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Ionic channels and conductance-based models for hypothalamic neuronal thermosensitivity

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Wechselberger, Martin, Chadwick L. Wright, Georgia A. Bishop, and Jack A. Boulant. Ionic channels and conductance-based models for hypothalamic neuronal thermosensitivity. Am J Physiol Regul Integr Comp Physiol 291: R518–R529, 2006. First published May 11, 2006; doi:10.1152/ajpregu.00039.2006.—Thermoregulatory responses are partially controlled by the preoptic area and anterior hypothalamus (PO/AH), which contains a mixed population of temperature-sensitive and insensitive neurons. Immunohistochemical procedures identified the extent of various ionic channels in rat PO/AH neurons. These included pacemaker current channels [i.e., hyperpolarization-activated cyclic nucleotide-gated channels (HCN)], background potassium leak channels (TASK-1 and TRAAK), and transient receptor potential channel (TRP) TRPV4. PO/AH neurons showed dense TASK-1 and HCN-2 immunoreactivity and moderate TRAAK and HCN-4 immunoreactivity. In contrast, the neuronal cell bodies did not label for TRPV4, but instead, punctate labeling was observed in traversing axons or their terminal endings. On the basis of these results and previous electrophysiological studies, Hodgkin–Huxley-like models were constructed. These models suggest that most PO/AH neurons have the same types of ionic channels, but different levels of channel expression can explain the inherent properties of the various types of temperature-sensitive and insensitive neurons.

Hodgkin-Huxley formalism; cationic channels; potassium leak currents

The rostral hypothalamus, especially the preoptic area and anterior hypothalamus (PO/AH), is an important integrative center in the regulation of body temperature. This neural area has partial control over both physiological and behavioral thermoregulatory responses (2, 3, 16). Depending on the species, PO/AH warming elicits various heat loss responses (e.g., skin wetting, panting, sweating, cutaneous vasodilation), whereas PO/AH cooling elicits heat retention and heat production responses (e.g., cutaneous vasoconstriction, shivering, increased metabolic endocrines, thermogenesis in brown adipose tissue). Electrophysiological studies show that some hypothalamic neurons sense changes in their own temperature (reviewed in Refs. 2, 3, and 5). About 20% of the spontaneously firing PO/AH neurons are classified as warm sensitive, because they significantly increase their firing rates during warming and decrease their firing rates during cooling. Conversely, a small population of neurons (i.e., less than 5%) is classified as cold sensitive, and their firing rates are inversely related to temperature. The remaining majority of spontaneously firing neurons are considered to be temperature insensitive and show little or no change in their firing rates during temperature changes. In addition to these spontaneous firing cells, in vitro intracellular studies classify another population as “silent” neurons because they only fire action potentials when electrically stimulated (12–14). The purpose of the present study was to use immunohistochemical labeling and mathematical models to explore the roles of the ionic channels responsible for these different PO/AH neuronal populations.

Electrophysiological studies have classified PO/AH neurons based on their thermal coefficient or slope of the linear regression of firing rate [i.e., action potentials/s (AP/s)] plotted as a function of hypothalamic temperature (2–5, 12–14). Warm-sensitive neurons have positive thermal coefficients that are at least +0.8 AP/s per °C, whereas cold-sensitive neurons have negative thermal coefficients that are at least −0.6 AP/s per °C. All other spontaneously firing neurons are classified as temperature insensitive. The criterion that distinguishes warm-sensitive and temperature-insensitive neurons is based on both physiological and morphological studies. Using the +0.8 AP/s per °C criterion for warm sensitivity, an early study showed that most warm-sensitive PO/AH neurons not only responded to hypothalamic temperature, but also received afferent synaptic input from skin and spinal thermoreceptors (4). PO/AH temperature-insensitive neurons, however, did not receive this afferent input. This suggests that the warm-sensitive neurons serve to integrate central and peripheral thermal information. Using the same criterion, Griffin et al. showed in a recent study that there are morphological differences between PO/AH warm-sensitive and temperature-insensitive neurons (14). Many warm-sensitive neurons orient their dendrites perpendicular to the midline. Presumably, this allows them to receive synaptic input from both medial and lateral ascending pathways carrying information from peripheral thermoreceptors. In contrast, temperature-insensitive neurons do not receive peripheral thermal information and, instead, often orient their dendrites parallel to the midline.

As shown in Fig. 1, previous intracellular recordings reveal that both warm-sensitive and temperature-insensitive neurons display slow prepotentials or pacemaker potentials that depolarize a neuron to a threshold to produce action potentials (13). Temperature-insensitive neurons (Fig. 1, A and C) tend to have slow firing rates, and temperature has little effect on the...
Vanilloid receptor transient receptor potential channels (TRPs; e.g., TRPV4) provide temperature-sensitive cationic currents in the dorsal root ganglion and peripheral receptors (1, 6, 15, 29). Little is known about the presence or role of these currents in determining the firing rates and thermosensitivity of hypothalamic neurons. Using immunohistochemical markers, we identified the extent of different ionic channels in PO/AH neurons. On the basis of these observations, Hodgkin–Huxley-like models were formulated to show how various neuronal types can be explained by different expression levels of identified ionic channels. These models may guide future experiments designed to understand the cellular mechanisms of neuronal thermosensitivity.

METHODS

Immunohistochemical Procedures and Analyses

All animal procedures were carried out under protocol approved by the National Institutes of Health and the Ohio State University Institutional Laboratory Animal Care and Use Committee.

Male Sprague-Dawley rats were anesthetized with intraperitoneal pentobarbital (100 mg/kg). Once anesthetized, the animals were perfused through the heart with phosphate-buffered saline (PBS) followed by ice-cold 4% paraformaldehyde/0.1 M PBS (fixative). The brains were removed and placed in fixative overnight at 4°C and then transferred to PBS that contained 30% sucrose until the brains sank. A tissue block containing the hypothalamus was cut, and a freezing microtome was used to cut 50-μm-thick sections in either coronal or horizontal planes. Sections containing the preoptic-anterior hypothalamus were pretreated with 5% normal sheep serum for rabbit primary antibodies or 5% normal rabbit serum for goat primary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) in PBS containing 0.5% Triton-X (PBT) for 2 h at room temperature. Sections were then incubated for 48 h at 4°C with constant agitation in various antibody solutions: 1) rabbit polyclonal antibody for HCN-2 (1:200, #APC-030, Alomone Labs, Jerusalem, Israel), 2) rabbit polyclonal antibodies for HCN-4 (1:200, #APC-052, Alomone Labs, Jerusalem, Israel), 3) rabbit polyclonal antibodies for TASK-1 (1:200, #APC-024, Alomone Labs, Jerusalem, Israel), and 4) goat polyclonal antibodies for TRAAK (either 1:100 or 1:200, SC-11324, Santa Cruz Biotechnology, Santa Cruz, CA), and 5) rabbit TRPV4 antibodies (generously provided by Dr. Michael J. Caterina, Johns Hopkins University). All antibodies were diluted in PBT (ICN Pharmaceuticals, Costa Mesa, CA). For rabbit primary antibodies, the sections were then rinsed in PBS and sequentially placed in sheep anti-rabbit IgG (1:500 in PBT) and rabbit peroxidase anti-peroxidase (1:500 in PBT) for 1 h each at room temperature with constant agitation. For goat primary antibodies, the sections were rinsed in PBS and sequentially placed in rabbit anti-goat IgG (1:500 in PBT) and goat peroxidase anti-peroxidase (1:1,000 or 1:500 in PBT) for 1 h each at room temperature with constant agitation. After a final rinse in PBS, the sections were processed using the glucose oxidase procedure (35) to visualize the distribution of HCN-2, HCN-4, TASK-1, TRAAK, and TRPV4. Tissue sections processed in parallel were placed in solutions containing 0.3% Triton-X (PBT) for 2 h at room temperature. Sections were then incubated for 48 h at 4°C with constant agitation in various antibody solutions: 1) rabbit polyclonal antibody for HCN-2 (1:200, #APC-030, Alomone Labs, Jerusalem, Israel), 2) rabbit polyclonal antibodies for HCN-4 (1:200, #APC-052, Alomone Labs, Jerusalem, Israel), 3) rabbit polyclonal antibodies for TASK-1 (1:200, #APC-024, Alomone Labs, Jerusalem, Israel), and 4) goat polyclonal antibodies for TRAAK (either 1:100 or 1:200, SC-11324, Santa Cruz Biotechnology, Santa Cruz, CA), and 5) rabbit TRPV4 antibodies (generously provided by Dr. Michael J. Caterina, Johns Hopkins University). All antibodies were diluted in PBT (ICN Pharmaceuticals, Costa Mesa, CA). For rabbit primary antibodies, the sections were then rinsed in PBS and sequentially placed in sheep anti-rabbit IgG (1:500 in PBT) and rabbit peroxidase anti-peroxidase (1:500 in PBT) for 1 h each at room temperature with constant agitation. For goat primary antibodies, the sections were rinsed in PBS and sequentially placed in rabbit anti-goat IgG (1:500 in PBT) and goat peroxidase anti-peroxidase (1:1,000 or 1:500 in PBT) for 1 h each at room temperature with constant agitation. After a final rinse in PBS, the sections were processed using the glucose oxidase procedure (35) to visualize the distribution of HCN-2, HCN-4, TASK-1, TRAAK, and TRPV4. Tissue sections processed in parallel were placed in solutions that did not contain the primary antibody to verify specificity of the reaction. In these cases, no labeling was observed. Tissue sections were then examined using a bright-field microscope, and the hypothalamic distributions of the various channel markers were recorded using a Zeiss AxioCam black and white digital camera attached to the microscope.

Model Development and Considerations

All simulations were performed on a LINUX workstation using the interactive differential equation simulation package XPPAUT (11).
Numerical integration has been done by a fourth-order Runge-Kutta method with adaptive step-size.

In vivo and in vitro studies have recorded different types of PO/AH neurons, including warm-sensitive, cold-sensitive, temperature-insensitive, and silent neurons (3). Some of these studies, however, suggest that cold sensitivity is not an inherent neuronal property but, rather, is due to synaptic inhibition from nearby warm-sensitive neurons. Intracellular recordings, for example, indicate that the firing activity of cold-sensitive neurons is strongly dependent on excitatory and inhibitory synaptic input (8). In addition, neuronal cold sensitivity is usually lost during synaptic blockade with high-Mg²⁺/low-Ca²⁺ media that block calcium entry at presynaptic terminals (9, 23). On the other hand, neuronal warm sensitivity and temperature insensitivity exist in the presence of high-Mg²⁺/low-Ca²⁺ media (9, 23) and in Ca²⁺-free media (21). For this reason, the models developed in this paper focus on the roles of Na⁺ and K⁺ currents to explain the minimal ionic channels necessary for neuronal warm sensitivity and temperature insensitivity.

Previous reviews have speculated that thermosensitive Ca²⁺ and Na⁺ TRPV4 channels may be a mechanism for PO/AH neuronal warm sensitivity (1, 6, 15, 29); however, this is not supported by our previous intracellular recording studies, which found no difference between temperature-sensitive and insensitive neurons in terms of temperature’s effect on resting membrane potentials and currents (12, 38). As reported below in the RESULTS, immunostaining of TRPV4 was not present in the cell bodies of PO/AH neurons. Accordingly, TRPV4 currents were not incorporated in the modeling.

A salient feature of the models is the effect of temperature on ionic conductances of the membrane. Hodgkin and Huxley (19) and Hodgkin et al. (20) showed that maximum conductances of the ionic channels in the squid giant axon are little altered by temperature, but the kinetics of activation and inactivation of the voltage-gated ion channels are significantly increased by increasing temperature. For different temperatures, Hodgkin and Huxley had to rescale these gating variables by the appropriate Q10-factor to combine different experimental data with their model. The rescaling factor is given by q = (Q10)ΔT/10 where ΔT denotes the temperature difference (18). Therefore, the Q10-factor for the gating dynamics is an important component in modeling thermosensitivity. In addition, temperature-dependent changes in ionic leak currents play an important role in our PO/AH neuronal models, and this is incorporated in a Q10-factor for the conductance of potassium leak channels.

Formulation of the Models

Figure 2 describes the currents presented in the models. These currents include the action potential’s fast, voltage-gated sodium current (I_Na) and delayed rectifier potassium current (I_K), as well as currents that determine the interspike interval between successive action potentials. These latter currents include the HCN sodium current (I_h), the potassium-A current (I_A), and various potassium leak currents (I_L). The models are based on a single compartment Hodgkin-Huxley formalism, and the dynamics are described completely by a set of autonomous differential equations. The time course of the membrane potential is obtained by applying Kirchhoff’s law to a single compartment neuron. In this case, the transmembrane current is equal to the sum of intrinsic currents, as follows

\[
\frac{dV}{dt} = I_{Na} + I_{K} + I_{A} + I_{h} + I_{L}
\]

where C is the whole cell capacitance (pF), V is the membrane potential (mV) and t is the time (ms). The ionic currents on the right-hand side of the equation are described in the following text. The capacitance C is set to 30 pF, which is estimated from previous studies (13).

The conductances of the ionic currents are regulated by voltage-dependent activation and inactivation variables called gating variables. The dynamics of a gate z for an ionic channel is described according to

\[
\frac{dz}{dt} = q_z \frac{z(V) - z}{\tau_z(V)}
\]

with

\[
z_z(V) = \frac{1}{1 + \exp[(V - \theta_z)/\sigma_z]} \\
\tau_z(V) = \sigma_z \cosh[(V - \theta_z)/2\sigma_z],
\]

where z(V) is the steady-state voltage dependent (in)activation function of z, and \(\tau_z(V)\) is the voltage-dependent time constant. The factor \(q_z = Q_{10}^{I(T-32)/10}\) denotes the temperature-dependent gating factor, where T is the temperature (°C) and \(Q_{10}\) is the Q10-factor of the speed of (in)activation of the ionic channel. Note that the Q10-factor is calibrated at T = 32°C where q_z = 1. This is the lower temperature limit of the model, which coincides with the lower temperature limits in most neurophysiological experiments. The term z(V) is a sigmoidal function with half (in)activation at V = \(\theta_z\) and a slope that is proportional to 1/\(\sigma_z\). The function \(\tau_z(V)\) is a bell-shaped curve that has a maximal value at V = \(\theta_z\) and a half-width determined by \(\sigma_z\). Each gating variable is described by only three parameters, which, in principle, can be measured experimentally.

The models incorporate Na⁺ and K⁺ channels, which are known to be expressed in the PO/AH (28, 34, 36, 37). Most of the physiological data on individual channels are obtained from the International Union of Basic and Clinical Pharmacology (IUPHAR) data base (7). The following ionic channels are used in the models.

\(Na^+\) channels. These voltage-gated sodium channels mediate the rapid increase in Na⁺ conductance during the initial phase of action potentials. The current through these sodium channels (7) is given by:

\[
I_{Na} = g_{Na} m_{Na} h (V - E_{Na})
\]

where parameters \(g_{Na} = 960\) nS, \(E_{Na} = 50\) mV.
50 mV. The equation for this current follows the classical Hodgkin-Huxley formalism (19). Because these sodium channels activate so fast (<0.5 ms), we assume instantaneous activation of the gating variable \( m \) without significantly altering the dynamics of the model. The parameters are \( \delta_m = -33 \text{ mV}, \sigma_m = -12 \text{ mV} \). The inactivation of the channel is modeled by the gating variable \( h \) with \( \delta_h = -72 \text{ mV}, \sigma_h = 5 \text{ mV} \), and \( \tau_h = 5 \text{ ms} \). The \( Q_{h} \)-factor for the inactivation of this sodium channel is given by \( Q_h = 3 \), following Hodgkin and Huxley (19).

\( K_v 1.1 \) channels. These voltage-gated nonactivating potassium channels (delayed rectifiers) are responsible for the shape of action potentials, as the outward current through these channels repolarizes the membrane potential. The current through these potassium channels (19) is given by \( I_k = g_K n^4(V-E_K) \) with parameters \( g_K = 540 \text{ nS}, E_K = -95 \text{ mV} \). Again, the equation for this current follows the classical Hodgkin-Huxley formalism (19). The activation of the channel is modeled by the gating variable \( n \) with \( \delta_n = -32 \text{ mV}, \sigma_n = -8.5 \text{ mV} \), and \( \tau_n = 5 \text{ ms} \). The \( Q_{n} \)-factor for the activation of this potassium channel is given by \( Q_n = 3 \) (19, 32).

HCN channels. The current through hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels acts as a pacemaker current. These HCN channels are considered to be nonselective cation channels; however, when activated and opened during membrane current. These HCN channels are considered to be nonselective cation channels; however, when activated and opened during membrane current. The \( Q_{a} \)-factor for the activation of HCN channel is given by \( Q_a = 3 \), following Hodgkin and Huxley (19).

\( K_{TP} \) or leak potassium channels. The tandem-pore (2P) channels are responsible for background potassium leak current (7, 10, 17, 22, 27, 30, 36) and contribute to the resting membrane potential. These channels lack intrinsic voltage sensitivity, and the currents generated are almost instantaneous and nonactivating. Hence, no gating variables are included in the model of leak channels. The conductance of leak channels is temperature sensitive however, and this is incorporated in the model by introducing an appropriate \( Q_{r} \)-factor for the leak conductance. The current through these potassium leak channels is given by \( I_L = (g_{task} g_{tr} + g_{or} g_{r1})(V-E_L) \) with \( E_L = -90 \text{ mV} \). Depending on the expression levels of the various leak channels (TASK, TREK, and TRAAK), the total leak conductance at 32°C in our model is given by \( g_L = (g_{task} + g_{or}) \leq 2.0 \text{ nS} \). The \( Q_{r} \)-factor of the conductance varies within the family of \( K_{TP} \) channels. TASK channels are not very thermosensitive and have a \( Q_{10} \)-factor of \( Q_{task} = 2 \), while TREK and TRAAK channels have a \( Q_{10} \)-factor of \( Q_{or} = 7 \). Because the temperature sensitivity of TREK and TRAAK (i.e., \( r \)) channels are the same, they are treated similarly in the model.

RESULTS

Immunohistochemistry

HCN ion channels. The current through these HCN channels is given by \( I_k = g_K n^4(V-E_K) \) with parameters \( g_K = 540 \text{ nS}, E_K = -95 \text{ mV} \). The activation of the channel is modeled by the gating variable \( n \) with \( \delta_n = -32 \text{ mV}, \sigma_n = -8.5 \text{ mV} \), and \( \tau_n = 5 \text{ ms} \). The \( Q_{n} \)-factor for the activation of this potassium channel is given by \( Q_n = 3 \) (19, 32).

Kv 4.1 or potassium-A channels. Our previous electrophysiological study has shown that potassium-A currents (7, 37) exist in all types of PO/AH neurons, including warm-sensitive, temperature-insensitive, and silent neurons (13). These channels evoke brief hyperpolarizing currents responsible for slowing neuronal firing rates. As indicated in Fig. 2, immediately following the action potential and AHP (i.e., afterhyperpolarizing potential), as the neuron begins to depolarize, A-currents \( (I_A) \) rapidly activate and prolong the membrane hyperpolarization. This serves as a damper on the generation of action potentials. After a short time, however, \( I_A \) inactivation begins, and this allows the membrane to slowly depolarize. The \( I_A \) inactivation lasts 50–100 ms and contributes to the pacemaker potential that leads to the next action potential. Previous studies have shown that \( I_A \) inactivation is strongly affected by temperature, such that slight warming greatly increases the inactivation rate (13, 25). The transient A-type potassium current is given by \( I_A = g_A \alpha_A (V)(V-E_A) b(V-E_A) \) with parameters \( g_A = 600 \text{ nS}, E_A = -95 \text{ mV} \), with a fourth-order Boltzmann function for the activation gate \( a \). Similar to the sodium channels (Na, 1.1), these channels activate very fast (<2 ms), and we assume instantaneous activation of the gate \( a \). Parameters are given by \( \delta_a = -48 \text{ mV}, \sigma_a = -24 \text{ mV} \). The inactivation of these channels is modeled by the gating variable \( b \) with \( \delta_b = -69 \text{ mV}, \sigma_b = 5.6 \text{ mV} \), and \( \tau_b = 18 \text{ ms} \). Most of experimental data indicate that \( \tau_b (V) \) is independent of the voltage \( V \) or that \( \tau_b (V) \) varies moderately with respect to voltage \( V \). Therefore, we assume that \( \tau_b (V) = \tau_0 \), which approximates experimental data better than the bell-shaped curve given by the formula for \( \tau_b (V) \) in our model. A similar modeling approach for \( \tau_r (V) \) was used in (33). The \( Q_{b} \)-factor for the inactivation of the A-type potassium channel is given by \( Q_b = 3 \) estimated from experimental studies (13, 25).

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beaded profiles in Fig. 6C, suggesting that TRAAK may also appear in axons.

**TRPV4 cationic channels.** Fig. 7 shows TRPV4 immunoreactivity in two different horizontal sections through the PO/AH. TRPV4 immunostaining is not present on hypothalamic neuronal cell bodies; but rather, it appears as punctate immunostaining throughout the neuropil. Because TRPV4 is not present in PO/AH neurons, this punctate staining does not appear to be linked with dendrites cut in cross section. Accord-
ingly, the punctate TRPV4 may be labeled axons that enter and traverse the hypothalamus from a remote source. In some cases, Fig. 7, C and D shows clusters of immunoreactive puncta (white arrows) that appear to overlie hypothalamic...
neurons, suggesting that they represent axonal terminal endings synapsing on hypothalamic neurons. Because PO/AH neurons do not show immunoreactivity, the neuronal modeling does not include TRP cationic channels.

**Neuronal Model Simulations**

Figure 2 shows a typical firing pattern with action potentials and the subsequent depolarizing prepotentials of a model PO/AH neuron. The action potentials are generated by the fast Na\(^+\) current \((I_{Na})\) and delayed rectifier K\(^+\) current \((I_K)\). The neuron’s pacemaking activity is partially due to the HCN current \((I_h)\) that depolarizes the membrane after an action potential and AHP. The present immunohistochemical studies show the expression of HCN-2 and HCN-4 subunits in PO/AH neurons, confirming in situ hybridization studies (28). HCN-2 channels are used in the model as their expression is highly enriched in the PO/AH region. The model neuron in Fig. 2 reflects the relatively low firing rates (<20 AP/s) seen in most PO/AH neurons. This low firing rate is partially due to the A-type potassium current \((I_A)\) that has been identified in PO/AH warm-sensitive, temperature-insensitive, and silent neurons (13). Potassium leak currents \((I_L)\) (through TASK, TREK, and TRAAK channels) also slow a neuron’s firing rate by hyperpolarizing the resting membrane potential. The following model simulations show that different expression levels of the leak channels may explain the various types of temperature-sensitive and insensitive neurons identified in the PO/AH.

**Changing the expression level of TASK-1 channels.** The presence of TASK-1 in PO/AH neurons is established by immunohistochemistry (see Fig. 5). The first model simulation focuses on TASK-1 leak channels as the sole potassium leak conductance. In this case, TASK-1 conductance constitutes the total leak conductance \(g_L = g_{task}\). This leak current varies, however, and the variation of the leak conductance corresponds to the different expression levels of TASK-1 channels. The \(Q_{10}\)-factor is \(Q_{task} = 2.0\) (10). Fig. 8 shows the voltage traces at three different temperatures for a model neuron having a low expression level of TASK-1, that is, \(g_{task} = 0.45\) nS. The model neuron’s firing rate increases from 8.6 AP/s at 32°C to 15.7 AP/s at 40°C, and the firing rate thermal coefficient is +0.9 AP/s per °C. Because the thermal coefficient is greater than 0.8 AP/s per °C, this meets the criterion for a warm-sensitive neuron and is qualitatively similar to the previously recorded warm-sensitive neuron shown in Fig. 1B. The basis of this warm sensitivity lies in the rate of activation of the HCN-2 channels which increases with temperature \((Q_r = 5)\), and the rate of inactivation of the A-type potassium channels, which also increases with temperature \((Q_b = 3)\). Accordingly, the depolarizing prepotential is due to both the pacemaker current \(I_h\) and the inactivation of the A-type potassium current \(I_A\). In this case, warming shortens the interspike intervals and
increases the firing rate. The conductance of TASK-1 channels increases only moderately with temperature ($Q_{\text{task}} = 2$), and the leak current $I_L$ is too weak to prevent the $I_h$ current from depolarizing the cell at higher temperatures. In the model, therefore, warm sensitivity is the interplay between the increased rate of activation of HCN-2 channels and the increased rate of inactivation of A-type potassium channels ($K_{v4.1}$) paired with a sufficiently small $K^+$ leak current through TASK-1 channels.

If there is an increase in the expression of TASK-1 leak channels, then neuronal thermosensitivity decreases. Figure 9 shows a model neuron in which the expression level of
TASK-1 channels is doubled, that is, the total conductance of the leak-channel at 32°C is doubled to $g_{\text{task}} = 0.9$ nS. Under these conditions, a temperature-insensitive neuron is obtained, where the cell’s firing rate is 6.7 AP/s at 32°C and 10.3 AP/s at 40°C; the thermal coefficient is +0.5 AP/s per °C. Thus an increased expression of TASK-1 channels can change the neuron from warm sensitive to relatively temperature insensitive, even when there are no changes in the properties of HCN-2 channels and A-type potassium channels (K,4.1). Although the activation and inactivation rates are still the same as for the model neuron in Fig. 8, the increased potassium leak current $I_L$ slows the spontaneous firing rate of the neuron in Fig. 9. This supports previous electrophysiological studies that find that temperature-insensitive neurons tend to have lower firing rates than warm-sensitive neurons (8, 12, 13).

If the expression of TASK-1 leak channels is further increased to $g_{\text{task}} = 1.1$ nS, then the neuron becomes extremely insensitive to temperature, and the thermal coefficient approaches zero. Fig. 10A shows neuronal thermal coefficients when TASK channels constitute all of the potassium leak currents. As long as these TASK leak conductances remain relatively low (i.e., <0.6 nS), neuronal warm sensitivity occurs (i.e., thermal coefficients are ≥ 0.8 AP/s per °C). However, further increases in the potassium leak conductance result in lower thermal coefficients, and temperature-insensitive neurons are produced. If the expression of TASK-1 channels continues to increase, the cell stops firing action potentials at 40°C; and when $g_{\text{task}} \geq 2.0$ nS, the neuron becomes silent over the entire 32° to 40°C range. The resting membrane potential for $g_{\text{task}} = 2.0$ nS is approximately −57 mV at 32°C. With increasing potassium leak conductance, $g_{\text{task}}$, the resting membrane potential becomes more hyperpolarized. Experiments show that the resting membrane potential of silent neurons is significantly more hyperpolarized, compared with the spontaneously firing warm-sensitive and temperature-insensitive neurons (12). Therefore, it is likely that silent neurons have a greater outward leak current $I_L$.

As illustrated in Figs. 8, 9, and 10A, the modeling shows that warm-sensitive, temperature-insensitive, and silent neurons can be obtained through variation in the expression level of TASK-1 potassium leak channels. These model simulations, however, do not produce neurons having cold sensitivity over the entire 32°C to 40°C temperature range, and this is in agreement with experimental studies concluding that cold sensitivity is not an inherent property of PO/AH neurons (9, 23).

Changing expression levels of TASK-1 vs. TREK-1/TRAAK channels. In addition to TASK, other potassium leak currents (e.g., TREK and TRAAK) exist in PO/AH neurons. Fig. 6 shows TRAAK labeling on the somas of PO/AH neurons, and in situ hybridization studies show expression of TREK-1 and TRAAK mRNA in the PO/AH (27, 36). Accordingly, our neuronal modeling compared the effects of different expression levels of $K_{2p}$ channels on a neuron’s response to temperature. As indicated above, TREK and TRAAK channels are much more sensitive to temperature than TASK channels; that is, the TREK/TRAAK Q10-factor $Q_{tr}$ = 7, whereas the TASK Q10-factor $Q_{\text{task}} = 2$ (27).

The effects of different proportions of these potassium leak currents on neuronal thermosensitivity are shown in Fig. 10, where TASK conductance is $g_{\text{task}}$, and the combined TREK and TRAAK conductance is $g_{\text{tr}}$. The three plots in Fig. 10 show how neuronal thermosensitivity is altered if the relative proportions of these leak currents vary. At any given total leak conductance, Fig. 10 shows the difference between a neuron having only TASK for its leak channels (Fig. 10A) compared with a neuron whose leak conductance is due half to TASK channels and half to TREK/TRAAK channels (Fig. 10B). In both cases, an increase in the total leak conductance causes a decrease in the thermal coefficient; however, if half of the leak conductance is due to TREK/TRAAK channels, Fig. 10B shows that there is a much greater decrease in the thermal coefficient at each level of total leak conductance. Furthermore, Fig. 10C shows that if only TREK/TRAAK leak channels are expressed, then there is an even greater reduction in thermosensitivity at each level of total leak conductance.

To illustrate, consider the example in Fig. 8 (and Fig. 12A) where a warm-sensitive neuron (0.9 AP/s per °C) has a total leak conductance due solely to TASK channels ($g_L = 0.45$ nS at 32°C). Fig. 11 shows what would happen to this neuron if the total leak conductance remains the same (0.45 nS), but half of this leak conductance is due to TASK channels, and the other half is due to TREK and TRAAK channels (i.e., $g_{\text{task}} = 0.225$ nS; $g_{\text{tr}} = 0.225$ nS). In this case (Fig. 11 and Fig. 12B), the neuron becomes temperature insensitive with a thermal coefficient of only 0.3 AP/s per °C. Moreover, if only TREK/TRAAK leak channels are expressed ($g_{\text{task}} = 0.0$ nS; $g_{\text{tr}} = 0.45$ nS), then the thermal coefficient reduces further to −0.5 AP/s per °C (Fig. 12C).
The conductance of TREK and TRAAK channels is much greater than TASK channels (7). TASK-1 channels have a single channel conductance of about 10 pS, whereas the conductance is about 50 pS for TRAAK channels and 100 pS for TREK-1 channels (7). Just a few open TREK-1/TRAAK channels can provide a leak conductance that is strong enough to completely shut down the neuronal firing rate. Therefore, the expression of TREK-1/TRAAK channels in a PO/AH neuron could be a determinant of silent neurons, in which the total outward leak current ($I_L$) could be sufficient to hyperpolarize the resting membrane potential and prevent the cell from firing. This would support a previous electrophysiological study that found that (compared to other PO/AH neurons) silent neurons have more hyperpolarized resting membrane potentials and smaller input resistances (i.e., larger resting conductances) (12).

TREK-1/TRAAK channels could act as a mechanism to shutdown a neuron’s firing rate when temperature rises too high. Furthermore, these channels provide the possibility for a neuron to show a type of cold sensitivity, at least at high temperatures. As previously mentioned, cold-sensitive neurons over the entire 32° to 40°C range are not found in the model, because none of the plots in Fig. 10 show activity with negative thermal coefficients near −0.6 AP/s per °C; i.e., the criterion for cold sensitivity. On the other hand, if a narrower temperature range is considered, then the model shows the possibility of cold sensitivity in the hyperthermic range from 36°C to 40°C. Fig. 12 shows the simulated firing rates of model neurons having different expression levels of TREK-1/TRAAK channels. In Fig. 12C, note that a neuron with only expression of TREK-1/TRAAK channels ($g_{task} = 0.0$ nS; $g_{tr} = 0.45$ nS), displays cold sensitivity in the hyperthermic range; that is, the neuron decreases its firing rate during warming from 36°C to 40°C and has a thermal coefficient of −1.0 AP/s per °C. This observation is in qualitative agreement with a hypothalamic tissue slice study (24), which found that some neurons showed cold sensitivity only in this hyperthermic range.
DISCUSSION

For many years, neurophysiological studies have sought to identify a unique mechanism to account for temperature sensitivity in hypothalamic thermoregulatory neurons. Recent reviews have suggested that the warm sensitivity of certain hypothalamic neurons is due to selective ionic channels, such as the thermosensitive cationic TRPV4 channel (1, 6, 15, 29). Whereas TRPV4 has been attributed to warm-induced membrane depolarization in dorsal root ganglion neurons and in peripheral receptors, there is evidence that this is not the mechanism for thermosensitivity in hypothalamic neurons. Our previous intracellular recordings of PO/AH neurons find that thermosensitivity is not attributed to thermally induced changes in resting membrane potential (8, 12), and a recent study found no differences in thermally induced resting currents between warm-sensitive, temperature-insensitive, and silent neurons (38). The present experiments underscore these previous studies. As shown in Fig. 7, TRPV4 immunoreactivity is not apparent in hypothalamic neurons, although it is present as punctate labeling, presumably in traversing axons and their synaptic terminals that innervate the hypothalamus.

The putative role of TRP in hypothalamic neuronal thermosensitivity was suggested in a previous study, which found TRPV4 immunoreactivity in the preoptic/anterior hypothalamus (15). In this study, the authors did not describe the immunoreactivity as being present in cell bodies, and a low-power image shows immunolabeling that has a diffuse distribution in the hypothalamic neuropil. Furthermore, this study acknowledged that the mRNA for TRPV4 was present in dorsal root and trigeminal ganglia but did not acknowledge mRNA for TRPV4 in hypothalamic neurons. This could support a hypothesis that TRPV4 channels are not expressed by hypothalamic neurons but rather are present on axons and axon terminals arising from other areas of the brain.

The importance of the models developed in the present study is their demonstration that a single, unique thermosensitive channel is not necessary to explain neuronal warm sensitivity. Rather, it is possible (even likely) that most PO/AH neurons contain the same basic set of ionic channels, and it is simply the variation in the proportions of expressed channels that determines whether a neuron will be warm sensitive or temperature insensitive or silent.

Although the results section stresses the critical role of potassium leak channels in determining neuronal thermosensitivity, variations in the expression of other channels can also significantly affect thermosensitivity. In the models, for example, the expression of HCN channels was kept constant; however, increasing HCN channel expression will increase both neuronal firing rate and thermosensitivity. For example, Fig. 9 shows a temperature-insensitive neuron having a thermal coefficient of 0.5 AP/s per °C. If the expression of HCN channels is increased by 40%, this neuron would become warm sensitive with a thermal coefficient of 0.9 AP/s per °C. Therefore, in the HCN-labeled preoptic neurons in Figs. 3 and 4, it is possible that the strongest labeled cells are more likely to display warm sensitivity.

The present study indicates that HCN channels, A-type potassium channels and K_{2P} potassium leak channels are all important in the activity of each type of PO/AH neuron. HCN and potassium-A currents play a crucial role in the pacemaker potentials that are a characteristic of spontaneously firing hypothalamic neurons. Despite the importance of HCN and potassium-A channels, the model also demonstrates the critical role of potassium leak channels. Variation in the expression levels of K_{2P} potassium leak channels is sufficient to produce either warm-sensitive, temperature-insensitive, or silent neurons. TASK-1 channels, in particular, are highly expressed in the PO/AH (Fig. 5), and these channels (along with TREK-1/TRAANK channels) are likely candidates to explain the different types of PO/AH neurons. As shown in Fig. 10, increasing the expression level of the K_{2P} potassium leak channels or increasing the relative expression levels of TREK-1/TRAANK vs. TASK-1 channels produces decreases in neuronal thermosensitivity (i.e., thermal coefficient). In general, the higher the conductance of the potassium leak current, the lower the thermal coefficient and the lower the spontaneous firing rate. This explains why temperature-insensitive neurons have lower firing rates compared with warm-sensitive neurons (12). In temperature-insensitive neurons, the high potassium leak current I_{L} produces greater hyperpolarization, thereby keeping the firing rate low. This also raises the possibility in Figs. 5 and 6 that the warm-sensitive neurons may be the cells that show little or no TASK and TRAANK labeling; whereas the neurons with the lowest thermosensitivity (and possibly silent neurons) may be the neurons showing the strongest labeling.

Our previous studies (3) suggest that there are interactions between hypothalamic neurons that regulate different homeostatic systems. Preoptic synaptic networks may be composed of various neuronal types that are either sensitive or insensitive to many endogenous factors (e.g., temperature, osmolality, glucose, and pH) (3). In addition to thermoregulation, this neural area controls several overlapping regulatory systems that together constitute homeostasis. Moreover, in addition to temperature, hypothalamic neurons sense endogenous factors that act as feedback signals in these regulatory systems. Previous studies have shown that a variety of these factors can alter the firing rate and thermosensitivity of PO/AH neurons, as well as influence thermoregulatory responses (3). Potassium K_{2P} channels may account for part of the interactions and overlap of neuronal regulatory systems. Potassium K_{2P} channels are responsible for K^{+} leak currents, and these channels are regulated by different physical and chemical stimuli, including membrane stretch, temperature, acidosis, lipids, and inhalational anesthetics (30). Furthermore, channel activity is tightly controlled by membrane receptors and second messenger phosphorylation pathways. Thus the same channels that determine neuronal thermosensitivity and firing rate are the channels that are influenced by various endogenous factors. This may be important in understanding the mechanisms underlying the interactions between different regulatory systems controlled by the hypothalamus.

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