The cold and menthol receptor, TRPM8, also designated CMR1, is a member of the transient receptor potential (TRP) family of excitatory ion channels. TRPM8 is a channel activated by cold temperatures, voltage, and menthol. In this study, we characterize the cold- and voltage-induced activation of TRPM8 channel in an attempt to identify the temperature- and voltage-dependent components involved in channel activation. Under equilibrium conditions, decreasing temperature has two effects. (i) It shifts the normalized conductance vs. voltage curves toward the left, along the voltage axis. This effect indicates that the degree of order is higher when the channel is in the open configuration. (ii) It increases the maximum channel open probability, suggesting that temperature affects both voltage-dependent and -independent pathways. In the temperature range between 18°C and 25°C, large changes in enthalpy (∆H = −112 kcal/mol) and entropy (∆S = −384 cal/mol K) accompany the activation process. The Q10 calculated in the same temperature range is 24. This thermodynamic analysis strongly suggests that the process of opening involves large conformational changes of the channel-forming protein. Therefore, the highly temperature-dependent transition between open and closed configurations is possible because enthalpy and entropy are both large and compensate each other. Our data also demonstrate that temperature and voltage interact allosterically to enhance channel opening.

Since the breakthrough identification of the vanilloid receptor, TRPV1, as the hot-capsaicin receptor (1), six temperature-dependent channels have been cloned during the last 7 years (1–8). Interestingly, all of the cloned channels belong to the extended transient receptor potential (TRP) family of excitatory channels (9, 10). TRP channels are membrane proteins constituted by subunits containing six-transmembrane domains, which assemble into tetramers forming conducting pores (11, 12). Heat-dependent channels belong to the vanilloid receptor TRPV subfamily and the cold-dependent channel TRPM8 belongs to the TRPM subfamily (13). Thermo-TRP channels have different temperature thresholds for activation, and their activity is modulated by several different agonists, allowing us to sense and differentiate a large spectrum of temperatures, from below 0°C to 50°C (14). Among those temperature-activated channels, TRPM8 was the first found to sense a cold stimulus (4, 7). TRPM8 is a nonselective outwardly rectifying channel. The channel opens at ~28°C, and its activity increases as the temperature diminishes and saturates at ~10°C (4). The channel is also able to respond to several agonists, including menthol (15).

Channel gating is known to be affected by temperature. Q10 value is used to estimate the temperature dependence of a given system. Channels with a Q10 > 2 are considered highly temperature-dependent (16). Thermo-TRP receptor channels present exceptionally high Q10 values (>20) (13). Several studies have addressed capsaicin binding (17, 18), pH dependencies (19), physiological relevance (13), and, recently, thermodynamics (20) of TRPV1, one of the molecular transducers of heat sensation. Large values for transitional entropy, enthalpy, and Q10 were found (20) indicating large rearrangements in the channel structure during activation. In contrast, little is known about regulation of its counterpart, TRPM8.

Here we show that the large changes in TRPM8 channel gating induced by temperature are mainly due to modifications of the maximum probability of opening and to a shift along the voltage axis of the conductance-voltage curves. Moreover, the results can be fully explained by using an allosteric model in which temperature has only a moderate effect on the voltage sensors (Q10 ~ 3) when channels are closed. Thus, temperature and voltage sensor activation act almost independently to promote channel opening.

### Methods

**Temperature Control and Ramps.** The recording chamber consisted of an electrically isolated bronze block with a hole of the appropriate dimensions to fit the coverslip containing the cell preparation. A Peltier device was attached to this bronze chamber and a heat dissipater was connected to the Peltier. The temperature feedback signal was obtained by using a miniature thermistor located in the solution and ~1 mm from the electrode, allowing us to measure 0.1°C changes. Temperature ramps were linear in the range that the recording of current or fluorescence was done, and the rate at which they were set (0.2°C/s) was sufficient to ensure steady-state conditions. This setting was tested by comparing the ramp results with those obtained in true equilibrium conditions (see Fig. 2A).

**HEK923 Electrophysiology.** Whole cell currents were obtained from HEK CR#1 cells. Gigaseals were formed by using 2–4 MΩ borosilicate pipettes (o.d., 1.5 mm; i.d., 0.86 mm; Warner Instruments, Hamden, CT). Whole cell voltage clamp was performed at different temperatures. Macroscopic currents were acquired at 10 kHz and filtered at 2 kHz. Single-channel currents were acquired at 20 kHz and filtered at 3 kHz. Borosilicate pipettes (7–10 MΩ) were used for single channels recordings. Custom-made software written in LABVIEW was developed for all electrophysiology data acquisition.

**Variance Analysis.** Nonstationary noise analysis (21, 22) was carried out at different temperatures. To estimate the maximum probability of opening, we collected 70 current records during activation of the channels by a depolarization voltage step from 0 to 120 mV with a 250-ms duration. Ensemble average current, ⟨I⟩, and its variance, σ2, on each isochrone were computed. The variance as a function of ⟨I⟩ data was fitted by the equation:

\[ \sigma^2 = A \cdot \langle I \rangle^B \]
\[ \sigma^2 = i(I) - \langle I \rangle^2 / N, \]  

where \( i \) is the single-channel unitary current and \( N \) is the number of channels in the patch. The maximum open probability, \( P_o^{\text{max}} \), was obtained according to the relationship \( P_o^{\text{max}} = I_{\text{max}} / (6N) \), where \( I_{\text{max}} \) is the maximum mean current measured in the experiment. For noise analysis, data were acquired at 20 kHz and filtered at 5 kHz.

**Fit to a Stationary Model.** For each temperature, a mean \( V_{0.5} \) (\( V_{0.5} \)) value was obtained. All of the corresponding \( I/I_{\text{max}} \) vs. voltage curves were displaced in the voltage axis by \( V_{0.5} - \langle V_{0.5} \rangle \), thus constructing an average curve that preserved the shape of the individual curves. Every set of points was converted to a 40-point set by a smoothing function to SIGMAPLOT 8.0. Average curves at four different temperatures were simultaneously fitted to Eq. 5 (see below) by least-squares fitting with the \( \text{SOLVER} \) function of \( \text{EXCEL} \) (Microsoft).

Methods for generation of cell line expressing TRPM8, cell culture, immunocytochemistry, calcium imaging, recording solutions used, and data analysis are provided in Supporting Text and Figs. 6 and 7, which are published as supporting information on the PNAS web site.

**Results**

**CR#1 Cell Line and Its Response to Temperature and Menthol.** To characterize the TRPM8 channel, we obtained a HEK293 cell clone expressing robust temperature- and menthol-activated currents, named CR#1. TRPM8 expression in CR#1 cells assessed by immunofluorescence is shown in Fig. 1A. Intracellular calcium (assayed with Fluo-4 fluorescence) of CR#1 cells increases upon stimulation with menthol (Fig. 1B) and low temperature (Fig. 1C), a characteristic of TRPM8-expressing cells (4, 7). Nontransfected cells did not respond to either stimulus (data not shown).

To obtain a complete temperature vs. activity relationship for TRPM8 channels, we exposed CR#1 cells to temperature ramps of 0.2°C/s, a ramp speed that assures steady-state conditions (see Methods and open symbols in Fig. 2A). Fig. 1D shows current vs. temperature plots obtained at two different holding potentials. As indicated in Fig. 1E, current at −60 mV is evoked at lower temperatures than current at +60 mV, a discrepancy that is due to the voltage dependency of the channel. In summary, the data shown in Fig. 1 is in good agreement with the results obtained previously by other groups (4, 7), and we conclude that the CR#1 cell line expresses normal TRPM8 channels.

**Thermodynamic Analysis of the Current vs. Temperature Curve.** Fig. 2A shows an averaged current vs. temperature plot (\( n = 7 \)) for the experiments at the +60-mV holding potential. We analyzed this data with two thermodynamical approaches. First, we used the 10-degree temperature coefficient (\( Q_{10} \)) to quantify the temperature sensitivity of the TRPM8 channel. The following equation describes the \( Q_{10} \) values’ meaning and its relationship to the observed current:

\[ Q_{10} = \left( \frac{T_2}{T_1} \right)^{10/(T_2 - T_1)}. \]  

In this equation, \( I_1 \) and \( I_2 \) are the measured currents at temperatures \( T_1 \) and \( T_2 \), respectively. The \( Q_{10} \) value is then obtained from the slope of a \( \log(I) \) vs. \( T \) plot or directly fitting the data by using Eq. 2. Fig. 2B shows a \( \log(I) \) vs. \( T \) plot in which we appreciate two temperature-dependent regimes (i.e., two linear components): a phase between 27°C and 18°C with \( Q_{10} = 23.8 \), and a shallower phase in the range from 18°C to 10°C with \( Q_{10} = 3.3 \), almost 1 order of magnitude lower than the first one. These two phases correspond to 68.5 and 25.8 kcal/mol, respectively. Channel gating has usually a \( Q_{10} \) of \( \approx 3 \), a value close to the \( Q_{10} \) of the shallower phase of the TRPM8 channel. The system saturates at \( \approx 10^4 \)°C.

The second thermodynamical analysis considers that the TRPM8 channel activation is well described by a two-state model: open (O) and closed (C). Defining the equilibrium constant as \( K_{eq} = O/C \), the open probability \( [P_{(O)}] \) is

\[ P_{(O)} = \frac{1}{1 + (K_{eq})^{-1}}. \]  

Therefore, \( K_{eq} = P_{(O)}/(1 - P_{(O)}) \). Because \( P_{(O)} = I/I_{\text{max}} \) we can use the data in Fig. 2A to obtain the equilibrium constant at 60 mV and any given temperature. Recalling that \( \Delta S = \Delta H / RT \) + \( \Delta S/R \), \( \Delta H \) and \( \Delta S \) for the channel opening can be obtained easily from a \( \ln(K_{eq}) \) vs. \( 1/T \) temperature plot or van’t Hoff plot, as shown in Fig. 2C. This plot shows again the two regimes for temperature dependency, with their corresponding \( \Delta H \) and \( \Delta S \) values. The activation process observed between 27°C and 18°C shows large transitional changes with an entropy change of \(-384 \text{ cal mol}^{-1} \cdot \text{K}^{-1} \) and enthalpy change of \(-112 \text{ kcal mol}^{-1} \). After this activation phase, there is a shallower, less
temperature-dependent phase with entropy and enthalpy changes of $-210$ cal·mol$^{-1}$·K$^{-1}$ and $-60$ kcal·mol$^{-1}$, respectively. As expected from the high temperature dependency, the enthalpy changes for channel opening are high. However, the free energy changes ($\Delta G$) are maintained at low levels due to the high entropic contribution (Fig. 2D). The change in entropy ($\Delta S$) is negative, meaning that the closed state has a greater entropy (is more disordered) than the open state. It is worthwhile to note that voltage-dependent Na$^+$ channels (23) and Shaker K$^+$ channels (24, 25) also have a negative $\Delta S$ for the closed-to-open transition.

As a third thermodynamic analysis of the TRPM8 channel, we studied the macroscopic kinetics of channel opening and closure. Both the activation and deactivation of the macroscopic currents exhibit a double exponential time course (Fig. 6). In particular, a double exponential time course for the deactivation process implies the existence of more than one open state or that we are in the presence of a closed-closed-open kinetic scheme where the closed-to-open rate constant is not zero (e.g., ref. 26).

Activation and deactivation rates were obtained from the inverse of the time constant ($\tau$) of the slow component. The temperature dependence of the activation and deactivation rates is shown in Fig. 2E and F. From these Arrhenius plots, where the rates are plotted against $1/T$, we can obtain activation and deactivation enthalpies, $\Delta H^\ddagger$, by using the relationship

$$\ln(k) = -\frac{\Delta H^\ddagger}{R} + \frac{\Delta S^\ddagger}{R} + \ln(v^*),$$

where $k$ is either the activation rate ($1/\tau_{activation}$) or the deactivation rate ($1/\tau_{deactivation}$), $\Delta S^\ddagger$ is the activation or deactivation entropy, and $v^*$ is the prefactor of the rate (16). Differentiating Eq. 4 with respect to $1/T$ we have $d\ln(k)/d(1/T) = -\Delta H^\ddagger/R$. The activation and deactivation $\Delta H^\ddagger$ are $3$ kcal·mol$^{-1}$ and $24$ kcal·mol$^{-1}$, respectively, corresponding to approximate $Q_{10}$ values of $1.4$ and $3.8$ (at room temperature). This means that the rate-limiting step for channel kinetics has much lower temperature dependence than the activation of the channel. Fig. 2E and F also show that channel kinetics is weakly voltage-dependent.

This finding was evaluated by fitting $\tau/V$ plots to a voltage-dependent function of the form $\tau = \tau_0 \exp(zFV/RT)$. Activation time constant is virtually voltage independent ($z = 0.05$), and deactivation time constant has a $z = 0.25$ (Fig. 7).

Voltage- and Temperature-Dependent Activation of TRPM8. The TRPM8 channel is activated not only by decreasing temperature but also by membrane depolarization. As shown in Fig. 1D and E, the membrane potential has an obvious effect on temperature dependence, affecting the temperature threshold for activation. To better understand the interaction between voltage- and temperature-dependence of TRPM8, we studied the steady-state voltage activation at fixed temperatures. Fig. 3A shows families of macroscopic current traces obtained from the same whole cell patch at $10^\circ$C, $20^\circ$C, and $31^\circ$C. The current magnitude increases when the temperature is decreased, and Fig. 3B shows that the steady-state current magnitude at $160$ mV increases $>2$-fold when the patch is cooled from $31^\circ$C to $10^\circ$C. At all of the temperatures studied, there is a strong outward rectification of the steady-state current. Fig. 3C and D show that, after a depolarizing pulse, the instantaneous tail current follows an ohmic relationship with respect to voltage, and that temperature does not affect this behavior. The outward rectification must therefore come from a genuine voltage-dependent gate similar to that of other voltage-dependent channels.

A more precise quantification of open probability is obtained from the tail currents, plotted against activation voltage in Fig. 3E. These plots are well fitted by a Boltzmann distribution of the form $I = I_{max}/(1 + \exp(-zF(V - V_{0.5})/RT))$, where $z$ is the voltage dependency, $V_{0.5}$ is the half-activation voltage, and $I_{max}$ is the maximum tail current. $F$, $R$, and $T$ have their usual meanings. $I_{max} = I \times N \times P_{(O)max}$, where $i$ is unitary current, $N$ is the number of channels, and $P_{(O)max}$ is the maximum open probability that can be achieved at large depolarizing voltages. When the tail currents are plotted as $I/I_{max}$ (Fig. 3F), it can be seen that, below $20^\circ$C, the curves are left-shifted. Above $20^\circ$C, there is little change in the $V_{0.5}$ of the curves, despite the fact that the tail currents keep increasing in the temperature range between $31^\circ$C and $20^\circ$C. This tail current increase between $31^\circ$C and $20^\circ$C must come from an increase in $I_{max}$ reflecting a change of either the maximum open probability $[P_{(O)max}]$, the number of channels in the patch ($N$), and/or the unitary current ($i$).

To address whether temperature changes $P_{(O)max}$, $I$, and/or $N$, we directly measured open probability at $120$ mV by using single channel recordings for high temperatures and nonstationary
noise analysis for the whole temperature range (21, 22). Both the noise analysis (n = 15) (Fig. 4 A and B) and the single channel (n = 6) (Fig. 4C) data show that temperature effectively changes the \(P_{\text{O}}\max\) at 120 mV, even at ranges where there is little change in the \(V_{0.5}\) of the \(G/V\) relationships. Neither the number of channels nor the unitary conductance was changed significantly by decreasing the temperature. Measured unitary conductance was \(\approx 60\) pS at 10°C and \(\approx 75\) pS at 30°C, similar to the 80 pS reported previously (4). Fig. 4D shows the \(P_{\text{O}}\) plotted as a function of temperature. To build the plot, we used noise analysis data for the whole range of temperatures, and single channel data to resolve lower probabilities. This was done because noise analysis is based on parabolic fitting to the data, and the fitting became stronger and more reliable when the \(P_{\text{O}}\) > 0.5.

Although 120 mV is not enough to reach the maximum open probability, the obtained \(P_{\text{O}}\) values forced us to normalize the \(G/V\) plots and express them as an actual open probability. Because the \(V_{0.5}\) value for any given temperature had a great variation between different patches, we averaged \(P_{\text{O}}\max/V\) curves by displacing the points in the voltage axis (see Methods), preserving the form of the curves. Fig. 5B shows averaged \(P_{\text{O}}\max/V\) curves, and Fig. 5C shows the same curves normalized by \(P_{\text{O}}\max\). In Fig. 5 D and E, \(P_{\text{O}}\max V_{0.5}\), and \(z\) values are plotted against temperature. Again, between 30°C and 20°C, the main effect is a change of \(P_{\text{O}}\max\) with little or no change of the \(V_{0.5}\) value. Below 20°C, both values change, until the effect apparently saturates at \(\approx 10\)°C. The voltage dependence of activation, measured as the \(z\) value (0.64 ± 0.1) of the Boltzmann distribution or the slope of the curve, changes little with temperature.

**Discussion**

In most channels the gating processes have \(Q_{10}\) values of \(\approx 3\), with some notable exceptions. In Shaker \(K^+\) channel inactivation is highly temperature-dependent (27). The inactivation rate increases with increasing temperature with a \(Q_{10}\) of 7, a result that has been interpreted in terms of a temperature-induced stabilization of some structure(s) of the inactivating peptide able to bind much more strongly to the channel than the other structures that the peptide can adopt (27, 28). It is difficult to imagine that a similar mechanism can explain the temperature sensitivity of TRPM8 channels, because the rates of activation or deactivation have lower \(Q_{10}\) values (\(\leq 3\)) than the overall channel gating (\(Q_{10} \approx 23\)). The slow gating of the ClC-\(\alpha\) channel shows a very strong temperature dependence (\(Q_{10} \approx 40\)), and this result has been interpreted in terms of a coupling of the slow gate with channel subunit interaction (29). In general, high \(Q_{10}\) for channel gating is compatible with large rearrangements of the protein induced by temperature. What makes the TRPM8 channel special is that, unlike the cases described above, in which rates of opening or closing are greatly affected, temperature mainly modifies equilibrium parameters of the activation pathway of TRPM8. In particular, temperature induces large changes in the maximum probability of opening and the half voltage.

**Possible Molecular Origin of the Large \(\Delta H\) and \(\Delta S\) Changes.**

The thermodynamic analysis of TRPM8 shows that this protein undergoes highly temperature-dependent rearrangements. The opening of the channel is accompanied by large enthalpy and entropy changes, although with low free energy changes that ensure the reversibility of the process. On the basis of the data presented here, we discuss below the possible molecular origin of the large \(\Delta H\) and \(\Delta S\) changes.

The most plausible explanation for the effect of temperature
an exposure to closed-to-open transition by temperature. For example, we can suggest that there is a net loss of hydrophobic interactions in the driven, and negative values of activated by heat. Hydrophobic interactions are entropically become zero could produce a channel activated by cold or one scenario, changing the temperature at which these parameters they become zero and then change their sign (29, 31). In such a situation decrease upon a drop in temperature, and at some point temperature-dependent process takes place only because the solvent of aliphatic and aromatic groups takes place. Restricting the temperature sensing to a discrete domain ensures the stability of the protein; otherwise the whole channel would “denature” by cold or heat.

A Model of Channel Activation by Voltage and Temperature. A recently published study by Voets et al. (32) describes the voltage and temperature dependence of the TRPM8 channel. We would like to point out here that our results differ from those of Voets et al. (32) in several important aspects. We found that the voltage dependence of the activation and deactivation rates does not account for the voltage-dependence of the channel. Also, the temperature dependence of the deactivation rate ($Q_{10} = 3.8$) is much smaller than the temperature dependence of the steady-state macroscopic currents ($Q_{10} = 24$). Therefore, neither the voltage- nor the temperature-dependence is contained in the activation or deactivation rates. This and the existence of two exponential components in the decay of the current make our results incompatible with a two-state model. The data presented here pose other restrictions that have to be accounted for. First, the existence of weakly voltage-dependent or -independent transitions between open and closed states needs to be postulated; otherwise, an open probability of 1 would always be reached at sufficiently high depolarizing pulses regardless of the temperature. Second, a decrease in temperature must increase the maximum open probability and must not affect the half-activation voltage until some threshold temperature is reached. Finally, the study of channel kinetics showed that there must be a rate-limiting step that is essentially voltage-independent and has low temperature dependence.

We found that a simple way to explain the data is to use an allosteric activation mechanism for both voltage and temperature. In this model, the activation of both sensors (voltage and temperature) and channel opening constitute three separate two-state equilibria that interact allosterically with each other. These equilibria can be represented as a cubic eight-state kinetic model (Fig. 5A). In absence of stimuli (e.g., at low voltages and high temperature), the channel is confined to the equilibrium between states $C_0$ and $O_0$, with a small equilibrium constant $L$ that makes channel opening very improbable. The equilibrium between states $C_0$ and $C_1$ represents the activation of voltage sensors and is governed by the voltage-dependent constant $J$. These two equilibria are coupled by the allosteric factor $D$, so that when voltage sensors are active, the opening of the channel (equilibrium between states $C_1$ and $O_1$) has a higher equilibrium constant, $L \times D$. When the channel is open, the activation of voltage sensors ($O_0$-$O_1$ equilibrium) is $J \times D$. Therefore, when the temperature is high the maximum open probability achievable by depolarization will be determined by the $LD$ value. The equilibrium between states $C_0$ and $C_1$ represents the activation of temperature sensors and is governed by the temperature-dependent constant $K$. $K$ is increased when the temperature is lowered. Activation of the temperature sensor is coupled to channel opening by the allosteric factor $C$, thus completing the cube depicted in Fig. 5A. In this way, when both stimuli (voltage and low temperature) are present, the open probability will be determined by the $LCD$ value, the equilibrium constant between states $C_3$ and $O_3$. We note that, being a homotetramer, the channel is likely to contain four voltage sensors. However, because there is no evidence about their molecular nature and whether they move independently, we are making a simplifying assumption that the voltage sensors move in concert. The existence of the temperature- and voltage-independent $C$-$O$ transitions in the allosteric model allow the rate-limiting steps for channel kinetics to have lower temperature and voltage dependence than the steady-state open probability. Finally, the existence of several kinetic paths

on the TRPM8 channel considers the existence of a temperature-sensing domain, a “temperature sensor” that would suffer large structural rearrangements upon temperature changes. It is interesting to recall that protein denaturation is also a process highly temperature-dependent, characterized by large entropic and enthalpic changes, whereas $\Delta G$ is relatively small (for example, see ref. 29). This is another case in which a highly temperature-dependent process takes place only because the large enthalpic change is compensated by a large change in entropy. The fact that denaturation can be induced by heat and by cold (30, 31) suggests that it is possible that, in the TRPM8 channel, certain specialized regions of the channel suffer severe rearrangements by cold, whereas in VR1 channels (20) the rearrangements of this hypothetical “temperature sensor” are driven by heat. This behavior is a peculiarity of protein thermodynamics, where both enthalpies and entropies of denaturation decrease upon a drop in temperature, and at some point they become zero and then change their sign (29, 31). In such a scenario, changing the temperature at which these parameters become zero could produce a channel activated by cold or one activated by heat. Hydrophobic interactions are entropically driven, and negative values of $\Delta S$ obtained for this process may suggest that there is a net loss of hydrophobic interactions in the closed-to-open transition by temperature. For example, we can visualize that, during the process of opening, an exposure to

![Figure 5](https://www.pnas.org/eutils/figure.fcgi?tool=eng&cmd=Retrieve&db=PubMed&dopt=new&ref=15498)
for channel activation and deactivation would explain the two exponential components of the current traces.

The equation for the open probability at any temperature and membrane potential is

$$P_{O} = \frac{1}{1 + J + K + JK}. \quad [5]$$

where $J = I_{O} \exp(zFV/RT)$ and $K = \exp(-(\Delta H - T\Delta S)/RT)$. $I_{O}$ is the equilibrium constant for the $C_0$ to $C_1$ transition at 0 mV, $z$ is the voltage dependency for this constant, $V$ is membrane potential, and $T$ is absolute temperature. $\Delta S$ and $\Delta H$ represent the difference of entropy and enthalpy between $C_1$ and $C_2$ states, respectively, $R$ is the universal gas constant, and $F$ is Faraday’s constant.

We selected four averaged and normalized $P_{O}/V$ curves corresponding to 31°C, 22°C, 18°C, and 10°C, to be simultaneously fitted to Eq. 5. The best fit was obtained with the following parameters: $L = 1.44 \times 10^{-4}$, $J_0 = 0.15$, $z = 0.6$, $D = 1,000$, $C = 3,047$, $\Delta H = -48$ kcal/mol, and $\Delta S = -177$ cal/(mol × K). Continuous lines in Fig. 5B and C show the best fit found by least-squares. In Fig. 5D and E, the lines are the prediction of $P_{O\text{max}}$ and $V_{0.5}$ as a function of temperature, and Fig. 5F shows simulated current vs. temperature curves at different holding potentials. Thus, the allosteric model can reproduce the steady-state behavior on TRPM8 over a wide range of conditions, with the assumption that activation of voltage sensors and temperature sensors additively affect the energy of the C–O transition. This model also gives transitional energies (see above) very similar to those obtained from the van’t Hoff plot. It is important to notice that the model is very sensitive to changes in these parameters.

It is noteworthy that an allosteric linkage between voltage sensor movement and channel opening, as opposed to a strict coupling, has been proposed for another channel activated by two stimuli, the high conductance, Ca$^{2+}$, and voltage-activated potassium (BK) channel. Detailed analysis of the voltage- and calcium-dependence of the BK channel showed that neither Ca$^{2+}$ nor voltage is strictly necessary for channel activation, and that both stimuli can act independently of each other (e.g., refs. 33 and 34). The present results indicate that neither temperature nor voltage is strictly necessary for TRPM8 channel activation and that they can be understood in an allosteric voltage- and temperature-gating kinetic scheme. Therefore, it is possible that, in the same way the voltage sensor of voltage-dependent channels converts the energy store in the membrane electric field into mechanical work, a temperature sensor in thermo-TRP channels converts thermal energy into mechanical work.

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Figure 6

Legend. Time course of macroscopic currents. Current traces for the time course of activation were elicited by a 160 mV step from a holding potential of 0 mV. The traces shown correspond to the same experiment at the indicated temperatures. Current traces for the time course of deactivation were elicited by a -130 mV step from a test pulse of 120 mV. The traces shown correspond to the same experiment at the indicated temperatures. Data was fitted to a double exponential function (red dotted lines).

Figure 7

Legend. Voltage dependence of activation and deactivation rates. The time course considered corresponds to the slower component detected in the activation and deactivation gating kinetic (see Fig. 6). (A) Lines are fit to the data using $1/\tau_{\text{act}} = C \exp(z_{\text{act}} F V / RT)$ where $\tau_{\text{act}}$ is the activation time constant, $C$ is a constant, and $z_{\text{act}}$ defines de voltage dependence of $1/\tau_{\text{act}}$. The values of $z_{\text{act}}$ were: 0.05, 0.03 and 0.04 at
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