Unique temperature-activated neurons from pit viper thermosensors

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Pappas, Todd C., Massoud Motamedi, and Burgess N. Christensen. Unique temperature-activated neurons from pit vipers. *Am J Physiol Cell Physiol* 287: C1219–C1228, 2004. First published June 22, 2004; doi:10.1152/ajpcell.00040.2004.—Rattlesnakes, copperheads, and other pit vipers have highly sensitive heat detectors known as pit organs, which are used to sense and strike at prey. However, it is not currently known how temperature change triggers cellular and molecular events that activate neurons supplying the pit organ. We dissociated and cultured neurons from the trigeminal ganglia (TG) innervating the pit organs of the Western Diamondback rattlesnake (*Crotalus atrox*) and the copperhead (*Agkistrodon contortrix*) to investigate electrophysiological responses to thermal stimuli. Whole cell voltage-clamp recordings indicated that 75% of the TG neurons from *C. atrox* and 74% of the TG neurons from *A. contortrix* showed a unique temperature-activated inward current (*I*<sub>ST</sub>). We also found an *I*<sub>ST</sub>-like current in 15% of TG neurons from the common garter snake, a species that does not have a specialized heat-sensing organ. A steep rise in the current-temperature relationship of *I*<sub>ST</sub> started just below 18°C, and cooling temperature-responsive TG neurons from 20°C resulted in an outward current, suggesting that *I*<sub>ST</sub> is on at relatively low temperatures. Ion substitution and Ca<sup>2+</sup>-imaging experiments indicated that *I*<sub>ST</sub> is primarily a monovalent cation current. *I*<sub>ST</sub> was not sensitive to capsaicin or amiloride, suggesting that the current did not show similar pharmacology to other mammalian heat-sensitive membrane proteins. Our findings indicate that a novel temperature-sensitive conductance with unique ion permeability and low-temperature threshold is expressed in TG neurons and may be involved in highly sensitive heat detection in snakes.

Snake; thermosensory; trigeminal; ion conductance

PIT VIPERS (Viperidae: Crotalinae, rattlesnakes, copperheads) have highly evolved sensitive receptors called pit organs, which receive and relay high-resolution thermal information to the brain. This thermal information is thought to be processed and integrated at the optic tectum (28) where it aids in detecting and striking prey (29). Although the pit organ has historically been studied as an infrared (IR) detector, thorough physiological (3, 9) and theoretical studies (17) suggest that the organ responds to thermal rather than photonic stimuli. Our investigations of the optical sensitivity and selectivity of this organ indicate that it is responsive to a broad range of radiation from IR to ultraviolet (26). The lack of spectral selectivity strongly supports the hypothesis that the pit is a highly sensitive thermoreceptor rather than photonic IR detector.

The pit organ shows structural specializations indicative of a high-resolution thermal detector. The temperature-sensing terminal nerve endings innervate an ultra-thin (<25 μm) pit membrane (29) that acts as the heat-sensing surface. This thin membrane and the associated terminal nerve mass are suspended in a facial cavity in front of an inner air space, giving the pit organ an extremely low thermal mass. The pit organ also has a rich capillary network that is thought to act as a rapid heat sink (12). This combination of low thermal mass and rapid heat dissipation contributes to the precise temporal and spatial resolution of thermal stimuli. Multiple- and single-unit neuronal activity recorded from pit organ afferents demonstrates that these receptors are sensitive to very small temperature changes (~0.003°C) (3) and can transmit this thermal information over a broad range of temperatures (15–37°C). This suggests that the pit organ must have highly sensitive neurons and ion channel mechanisms capable of receiving and transmitting thermal information and that these complement the anatomic specializations of the pit organ for heat reception. However, the cellular and molecular machinery responsible for converting temperature information into neuronal signals in the pit organ is completely unknown.

A generator potential for thermal stimuli at the pit organ has been identified using extracellular recordings (34), and this supports the hypothesis that the terminal nerve mass may express temperature-sensitive integral membrane proteins. This is also consistent with reports on mammalian thermoreceptors, especially thermal nociceptors, where the activation-temperature threshold, ion conductance, and even behavioral features (6, 7) have been correlated to the presence of a temperature-gated cation channel TRPV1. Thermal nociceptive neurons are fairly plentiful and thus benefited studies linking TRPV1 to thermal responses. The snake pit organ is a unique system for the study of these lower temperature-threshold thermoreceptors because it has the highest known density of warm receptors (4) and there are far fewer “warm” receptors in mammals (3, 15, 24). By analogy to TRPV1, ion conductances corresponding to warm receptors may be abundant in neurons that project to the pit organ.

Here, we demonstrate for the first time that dissociated neurons from the pit viper trigeminal ganglia (TG), which supplies the pit organ (4), show a heat-sensitive current with temperature-threshold and biophysical properties unlike those identified in mammalian neurons. Voltage-clamp recordings revealed an inward monovalent cation current (*I*<sub>AT</sub>) that increased with heating and tracked temperature change. *I*<sub>AT</sub> was found in a large proportion of TG neurons that were isolated and had a threshold of ~18°C.

MATERIALS AND METHODS

Animals. Copperheads (*Agkistrodon contortrix*), Western Diamondback rattlesnakes (*Crotalus atrox*), and common garter snakes (*Thamnophis sirtalis*) were caught wild and obtained from a commercial...
RESULTS

We performed whole cell voltage-clamp recordings on neurons cultured from the TG. Our primary culture technique yielded neurons and other cells that were <60 μm in diameter; the size of neurons we recorded from was 30 ± 9 μm (SD, n = 157). Neurons were identified morphologically as phase-bright cells that showed voltage-dependent conductances when 10-mV voltage steps from -90 to +20 mV (100 or 300 ms) were applied (Fig. 1). Under our culture conditions, neurons did not typically express neurites. Table 1 presents general characteristics recorded from these cells. Figure 1, A and C, shows the two general current responses we recorded from these neurons, with the current-voltage relationships shown in Fig. 1, B and D, respectively. These currents differed only in having a rapid inward component following voltage-dependent outward currents (Fig. 1A, asterisk). The input resistance (R) of neurons of the three species tested was determined from the slope of current-voltage relationships from -90 to -60 mV and is presented in Table 1. A two-way analysis of variance shows no significant difference in input R among species tested (F2,46 = 0.69, P > 0.05) or between temperature-unresponsive and temperature-responsive neurons (see below for criteria, F1,46 = 0.51, P > 0.05).

We did not observe cells showing spontaneous action potentials (APs) under current clamp. However, in 14 of 19 neurons (74%) from A. contortix, we were able to elicit APs under current clamp with a 50- to 100-pA, 100-ms anodic current injection (Fig. 1E). We recorded two types of APs, which could be distinguished by having a short (mean = 5.4 ± 1.2 ms, SD, n = 9) or long (mean = 17.1 ± 1.7 ms, SD, n = 5) spike duration. Due to the low number of temperature-unresponsive neurons in this sample, we were unable to determine if the distribution of AP type was nonrandom with respect to the temperature response characteristics of the neuron.

Temperature responses were elicited by applying heated or chilled HBS as close as possible to the voltage-clamped neuron using a polypropylene Pasteur pipette. A thermocouple with rapid temperature response (Physitemp, Clifton, NJ) placed within 500 μm of the cell was used to estimate the thermal stimulus delivered to the cell. We determined heating and cooling parameters from 30 representative temperature records in which we sought to determine the current response of neurons to a temperature step. Representative temperature traces are presented in Fig. 2, A–C, top. The cells were heated from a resting room temperature of 20.4 ± 1.4°C (SD) to a final temperature of 34.5 ± 4.8°C (range 27–49°C) at a mean rate of 3.3 ± 1.8°C/s. The duration of the elevated temperature step was variable but always greater than 10 s, during which there was ambient cooling at a rate of 0.17 ± 0.05°C/s. The cells were then cooled with chilled HBS at a rate of 2.0 ± 1.0°C/s back to baseline temperature to show reversal of the response.

Temperature-responsive neurons were defined as having consistent, temporally correlated responses to stimulation and having an inward current of at least 2.0 pA/°C when heated to a minimum of 10°C from room temperature (~20°C). These currents also needed to reverse upon cooling. Using these criteria, we identified 75% (18 of 24) of the TG neurons in C. atrox and 74% (90 of 122) in A. contortix as temperature responsive (Table 1 and Fig. 2, A and B). Figure 2, A–C, shows
representative inward current responses to application of warmed HBS to TG neurons from all species tested. We called this current $I_{\Delta T}$. The current responses were rapid, temperature dependent, and followed temperature changes closely. Figure 2D shows a representative response from a temperature-unresponsive cell, showing no significant current change due to the application of heated HBS. In temperature-responsive cells, inward current in response to heat was accompanied by a

Table 1. Characteristics of TG neurons

<table>
<thead>
<tr>
<th>Species</th>
<th>C. atrox</th>
<th>A. contortix</th>
<th>T. sirtalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of total neurons</td>
<td>30</td>
<td>165</td>
<td>20</td>
</tr>
<tr>
<td>Temperature responsive</td>
<td>18 (75%)</td>
<td>90 (74%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Temperature unresponsive</td>
<td>6</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Responders</td>
<td>32±12 (16)</td>
<td>30±8 (76)</td>
<td>47±6 (3)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>33±12 (6)</td>
<td>30±8 (25)</td>
<td>35±10 (16)</td>
</tr>
<tr>
<td>Input resistance, * MΩ</td>
<td>354±246 (11)</td>
<td>540±259 (19)</td>
<td>371±272 (3)</td>
</tr>
<tr>
<td>Responders</td>
<td>392±151 (2)</td>
<td>681±160 (4)</td>
<td>468±266 (13)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>15.6±11.7 (13)</td>
<td>11.2±14.1 (36)</td>
<td>3.5±2.5 (3)</td>
</tr>
<tr>
<td>$I_{\Delta T}$ current magnitude, pA°C</td>
<td>514.4±639.3 (11)</td>
<td>611.6±649.3 (20)</td>
<td>46.9±3.0 (3)</td>
</tr>
</tbody>
</table>

Values are means ± SD; * is shown in parentheses. *Two-way ANOVA showed no significant effects of species ($F_{2,46} = 0.69, P > 0.05$) or responsiveness ($F_{1,40} = 0.51, P > 0.05$) and no interaction effect ($F_{2,40} = 0.24, P > 0.05$). † ANOVA showed a significant effect of species on temperature-activated inward current ($I_{\Delta T}$) current density ($F_{2,31} = 6.65, P < 0.01$). Scheffe’s multiple contrast showed that current density was significantly different in neurons from $T$. sirtalis when compared with those of either $C$. atrox ($P < 0.01$) or $A$. contortix ($P < 0.01$). TG, trigeminal ganglia.
I. Introduction

Temperature-sensitive neurons in the pit organ (PO) are thought to play a role in thermoregulation. The pit organ is a sensory structure in snakes, particularly pit vipers, that detects temperature changes in the environment. These neurons are activated by both heating and cooling, and their currents show a temperature-dependent response.

II. Materials and Methods

Temperature-sensitive currents were recorded from TG neurons in three species of snakes: the common garter snake (Thamnophis sirtalis), the rough green snake (Opheodrys aestivus), and the eastern massasauga rattler (Sistrurus miliaris). Current-voltage (I-V) relationships were determined, and statistical comparisons were made using the Wilcoxon rank-sum test.

III. Results

Figure 2 shows the temperature-sensitive currents in TG neurons from all species tested. A–C: temperature-activated inward current ($I_{\text{ST}}$) in representative TG neurons from all species tested (top trace), showing temperature-dependent response (bottom trace) to bath heating (temperature profile in top trace). D: current trace from A. contortix TG neuron that did not express $I_{\text{ST}}$. E: under current-clamp TG neurons expressing $I_{\text{ST}}$ show a depolarization (bottom trace) in response to heating, as shown in this representative trace from an A. contortix TG neuron.

IV. Discussion

The magnitude of $I_{\text{ST}}$ varied among species, with the common garter snake showing the highest current density (15.6 ± 11.7 pA/°C) and the eastern massasauga rattler showing the lowest (3.9 ± 4.0 pA/°C). There was a weak but statistically significant negative correlation (r = -0.49, P < 0.05) between neuron surface area and the log$_{10}$-transformed current density value, indicating that smaller cells tend to express more $I_{\text{ST}}$.

V. Conclusion

In conclusion, the temperature-sensitive currents in TG neurons from different species of snakes show a high degree of variability, with the common garter snake having the highest current density and the eastern massasauga rattler having the lowest. A weak but statistically significant negative correlation was found between neuron surface area and the log$_{10}$-transformed current density value, suggesting that smaller cells express more $I_{\text{ST}}$.
range of temperatures, and current values were subtracted from the baseline that represented the lowest value over the record. The $I_{\Delta T}$ vs. $T$ relation showed a nonlinear response, with little current increase at lower temperatures (10–15°C) transitioning to a more rapid rate of increase at just below 18°C. Regression lines for the two components were predicted by least-squares method minimizing the trend in residuals (40), and the threshold was determined to be 17.8°C for the steeper current gain per unit temperature.

The RP of $I_{\Delta T}$ was determined for TG neurons from A. contortix by subtracting currents generated from increasing voltage steps at 15°C from those resulting from heating neurons to 26–35°C (Fig. 4A). We use CsCl electrodes to reduce the contribution of voltage-activated K+ channels, which may influence the value of the RP (1). The RP of $I_{\Delta T}$ was $-12.7 \pm 9.6$ mV (SD, $n = 9$). Similarly, cells held at and above 0 mV showed a reversal of the heat-induced current (Fig. 4B). Consistent with the RP, ion substitution experiments indicated that heat may activate a monovalent cation channel with K+ permeability. Substitution of NaCl with equimolar N-methyl-D-glucamine resulted in a 49% (±26% SD, $n = 4$) decrease in the magnitude of the integrated $I_{\Delta T}$ current per °C (Fig. 5A). More detailed ion substitution experiments are summarized in Table 2. Voltage ramps were used to examine RP of neurons in varying ionic conditions. The RP of $I_{\Delta T}$ in 10 mM NaCl shifted to $-27.9 \pm 4.0$ mV (Table 1) from $-12.7$ mV. Interestingly, increasing CaCl$_2$ from 2 to 10 mM had no effect on the RP of $I_{\Delta T}$ ($-24.8 \pm 3.2$ mV, SD, $n = 3$) from cells in 10 mM NaCl. Similarly, a 10-fold reduction in Ca$_2^+$ did not affect the RP in 120 mM NaCl. From these data, and estimated intracellular concentrations of Na$^+$ and K$^+$ of 4 and 140 mM, respectively, we determined that for $I_{\Delta T}$ the relative permeability $P_{K_-}/P_{Na^+} \approx 1.16$ from the Goldman, Hodkin, and Katz voltage equation (16).

To verify the lack of Ca$_2^+$ permeability in $I_{\Delta T}$ suggested by ion substitution experiments, we measured intracellular Ca$_2^+$ using fura-2 in 43 voltage-clamped TG neurons from A. contortix. $I_{\Delta T}$-expressing TG neurons did not show an increase in intracellular Ca$_2^+$ (Fig. 5B), further indicating that these heat-activated channels are impermeable to Ca$_2^+$. From these data, it can be assumed that $I_{\Delta T}$ is primarily a monovalent cation conductance.

Previous research established the pharmacological identity of several temperature-sensitive ion channels (7, 20, 23, 25). We exposed $I_{\Delta T}$-expressing TG neurons from A. contortix to 10 μM capsaicin [in 0.001% (vol/vol) ethanol] to determine if a pharmacologically active TRPV1 homologue is present in these cells (7). Cells did not respond to capsaicin nor was the magnitude of response to heat stimulus changed ($n = 4$). Similarly, amiloride, which blocks temperature-sensitive epithelial Na channels (2), had no effect on the magnitude of $I_{\Delta T}$ in TG neurons ($n = 5$).
DISCUSSION

We recorded a novel temperature-activated current in neurons that supply the pit organ of *A. contortix* and *C. atrox*. The current was novel in that it had the lowest threshold of activity of any known heat-activated conductance and had ion permeability unlike that of any characterized temperature-sensitive channel. $I_{AT}$ was a monovalent cation conductance that was active at ambient temperatures and increased in response to heating. The effective temperature range of $I_{AT}$ was consistent with having a role in sensitive thermal detection of prey and other environmental stimuli. $I_{AT}$ was found in a large proportion of the neurons in the TG of *C. atrox* and *A. contortix*. Temperature-activated neurons were also found in the TG of snakes lacking the pit organ (*T. sirtalis*) but at a much lower frequency and with lower current density. This suggests that crotaline snakes may have adapted a general warm thermosensor to a specialized function as neuronal signal transducers in a highly sensitive thermoreceptive organ. The ion dependence of $I_{AT}$ was found to be different from other known temperature-sensitive channels (7, 13, 25, 39), further illustrating the unique nature of these temperature-gated conductances. The presence of a large number of neurons expressing temperature-gated currents in these cells further strengthens the hypothesis that heat detection in the pit organ is not due to photonic IR detection but is a result of highly sensitive heat detection (3, 9, 26).

*Use of in vitro preparations to study thermoreceptors.* A generator potential for pit thermoreceptors has been recorded from crotaline snakes including *Agkistrodon* (34). Terashima and colleagues (34) recorded both voltage spikes and slow potentials using extracellular electrodes inserted just below the pit membrane into the terminal nerve mass. These potentials were proportional to stimulus intensity and duration but drifted in direction due to the instability of the preparation. We chose to use a voltage clamp to obtain more detailed biophysical information on temperature-activated currents, but we were unable to clamp the terminal nerve mass, which probably contains the highest density of cellular structures that cause temperature-dependent currents (i.e., heat-sensitive ion channels). Recordings were taken from cell bodies because of their ease of preparation and because temperature responses of neurons have been shown to be accurately replicated in cultured trigeminal and dorsal root ganglion (DRG) neuron cell bodies (8, 23, 27, 31). This is because heat-sensitive ion channels are expressed and active at the cell body in cultured neurons.

Neurons were subjected to a wide range of temperatures during these recording procedures, and some of these neurons

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**Fig. 4.** *A:* current-voltage relationship of $I_{AT}$ from a representative cell recorded with CsCl electrodes. *Insets:* currents at 15 and 35°C resulting from current steps depicted beneath them. The current for the plot was measured at 100 ms from the start of the voltage step. The current-voltage plot shows the difference current resulting from the subtraction of currents at 15°C from those at 35°C. *B:* $I_{AT}$ recorded from a TG neuron held at +20 and −65 mV. The temperature trace is representative of the heating protocol applied at both holding potentials.
showed no response to temperature change. This is consistent with findings in ganglion cultures from mammalian sensory neurons where there was a clear distinction between temperature-sensitive and -insensitive cells and suggests that there was no artifactual temperature-induced activation of resting leak or voltage-activated currents with thermal stimulus. Cells expressing $I_{\Delta T}$ showed characteristic nonlinear relationship between temperature and currents that indicated a threshold just below $18^\circ C$. This type of current-temperature relationship has been seen for temperature-activated currents (36) and for isolated temperature-gated ion channels from mammals (7, 25, 39). The transition from slow to rapid current change as a function of temperature is thought to happen at the temperature in which there is a rapid and reversible change in the ion channel, possibly associated with temperature-dependent gating of the channel (36).

We could not record APs in response to heating of TG neurons, although it is clear from studies on intact preparations that heat sensation in the pit organ is transmitted via APs. It is possible that the rate of heat stimulus we presented to these cells was too slow to generate APs. Temperature changes in the low thermal mass pit organ should be nearly instantaneous, even if they are small. With slow changes, however, ion channels responsible for stimulating regenerative changes in membrane potential might be inactivated before the AP could take place or voltage-activated channels responsible for terminating APs might be activated before the $I_{\Delta T}$ could significantly change the membrane potential. Two previous studies (3, 9) suggest that pit afferents respond to the rate of temperature change as well as the stimulus intensity. However, changes in AP frequency could be distinguished even with very long temperature rise times (3). It is also possible that the density of $I_{\Delta T}$ in dissociated TG neurons was not sufficient to give rise to AP-producing depolarization of membrane potential or that accessory channels involved in AP production in these cells were not adequately expressed. This could be the

Table 2. Ion substitution and reversal potentials of $I_{\Delta T}$

<table>
<thead>
<tr>
<th>External Cations, mM</th>
<th>$Na^+$</th>
<th>NMDG</th>
<th>Ca$^{2+}$</th>
<th>Reversal Potential, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>0</td>
<td>2</td>
<td>-12.7±9.6</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>0.2</td>
<td>-11.7±5.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>110</td>
<td>2</td>
<td>-27.9±4.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>110</td>
<td>10</td>
<td>-24.8±3.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. NMDG, $N$-methyl-$D$-glucamine.

Fig. 5. $A$: sodium dependence of $I_{\Delta T}$. TG neurons were voltage clamped at $-65 \text{ mV}$ in HEPES-buffered saline (HBS), and $I_{\Delta T}$ was recorded in response to application of warmed HBS. The recording media were changed to one in which $120 \text{ mM}$ $N$-methyl-$D$-glucamine was substituted for $NaCl$, and the cell was heated by application of the substituted media. $B$: calcium permeability of the heat-activated current was monitored using fura-2 spectrophotometry and whole cell voltage clamping simultaneously. $B$ shows simultaneous fura-2, temperature, and current data for a representative heat-responsive TG neuron. TG neurons loaded with fura-2 show no heat-induced change in the ratio (stimulation at 340 nm/380 nm) of fluorescence emission at 510 nm. The neuron was depolarized (bar) by turning off the holding potential, and this resulted in a strong signal, indicating that cells were loaded and responsive to intracellular Ca$^{2+}$ level changes. Temperature and current data were not taken just before and after the positive control depolarization, but off-line monitoring of temperature shows there was no deviation from baseline ($20^\circ C$).
result of low or altered expression of putative ion channel(s) responsible for \( I_{\text{AT}} \) or lowered or altered AP-associated ion channels in dissociated cell culture. Finally, it is possible that the role of \( I_{\text{AT}} \) is not to produce fast enough or robust enough depolarization for AP activation but instead modulate ongoing changes in membrane potential. This is supported by studies from pit viper trigeminal nerve (3, 9) and TG neurons (33) showing that these elements are spontaneously active and that temperature change is signaled by alterations in ongoing spike activity. We did not see spontaneous APs in our cells, possibly as a result of dissociated culture, but we speculate that one mechanism by which \( I_{\text{AT}} \) might signal heat is to produce a slow depolarization that increases spike frequency, as has been described for other sensory systems (5, 10).

Neurons expressing a temperature-sensitive current similar to \( I_{\text{AT}} \) were also found in the TG from the common \( T. \) sirtalis, a snake that does not have a specialized organ for sensitive heat detection, but at a significantly lower frequency and with lower current density. It is not surprising that we found thermosensitive cells in the TG from this snake, as this ganglion supplies more general temperature sensors to the face (4). It is also possible that some of the temperature-responsive neurons we recorded from the \( C. \) atrox and \( A. \) contortrix TG are actually cutaneous warm receptors that did not send projections to the pit organ but to other parts of the face. If this is the case, then the frequency of cells expressing \( I_{\text{AT}} \) we observed in the TG is not an accurate estimate of the frequency of warm-receptive neurons sending projections to the pit organ but instead to the entire facial region innervated by the trigeminal nerve. The frequency of TG cells showing various temperature-activated currents that actually send afferents to the pit organ will have to be determined using retrograde labeling from the pit organ in conjunction with physiological recordings in the TG. However, a comparison can be made with the frequency of warm-receptive neurons found in the TG of the \( T. \) sirtalis, as both estimates were made using the same sampling procedure. The fact that \( I_{\text{AT}} \) in \( T. \) sirtalis was qualitatively similar to that of pit vipers has interesting implications in the evolution of the pit organ and its thermosensitivity. The pit afferents of crotaline snakes have been shown to have a higher number of warm-sensitive fibers than any known animal (3). Our data on proportion of heat-sensitive neurons in pit vipers vs. \( T. \) sirtalis are consistent with these findings. Additionally, our data also showed that the current density of \( I_{\text{AT}} \) is much higher in pit vipers than in \( T. \) sirtalis. It is possible that pit viper thermosensors evolved as specializations of general cutaneous warm receptors like those that would be found in all snakes. The evolution of a specialized pit organ was accompanied by an expansion of numbers of warm-sensitive neurons supplying the area, resulting in very high thermal sensitivity of the pit organ. Additionally, individual neurons may be more sensitive to temperature, and this would result in these neurons having greater electrophysiological responses to temperature change further enhancing sensitivity of an integrated thermoreceptor like the pit.

\( I_{\text{AT}} \) correlates with behavioral data on snake thermosensing.

The concordance in temperature-response characteristics between behavioral or nerve recordings and cultured neuronal preparations has often been used to establish a causal link between temperature sensation and temperature-activated currents (8). Our data from snake TG neurons correlated with features discovered from behavioral, whole nerve, or single-fiber recordings from pit vipers. Most notably, the temperature threshold for \( I_{\text{AT}} \) in pit vipers was in good agreement with recordings from the pit afferent nerves that showed loss of spontaneous spiking activity at 10–15°C (9). Above this threshold, there was a constitutive inward current, which may be related to “background” activity of nerves seen above 18°C. The temperature-tracking characteristics of \( I_{\text{AT}} \) may also be related to the nonadapting spike discharge seen in pit afferents when the pit is heated. The detailed investigations of de Cock Bunning et al. (9) demonstrate that rapid adaptation of spiking frequency is a property of rapid cooling in the low thermal mass pit organ and not an adaptation at the nerve itself. Adding water to the pit increased its thermal mass and resulted in a nonadapting discharge of pit afferents. This suggests that the neural response is constant throughout the thermal stimulus and is in agreement with the behavior of \( I_{\text{AT}} \). The temperature-tracking characteristics of \( I_{\text{AT}} \) were also consistent with the slow, continuous temperature-dependent changes seen in the extracellular generator potentials recorded at the pit membrane (34).

Behavioral data also show that pit afferents stop firing above 37°C (9), and this does not correlate well with the behavior of \( I_{\text{AT}} \), which showed responses even at and above 40°C. This raises the possibility that there may be other temperature-sensitive currents that mediate thermosensitivity at the pit organ. It is possible that there may be a high-threshold temperature-sensitive current that responds at temperatures above 37°C and may be involved with the cessation of the pit response at these temperatures. We already have preliminary data that suggest that the TG may contain neurons that have a transiently activated cooling-sensitive current that may contribute to the sensitivity described in snake thermosensing (30).

Similarities and differences between \( I_{\text{AT}} \) and currents from temperature-sensitive ion channels. The hypothesis that a heat-sensitive ion conductance, similar to the one found here for the snake, is a transducer of thermal information has also been proposed and investigated for mammalian primary thermosensory neurons in vitro (23, 27, 31). In some neurons, currents evoked at the level of thermal nociception (\( \approx 42^\circ \text{C} \)) can also be mimicked by capsaicin (7, 23, 27), the noxious ingredient of hot peppers that can elicit a similar thermal sensation and can be partially blocked by antagonists for capsaicin binding (32). This feature has allowed investigators to isolate a temperature-responsive ion channel, TRPV1, by expression cloning (7) and identify a cold-sensitive channel by similar methods (25). Homology screening has revealed a number of temperature-sensitive channels in the transient receptor potential (TRP) family of integral membrane proteins that respond over a broad range of temperatures (7, 13, 25, 39). Although neither the pit organ (26) nor our \( I_{\text{AT}} \)-expressing TG neurons show capsaicin sensitivity, this does not preclude that \( I_{\text{AT}} \) could arise from a TRPV1 homolog or ortholog, as shown within chicken (18) and bullfrog (21) DRG neurons, respectively. The similarity of some temperature-response characteristics of \( I_{\text{AT}} \) to those of members of the TRP family suggests that \( I_{\text{AT}} \) may arise from a single, temperature-gated ion channel homologous to one of the TRP proteins. \( I_{\text{AT}} \) shows a similar current-voltage relationship both in terms of its RP and its rectification (7, 13, 39).
In stark contrast to the TRP channels, our data indicated that $I_{\Delta T}$ showed no Ca$^{2+}$ permeability. TRP channels, including the temperature-activated family members, show very high Ca$^{2+}$ permeability (7, 13, 25, 39). We believe that because a snake’s body temperature is often above the threshold of $I_{\Delta T}$, the current would be active a great deal of time and high Ca$^{2+}$ permeability could result and Ca$^{2+}$ induced toxicity like that found for TRPV1 (19). This does not completely rule out a role mutants of TRPV1 have been shown to have altered Ca$^{2+}$ detection in snakes.

Our investigations suggest that thermosensation at the pit organ may, in part, be mediated by $I_{\Delta T}$, but the behavioral data suggest that the cellular and molecular entities that mediate these processes may be more complex. Thermosensation is probably a complex integrated product of multiple temperature-sensitive currents, possessing unique temperature activation profiles and activation kinetics. The currents we recorded in these TG neurons may represent only part of the complete array of thermosensitive currents in the pit organ. Our continued investigations will focus on elucidating details such as sensitivity and response time of $I_{\Delta T}$ to allow us to further characterize the contribution of $I_{\Delta T}$ to highly sensitive thermodetection in snakes.

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GRANTS

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