Halothane Modulates Thermosensitive Hypothalamic Neurons in Rat Brain Slices

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Background: In vitro, halothane alters spontaneous firing in and thermosensitivity of neurons in the preoptic region of the anterior hypothalamus. To better understand the mechanisms by which halothane specifically disrupts normal thermoregulatory function this investigation examined the effects of halothane on thermosensitive preoptic region neurons in isolated hypothalamic tissue slices.

Methods: Brain slices were obtained and prepared from Sprague-Dawley rats. Preoptic region neurons were characterized by extracellular recording of spontaneous firing rates and thermosensitivity to localized heating and cooling, before, during, and after halothane equilibrated in the perfusate and carrier gas.

Results: One hundred sixteen neurons were characterized by their thermosensitivity as: 29% warm-sensitive (>-0.8 spikes s⁻¹ °C⁻¹); 14% cold-sensitive (<-0.6 spikes s⁻¹ °C⁻¹); and 57% temperature-insensitive. Halothane significantly reduced the spontaneous firing rates to 64% of control and the thermosensitivity to 55% of control for warm-sensitive neurons at 1% halothane. Halothane significantly reduced the spontaneous firing rate of cold-sensitive neurons to 24 and 40% of control, and the thermosensitivity to 61 and 36% of control at 0.5, and 1% halothane, respectively. Spontaneous firing rates and thermosensitivity returned toward control values in warm-sensitive neurons (92 and 122% of control, respectively) after discontinuation of halothane, which did not occur in cold-sensitive neurons (49 and 36% of control, respectively). Halothane did not alter the thermosensitive temperature range or the set point temperature at which neurons became most thermosensitive. Halothane also did not affect the firing rates of temperature-insensitive neurons.

Conclusions: Halothane alters the firing rate and thermosensitivity of individual temperature-sensitive neurons in in vitro slices of the preoptic region of the anterior hypothalamus in the absence of afferent modulation. This disruption may result in an impairment of thermoregulatory responses locally within the preoptic region, to thermal challenges and represents a potential mechanism by which halothane widens the thermoregulatory range. (Key words: Anesthetics, volatile; halothane. Brain: anterior hypothalamus; brain slices; preoptic region. Single-unit activity, temperature; thermoregulation.)

VOLATILE anesthetics disrupt thermoregulation and diminish autonomic and behavioral responses to thermal challenges. Alterations in thermal homeostasis produced by the volatile agents interfere with both heat loss and heat gain, and may profoundly affect cardiovascular, musculoskeletal, renal, and respiratory systems as well as coagulation pathways.¹,² Multiple sites within the central nervous system (CNS) are involved in normal thermoregulation. Peripheral and deep thermal receptors converge on the neural substrates of the spinal cord and higher CNS thermoregulatory areas, and are essential to the physiologic processing of thermal input and initiation of appropriate thermoregulatory responses.³ However, specific neurons in the preoptic region of the anterior hypothalamus appear to be most intimately involved at the highest CNS level in the initiation and maintenance of a normal thermoregulatory response.⁴ A hierarchical model has been proposed in which phylogenetically older centers subserve newer, more precise regions and signal processing occurs suc-
cessively in the spinal cord, mid-brain, and hypothalamus. Disrupting normal preoptic region neuronal function alters the extent of thermoregulatory responses. Partial ablation of these neurons attenuates the magnitude of a response to a thermal challenge, whereas complete ablation of these neurons severely blunts normal thermoregulatory responses. Local preoptic region heating increases body heat loss through various mechanisms including vasomotor adjustments, sweating, and respiratory and behavioral alterations. Similarly, local preoptic region cooling results in decrease in the rate of body heat loss or increase in the rate of heat production by vasomotor alterations and the initiation of shivering. Previous studies from this laboratory have demonstrated that in chronically instrumented cats, volatile anesthetics produce an improvement in the control of thermoregulatory responses at the level of the anterior hypothalamus at the preoptic region. Halothane, isoflurane, and enflurane abolished normal thermoregulatory responses to preoptic region thermal challenges and preoptic region heating and cooling was found to appropriately modulate postanesthetic shivering. The presence of specific preoptic region neurons that alter firing rates and patterns in response to local thermal challenges is well established. While the ability of anesthetics to alter normal thermoregulation is well documented, only a previous study from this laboratory has specifically examined the effects of volatile anesthetics on single thermosensitive neurons in the CNS. Poterack and coworkers demonstrated that halothane decreases both the spontaneous firing rate and thermosensitivity of warm-sensitive preoptic region neurons in cats anesthetized with chloralose and urethane. However, the effects of the baseline anesthetics and the confounding influence of potent afferent thermal input from peripheral or lower CNS sites was not eliminated. Studies using preoptic region tissue slice preparations have confirmed the existence of distinct populations of warm- and cold-sensitive and temperature-insensitive neurons. Subtypes of preoptic region neurons differ in terms of morphology, intracellular second messengers, synaptic dependency, and ionic conductances. There are no previous studies examining the effects of halothane on these preoptic region subpopulations.

The purpose of the current investigation was to examine the effects of graded concentrations of halothane on firing rates and thermosensitivity of temperature-sensitive and temperature-insensitive preoptic region neurons in tissue slices, while eliminating confounding thermal afferent modulation and additional baseline anesthetics. In addition, the differential effects of halothane on warm-sensitive and cold-sensitive neurons was examined.

Materials and Methods

Experimental procedures were approved by the Medical College of Wisconsin Animal Use and Care Committee, and protocols were completed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals of the American Physiological Society and in accordance with National Institutes of Health guidelines. All animals were housed within the animal facilities of the Medical College of Wisconsin, accredited by the American Association for the Accreditation of Laboratory Care.

General Preparation

Male Sprague-Dawley rats, weighing 250–325 g, with no significant weight differences between the groups, were used in these experiments. Many of the techniques used in this tissue slice preparation are similar to those described by Hatton et al. Rats were placed in a chamber and lightly anesthetized with halothane (less than 1% on vaporizer dial), decapitated, and their brains were quickly removed and rinsed with nutrient medium of the following composition (in mM): NaCl 124, KCl 5, CaCl₂ 2.4, MgCl₂ 1.3, glucose 10, KH₂PO₄ 1.24, NaHCO₃ 26, gaseous equilibration with 95% O₂ and 5% CO₂, pH 7.4, 300 mOsm/kg. Nutrient medium was prepared daily and all electrophysiologic recordings were performed on the same day as tissue slices preparation. Brains were cut freehand to small blocks containing the ventrostral forebrain. Coronal oriented tissue slices (300-μm thick) containing the preoptic region were sectioned with a vibratome mechanical tissue slicer. Typically, two tissue slices containing both the anterior commissure and the optic chiasm, in addition to at least one slice immediately caudal and/or rostral were evaluated for recordings. During the slicing procedure, tissue remained bathed in the oxygenated nutrient medium at or slightly below room temperature. Time from decapitation to placement in nutrient medium was less than 5 min. Subsequently, the slices were transferred to a thin nylon mesh, which rested on the methyl methacrylate polymer slice chamber (fig. 1).
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Fig. 1. Schematic representation of the brain slice preparation used. See text for specific details.

The slice chamber employed in these studies was a Hass type chamber (Medical Systems, Greenvale, NY), physically modified after the method of Kelso et al.14 With this modification, selective and precise thermal challenges of very small portions of the neuronal tissue being concurrently used for extracellular neuronal recording may be performed. The center recording chamber containing the slices was surrounded by an outer, temperature-controlled water bath to provide a humidified oxygenated atmosphere above the slice. Slice temperature was maintained at 37°C by autoregulation heating of the water bath and perfusion by a sensing thermistor throughout the experiment. Slices were maintained in this chamber, continuously perfused with the oxygenated nutrient medium for 1–2 h before the initiation of the experimental protocol to allow for neuronal recovery after decapitation and slicing. The nutrient medium flowed at a rate of 1.5–3.0 ml/min, under and around the tissue slices, which allowed the volume of the slice chamber to be completely exchanged 2–3 times/min by switching the perfusion medium via solenoid controlled valves. Filter paper was placed at the edge of the nylon mesh to direct the perfusion medium out of the chamber. Precise, rapid, local preoptic region tissue temperature was controlled by passing water through a thermoelectric Peltier device (Melcor, Trenton, NJ) coupled to the thermode cannula. The thermode cannula was located within the perfusion chamber methyl methacrylate polymer base immediately below the preoptic region of the brain slice (as modified and described by Kelso et al.14) This allowed local, precise cooling and
heating of the preoptic region neurons without altering
the temperature of the perfusion medium or the sur-
rounding areas of the slice. A complete heating and
cooling thermal challenge was performed over ap-
proximately 10 min.

After the slices had equilibrated, single-unit neuronal
activity was recorded with 1- to 2-μm tip, glass carbon
and filament microelectrodes or 10-μm tip polytetra-
fluorethylene-insulated microelectrodes. Electrodes
were mechanically advanced (Burleigh Instruments,
Fishers, NY) within tissue slices at 5-20-μm increments
until a consistent single extracellular single unit was
identified. Unit activity was sequentially amplified at
10-20,000 times by alternating current coupled ampli-
fiers. A microthermocouple was inserted into the
slice tissue for precise measurement of local temper-
ature at the single unit recording site. Amplified elec-
trical activity was displayed on an oscilloscope and
monitored by a speaker. Voltage and time/voltage win-
dow discriminators were used for single-unit isolation
and elimination of background noise and the subse-
quent window discriminator directed to an 8-bit fre-
quency to voltage converter to generate a spike his-
togram. Halothane concentrations in the artificial cere-
brospinal fluid bath were intermittently sampled and
measured with the use of a Perkins Elmer (Norwalk,
CT) model Sigma 3B gas chromatography system.

**Experimental Protocol**

After single neuronal identification, specific heating and
cooling of the preoptic region (30–40°C) was performed
as described earlier, and control measurements of firing
rates at 36°C and throughout the thermal challenge were
recorded. Graded concentrations of halothane (0.25%,
0.5%, and 1.0%) were introduced into the perfusion me-
dium and into the humidified oxygenated environment
immediately surrounding the slice chamber. After intro-
duction of halothane or any change in concentration, 30
min was allowed for equilibration. Preliminary experi-
ments in this system demonstrated that 15–22 min was
required to achieve steady-state halothane concentrations
within the slice chamber. After halothane equilibration,
heating and cooling temperature curves were again per-
formed and firing rates recorded. At the end of the ex-
periments, halothane was discontinued for 30–45 min
and temperature curves again repeated and the results
quantified.

**Data Analysis**

All data were recorded on FM tapes using a Vetter
tape deck (Rebersburg, PA). In addition to the raw
spike firing rate, integrated firing rate, spike histogram,
and tissue slice temperature were recorded continu-
onously on a polygraph. Data were subsequently analyzed
on an IBM-compatible microcomputer equipped with
a Metrabyte Dash-16 12-bit analog-to-digital convertor
and Asyst analysis software (Keithly Metrabyte, Taun-
ton, MA) developed in this laboratory. Thermosensitiv-
ity was calculated as impulses or spikes · s⁻¹ · °C⁻¹.
Neurons were classified as warm-sensitive; generally
increasing firing rates with increasing temperature
(>0.8 spikes · s⁻¹ · °C⁻¹), cold-sensitive; generally in-
creasing firing rates with decreasing temperature (<0.6
spikes · s⁻¹ · °C⁻¹) and temperature-insensitive based on
well-accepted criteria. After establishing thermo-
sensitive neuronal responses, linear regression was de-
termined over the most thermosensitive temperature
range. Thermosensitivity was determined over a 2–5°C
temperature range which included 37°C during the con-
trol, prehalothane conditions. Linear regression was
used to analyze and describe the thermosensitivity for
all neurons evaluated. Although several units exhibited
more nonlinear curves throughout the entire temper-
range tested, complex computer-assisted analysis of
these various order exponential and logarithmic regres-
sions, although more precisely descriptive of firing
patterns, resulted in data that were difficult to av-
verage and made interneuronal comparisons impossible.
Therefore, whereas many units were analyzed in this
fashion, the data are not presented here because of their
complexity. Linear regression curves were performed
over a 2–5°C temperature range in which these neurons
exhibited their greatest thermosensitivity. The specific
range was determined by computer regression analysis
of multiple temperature ranges before determination of
the maximum thermosensitive range. Linear regres-
sion correlation (R value) of >0.6 was required to be
considered temperature-sensitive. During each exper-
imental condition, linear response curves were the re-
sult of 2 or 3 computerized merged heating and cooling
cycles from an individual neuron. As evaluations were
performed over the most thermosensitive temperature
range, the temperature ranges were not necessarily
consistent between experimental conditions, i.e., con-
tral or halothane concentrations. Therefore, the
threshold temperatures were also recorded and com-
pared between experimental conditions. These thresh-
old temperatures represent the lowest temperature at
which a warm-sensitive neuron entered the most ther-
mosensitive range and the highest temperature at which
a cold-sensitive neuron entered the most thermosen-

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sitive range. All thermosensitivity calculations were
determined by 2 or 3 evaluators, one of whom was
blinded to the experimental conditions.

Spontaneous firing rate at 36°C, firing rate at 37°C,
maximum firing rate within the most thermosensitive
temperature range, and threshold temperatures, were
calculated as a mean of 2 or 3 samples, for each neuron,
for each experimental condition. The spontaneous fir-
ing rate at 36°C was obtained by an average of 5–7
samples of 10-sec counts on a ratemeter from the win-
dow discriminator output, while the firing rate at 37°C
and the maximum firing rate within the most ther-
mosensitive temperature range were obtained by graphic
analysis of the linear regression curves.

The millimolar concentrations of halothane measured
in the bath were converted to equivalent partial pres-
sures in the solution and expressed as percentages of
the volatile agent in the gas phase15 (table 1).

**Table 1. Concentrations of Halothane**

<table>
<thead>
<tr>
<th>Vaporizer Setting (%)</th>
<th>Concentration in Bath (mm)</th>
<th>Calculated Partial Pressure (vol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.08 ± 0.01</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>0.5</td>
<td>0.18 ± 0.01</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>1.0</td>
<td>0.35 ± 0.01</td>
<td>1.12 ± 0.05</td>
</tr>
<tr>
<td>0% (after halothane)</td>
<td>0.01 ± 0.01</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 23. Halothane concentrations as set by vaporizer
dial, in bath (measured by gas chromatography) and calculated partial pressures
in the solution (percent of volatile agent in the gas phase). Pilot data had previously
shown that 30 min was sufficient for equilibration at a particular halothane con-
centration.

either lost before a temperature change was completed
or their response was not repeatable on successive
temperature challenges. Of the 116 characterized neu-
rons, 34 (29%) were warm-sensitive, 16 (14%) were
cold-sensitive, and 66 (57%) were temperature-insen-
sitive. Of these 116 neurons, 13 warm-sensitive, 6 cold-
sensitive, and 5 temperature-insensitive neurons were
successfully challenged with halothane and completed
the experimental protocol.

**Warm-sensitive Neurons**

Successful experiments were completed with haloth-
ane in 13 warm-sensitive neurons. The control spont-
aneous firing rate at 36°C of the warm-sensitive neu-
rons was 11.2 ± 2.1 Hz and the firing rate at 37°C,
(measured graphically from the linear regression
curve), was 12.9 ± 2.0 Hz (table 2). Responses to
thermal challenges varied from linear over the entire
temperature range (31–41°C) to biphasic increases,
with threshold temperatures (initiation of the most
thermosensitive temperature range) commonly at
35.5–36.5°C. Administration of 0.25% halothane did
not change the spontaneous firing rate at 36°C (fig. 2)
or the firing rate at 37°C (table 2). Increasing concen-
trations of halothane resulted in firing rates of 84% ±
11 at 36°C; 83% ± 15 at 37°C (0.5% halothane) and
64% ± 12 (P < 0.05) at 36°C; and 58% ± 12 (P <
0.05) at 37°C (1% halothane) as % of control. The
firing rates returned to 92–95% of control levels after
halothane was discontinued.

The temperature range in which warm-sensitive neu-
rons were most thermosensitive was 35.8 ± 0.3°C to
38.6 ± 0.4°C at control. Neither the temperature range
nor the threshold temperature (lowest temperature
within the most thermosensitive temperature range)
was significantly altered by halothane administration. The maximum firing rate within the thermosensitive temperature range evaluated (19.6 ± 2.6 Hz at control) was significantly decreased to 66 ± 10% of control at 1% halothane, and returned to 94 ± 9% of control after discontinuation of halothane administration. The calculated thermosensitivity of the warm-sensitive units before halothane administration was 2.9 ± 0.5 spikes·s⁻¹·°C⁻¹ (fig. 3). Thermosensitivity was significantly attenuated (55 ± 16% of control) by halothane administration only at the highest concentration used (1%). After discontinuation of halothane, thermosensitivity returned to control (122 ± 26% of control). Figure 4 shows representative responses to thermal challenges in a single warm-sensitive neuron.

**Cold-sensitive Neurons**

Successful experiments were completed with halothane in six cold-sensitive neurons. The control spontaneous firing rate at 36°C of the cold-sensitive neurons was 11.9 ± 2.6 Hz and the firing rate at 37°C was 9.0 ± 2.9 Hz (table 3). Responses to thermal challenges varied from linear (2 of 6) over the entire temperature range (31–41°C) to previously well-described, bell-shaped curves with the greatest thermosensitivity above 36°C. Administration of 0.25% halothane did not significantly affect the spontaneous firing rate at 36°C or the firing rate at 37°C. However, further increases in halothane decreased firing rates to 24 ± 9% at 36°C (0.5% halothane) and 40 ± 18% at 36°C (1% halothane) of control (table 3, fig. 2). In contrast to the recovery of firing in warm-sensitive neurons on discontinuation of halothane, the spontaneous firing rate of cold-sensitive neurons only returned to 49 ± 11% of control 30 min after discontinuation of halothane.

The temperature range in which cold-sensitive neurons were most thermosensitive was 36.5 ± 0.5°C to 38.8 ± 0.4°C at control. As observed with the warm-sensitive neurons, neither the most thermosensitive temperature range nor the threshold temperature

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**Table 2. Effects of Halothane on Warm-sensitive Neurons**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low temp (°C)</td>
<td>35.8 ± 0.3</td>
<td>36.0 ± 0.5</td>
<td>35.9 ± 0.4</td>
<td>38.5 ± 0.3</td>
<td>36.1 ± 0.4</td>
</tr>
<tr>
<td>High temp (°C)</td>
<td>38.6 ± 0.4</td>
<td>38.9 ± 0.5</td>
<td>38.7 ± 0.4</td>
<td>38.5 ± 0.3</td>
<td>38.5 ± 0.4</td>
</tr>
<tr>
<td>SFR at 36°C (spikes/s)</td>
<td>11.2 ± 2.1</td>
<td>10.5 ± 2.0</td>
<td>9.2 ± 1.8</td>
<td>6.5 ± 1.8*</td>
<td>10.3 ± 3.3</td>
</tr>
<tr>
<td>FR at 37°C (spikes/s)</td>
<td>12.9 ± 2.0</td>
<td>13.7 ± 2.3</td>
<td>10.7 ± 2.1</td>
<td>7.4 ± 1.8*</td>
<td>12.5 ± 3.3</td>
</tr>
<tr>
<td>MFR (spikes/s)</td>
<td>19.6 ± 2.6</td>
<td>21.0 ± 2.7</td>
<td>17.9 ± 2.9</td>
<td>12.1 ± 2.0*</td>
<td>18.2 ± 3.6</td>
</tr>
<tr>
<td>Thermosensitivity</td>
<td>2.9 ± 0.5</td>
<td>2.8 ± 0.5</td>
<td>1.9 ± 0.4</td>
<td>1.2 ± 0.3*</td>
<td>2.6 ± 0.4</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Post = 30–45 min post halothane discontinuation; Low temp and High temp = lowest and highest temperature, respectively, in most thermosensitive temperature range; FR = firing rate; SFR and MFR = spontaneous and maximum firing rate, respectively.

*P < 0.05 versus control (n = 13). Note the decrease in FR at 36°C and 37°C, MFR, and thermosensitivity during 1% halothane administration. There is no change in the thermosensitive range or threshold temperature (low temp).

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(highest temperature within the thermosensitive temperature range) was significantly altered by the administration of halothane. The maximum firing rate within the thermosensitive temperature range evaluated was 20.5 ± 2.9 Hz at control. Halothane administration significantly decreased the maximum firing rate (expressed as % of prehalothane control to 54 ± 7% (P < 0.05), and 61 ± 17% (P < 0.05) at 0.5, and 1% halothane, respectively. Thirty minutes after discontinuing halothane, the maximum firing rate did not return toward control values and remained significantly attenuated at 59 ± 15% of control. The average calculated thermosensitivity during control conditions of the cold-sensitive units was −5.3 ± 0.8 spikes·s⁻¹·°C⁻¹. Maximum thermosensitivity was blunted by all concentrations of halothane administered (61 ± 12% and 36 ± 9% of control at 0.5, and 1% halothane, respectively) (fig. 3). After discontinuation of halothane, thermosensitivity did not recover and remained significantly decreased from control values (36 ± 7% of control). Figure 5 illustrates representative responses to thermal challenges in a single cold-sensitive neuron.

Temperature-insensitive Neurons

Halothane was administered to five neurons classified as temperature-insensitive, which subsequently completed the experimental protocol. The effects of halothane on various firing rate and thermosensitivity parameters in temperature-insensitive neurons are found in table 4. The spontaneous firing rate of these neurons at 36°C during control conditions was similar to the firing rate observed at 37°C during a thermal challenge (4.1 ± 0.6 Hz and 3.7 ± 0.6 Hz, respectively). Maximum firing rate during prehalothane control was 7.2 ± 1.1 Hz and varied both between and within neurons as to the temperature at which the maximum firing rate occurred. The maximum firing rate was not associated with specific temperatures in this subset of neurons, but rather was a random occurrence. Administration of increasing concentrations of halothane did not significantly affect the thermosensitivity of these cells. In addition, halothane administration did not significantly modulate the spontaneous or maximum firing rate (table 4) of temperature-insensitive neurons during a thermal challenge.

Discussion

Temperature-sensitive neurons exist in the preoptic region of the anterior hypothalamus and are intimately involved in thermoregulatory functions. The role of the anterior hypothalamus in thermoregulation is well documented. Previous investigations have demonstrated a system in which a subpopulation of these neurons respond both to extrahypothalamic thermal challenges and, appropriately, to the administration of a variety of pharmacologic agents. These findings have led to the widely accepted concept that an essential role for these neurons is the initiation and modulation of thermoregulatory responses. The current investigation demonstrates that the volatile anesthetic, halothane, significantly alters both the spontaneous and maximum firing rate and the thermosensitivity of single, isolated temperature-sensitive preoptic region neurons in an in vitro preparation without afferent modulation. The alterations in thermosensitivity by halothane were not accompanied by modifications in the threshold temperatures or thermosensitive temperature range. In addition, halothane differentially affects warm-sensitive and cold-sensitive neurons and only minimally alters firing of temperature-insensitive neurons in the preoptic region of the anterior hypothalamus.

It is widely believed that volatile anesthetics, including halothane, produce a poikilothermic state in which
cision in thermoregulatory responses. Farber et al. have shown that volatile anesthetics abolish the thermoregulatory responses that typically occur during heating and cooling of the preoptic region in chronically prepared cats. Also, postanesthetic shivering during emergence was attenuated or augmented by preoptic region heating or cooling, respectively. Previous investigations have shown that general anesthetics disrupt normal thermoregulatory responses by inhibiting shivering and sweating, attenuating peripheral vasoconstriction, disrupting basal metabolism and altering behavioral thermoregulation. However, only one previous study has attempted to delineate the actions of halothane specifically on the CNS neurons that mediate and modulate these thermoregulatory responses. Poterack et al. examined the direct effects of halothane on thermosensitive neurons in the preoptic region of cats anesthetized with α-chloralose and urethane. Halothane administration produced progressive decreases in spontaneous firing rate and calculated thermosensitivity of preoptic region, warm-sensitive neurons without changing the periodicity of firing. These findings supported those of multiple clinical studies demonstrating the disrupting effects of volatile anesthetics on thermoregulation and provided the first evidence that these alterations are also observed in thermosensitive units within the preoptic region. However, in that previous study, possible confounding effects of additional baseline anesthetics and the extrahypothalamic afferent thermal and nonthermal input into the CNS or on other thermoregulatory centers did not allow definitive conclusions to be drawn about the specific effects of halothane directly on these thermosensitive neurons. In addition, only one cold-sensitive neuron was completely evaluated in that study and thus differential effects on warm and cold-sensitive neurons could not be evaluated.

To evaluate the direct role of halothane on preoptic region neurons, the current studies were performed in tissue slices devoid of distant afferent inputs, yet the neurons remained embedded in and affected by their normal microenvironment (neuronal and glial framework). This technique obviates the need for additional baseline anesthetics and allows precise localization of brain regions and subsequent placement of recording electrodes, which is not possible with in vivo studies. Numerous investigations have demonstrated that hypothalamic tissue slices contain the same proportions of warm-sensitive, cold-sensitive, and temperature-insensitive neurons as those recorded in intact animals.
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Table 3. Effects of Halothane of Cold-sensitive Neurons

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low temp (°C)</td>
<td>36.5 ± 0.5</td>
<td>36.6 ± 0.7</td>
<td>35.7 ± 0.7</td>
<td>36.2 ± 0.9</td>
<td>35.6 ± 0.9</td>
</tr>
<tr>
<td>High temp (°C)</td>
<td>38.8 ± 0.4</td>
<td>39.1 ± 0.5</td>
<td>37.8 ± 0.8</td>
<td>38.5 ± 0.9</td>
<td>38.0 ± 0.6</td>
</tr>
<tr>
<td>SFR at 36°C (spikes/s)</td>
<td>11.9 ± 2.6</td>
<td>8.9 ± 0.1</td>
<td>2.2 ± 0.7*</td>
<td>4.5 ± 2.2*</td>
<td>5.2 ± 1.9*</td>
</tr>
<tr>
<td>FR at 37°C (spikes/s)</td>
<td>9.0 ± 2.9</td>
<td>10.8 ± 1.9</td>
<td>2.6 ± 1.2*</td>
<td>5.0 ± 3.2</td>
<td>6.9 ± 3.1</td>
</tr>
<tr>
<td>MFR (spikes/s)</td>
<td>20.5 ± 2.9</td>
<td>16.0 ± 1.2</td>
<td>12.2 ± 3.4*</td>
<td>11.8 ± 2.8*</td>
<td>12.1 ± 3.5</td>
</tr>
<tr>
<td>Thermosensitivity (spikes - s⁻¹ - °C⁻¹)</td>
<td>-5.3 ± 0.8</td>
<td>-3.8 ± 0.1</td>
<td>-2.8 ± 0.5*</td>
<td>-2.0 ± 0.6*</td>
<td>-2.0 ± 0.5*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.
Post = 30–45 min post halothane discontinuation; Low temp and High temp = lowest and highest temperature, respectively, in most thermosensitive temperature range; FR = firing rate; SFR and MFR = spontaneous and maximum firing rate, respectively.

* P < 0.05 versus control (n = 6). Note the decrease in FR at 36°C and 37°C, MFR, and thermosensitivity during 0.5 and 1% halothane administration. Note also the poor recovery after halothane discontinuation.

While preoptic region neurons receive extensive thermal afferent input, they have also been shown to have an integral role in osmolar, glucose, steroid regulation, and cardiovascular pressure/volume responses. Further, it is not only the thermosensitive neurons that interact with other regulatory systems, but nearly half of the thermosensitive neurons respond to nonthermal stimuli. As an integrative structure important in the control of several homeostatic systems including body temperature regulation, and fluid and metabolite balance, the direct effects of volatile anesthetics on the preoptic region is highly consequential. The actions of halothane on preoptic region neurons are thus important both in terms of mechanisms of thermoregulatory control as well as a potential model for cellular actions of volatile anesthetics on other regulatory or neuronal functions that may be more difficult to examine in vitro.

Three populations of preoptic region neurons were identified in this study using well-accepted criteria: warm-sensitive, cold-sensitive, and temperature-insensitive. Further subsets of thermosensitive neurons have been previously characterized, including primary preoptic region thermoreceptors with linear relationships between firing rate and temperature, those with thermosensitivity above or below a certain threshold, and those with bell-shaped thermosensitive curves. While all of these types of firing responses were observed in the current investigation, there were too few in each classification to allow evaluation of the differential effects of halothane on these subpopulations of preoptic region neurons. The results of the current study demonstrate that halothane differentially disrupts warm and cold neuronal thermosensitivity, in that cold-sensitive neurons were more susceptible to the effects of lower halothane concentrations.

It has been suggested that neurons with the highest firing rates are most thermosensitive in the hypothermic range, may be most influenced by extrahypothalamic temperature changes and are primarily responsible for heat production responses. In contrast, peripheral temperatures may have limited influence on heat-loss responses, which are regulated by hypothalamic neurons receiving relatively little synaptic input or the input is primarily inhibitory in nature. While a single central thermoregulatory integrator with multiple inputs and output may exist, a multiple thermostat system, in which warm and cold afferent information elicits responses from separate neural networks, has been postulated. Within this framework, warm-sensitive neurons would subserve heat loss mechanisms while cold-sensitive neurons may modulate heat gain mechanisms. Several studies have shown that the local application of various pharmacologic agents results in changes in the activity of thermosensitive preoptic region neurons that are appropriate to those observed in whole body thermoregulatory responses. Microinjection of a variety of pyrogens into the preoptic region attenuates heat loss and augments heat production responses. Similarly, local application of these substances inhibited warm-sensitive neuronal firing and excited cold-sensitive preoptic region neurons. Conversely, substances such as serotonin and Δ⁹-tetrahydrocannabinol, which evoke hypothermic responses in terms of whole body thermoregulation, increased the thermosensitivity of heat-sensitive neurons.
while diminishing both the thermosensitivity and spontaneous firing of cold-sensitive neurons.\textsuperscript{30–32}

Results of the current investigation support the findings of Poterack et al.\textsuperscript{9} that halothane attenuates the thermosensitivity and spontaneous firing rate of warm-sensitive preoptic region neurons in intact anesthetized animals. Also, the current finding that cold-sensitive neurons are more sensitive to the inhibitory effects of halothane suggests a mechanism for the clinical observation that warm defense thresholds may not decrease to the same extent as cold defense thresholds.\textsuperscript{19} Similarly, the halothane-mediated attenuation of both cold- and warm-sensitive neuronal firing and thermosensitivity may be associated with the widening of the interthreshold thermoregulatory range seen clinically during the administration of the volatile anesthetics.\textsuperscript{19,33} However, several clinical studies have demonstrated that although halothane disrupts threshold temperatures, thermosensitivities and maximum response intensities remain relatively well preserved.\textsuperscript{33,34} The reason for the differences between those of Sessler and colleagues and those of the current study are unclear, but may be related to the lack of distant afferent input in our study, the possibility that the altered thermosensitivity (gain) and firing rates in isolated neurons does correlate with a wider threshold range in an intact organism, or that thermomodulatory changes by halothane are also mediated at centers other than the anterior hypothalamus.\textsuperscript{8} Halothane has been shown to dose-dependently attenuate thermoregulatory responses to spinal cord cooling in brain-stem-transected cats,\textsuperscript{8} suggesting a spinal cord or lower brain stem site of thermomodulatory action. Studies have previously demonstrated convergence of thermal information at mid-brain and medullary areas where thermoregulatory, vasomotor, respiratory, and somatic responses may be initiated and/or integrated.

Neuronal thermosensitivity may be either an intrinsic excitability or a characteristic dependent on local synaptic circuits. While the firing rate of some warm-sensitive preoptic region neurons is inhibited by synaptic blockade, the thermosensitivity of all warm-sensitive preoptic region neurons is preserved, suggesting that these neurons possess an intrinsic thermosensitivity. In contrast, cold-sensitive preoptic region neuronal thermosensitivity was abolished during synaptic blockade, indicating that cold sensitivity may be due to local synaptic input and inhibition from local warm-sensitive neurons.\textsuperscript{11,35} The differential effects of halothane on these neuronal subpopulations may be related to these specific characteristics. Analyses of thermosensitive neurons in other diencephalic regions have shown that populations of both intrinsically and synaptically mediated warm- and cold-sensitive neurons exist.\textsuperscript{35}
HALOTHANE AND THERMOSENSITIVE NEURONS

Table 4. Effects of Halothane on Thermoinensitive Neurons

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low temp (°C)</td>
<td>35.0 ± 0.6</td>
<td>34.7 ± 0.8</td>
<td>35.5 ± 0.4</td>
<td>35.7 ± 0.2</td>
<td>35.7 ± 0.3</td>
</tr>
<tr>
<td>High temp (°C)</td>
<td>38.8 ± 0.3</td>
<td>38.6 ± 0.5</td>
<td>38.8 ± 0.3</td>
<td>38.3 ± 0.5</td>
<td>38.7 ± 0.5</td>
</tr>
<tr>
<td>SFR at 36°C (spikes/s)</td>
<td>4.1 ± 0.6</td>
<td>3.7 ± 0.8</td>
<td>3.5 ± 0.6</td>
<td>3.2 ± 0.9</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>FR at 37°C (spikes/s)</td>
<td>3.7 ± 0.6</td>
<td>3.0 ± 0.8</td>
<td>3.5 ± 0.6</td>
<td>3.3 ± 1.3</td>
<td>4.1 ± 1.1</td>
</tr>
<tr>
<td>MFR (spikes/s)</td>
<td>7.2 ± 1.1</td>
<td>6.4 ± 1.3</td>
<td>6.4 ± 1.3</td>
<td>6.2 ± 1.9</td>
<td>6.9 ± 1.6</td>
</tr>
<tr>
<td>Thermosensitivity (spikes·s⁻¹·°C⁻¹)</td>
<td>-0.1 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.3</td>
<td>0.0 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Post = 30–45 min post halothane discontinuation; Low temp and High temp = lowest and highest temperature, respectively, in temperature range evaluated; FR = firing rate; SFR and MFR = spontaneous and maximum firing rate, respectively.

* P < 0.05 versus control (n = 5). Note the lack of effect of halothane on FR, SFR, MFR, and thermosensitivity.

Previous findings using extracellular recordings have been confirmed with intracellular analysis of warm- and cold-sensitive neurons. 36 Warm-sensitive neurons exhibit a slow, depolarizing temperature-dependent prepotential, which disappeared during hyperpolarization, suggesting intrinsic thermosensitivity. Indeed, a halothane-mediated hyperpolarization 37,38 may be the mechanism for the attenuation of thermosensitivity. Activity of cold-sensitive preoptic region neurons correlated with excitatory and inhibitory postsynaptic potentials, suggesting synaptic-dependent thermosensitivity. Such an interpretation, that cold-sensitive, preoptic region neurons represent synaptically mediated interneurons, is supported by the observation that halothane produced greater and more prolonged depressant effects on these cells, as compared to effects on warm-sensitive neurons, in as much as halothane has been previously demonstrated to attenuate synaptic transmission in some but not all, neuronal models. 39

The prolonged effects of halothane on cold-sensitive neurons, and thus an attenuation of heat gain rather than heat loss responses, may be related to the findings of Sessler and coworkers that thermoregulatory vasoconstriction was sufficient to reduce the magnitude of core cooling, despite a continually decreasing body heat content. 40 If postanesthetic shivering were mediated at the level of the anterior hypothalamus, one would predict a hypersensitivity of cold-sensitive neurons or a prolonged depressant effect on warm-sensitive neurons after discontinuing halothane. In contrast, our results provide further evidence that postanesthetic tremor may arise as a result of either disinhibition of, or direct action on other CNS or spinal cord thermoregulatory sites.

In contrast to the modulation of thermosensitive neurons, halothane did not alter the firing frequency of thermoinsensitive neurons. While some temperature-insensitive neurons may display warm-sensitive characteristics when depolarized, 46 temperature-insensitivity appears to be maintained by hyperpolarization and a Na⁺-K⁺ pump. 41 While the functional importance of temperature-insensitive neurons is poorly understood, early studies suggesting a primary role in modulating nonthermoregulatory homeostatic systems, is unlikely because these neurons do not represent a majority of osmosensitive, glucosensitive, steroid-sensitive, or baro/volume sensitive neurons. 22,30

In summary, various intravenous anesthetics have been found to decrease the thermosensitivity and firing rate of warm-sensitive preoptic region neurons 42 and cold-sensitive neurons in the reticular formation. 43 The results of the current study show that halothane alters the firing rate and thermosensitivity of individual temperature-sensitive preoptic region neurons. This modulation of neuronal sensitivities may result in an imprecision of thermoregulatory responses to thermal challenges. In addition, these findings strongly suggest that effects on preoptic region neurons represent an important mechanism by which volatile anesthetics disrupt normal thermoregulatory responses. Further studies are needed to delineate whether halothane alters the thermosensitivity of these cells by direct cellular action or an effect on neurotransmission.

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