al. (1996), done at various temperatures.

Temperature serves as a useful non-invasive biophysical tool to understand the gating and activation of voltage-gated ion-channels in cases where the model used for kinetic analysis is consistent over the range of temperatures, as shown for K^+ channels in cardiac and human T-lymphocytes (Tsien and Noble 1969; Pahapill and Schlichter 1990) and the one reported here. The channel reported here has a temperature-sensitive, voltage-dependent rate-limiting step associated with channel opening, at low intracellular calcium. The negative entropy of the channel in the transition state agrees with the prevalent observation of a constrained transition state compared to the stable states. Our study shows physiological temperature for mammalian K^+ channels is important for channel function, because at these temperatures the channel overcomes the pull or restraint on the voltage sensor by other parts of channel, with much lower input of energy in the form of voltage change. Thermodynamic studies with mutant channels is a potential area for a better understanding of channel activation and gating.

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