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Thermodynamically Specific Gating Kinetics of Cardiac Mammalian $K^+_{(ATP)}$ Channels in a Physiological Environment Near 37°C

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Abstract. Elementary K^+ currents through isolated ATP-sensitive K^+ channels from neonatal rat cardiocytes were recorded to study their temperature dependence between 9°C and 39°C. Elementary current size and, thus, K^+ permeation through the open pore varied monotonically with temperature with a Q_{10} of 1.25 corresponding to a low activation energy of 3.9 kcal/mol. Open-state kinetics showed a complicated temperature dependence with Q_{10} values of up to 2.94. Arrhenius anomalies of $\tau_{open(1)}$ and $\tau_{open(2)}$ indicate the occurrence of thermally-induced perturbations with a dominating influence on channel portions that are involved in gating but are obviously ineffective in altering pore-forming segments. At 39°C, open-state exit reactions were associated with the highest activation energy (O_2 exit reaction: 12.1 kcal/mol) and the largest amount of entropy. A transition from 19°C to 9°C elucidated a paradoxical kinetic response, shortening of both O-states, irrespective of the absence or presence of cAMP-dependent phosphorylation. Another member of the K^+ channel family and also a constituent of neonatal rat cardiocyte membranes, 66 pS outwardly-rectifying channels, was found to react predictably since τ_{open} increased on cooling. Obviously, cardiac $K^+_{(ATP)}$ channels do not share this exceptional kinetic responsiveness to a temperature transition from 19°C to 9°C with other K^+ channels and have a unique sensitivity to thermally-induced perturbations.

Key words: K^+ permeation — Open state kinetics — Q_{10}—Arrhenius anomalies — Temperature dependence — cAMP-dependent phosphorylation

Introduction

Membrane conductance in many hypoxic cells may be dominated by activated ATP-sensitive K^+ channels. First identified in ATP-depleted mammalian cardiocytes (Noma, 1993), $K^+_{(ATP)}$ channels play a role in coupling energy metabolism with the electrophysiological status of the cell by setting the resting potential and, particularly in myocardium, by shaping the action potential. They belong to a group of ligand-operated, voltage-independent K^+ channels characterized by a unique transmembrane topology when compared with voltage-dependent K^+ channels, namely two membrane-spanning (M1 and M2) domains and connected with the conserved H5-region (Ho et al., 1993; Ashford et al., 1994). The latter seems likely to be pore-forming. The structural equivalent of the gating process and its possible location within the channel protein remains to be elucidated. Conformational changes allow the channel to switch between several closed and, as often reported from cardiac $K^+_{(ATP)}$ (for review *see* Noma & Takano, 1991), two open states. These and other elementary properties together with the modulation of channel activity by cytosolic factors including nucleosides and nucleotides (Parent & Coronado, 1989) and a G-protein (Kirsch et al., 1990) have been analyzed in great detail during the last decade to provide a comprehensive picture of channel function but, with respect to the core temperature in birds and mammals, at a thermally unphysiological environment, near 20°C.

Ionic channel function can be sensitively influenced by heat but Arrhenius anomalies complicate the temperature dependence in ligand-operated (Dreyer et al., 1976; Lass & Fischbach, 1976; Anderson et al., 1977) as well as in voltage-gated (Schwarz, 1979; Romey et al., 1980) channels. Thus, permeation and gating properties of mammalian $K^+_{(ATP)}$ channels will be hardly predictable for the physiologically relevant temperature near 37°C.

The present patch clamp experiments with isolated $K^+_{(ATP)}$ channels from rat cardiocytes focus on this problem and should define their elementary properties over a broad range, between 9°C and 39°C, thereby including

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the physiologically relevant mammalian *in situ* temperature. In fact, specific thermodynamics determine the exit reaction from the both O-states when the channel operates near 37°C. Open-state kinetics, but not K^+ permeation, are complicated by Arrhenius anomalies, the other important aspect, suggesting that the pore-forming channel region is somehow shielded from thermally-induced perturbations.

Materials and Methods

Elementary K^+ currents through two types of cardiac K^+ channels from cultured neonatal rat cardiocytes were recorded in the inside-out mode by employing the standard patch clamp technique (Hamill et al., 1981) and an L-M/EPC5 amplifier. In the present recording conditions, both channel types of interest can be easily distinguished even when coexisting in an individual inside-out patch: $K^+_{(ATP)}$ channels exhibit a smaller conductance of 23 pS and characteristic open-state kinetics with two O-states, in contrast to the less abundant 66 pS outwardly-rectifying K^+ channel (Benz, Fröbe & Kohlhardt, 1991). Tissue disaggregation as well as cultivating and handling of the short-time (18–24 hours) cardiocytes were essentially identical with procedures described on detail earlier (Kohlhardt et al., 1989). Exclusively rod-shaped cardiocytes were selected for the patch clamp experiments, the cell type in the more advanced developmental stage in this primary culture.

Elementary K^+ currents were recorded at –7 mV and an asymmetrical cationic milieu with a quasi-physiological K^+ concentration gradient across the membrane (5 mmol/l external K^+; 140 mmol/l internal K^+) as outward currents. The records were filtered at 1 kHz with a 8-Pole Bessel filter, stored on tape and digitized with a sampling rate of 5 kHz (dead time of the apparatus 0.2 msec) to be analyzed. Analysis concentrated on i_{unit}, NP_o and open time. Closed time could not be determined since any patch contained multiple (2–4) $K^+_{(ATP)}$ channels. Open-time analysis was based on the 50% threshold method (Colquhoun & Sigworth, 1983) and, by neglecting the first bin of 0.4 msec, τ_{open} resulted from the best fit of probability density functions. i_{unit} was obtained from Gaussian event distributions and NP_o from 30 sec recording intervals.

The environmental temperature of the inside-out patches was controlled by means of a Peltier element device. Temperature transitions were performed in 10°C steps with an accuracy of ±0.25°C, starting from an initial equilibration temperature of 19°C or 29°C and needed less than 60 sec to be accomplished. A further equilibration of 90 sec preceeded the collection of single-channel events for analysis.

Solutions (composition in mmol/l)

Isotonic K^+ solution (A) was used as the bathing solution of the cardiocytes and faced the cytoplasmic membrane surface in the inside-out recording mode and contained (in mM): KCl 140; $MgCl_2$ 2; glucose 20; HEPES 10; EGTA 2; pH 7.4.

Pipette solution (B) faced the external side of the membrane and contained (in mM): KCl 5; NaCl 135; $MgCl_2$ 2; HEPES 10; pH 7.4. Solution A was supplemented with a cocktail of nucleotides (10 µmol/l ATP, 100 µmol/l ADP, 100 µmol/l GDP) to stabilize $K^+_{(ATP)}$ channel activity in cell-free conditions.

Compounds

ATP, ADP and cAMP were purchased from Sigma Chemie, München, and ATP-γ-S from Boehringer Mannheim. All compounds were freshly dissolved just before use in solution A.

Whenever possible, the data are expressed as mean ± SEM. Temperature coefficients (Q_{10}) were conventionally calculated with the premise that a rise in temperature causes an increase in i_{unit} and a decrease in $\tau_{open(1)}$ and $\tau_{open(2)}$.

Results and Discussion

Neonatal cardiac $K^+_{(ATP)}$ channels became activated just after patch excision when exposed to an artificial cytosolic environment with critically reduced ATP concentration. Despite the presence of stimulating nucleotides (ADP and GDP) and ATP in a residual concentration (50 µmol/l), rundown of channel activity could not be completely prevented. NP_o declined to some degree within the first few minutes after patch excision sometimes accompanied by a decrease of $\tau_{open(2)}$ indicating a shortening of the O_2-state. An initial equilibration period (at 19°C or 29°C) was, therefore, expected to ensure that channel activity had attained a steady state. Otherwise or in a few cases with strong deactivation, the inside-out patch was discarded.

Figure 1 demonstrates the temperature sensitivity of $K^+_{(ATP)}$ channels between 9°C and 29°C. Cooling from 19°C to 9°C reduced i_{unit} from 1.60 pA to 1.17 pA and, as it is also evident from the amplitude histograms in Fig. 1, diminished P_o significantly. More interesting is the response of open state kinetics. Opposite to the expected reaction, $\tau_{open(1)}$ and $\tau_{open(2)}$ tended to decline, from 1.8 msec to 1.0 msec and from 14.8 msec to 12.0 msec, respectively. Rewarming to 19°C promptly restored the initial values for i_{unit} and open state kinetics. Only warming to 29°C influenced $\tau_{open(1)}$ and $\tau_{open(2)}$ in the predicted fashion causing a decrease to 0.5 msec and 9.0 msec, respectively whilst i_{unit} rose from 1.62 pA to 2.00 pA.

Warming from 19°C to 29°C or, in another series of experiments, from 29°C to 39°C consistently enhanced channel activity. Thus, NP_o increased but the maximum number of simultaneously open channels, the best estimator for $N \leq 4$ (Horn, 1991), proved temperature-insensitive. A recruitment of sleepy channels similar to observations after warming of voltage-dependent K^+ channels from human T lymphocytes (Pahapill & Schlichter, 1990) is, therefore, very unlikely to occur.

Cooling may have a more substantial influence on the gating process since, as evidenced in K^+ channels from skeletal muscle (Beam, 1981) or voltage-dependent cardiac Na^+ channels (Kohlhardt, 1990) kinetic states can be observed that are not evident at higher temperatures. Importantly, bimodal distributed open-time probability density functions were consistently found to indicate that $K^+_{(ATP)}$ channels likewise attain at 39°C two open (O_1 and O_2) states. The valid Markovian reaction scheme, R-O_1-O_2 or O_1-R-O_2 (where R combines several closed states, for review *see* Noma & Takano, 1991) still

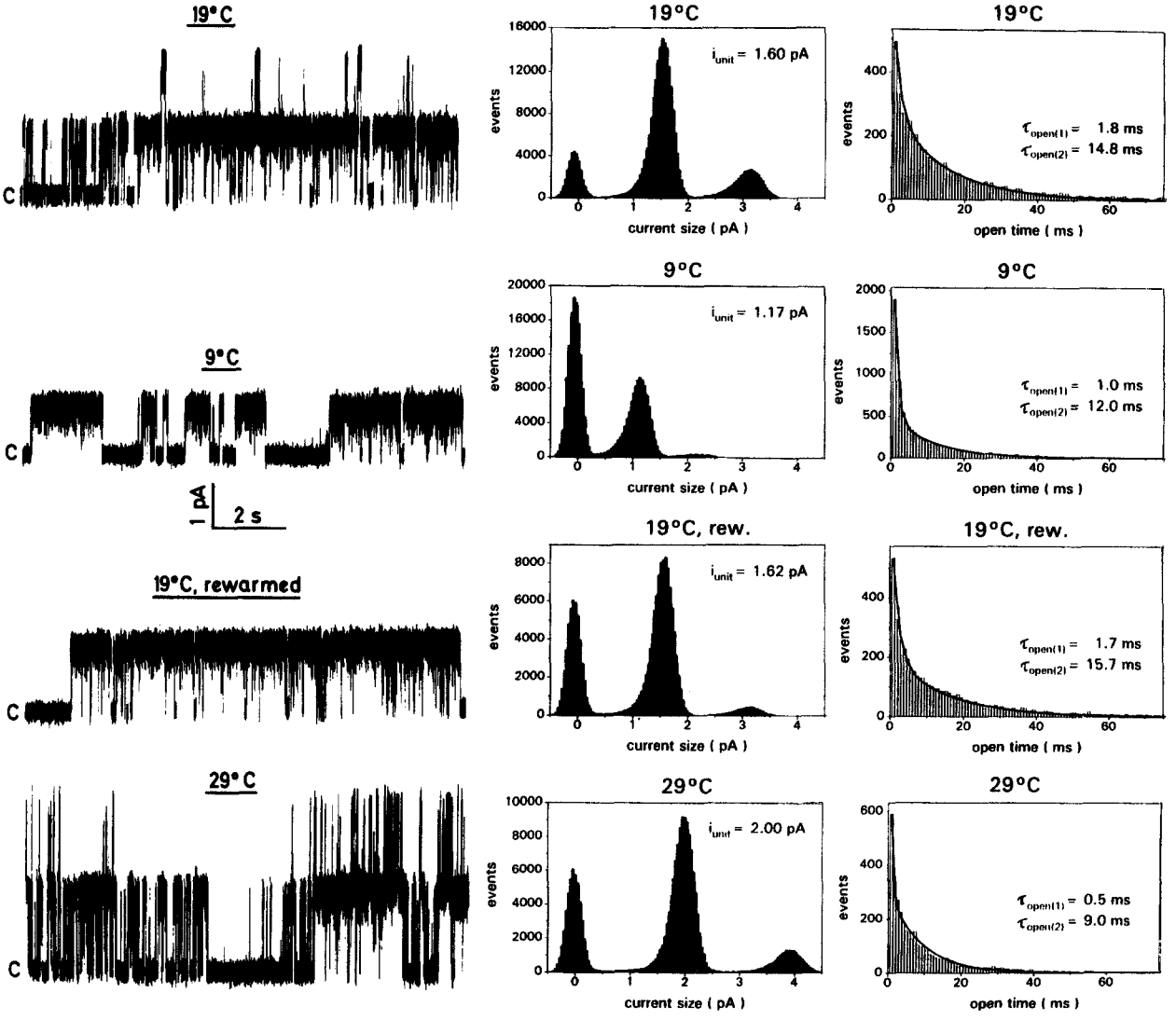

Fig. 1. Selected recordings of elementary K^+ currents through $K^+_{(ATP)}$ channels (left), amplitude histograms (middle) and open time histograms (right) from an inside-out patch at 19°C, 9°C, after rewarming to 19°C, and at 29°C. Disregarding the first bin of 0.4 msec, the best fits of the open time histograms were as follows: 19°C (control): $N(t) = 383\exp(-t/0.0018) + 272\exp(-t/0.0148)$; 9°C: $N(t) = 3574\exp(-t/0.0010) + 485\exp(-t/0.0120)$; 19°C (rewarming): $N(t) = 578\exp(-t/0.0017) + 197\exp(-t/0.0157)$ 29°C: $N(t) = 1841\exp(-t/0.0005) + 302\exp(-t/0.0090)$ Patch 864, membrane potential −7 mV.

awaits clarification in a maximum-likelihood analysis. Consequently, only $1/\tau_{open(2)}$ reflects unambiguously the rate constant governing the exit rate from the O_2-state, in contrast to the significance of $1/\tau_{open(1)}$ which depends on the valid reaction scheme. Although a transition from 29°C to 39°C shortened in 7 experiments both O-states with a decline of $\tau_{open(2)}$ from 14.1 ± 2.8 ms to 5.1 ± 0.9 ms, the ratio O_2-events to O_1-events (which was calculated from the area under both exponentials of the open time probability functions) may rise. In three experiments, O_2-events dominated as if $K^+_{(ATP)}$ channels favor this conductive configuration, an observation which is suggestive for a gating shift that occurs at 39°C.

K^+ permeation through open cardiac $K^+_{(ATP)}$ channels follows the principles of diffusion. i_{unit} increased monotonically from 1.46 ± 0.02 pA (n = 5) at 9°C to 2.19 ± 0.01 pA (n = 7) at 39°C and, consequently, was linearily related to temperature in an Arrhenius plot (Fig. 2*A*). The slope of the latter corresponds to a mean Q_{10} of 1.25 ± 0.03 (n = 19). Consistent with the behavior in other ionic channels including the artificial gramicidin channel, low values for activation energy (3.9 kcal/mol) and for the amount of enthalpy (3.3 kcal/mol) were calculated, in agreement with the enthalpy of K^+ diffusion in water (Robinson & Stokes, 1955). The uniform slope of the Arrhenius plot seems not trivial with respect to Arrhenius anomalies reported in the literature (Fischbach & Lass, 1978; Pahapill & Schlichter, 1990) to occur as

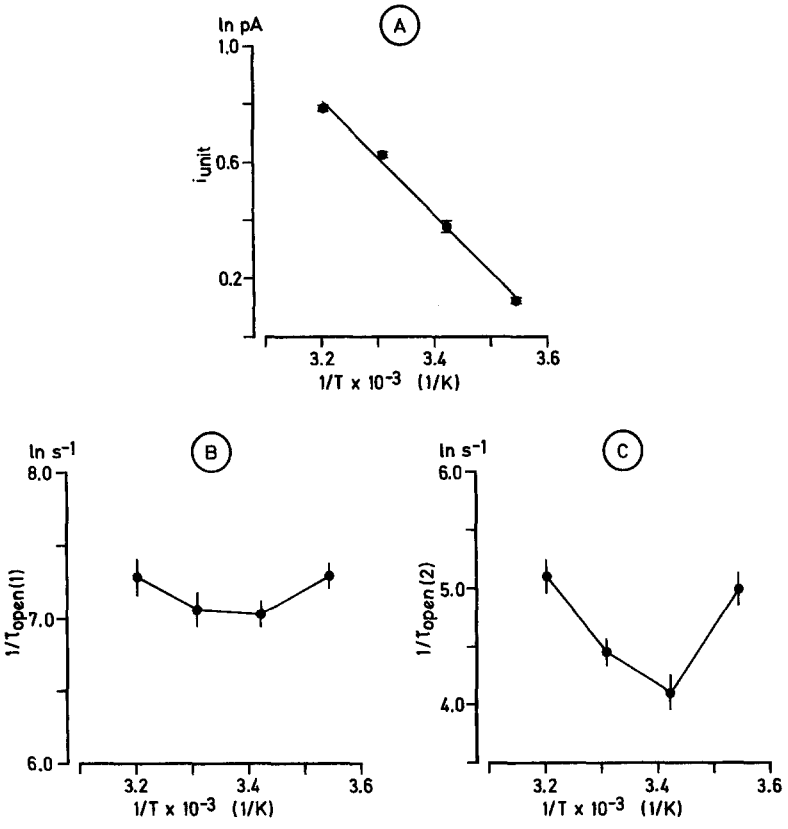

Fig. 2. Arrhenius plots of i_{unit} (*A*), $1/\tau_{open(1)}$ (*B*) and $1/\tau_{open(2)}$ (*C*) *vs.* temperature. Each data point symbolizes the mean of 7 (at 39°C), 14 (at 29°C), 12 (at 19°C), and 5 (at 9°C) inside-out patches with at least two $K^+_{(ATP)}$ channels, vertical bars indicate SEM. For simplicity, the data points in *B* and *C* were connected with each other by straight lines. Note that the temperature 3.2 K^{-1} on the abscissa refers to 39°C.

breaks or nonlinearities in a very similar temperature region around 20°C.

Open-state kinetics, however, were found to have a fundamentally distinct temperature dependence. Arrhenius plots of $\tau_{open(1)}$ and $\tau_{open(2)}$ revealed multiple slopes (Fig. 2*B* and *C*), corresponding to individual Q_{10} values for a transition from 19°C to 9°C, from 19°C to 29°C, and from 29°C to 39°C. Most surprising is the slope reversal below 19°C and the Q_{10} values below unity: Q_{10} for $\tau_{open(1)}$ amounted to 0.65 ± 0.14 (n = 5) and for $\tau_{open(2)}$ to 0.60 ± 0.16 (n = 5). This completely disagrees with recent observations of McLamon et al. (1993). Likewise, in neonatal cardiac $K^+_{(ATP)}$ channels where cooling from 20°C to 10°C had a predictable influence and prolonged the conductive state with a Q_{10} of 2.3. It seems less plausible to relate this discrepancy to the specific environmental conditions in the present experiments, particularly to the cytosolic presence of nucleotides. Interestingly, for a transition from 19°C to 29°C, the Q_{10} values for $\tau_{open(1)}$ (1.43 ± 0.12; n = 7) and for $\tau_{open(2)}$ (1.52 ± 0.32; n = 7) perfectly agree with those reported by McLamon et al. (1993) for a transition from 20°C to 30°C. A transition from 29°C to 39°C revealed the highest temperature coefficients: the Q_{10} for $\tau_{open(2)}$, for example, amounted to 2.94 ± 0.4 (n = 7) and was significantly larger ($P < 0.01$) than the value obtained for the transition from 19°C to 29°C. Accordingly, the steepest positive slope in the Arrhenius plot was found between 29°C and 39°C.

Cardiac $K^+_{(ATP)}$ channels share with voltage-dependent K^+ channels (Beam & Donaldson, 1983; Pahapill & Schlichter, 1990) the common peculiarity of exhibiting specific, unpredictable gating kinetics at a physiological temperature near 37°C, despite an only small consensus degree in the molecular channel architecture. Near 37°C, the exit reaction from both O-states was thermodynamically characterized (Table) by the highest activation energy and the highest amount of entrophy. The numerical results of this thermodynamic analysis should be considered with some reservation for reasons arising from the so-far undefined valid Markovian reaction scheme as mentioned above. Leaving the O_2-state was accompanied by about a 4-fold larger amount of entrophy when compared with the exit reaction from the O_1-state, suggesting that the former transition is related to a more rigorous conformational change of the channel protein. Interestingly, in voltage-dependent cardiac Na^+ channels, a similar phenomenon has been recently observed (Benndorf & Koopmann, 1993) in that deactivation proceeds with a greater entrophy than inactivation, two kinetic processes with a distinct structural equivalent at the α-subunit. Less advanced knowledge of the structure-function relationship in cardiac $K^+_{(ATP)}$ channels, particularly the unknown

Table 1. Thermodynamics of the exit reaction from the O_1-state and from the O_2-state of cardiac $K^+_{(ATP)}$ channels

	Temperature range (19°C → 9°C) (reference temperature 19°C)		Temperature range (19°C → 29°C) (reference temperature 19°C)		Temperature range (29°C → 39°C) (reference temperature 39°C)	
	Exit reaction from O_1	Exit reaction from O_2	Exit reaction from O_1	Exit reaction from O_2	Exit reaction from O_1	Exit reaction from O_2
Activation energy (E_a)	−4.3 kcal/mol	−14.6 kcal/mol	0.5 kcal/mol	6.2 kcal/mol	4.2 kcal/mol	12.1 kcal/mol
Enthalpy (ΔH)	−4.9 kcal/mol	−15.2 kcal/mol	−0.1 kcal/mol	5.6 kcal/mol	3.6 kcal/mol	11.5 kcal/mol
Free energy (ΔG)	13.0 kcal/mol	14.7 kcal/mol	13.0 kcal/mol	14.7 kcal/mol	13.8 kcal/mol	13.8 kcal/mol
Entropy (ΔS)	−61.2 e.u.	−102.2 e.u.	−44.7 e.u.	−31.1 e.u.	−32.7 e.u.	−7.3 e.u.

The thermodynamic parameters were conventionally calculated taking the mean values of $\tau_{open(1)}$ and $\tau_{open(2)}$ as rate constants (k_{-1}) and (k_{-2}) tentatively supposing a Markovian reaction scheme O_1-R-O_2. The activation energy (E_a) was determined from the slopes of the Arrhenius plots of $1/\tau_{open(1)}$ and $1/\tau_{open(2)}$, respectively. Enthalpy (ΔH) results from $\Delta H = E_a - RT$, free energy (ΔG) from $\Delta G = -RT \ln k_{-1} + RT \ln k_B T/h$, and entropy ($\Delta S$) from $\Delta S = (\Delta H - \Delta G)/T$. R is the universal gas constant, k_B is Boltzmann's constant and h is Planck's constant. The three temperature ranges refer to obviously three distinct slopes in the Arrhenius plots (*see* Figs. 2*B* and 2*C*). Note the preliminary character of the data since the validity of the O_1-R-O_2 scheme is unproven.

structural substrate of gating processes, invalidates any structural interpretation of the observed entrophy difference.

Heat seems a major energy source for gating in inward-rectifying K^+ channels. The facilitated exist reaction from both O-states on cooling with a negative enthalpy represents *per se* a thermodynamic paradox and indicates the influence or the dominance of one or more "hidden" factors. Their nature remains hypothetical. By thermodynamic phase transitions as they are known to occur in response to a temperature fall below a critical level, about 18°C (Chapman, 1975; Sandermann, 1978), membrane lipids can attain a more ordered gel-like structure. Since the function of integral proteins depends *sensitively* on the microviscosity of the surrounding lipids (Overath et al., 1970), such a thermally-induced microviscosity change can, by a so-far poorly understood lipid-protein interaction, evoke Arrhenius anomalies (Kumamoto et al., 1971). In addition, a thermally-induced intrinsic change of the channel protein with conformational consequences as in enzymes (Levy et al., 1959) could also be envisaged. Both possibilities are consistent with the notion that portions of the cardiac $K^+_{(ATP)}$ channels which are involved in forming the central pore remain highly protected against such perturbations. It is, therefore, tempting to assume that the structural equivalent of the gating process is located in a channel domain at some spatial distance from the H5-segment.

Since cardiac $K^+_{(ATP)}$ channel activity involves phosphorylation processes (for review *see* Noma & Takano, 1991), one might argue that the thermodynamically paradoxical facilitation of the exit reaction from O-states could have a metabolic reason or, more precisely, could be related to the degree of cAMP-dependent phosphorylation. A first series of experiments, therefore, tested the channel response under the influence of the nonhydrolyzable ATP-γ-S (10–60 μmol/l). Despite the cytosolic presence of this ATP analogon, $\tau_{open(1)}$ and $\tau_{open(2)}$ decreased on lowering the temperature from 19°C to 9°C (Fig. 3*A*); rewarming promptly restored the initial values. With cAMP-dependent phosphorylation, i.e., exposing the channels cytosolically to 40 μmol/l ATP-γ-S plus 10–60 μmol/l cAMP, essentially the same shortening of both O-states occurred on cooling in three series of experiments each consisting of four inside-out patches and treated with increasing cAMP concentrations. The Q_{10} values for $\tau_{open(1)}$ and $\tau_{open(2)}$ (0.65 ± 0.14 and 0.60 ± 0.16, respectively, n = 12) correspond to the temperature coefficients obtained without cAMP-dependent phosphorylation and mentioned above. Thus, thermally-induced perturbations of membrane lipids or of the channel protein itself can most plausibly explain the observed Arrhenius anomalies.

It is important to see that another member of the cardiac K^+ channel family, the 66 pS outwardly-rectifying K^+ channel, exhibited a conventional temperature sensitivity. Stable channel activity was expected during an initial equilibration period at 19°C after patch excision before temperature experiments began. Cooling to 9°C caused a typical prolongation of the conductive state (Fig. 3*B*). Open-time probability density functions remained monoexponentially distributed, which largely excludes the idea that cooled channels might attain more than only one open configuration. Thus, τ_{open} increased with a mean Q_{10} of 1.54 from 1.54 msec to 2.41 ± 0.34 msec (n = 3), although the 66 pS K^+ channel faced the same membrane lipid matrix with potentially the same thermally-induced microviscosity alterations as $K^+_{(ATP)}$ channels. The exceptional kinetic reaction of the latter on cooling to 9°C would have, therefore, an interesting implication, namely that a so-far hypothetical but most likely structural peculiarity makes cardiac $K^+_{(ATP)}$ channels highly sensitive to thermally-induced perturbations at an abnormal environmental temperature.

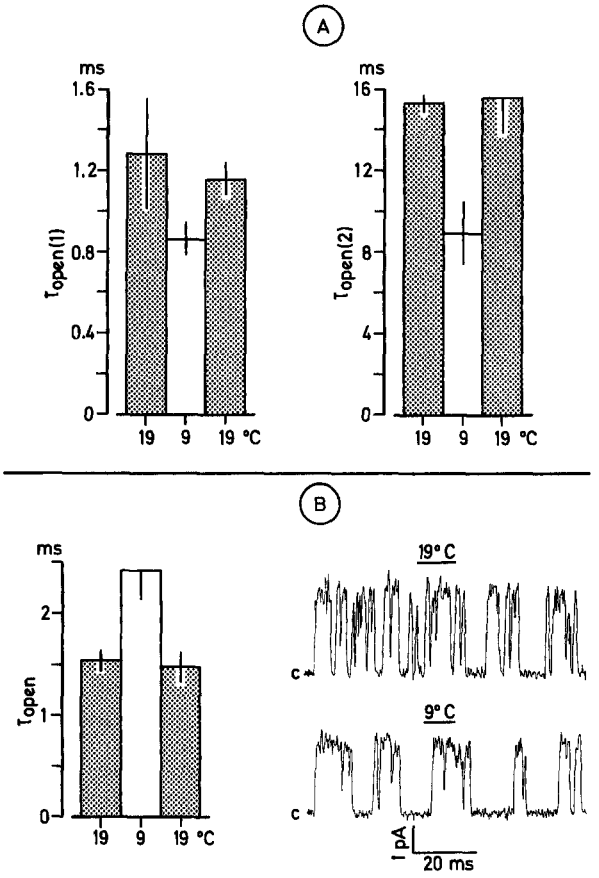

Fig. 3. (*A*) The response of $\tau_{open(1)}$ (left) and $\tau_{open(2)}$ (right) to a temperature transition from 19°C to 9°C and back to 19°C in cardiac $K^+_{(ATP)}$ channels exposed cytosolically to ATP-γ-S (20 μmol/l). Each column represents the mean of 4 inside-out patches, vertical bars indicate SEM. (*B*) The response of τ_{open} in 66 pS outwardly-rectifying cardiac K^+ channels to a temperature transition from 19°C to 9°C and back to 19°C. Each column represents the mean of 3 inside-out experiments, vertical bars indicate SEM. On the right, selected elementary current records through 66 pS K^+ channels at 19°C (upper row) and at 9°C (lower row) are depicted (patch 856, membrane potential –7 mV).

References

Anderson, C.R., Cull-Candy, S.G., Miledi, R. 1977. Potential-dependent transition temperature of ionic channels induced by glutamate in locust muscle. *Nature* **268:**663–665

Ashford, M.L.J., Bond, C.T., Blair, T.A., Adelman, J.P. 1994. Cloning and functional expression of a rat heart K_{ATP} channel. *Nature* **370:**456–459

Beam, K.G. 1981. Potassium currents in mammalian muscle. *Biophys. J.* **33:**72*a* (*Abstr.*)

Beam, K.G., Donaldson, P.L. 1983. A quantitative study of potassium channel kinetics in rat skeletal muscle from 1 to 37°C. *J. Gen. Physiol.* **81:**485–512

Benndorf, K., Koopmann, R. 1993. Thermodynamic entrophy of two conformational transitions of single Na^+ channel molecules. *Biophys. J.* **65:**1585–1589

Benz, I., Fröbe, V., Kohlhardt, M. 1991. Single cardiac outwardly rectifying K^+ channels modulated by protein kinase A and a G-protein. *Eur. Biophys. J.* **20:**281–286

Chapman, D. 1975. Phase transitions and fluidity characteristics of lipids and cell membranes. *Quart. Rev. Biophys.* **8:**185–235

Colquhoun, D., Sigworth, F. 1983. Fitting and statistical analysis of single channel records. *In:* Single channel recordings. B. Sakmann and E. Neher, editors. pp. 191–264 Plenum, New York

Dreyer, F., Müller, K.D., Peper, K., Sterz, R. (1976). The *M. omohyoideus* of the mouse as a convenient mammalian muscle preparation. A study of junctional and extrajunctional acetylcholine receptors by noise analysis and cooperativity. *Pfluegers Arch.* **367:**115–122

Fischbach, G.D., Lass, Y. 1978. A transition temperature for acetylcholine channel conductance in chick myoballs. *J. Physiol.* **280:**527–536

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cell and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Ho, K., Nichols, C.G., Lederer, W.J., Lytton, J., Vassilev, P.M., Kanazirska, M.V., Hebert, S.C. 1993. Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature* **362:**31–37

Horn, R. 1991. Estimating the number of channels in patch recordings. *Biophys. J.* **60:**433–439

Kirsch, G.E., Sykes, J.S. 1987. Temperature dependence of Na currents in rabbit and frog muscle membranes. *J. Gen. Physiol.* **89:**239–251

Kohlhardt, M., Fichtner, H., Fröbe, U. 1989. Gating in iodate-modified single cardiac Na^+ channels. *J. Membrane Biol.* **112:**67–78

Kohlhardt, M. 1990. Different temperature sensitivity of cardiac Na^+ channels in cell-attached and cell-free conditions. *Am. J. Physiol.* **259:**C599–C604

Kumamoto, J., Rainson, J.K., Lyons, J.M. 1971. Temperature "breaks" in Arrhenius plots: a thermodynamic consequence of a phase change. *J. Theor. Biol.* **31:**47–51

Lass, Y., Fischbach, G.D. 1976. A discontinuous relationship between the acetylcholine-activated channel conductance and temperature. *Nature* **263:**150–151

Levy, H.M., Sharon, N., Koshland, D.E. 1959. Purified muscle proteins and the walking rate of ants. *Proc. Natl. Acad. Sci. USA* **45:**785–791.

McLarnon, J.G., Hamman, B.N., Tibbits, G.F. 1993. Temperature dependence of unitary properties of an ATP-dependent potassium channel in cardiac myocytes. *Biophys. J.* **65:**2013–2020

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:**147–148

Noma, A., Takano, M. 1991. The ATP-sensitive K^+ channel. *Jap. J. Physiol.* **41:**177–187

Overath, P., Schairer, H., Stoffel, W. 1970. Correlation of in vivo and in vitro phase transitions of membrane lipids of Escheria coli. *Proc. Natl. Acad. Sci. USA* **67:**606–612

Pahapill, P.A., Schlichter, L.C. 1990. Modulation of potassium channels in human T lymphocytes: effects of temperature. *J. Physiol.* **422:**103–126

Parent, L., Coronado, R. 1989. Reconstitution of the ATP-sensitive potassium channel in skeletal muscle. *J. Gen. Physiol.* **94:**445–463

Robinson, R.A., Stokes, R.H. 1955. Electrolyte solutions. Academic, New York

Romey, G., Chichreportchiche, R., Lazdunski, M. 1980. Transition temperatures of the electrical activity of ion channels in the nerve membrane. *Biochim. Biophys. Acta.* **602:**610–620

Sandermann, H. (1978) Regulation of membrane enzymes by lipids. *Biochim Biophys Acta* **515:**209–237

Schwarz, W. 1979. Temperature experiments on nerve and muscle membranes of frogs. *Pfluegers Arch.* **382:**27–34