# Temperature Dependence of Unitary Properties of an ATP-dependent Potassium Channel in Cardiac Myocytes

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ABSTRACT The temperature dependence of the properties of unitary currents in cultured rat ventricular myocytes has been studied. Currents flowing through an ATP-dependent $K^+$ channel were recorded from inside-out patches with the bath temperature varied from 10° to 30°C. The channel conductance was 56 pS at room temperature (22°C), and the amplitudes of unitary currents and the channel conductance exhibited a relatively weak ($Q_{10}$ from 1.4 to 1.6) dependence on temperature. The temperature dependence of channel mean open times was biphasic with the low temperature (10–20°C) range showing a relatively stronger temperature dependence ($Q_{10}$ of 2.3) than the high temperature (20–30°C) range ($Q_{10}$ of 1.6). The activation energies for the two regions were determined from an Arrhenius plot with the activation energy, corresponding to the lower temperature range, near 16 kcal/mol. Thermodynamic analysis, using transition rate theory, indicated that the formation of a transition state prior to channel closure to be associated with a positive entropy component for the high $Q_{10}$ region.

## INTRODUCTION

A number of studies have used variable temperature and thermodynamic equations in the analysis of agonist and antagonist binding to receptors (Weiland and Molinoff, 1981; Contreras et al., 1986; Hitzemann, 1988; Raffa and Porreca, 1989). At present, however, the use of similar procedures to study the temperature dependence of the properties of ion channels are not common. However, altered temperature would be expected to perturb the function of ion channel proteins and thus have general utility as a biophysical probe for characterization of properties and function of ion channels. Another consideration, relevant to the use of variable temperature, are the recording of data which more closely approximates normal physiological conditions. In general, it may not be warranted to simply extrapolate properties of ion channels, determined at room temperature, to describe function at normal (body) temperature.

Some examples can be cited in the measurement of the temperature dependence of ion channel properties. In skeletal muscle, Beam and Donaldson (1983) described the effects of variable temperature (1–37°C) on various components involved in the Hodgkin-Huxley description of potassium currents. The results showed considerable effects of temperature to alter activation parameters with the time constant of activation markedly changed at low temperature. The overall conclusion was that room temperature measurements would serve as a relatively poor indicator for the physiological function of $K^+$ channels at normal temperature. A similar conclusion was also reached in a recent study which determined the temperature dependence (from 5° to 42°C) of the activation, inactivation, and conductance associated with repolarizing $K^+$ channels in human T lymphocytes (Pahapill and Schlichter, 1990). Variable temperature caused significant changes in channel kinetic behavior with the degree of the effects also dependent on temperature. For both studies (Beam and Donaldson, 1983; Pahapill and Schlichter; 1990), Arrhenius plots (magnitude of kinetic component vs the inverse of temperature) were curvilinear with no apparent discontinuities in the slopes. In the study using T lymphocytes (Pahapill and Schlichter, 1990), thermodynamic quantities, such as changes in free energy and entropy, were also determined for channel activation and inactivation. Thermodynamic analysis, using transition state theory, has also been applied to the temperature dependence of drug (*n*-alkanol) block of end-plate channels (McLarnon, and Quastel, 1984) and drug (antiarrhythmic) block of calcium-activated $K^+$ channels in hippocampal neurons (McLarnon and Wang, 1991). In both cases, drug actions were associated with increases in system entropy consistent with conformational changes in segments of the ion channel.

In the present study we have examined the effects of both cooling and heating the bath solution from room temperature (range of temperature 10–30°C) on properties of unitary currents (amplitude, conductance, and mean open time) of an ATP-dependent potassium channel K(ATP) in cultured rat myocytes. The experiments have used inside-out patches for the measurements since such patches were stable over the relatively long durations required for data recording. With high internal $Na^+$, as used presently, the ATP-dependent $K^+$ channel exhibits inward rectification in current/voltage plots (Trube and Hescheler, 1984; Davies et al., 1991). The kinetic behavior of the K(ATP) channels are generally complex and include voltage-dependent block by internal cations (Noma, 1983; Horie et al., 1987; Spruce et al., 1987; Woll et al., 1989). Other factors, including modulation of channel gating by internal and external $K^+$ such as found with the conventional inward rectifier channel (IK1) (Cohen et al., 1989; Pennefather et al., 1992), may also be involved (Ashcroft,

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1988). Thus, it was possible that the use of variable temperature would have some utility in the description of underlying kinetic factors involved in the gating of the ATP-dependent $K^+$ channel.

## MATERIALS AND METHODS

### Preparation of cultured cells

Myocytes were isolated from the hearts of Sprague-Dawley rats by modifications of the procedures previously described by Wittenberg et al. (1986) and Langer et al. (1987) and are briefly described below. Following removal of the heart, solution A (nominally calcium-free minimal essential medium-Joklik's modified supplemented with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 21.1 mM), $NaHCO_3$, and 5% bovine serum albumin) was forced through coronary arteries with a syringe. After arresting the heart, the aorta was cannulated and perfused with solution A at 37°C. This was followed by a 40–45-min perfusion with solution B (solution A with $CaCl_2$ (50 $\mu$M) and type II collagenase (1.5 mg/ml, 189 units/mg Worthington Biochemical Corp., Freehold, N.J.). The ventricles were then removed and teased apart in 10 ml of solution B. Following further agitation the cell suspension was passed through a nylon mesh into a centrifuge tube to allow the cells to pellet. The supernatant was removed and the cells resuspended in solution A supplemented with $CaCl_2$ (200 $\mu$M). This procedure was repeated two additional times with $CaCl_2$ at 0.5 and 1 mM. The final pellet was resuspended in Medium-199 (M-199) (1.8 mM $CaCl_2$) supplemented with HEPES (21.1 mM), $NaHCO_3$ (4.4 mM), fetal bovine serum (5%), penicillin (100 units/ml), and streptomycin (0.1 mg/ml). Myocytes were then plated on glass coverslips previously coated with M-199 containing 20 mg/ml laminin (Haddad et al., 1988). Cells were allowed to plate for 1 h at room temperature after which the glass coverslips were washed twice and bathed in fresh M-199 (containing no added fetal bovine serum) for 2–4 h prior to experiments.

### Electrophysiology

Unitary currents were recorded from inside-out patches obtained from rat myocytes. The bath solution had the following composition (in millimolar): NaCl, 140; KCl, 5; $CaCl_2$, 1.2; HEPES, 10; pH 7.3. The patch pipette contained the following (in millimolar): KCl, 140; NaCl, 5; $CaCl_2$, 0.5; HEPES, 10. The high pipette $K^+$ was used to enhance the conductance of inward rectifier channels in the patch. In some experiments ATP was also included in the internal (bath) solution at a concentration of 2 mM.

Recording of unitary currents utilized a patch-clamp amplifier (Axopatch, Axon Instruments, Foster City, CA), with internal low pass filter set at 2 kHz. All data were sampled at 5 kHz and stored on computer or videotape for subsequent analysis. The routines used for data analysis included programs on pClamp (Axon Instruments). Distributions for channel amplitudes and open and closed times were constructed from analysis of unitary events and generally consisted of a minimum of 200 events. In several experiments it was possible to maintain stable recording over considerable time and in such cases it was possible to record in excess of 1000 events for distributions.

The temperature control system and data recording procedure with variable temperature has been described previously (McLarnon and Wang, 1991) and is briefly discussed. The hardware consisted of a proportional control system incorporating thermistors and Peltier devices and was similar in design to that outlined by Chabala et al. (1985). Data were initially recorded at a set-point of 24°C and at 2°C intervals during cooling to 10°C. The cooling rate was set so that data could be recorded at intermediate temperatures over 10-s periods. After data were gathered at 10°C, a heating run was done (same rate as cooling run) up to a temperature of 30°C. The magnitudes of current amplitudes and mean open times showed no significant differences during cooling and heating trials and consequently were grouped together for the individual inside-out patches. The procedure outlined above served as the minimum requirement to determine the temperature sensitivity of unitary currents, however, in several instances patch activity was sufficiently stable that additional cooling and heating runs were possible. Such instances are specified in Results. A measure of the temperature dependence of unitary properties of inward rectifier currents included determination of $Q_{10}$ values. The $Q_{10}$ is an expression of the ratio of the values of a quantity over a 10°C change in temperature. As such, the magnitude of $Q_{10}$ is dependent on the specific temperature range used in the calculation.

A thermodynamic analysis has been applied to the rate constant $k_{-1}$ which describes transitions from the open to closed states of the system. In this analysis, the temperature dependence of $k_{-1}$ has been determined using an Arrhenius plot (graph of ln $k_{-1}$ versus $T^{-1}$). The transition state activation energy $E_a$, which is a measure of the energy barrier in the transition between states, was then determined from the slope of the Arrhenius plot (product of the slope and universal gas constant $R$). Standard thermodynamic transition rate theory (Minneman et al., 1980; Weiland and Molinoff, 1981) was then applied to evaluate enthalpy $\Delta H^\dagger$ ($\Delta H^\dagger = E_a - RT$) and free energy $\Delta G^\dagger$ ($\Delta G^\dagger = -RT \ln k_{-1} + RT \ln k_B T/\hbar$), where $k_B$ is Boltzmann's constant and $\hbar$ is Planck's constant. The entropy $\Delta S^\dagger$ was then found using the relation ($\Delta G^\dagger = \Delta H^\dagger - T\Delta S^\dagger$).

## RESULTS

In these experiments inside-out patches were isolated from cultured rat ventricular myocytes. A high pipette $K^+$ (140 mM) was used to enhance the conductance of an inwardly rectifying $K^+$ channel in the patches. Typical currents recorded from excised patches (at room temperature, 24°C) are shown in the top traces of Fig. 1 *A*; channel events generally occurred in bursts as has been noted in previous studies on inward rectifier currents in cardiac cells (Sakmann and Trube, 1984; Trube and Hescheler, 1984; Payet et al., 1985) and ATP-dependent $K^+$ channels in muscle (Spruce et al., 1987; Woll et al., 1989). Unitary inward currents were reduced in amplitude when bath $K^+$ was increased (replacement for $Na^+$) and were not evident when 140 mM $K^+$ was present in the bath solution; in this case symmetrical $K^+$ was across the inside-out patches. Channel activity was significantly reduced when ATP was introduced into the bath solution. As shown in the second trace from the top (Fig. 1 *A*), typical current records had no channel activity with the occasional opens having amplitudes the same as control. Prior to the addition of ATP, channel open probability was in excess of 0.3 ($n$ = 3 patches). After the addition of ATP (at 2 mM) channel openings were infrequent with the open probability less than 0.02; the actions of ATP were completely reversible.

A current-voltage ($I/V$) relation for the data shown in Fig. 1 *A* is presented in Fig. 1 *B*. Over the hyperpolarization range from −20 to −100 mV, the $I/V$ relation was linear with a slope conductance of 54 pS. The mean value of slope conductance was 56 ± 4 pS ($n$ = 6 patches). At potentials near 0 mV considerable rectification in the $I/V$ plot was found; no unitary currents were evident with depolarizing patch potentials. The rectification properties of this channel in rat myocytes were very similar to those described (Fig. 6, Trube and Hescheler (1984)) for an ATP-sensitive inward rectifier $K^+$ channel with inside-out patches obtained from guinea pig ventricular myocytes and also for an ATP-sensitive $K^+$ channel in mouse skeletal muscle (Woll et al., 1989). The single channel conductance of this channel in guinea pig myocytes was 80 pS (with 147 mM $K^+$ in the patch pipette) and was

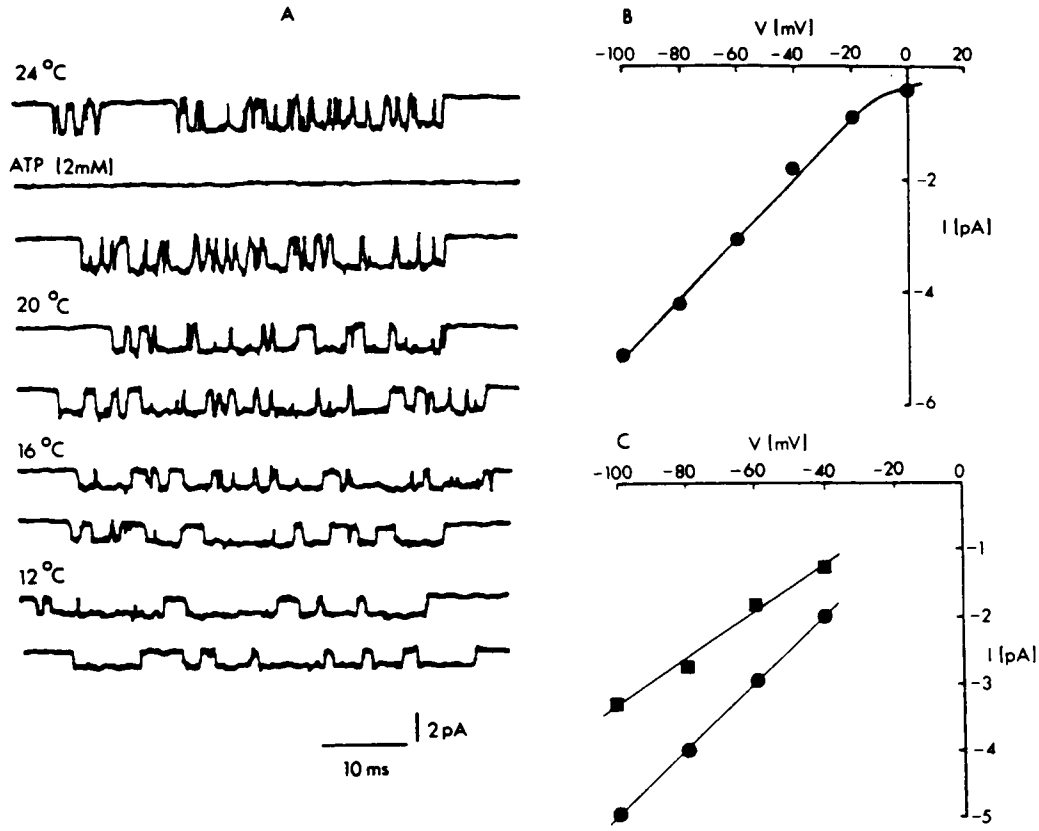


FIGURE 1 Temperature dependence of unitary currents recorded from a single patch. (*A*) Top three traces (*downward openings* from the closed state) show typical currents recorded at 24°C (*first* and *third traces*) and effects of ATP (at 2 mM) in the second trace from the top. Lower records from the same patch are contiguous traces of unitary events at the temperatures shown. The patch potential was −40 mV. (*B*) Current-voltage plot for the patch activity shown in *A*. The slope conductance was 54 pS over the voltage region from −20 to −100 mV. The holding temperature was 22°C. (*C*) Temperature dependence of channel conductance. Unitary current amplitudes recorded at the potentials shown for $T$ = 22°C (●) and $T$ = 12°C (■). The data are mean values from $n$ = 3 patches. Slope conductance was 53 pS at 22°C and was 35 pS at 12°C. The error bars are approximately the size of the symbols shown and the linear relationships were drawn by eye.

55 pS (with extracellular $K^+$ at 70 mM). The unitary conductances of ATP-dependent $K^+$ channels are generally in the range 50–80 pS when external $K^+$ is near 140 mM (Ashcroft, 1988). Earlier work had also documented properties of an ATP-regulated $K^+$ channel in atrial and ventricular cardiac cells (Noma, 1983). However, with physiological $K^+$ across patches, inward rectification was not observed (Noma, 1983).

Contiguous traces, at four temperatures, illustrate the effects of temperature on unitary currents in Fig. 1 *A*. Diminishing bath temperature, in increments of 4°C from 24°C, were associated with reduced current amplitudes and prolonged time courses. In addition, the frequency of channel events was lower at cold temperature. The $Q_{10}$ is a measure of the temperature dependence of a quantity and is expressed as a ratio of the values over a 10°C change in temperature; thus, the magnitude of $Q_{10}$ is dependent on the specific 10°C span considered. With regard to the amplitudes of unitary currents, the $Q_{10}$ ($n$ = 6 patches) was equal to 1.6 (20°-10°C) or 1.4 (30°-20°C). These magnitudes of $Q_{10}$ would indicate a relatively low temperature dependence for current heights and thus channel conductance. In three of the six inside-out patches, a high number of events (in excess of 600) were recorded for patch potentials from −40 to −100 mV (increments of 20 mV) at the two temperatures, 12° and 22°C. This procedure allowed for a determination of the temperature dependence of channel conductance. The averaged unitary currents for the patches are plotted as a function of patch potential at the two temperatures in Fig. 1 *C*. At 22°C the channel conductance was 53 pS and at 12°C the channel conductance was decreased to 35 pS. The $Q_{10}$ associated with the temperature dependence of channel conductance was 1.5. Thus the amplitudes of unitary currents and the conductance of the inward rectifier $K^+$ channel were not strongly dependent on temperature.

In order to determine the actions of temperature on channel kinetics, distributions for channel open and closed times were constructed. As shown in Fig. 2 *A*, at room temperature ($T$ = 22°C) the open distribution (*top trace*) was fit with a single time component, and the closed time distribution (*lower trace*) required a fit with a two-component exponential function. Similar distributions are shown in the upper (open time histogram) and lower (closed time histogram) traces of Fig. 2 *B* with the bath temperature reduced to 12°C. For this patch the mean open time was prolonged (by a factor of 3) at $T$ = 12°C compared with the $T$ = 22°C value and

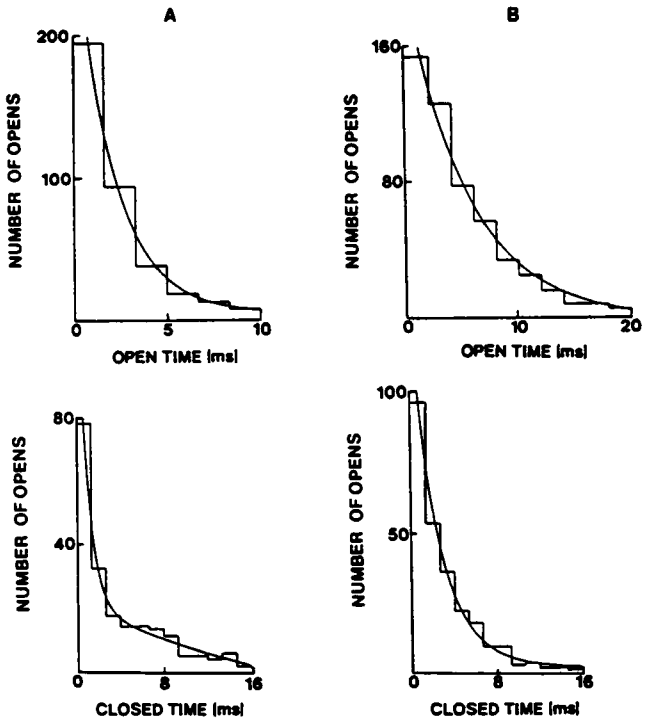


FIGURE 2 Distributions for open and closed times at different temperatures. (*A*) Temperature of 22°C. Top is distribution of open times with mean 1.7 ± 0.3 ms (610 events); Bottom is distribution of closed times with means of 0.8 ± 0.5 ms and 19.4 ± 3.4 ms (575 events). (*B*) Temperature of 12°C. Top is distribution of open times with mean 4.7 ± 0.5 ms (520 events); Bottom is distribution of closed times with means of 1.4 ± 0.6 ms and 30.8 ± 7.2 ms (480 events). The patch potential was −60 mV.

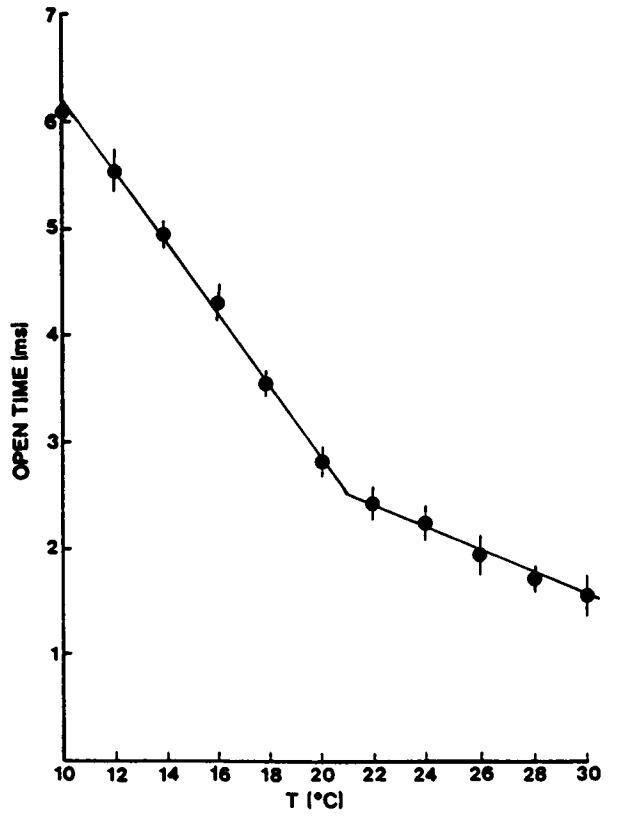


FIGURE 3 Temperature dependence of mean open time. The magnitudes of open times are mean values from $n = 6$ patches with $V = -60$ mV. The lines shown represent visual fits to the data.

the two closed time components were also lengthened at the colder temperature. For all patches studied, single component $\tau$ (open) and double component $\tau$ (closed) functions gave adequate fits to the respective open and closed time distributions. The same number of components have been used to fit distributions for ATP-dependent $K^+$ channels in cardiac cells (Trube and Hescheler, 1984; Kakei and Noma, 1985) and mouse skeletal muscle (Woll et al., 1989). However, additional time components were required in the fit of open and closed time distributions in frog skeletal muscle (Spruce et al., 1987). In general, the two components for closed time distributions showed considerable variability between patches for the same temperature and patch potential. Since open times were highly reproducible from patch-to-patch (for the same $T$ and $V$), the effects of temperature on channel kinetics concentrated on the temperature dependence of open times.

A graph of the temperature dependence of channel mean open time ($n = 6$ patches) is shown in Fig. 3. Evidently a single linear relationship would not provide an adequate fit to the points. In addition, a curvilinear relationship was also not indicated from the data. The results were fit with two separate linear functions with the inflection point close to a temperature of 21°C. Over the temperature range from 10° to 20°C the slope was considerably larger ($Q_{10}$ value of 2.3) compared with the slope measured from 20° to 30°C ($Q_{10}$ value of 1.6). Consideration of only the data recorded over the lower temperature range (10–20°C) would also have led to the conclusion that open times must be less sensitive to temperature for $T$ in excess of 20°C. This was so since extrapolation of the low temperature slope (10–20°C) to normal (physiological) temperature would yield a negative mean open time. If the slope of the graph from 22° to 30°C (Fig. 3) is extended to 37°C, then a mean open time for the inward rectifier $K^+$ channel of near 1 ms is found at normal body temperature.

In one particular patch it was possible to record unitary currents over three complete heating and cooling trials. The longevity of this patch allowed for accumulation of a high number of events at intermediate temperatures and at different patch potentials. In this patch (Fig. 4), the temperature dependence of open times was determined for three different patch potentials (−40, −60, and −80 mV). The temperature dependence of mean open times showed the characteristic biphasic slope (as shown in Fig. 3) with the inflection points, for all potentials, close to 21°C. Over the lower temperature range (≤20°C), the slopes of the linear relations were −0.52 ms/°C at −40 mV, −0.53 ms/°C at −60 mV, and −0.58 ms/°C at −80 mV. All three slopes were fit with a linear relation with a $Q_{10}$ of 2.7. For $T$ in excess of 20°C, the slopes of the lines were −0.1 ms/°C corresponding to a $Q_{10}$ value of 1.6. At a given temperature, the mean open times were diminished

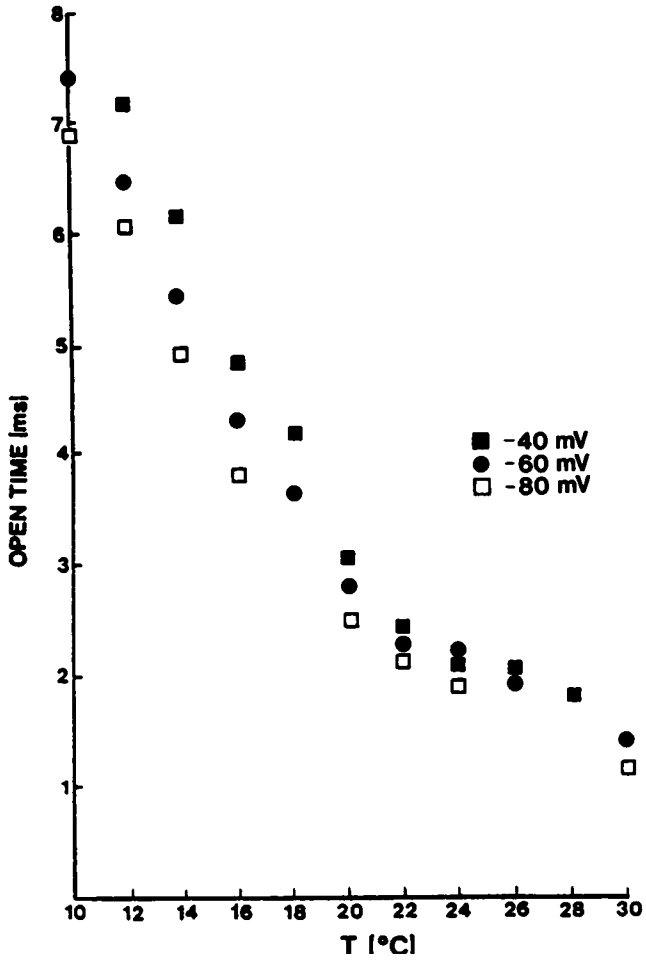


FIGURE 4 Temperature dependence of mean open times at different potentials. The data shown were recorded from the same patch with $V = -40$ mV (■), $V = -60$ mV (●), and $V = -80$ mV (□). At some temperatures only one or two of the potentials were studied.

with increased patch hyperpolarization which is a characteristic property of inward rectifier $K^+$ channels in a variety of cells including cardiac (Sakmann and Trube; 1984b, Kurachi, 1985), and glial cells (McLarnon and Kim, 1989). The relatively high magnitude of $Q_{10}$, associated with temperature less than 20°C, is higher than would be expected if diffusion was rate limiting. This result would suggest that channel kinetics were regulated by a nondiffusional process and that thermodynamic analysis could have some utility in the description of underlying mechanisms.

### Thermodynamic analysis

The distributions for channel open times show a single open state for the K(IR) channel (Fig. 2). The Arrhenius equation relates the rate constant $k_{-1}$ for the transition from the single open state to a closed state (or states) to temperature $T$ by:

$$k_{-1} = Ae^{-E_a/RT}, \quad (1)$$

where $E_a$ is the activation energy, $R$ is the gas constant, and $A$ is a constant associated with the kinetic process. An Arrhenius plot is a graph of the ln $(k_{-1})$ dependence on the inverse of $T$, where $T$ is expressed in units of °K. The Arrhenius plot for the rate constant $k_{-1}$ is shown in Fig. 5 with an apparent inflection point between the two regions of temperature sensitivity close to a $T^{-1}$ of 0.00340 °$K^{-1}$. The transition state activation energy Ea can be found from the product of the slope of the Arrhenius plot and $R$. Denoting the slope of the low temperature dependence (20–30°C) as slope 1 and of the high temperature dependence (10–20°C) as slope 2 then the respective activation energies were $E_a(1)$: 8.7 kcal/mol and $E_a(2)$: 16.1 kcal/mol. The corresponding values of enthalpy $\Delta H^\dagger$ were then determined (see Methods) to be $\Delta H^\dagger(1)$: 8.1 kcal/mol and $\Delta H^\dagger(2)$: 15.5 kcal/mol. The magnitudes of the transition state entropy $\Delta S^\dagger$ were then found from subtraction of free energy (estimated at $T = 22$°C for both regions of temperature) from enthalpy. For the low temperature dependence (20–30°C) region the entropy change was −18.9 e.u. and for the high temperature region (10–20°C) the entropy change was +5.7 e.u. The magnitudes of the transition state thermodynamic parameters, including free energy, are listed in Table 1. A positive value for entropy is associated with increased disorder accompanying the formation of the transition state prior to channel closure, and a negative value for entropy indicates an increase in the order is associated with the transition state for the temperature region 20–30°C. The results could indicate that different mechanisms may be rate-limiting at low and higher temperatures during the formation of the activated transition states subsequent to the channel-closing step. It should be noted that since the activation energy, free energy, enthalpy, and entropy described above are transition state thermodynamic parameters, they do not provide any information regarding differences between channel open and closed states. As such, the parameters do not represent equilibrium values; this point is considered in more detail, with regard to free energy, in the discussion.

## DISCUSSION

The use of variable temperature as a probe for the measurement of properties of ion channels is not a commonly utilized biophysical tool. However, such measurements could serve a useful function since altered temperature leads to perturbation of channel properties which in turn may provide information regarding underlying mechanisms. Furthermore, details regarding the properties of ion channels at normal (body) temperature may be provided; unless otherwise shown it perhaps is best assumed that data recorded at a convenient temperature (for example, room temperature) may not serve as a realistic indicator of normal channel function. This point is considered below.

In this work the temperature dependence, over a range from 10° to 30°C, of an inwardly rectifying, ATP-dependent $K^+$ channel was studied in patches excised from rat myocytes. The inward rectification property of this channel is most prominent when extracellular $K^+$ is near 140 mM and the intracellular solution contains $Na^+$ or $Mg^{2+}$ (Trube and

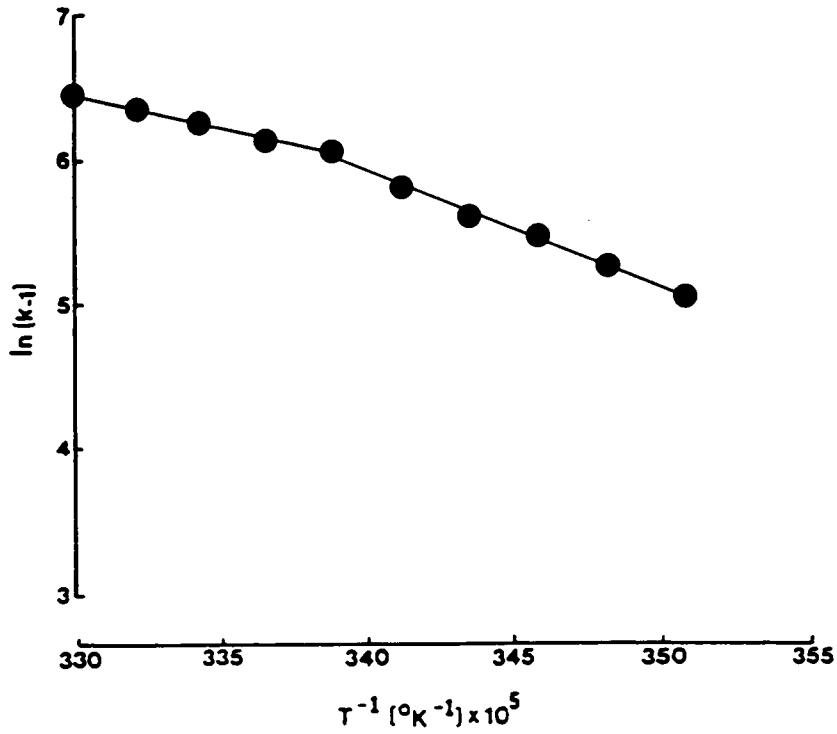


FIGURE 5 Arrhenius plot for mean open time. The graph shown is ln $k_{-1}$ (where $k_{-1}$ is the rate constant for the open to closed transition) vs. $T^{-1}$. The lines shown are fits using linear regression analysis with activation energies proportional to the slopes of the linear relations.

**TABLE 1 Temperature dependence of transition state parameters**

| | Slope 1 (20–30°C) | Slope 2 (10–20°C) |
|---|---|---|
| $Q_{10}$ | 1.6 ± 0.1 | 2.3 ± 0.2 |
| $E_a$ | 8.7 ± 1.6 kcal/mol | 16.1 ± 2.4 kcal/mol |
| $\Delta H^\dagger$ | 8.1 ± 1.2 kcal/mol | 15.5 ± 2.3 kcal/mol |
| $\Delta G^\dagger$ | 13.8 ± 1.5 kcal/mol | 13.8 ± 1.5 kcal/mol |
| $\Delta S^\dagger$ | −18.9 ± 5 e.u. | 5.7 ± 2.0 e.u. |

The magnitudes of thermodynamic parameters are with $V$ = −60 mV and $T$ = 22°C.

Hescheler, 1984; Woll et al., 1989; Davies et al., 1991). Activation of this channel (unitary conductance of 56 ± 4 pS; $n$ = 6) was dependent on the absence of ATP in the bath (intracellular) solution. A plot of the temperature dependence of the amplitudes of the currents was linear over the full temperature range (10–30°C) with a slope ($Q_{10}$) of 1.5 ± 0.2 ($n$ = 6) from 12° to 22°C. In three patches, sufficient data were recorded with both variable temperature and potential to determine the dependence of unitary conductance on temperature. At 22°C the channel conductance was 53 pS, and at 12°C the channel conductance was 35 pS ($Q_{10}$ of 1.4). Thus, both current amplitudes and channel conductance (derived from the voltage dependence of amplitudes) were relatively weakly dependent on temperature. The low magnitudes of $Q_{10}$ would suggest that processes which determine the conductance of the inward rectifier channel were not strongly dependent on temperature. For example, simple diffusion of ions through the pore would be consistent with this magnitude of $Q_{10}$ (Hille, 1975). Furthermore, the results would indicate that the mechanisms which determine channel conductance were not coupled to the processes involved in the establishment of mean open time at low temperature (see below).

Previous measurements on the temperature dependence of the amplitudes of channel currents or conductance would generally accord with rate-limiting diffusion of ions in an aqueous environment. For example, the whole-cell conductance of $K^+$ channels in T lymphocytes had a $Q_{10}$ of 1.7 (at 20°C); the unitary conductance at the same temperature had a lower $Q_{10}$ of 1.2 (Pahapill and Schlichter, 1990). Rate-limiting diffusion has been associated with low $Q_{10}$ (≤1.5) and activation energies near 4 kcal/mol for gramicidin channels in membrane (Hladky and Haydon, 1972). At the neuromuscular junction the amplitudes of miniature end-plate currents were found to have a $Q_{10}$ of 1.5 (Gage and McBurney, 1975).

The characterization of the effects of temperature on channel kinetics focussed on the temperature sensitivity of mean open times. Channel closed rate constants were somewhat variable between patches held at the same temperature; thus, open probability was not a reproducible measure of kinetic behavior. The graph of the dependence of mean open time on temperature (Fig. 3) exhibited two distinct slopes with an inflection point near $T$ = 21°C. The occurrence of a lower temperature sensitivity for open times as bath temperature was elevated above 20°C was predictable from observation of the data recorded from 10° to 20°C. This was so since extrapolation of the linear relation for mean open time dependence on temperature (from 10° to 20°C; Fig. 3) to body temperature would yield a negative value for open time at 37°C. However extrapolation of the mean open times measured from 20° to 30°C, would give a magnitude for this

quantity of about 1 ms at body temperature. The results would suggest that kinetic properties of the ATP-dependent $K^+$ channel were rate-limited by different processes over the temperature range studied.

Distributions for open times were fit with single exponential functions at all temperatures. Thus the relation between open time $\tau$ and temperature $T$ could be expressed by $-\ln \tau = \ln A - E_a/RT$ where $\tau^{-1}$ is the rate constant ($k_{-1}$) for the open to closed transition and $E_a$ is the activation energy for the process. An Arrhenius plot was constructed, and two distinct regions with different temperature sensitivity were apparent (Fig. 5). The values of $E_a$ were 16.1 and 8.7 kcal/mol for the respective temperature regions of 10–20°C and 20–30°C. The magnitude of $E_a$ for the higher temperature range is in reasonable accord with rate-limiting diffusion as noted above to describe the temperature dependence of current amplitudes and conductance. Activation energies less than 10 kcal/mol are consistent with diffusion in an aqueous medium (Hille, 1975).

The high value of activation energy determined for the temperature dependence of mean open time at lower temperatures (10–20°C) is higher than expected for a simple diffusional process. Transition rate theory relates activation energies to the thermodynamic quantities of free energy $\Delta G^\dagger$, enthalpy $\Delta H^\dagger$, and entropy $\Delta S^\dagger$ (see Methods) where $\Delta G^\dagger = \Delta H^\dagger - T\Delta S^\dagger$. It would seem possible from the results (Table 1) that different rate-limiting processes were involved for the two regions of temperature sensitivity. In particular, the higher temperature sensitivity (10–20°C) was associated with a positive entropy term. Thus the formation of the transition state was accompanied by an increased entropy signifying an increase in the disorder of the system. If, for example, this step involved isomerization of the protein then the protein in the activated state subsequent to channel closure is in a less-ordered configuration. The formation of an activated transition state was not supported energetically by molecular bonds (such as covalent or hydrogen) or by electrostatic interactions since these would be associated with negative enthalpy.

A qualitative consideration of the thermodynamic parameters involved in channel activation is possible. An estimate for the forward rate constant for activation of K(ATP) can be found from the inverse of the fast component of closed time distributions in this work and in previous studies of a K(ATP) channel in ventricular myocytes (Trube and Hescheler, 1984) and an inward rectifier $K^+$ channel in ventricular cell membrane (Kurachi, 1985). A time component near 0.5 ms has been found which gives an activation rate constant of $2 \times 10^3$ $s^{-1}$. With this estimated value of rate constant, then the free energy associated with channel opening is about +13 kcal/mol (using the equation for the relation between rate constant and free energy given in Methods). Thus, the enthalpy associated with channel opening is more positive, by 13 kcal/mol, than the term $T\Delta S^\dagger$. In this regard, Pahapill and Schlichter (1990) have determined similar values for changes in free energy (near 13 kcal/mol and essentially independent of temperature over 5–40°C) for the activation of $K^+$ channels in T lymphocytes. If this result is applied to K(ATP), then the net change in free energy can be found by subtracting the magnitude of $\Delta G^\dagger$(Table 1) from 13 kcal/mol giving a value of about −1 kcal/mol. In essence this is the change in free energy which drives the isomerization and represents an equilibrium value if the same rate-limiting step underlies channel opening and closing.

On the basis of the measurement of the temperature dependence of mean open times, the extrapolation of room temperature data to describe the kinetic behavior of the ATP-dependent $K^+$ channel at physiological temperature would not be warranted. A similar conclusion has been reached regarding properties of $K^+$ channels in rat skeletal muscle (Beam and Donaldson, 1983) and in human T lymphocytes (Pahapill and Schlichter, 1990). In both cases the temperature range studied was wider than used presently and included 37°C. Arrhenius plots of the temperature dependence of channel activation in muscle (Beam and Donaldson, 1983) and of the conductance and kinetic parameters of $K^+$ channels in T lymphocytes (Pahapill and Schlichter, 1990) were curvilinear. The lack of discontinuities in these studies contrasts with the evident inflection point seen in Fig. 5 in the present work. In T lymphocytes, the channel conductance and activation kinetics exhibited a stronger temperature dependence for low temperature (10–20°C) relative to high temperature (30–40°C), whereas channel inactivation kinetics showed the opposite behavior (Pahapill and Schlichter, 1990). In skeletal muscle (Beam and Donaldson, 1983), the activation kinetics were most sensitive to cold temperature (below 20°C). The essential conclusion from the studies on voltage-dependent $K^+$ channels in muscle and T lymphocytes was that considerable caution should be exercised in the extrapolation of room temperature data to describe channel function at physiological temperature. A similar conclusion is possible from the present data with the proviso that the study of K(ATP) represented the use of nonphysiological conditions, including excised patches (for stability during temperature changes) and high external $K^+$ (for the elevated unitary conductance).

Although variable temperature constitutes a biophysical probe for ion channel properties, the interpretation of data and the concomitant use of thermodynamic analysis in the description of underlying mechanisms of action are, at present, of limited utility. For example, while it may be reasonable to suggest that the two-component Arrhenius plot for mean open time is indicative of separate rate-limiting processes over the different temperature ranges, it is difficult to relate either temperature region to a specific process. The results would also be consistent with a phase transition in the membrane, giving rise to the observed discontinuity seen in Fig. 5. At most one could state that the weak temperature dependence of open times at higher temperature (20–30°C) and the weak temperature dependence of unitary currents and conductance at all temperatures (10–30°C) were consistent with rate-limiting diffusion. It seems premature to attempt to relate the relatively high activation energy and positive entropy, associated with the temperature dependence of mean

open time at lower temperatures (10–20°C), to a particular process. The kinetic behavior of K(ATP) channels is modulated by a number of factors, including channel block by internal monovalent and divalent cations (Horie et al., 1989; Woll et al., 1989) and isomerization of the protein. Such quantities would be expected to have both temperature- and voltage-dependence. It should be noted that in the intact cell the metabolic actions of ATP, in the regulation of the K(ATP) channel, would also be expected to be characterized by a strong dependence on temperature.

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