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Ion channel proteins in neuronal temperature transduction: from inferences to testable theories of deep-body thermosensitivity

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THE RECENT MODEL ANALYSIS of hypothalamic neuronal thermosensitivity by Wechselberger et al. (this issue) highlights the progress made in identifying ion channels and in elucidating temperature dependencies of their properties as a new perspective for unraveling the neuronal network of temperature regulation (28). Proceeding from sequence homologies, (super-)families of genes encoding a multitude of ion channel proteins have been identified. So far, the mechanisms underlying temperature transduction by a peripheral or a central nervous system (CNS) neuron could only be inferred from the temperature coefficients of the classical triad of 1) voltage-gated, rapidly inactivating sodium and 2) delayed noninactivating potassium channels and 3) sodium-potassium ATPase (21) supplemented later by the K_4.1 (potassium A) channel, which slows repetitive neuronal excitation and displays a Q_{10} >> 1 of its inactivation rate (6). Additional immunocytotoxic evidence is now presented by Wechselberger et al. (28) for the presence in hypothalamic neurons of hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels (12), accounting for inward currents at normal membrane potential and K_2P (tandem-pore potassium-selective) “background” or “leak” channels (10, 13, 14, 22) acting to stabilize the resting membrane potential (17). Proceeding from the temperature dependencies of HCN channel activation and K_4.1 channel inactivation rate, respectively, the temperature influence on conductance of K_2P channel subtypes and their relative expression levels are considered in model calculations to assess their combined influence on neuronal thermosensitivity.

Subtypes of the transient receptor potential (TRP) cation channel superfamily (5, 15), may additionally contribute to neuronal thermosensitivity, according to studies on model cells or neurons in which they are expressed. TRPV3/TRPV4 channels have positive temperature coefficients (2). The TRPM8 channel exhibits a negative temperature coefficient (20).

The study by Wechselberger et al. (28) is exemplary in that it shows for CNS neurons how knowledge about the channel expression pattern of a particular neuron may be combined with results of electrophysiological channel function analysis to predict the neuron’s temperature-transducing property. However, elucidation of the roles played by the aforementioned channel proteins in peripheral temperature perception and, in particular, in neurons sensing deep-body temperature, requires observation of several preconditions. First, as documented by various references listed below, the distribution of channels is 1) ubiquitous, more or less, both in nonexcitable cells and in neurons and 2) they respond to a multitude of physical and chemical stimuli, including endogenous signal molecules. Therefore, independent evidence is required for the thermoafferent or thermointegrative function of a neuron with known temperature dependence of its activity. Second, the proportions of different membrane channel subtypes expressed in a given neuron may be critical. The above model (28) proposes, for instance, that a high-density of K_2P channels with a Q_{10} >> 1 would tend to hyperpolarize a CNS neuron with rising temperature, thereby stabilizing it against depolarizing temperature effects transmitted by, e.g., HCN (and/or TRPV3/ TRPV4) channels with a Q_{10} < 1. Conversely, their inactivation by decreasing temperature would enhance a neuron’s cold sensitivity in the presence of simultaneously expressed TRPM8 channels with Q_{10} < 1. To consider the other extreme, fewer and/or less temperature-dependent K_2P channels, e.g., TASK-1 (28), would increase a neuron’s activation by rising temperature, if K_4.1, HCN, or TRPV3/TRPV4 channels are simultaneously present, whereas simultaneously present TRPM8 channels would become less effective in activating a neuron by cooling.

Analysis of peripheral thermoreception has met the difficulty that the free nerve endings of skin’s cold and warm receptors are barely accessible to combined histochemical and electrophysiological analysis. Therefore, heterologous expression of channel proteins and studies on dorsal root ganglion cells expressing defined receptors are in the focus of analysis. For “cold transduction” in rat primary sensory neurons, inhibition of background potassium conductance was proposed (22). The involvement of TRPM8 channels in cold signal transduction of a cell is strongly suggested by the similarity of their responses to those of cold fibers and by their combined cold and menthol sensitivity and inactivation by calcium entry, as it has also been found in populations of cultured rat dorsal root ganglion neurons (18, 23). For innocuous heat perception, the K_2P channel family may be important in view of evidence for warmth sensitivity of the TREK-1, TREK-2, and TRAAK subtypes and a lesser temperature dependence of the TASK-1 subtype (10, 13, 14, 28). Among the TRP channels, TRPV3/TRPV4 have been implicated in transduction of touch and noxious heat. Their involvement in neuronal warmth percep-
tion is not yet established (2, 27), but keratinocytes expressing these channels might signal temperature increases to adjacent warm fibers (4). Evidence for TRPV3/TRPV4 receptor expression in dorsal root ganglion (DRG) cells is still equivocal, but receptor gene transcripts were detected in analogous neurons (30).

As a provisional conclusion, DRG cell bodies appear to represent a convenient model to elucidate the molecular basis of peripheral cold and warm signal generation, because function and structure are associated. 1) DRG neurons are afferent. 2) Their cell size permits us to attribute to them, with statistical reliability, either protopathic (temperature or pain perception) or proprioceptive functions. 3) It appears legitimate to extrapolate from cell body current-voltage relationships to those of the nerve endings. 4) There is awareness of the problems concerning specificity and sensitivity of channel protein identification by immunocytochemistry in comparison to the detection of corresponding gene transcription products.

For the analysis of deep-body temperature sensing by CNS neurons, it would seem plausible if they were functioning in analogy to DRG neurons. Indeed, this was an early suggestion after the discovery of deep-body temperature perception in the vertebral canal, because its cooling and heating not only affected the spinal cord itself but also the adjacent dorsal root ganglia. However, the thermophysiological responses were preserved when peripherally deafferentated spinal cord sections were heated or cooled and, thus, indicated that the spinal cord itself was the main source of temperature signals (9, 16). Moreover, cold signals originating in the skin and in the spinal cord were shown to converge upon the same set of spinal afferent anterolateral tract neurons but were found to be differently affected by descending inhibitory control (7). Thus peripheral and CNS thermoreception are not directly comparable, and criteria for afferent specificity, as they are deducible for an afferent DRG neuron from location and size, are not readily available for a CNS neuron. Moreover, temperature dependence of a neuron is not necessarily specific. Therefore, attributing thermosensory specificity to such a neuron requires knowledge about its affiliation to either one of the two bimodally organized thermoafferent pathways (24), the existence of which was earlier proposed (3, 8) and experimentally confirmed later (11, 26).

Defining CNS neurons as “thermosensitive” according to a predetermined temperature coefficient (e.g., ≥ 0.8 impulses/s per °C) (28) does not rule out thermoafferent signal processing by less temperature-dependent thermosensitive hypothalamic neurons. In fact, their early consideration in the seminal “high/low $Q_{10}$” theory of hypothalamic thermoregulation (8) has, so far, most plausibly explained species differences and nonlinearities of stimulus-response relationships in homeothermic thermoregulation (25).

Intrinsic thermosensitivity has been attributed preferably to CNS neurons with positive temperature coefficients (28), but the existence of intrinsically cold-sensitive neurons is not definitively excluded (29). A recent study demonstrated cold sensitivity of dissociated hypothalamic neurons (1), but the low threshold temperatures seem to preclude a role in physiological temperature regulation. Lack of evidence for intrinsic neuronal cold sensitivity at the spinal level (19) has suggested that cold signals transmitted afferently from the spinal cord to the brain are generated indirectly.

Further progress in elucidating thermosensory specificity at the single neuron level must consider the multimodality of most of the channel subtypes listed above. This requires experimental evidence for consistency between a CNS neuron’s channel distribution pattern predicting its thermosensitivity and its affiliation to either one of the two thermoafferent systems. Accepting this premise would define the direction of future experiments. First and foremost, channels would have to be identified. However, because of problems of specificity and sensitivity associated with the immunocytochemical approach, positive or negative findings obtained with a single antibody for a particular channel are not conclusive a priori. The search for the most suitable antibody may require several trials, and alternative techniques of channel marking may have to be considered, for example, second messenger detection after specific channel stimulation and blockade. Second, in line with the model calculations of Wechselberger et al. (28) channel interactions have to be taken into consideration, depending on the channel types expressed in a neuron and on their relative densities. Multilabeling of channel proteins appears necessary, and finding a set (or sets) of neurons classifiable according to their channel expression patterns would be an exciting discovery. Third, electrophysiological assessment of thermosensitivity would help to establish consistency between actual temperature coefficients and channel expression patterns of neurons. Finally, demonstrating their connectivities, for example, by tracing techniques, would be desirable.

As a conclusion, this commentary is obviously proposing an idealized approach to elucidate the thermosensory functions of CNS neurons by combining molecular and electrophysiological tools with topographical analysis. Whoever has done experiments to assess deep-body thermosensitivity knows too well that a “success” will fall short, more or less, of the ideal goal and, for this reason, the above considerations would miss their purpose, if they were discouraging. Rather, they are meant to transmit the message that enthusiastic speculations are the source of new ideas, provisional as they may be, to search for new experimental approaches to problems that still remain to be solved.

REFERENCES


