Carbon dioxide and pH effects on temperature-sensitive and -insensitive hypothalamic neurons

Wright CL, Boulant JA. Carbon dioxide and pH effects on temperature-sensitive and -insensitive hypothalamic neurons. J Appl Physiol 102: 1357–1366, 2007. First published November 30, 2006; doi:10.1152/japplphysiol.00303.2006.—The preoptic-anterior hypothalamus (POAH) controls body temperature, and thermoregulatory responses are impaired during hypercapnia. If increased CO2 or its accompanying acidosis inhibits warm-sensitive POAH neurons, this could provide an explanation for thermoregulatory impairment during hypercapnia. To test this possibility, extracellular electrophysiological recordings determined the effects of CO2 and pH on the firing rates of both temperature-sensitive and -insensitive neurons in hypothalamic tissue slices from 89 male Sprague-Dawley rats. Firing rate activity was recorded in 121 hypothalamic neurons before, during, and after changing the CO2 concentration aerating the tissue slice chamber or changing the pH of the solution bathing the tissue slices. Increasing the aeration CO2 concentration from 5% (control) to 10% (hypercapnic) had no effect on most (i.e., 69%) POAH temperature-insensitive neurons; however, this hypercapnia inhibited the majority (i.e., 59%) of warm-sensitive neurons. CO2 affected similar proportions of (non-POAH) neurons in other hypothalamic regions. These CO2 effects appear to be due to changes in pH since the CO2-affected neurons responded similarly to isocapnic acidosis (i.e., normal CO2 and decreased pH) but were not responsive to isohydric hypercapnia (i.e., increased CO2 and normal pH). These findings may offer a neural explanation for some heat-related illnesses (e.g., exertional heat stroke) where impaired heat loss is associated with acidosis.

neuronal thermosensitivity; hypercapnia; isocapnic acidosis; isohydric hypercapnia

THE PREOPTIC AREA and anterior hypothalamus (POAH) plays an important role in thermoregulation. This neural area senses core temperature, integrates central and peripheral thermal information, and evokes a variety of physiological and behavioral thermoregulatory responses that maintain body temperature constant (3, 4, 19, 23). Depending on the species, POAH warming elicits heat-loss responses (e.g., sweating, panting, skin wetting), and POAH cooling elicits responses that promote heat retention (e.g., cutaneous vasoconstriction) and heat production (e.g., shivering and nonshivering thermogenesis). Both in vivo and in vitro electrophysiological studies have identified different POAH neuronal types on the basis of their firing rate responses to changes in hypothalamic temperature. Although the majority of POAH neurons are classified as temperature insensitive, ~20% of the neurons are considered to be warm sensitive because their firing rates significantly increase during warming and decrease during cooling (3, 4, 17, 18). These warm-sensitive neurons not only sense their own temperature but, in addition, they are integrative and receive synaptic afferent inputs from skin and spinal thermal receptive pathways (5). For this reason, some POAH warm-sensitive neurons are thought to be responsible for eliciting heat-loss responses and suppressing heat retention and/or production responses.

Hypercapnia (i.e., increased CO2) and its accompanying tissue acidosis impair thermoregulation (36). When exposed to elevated (e.g., 10%) CO2, rats show significant increases in body temperature in warm environments and significant decreases in body temperature in cool environments (36). Much of this thermoregulatory impairment may be due to the effects of elevated CO2 and reduced pH on hypothalamic neurons controlling heat loss and heat production. While hypercapnia and acidosis often inhibit spontaneous neuronal activity in different parts of the central nervous system (6, 15, 22, 26, 29), previous electrophysiological recordings in anesthetized rats found that warm-sensitive POAH neurons were generally excited by brief periods of CO2 inhalation (38, 40). This excitation, however, appears to be due to afferent input from peripheral chemoreceptors and baroreceptors (39) since extracellular recordings in rat hypothalamic tissue slices found the opposite response; i.e., in vitro POAH neurons tended to decrease their firing rates during perfusions with hypercapnic solutions (28). Because of this disparity in neuronal responses to hypercapnia, further investigation is warranted.

If hypercapnia inhibits POAH warm-sensitive neurons, this could lead to reduced heat-loss responses, producing hyperthermia in warm environments. To examine this possibility, the present study characterized the effect of elevated CO2 on the firing rate activity of neurons in rat hypothalamic tissue slices. Since the POAH plays an important role in thermoregulation, these experiments sought to determine if CO2 has different effects on POAH neurons compared with neurons in other hypothalamic regions. Similarly, since warm-sensitive neurons have putative thermoregulatory roles, the effects of CO2 were compared between warm-sensitive and temperature-insensitive neurons. Finally, since CO2 lowers extracellular and intracellular pH, these experiments sought to understand the mechanisms of neuronal CO2 sensitivity by comparing firing rate responses to both hypercapnia and perfusions having reduced pH.

METHODS

Tissue slice preparation. As in previous studies (8, 17, 18), horizontal hypothalamic tissue slices were prepared from 89 male Sprague-Dawley rats. The mean age of these rats was 48 ± 10 (SD) days old. Each rat was quickly decapitated using a guillotine, according to procedures approved by the National Institutes of Health and the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: J. A. Boulant, Dept. of Physiology and Cell Biology, 201 Hamilton Hall, Ohio State Univ., 1645 Neil Ave., Columbus, OH 43210 (e-mail: boulant.1@osu.edu).

http://www.jap.org
8750-7587/07 $8.00 Copyright © 2007 the American Physiological Society
First published November 30, 2006; doi:10.1152/japplphysiol.00303.2006.
Neuronal thermosensitivity was determined over a minimal 3°C temperature range in which the neuron was most thermosensitive. Figure 2 shows examples of temperature-sensitive and temperature-insensitive neurons recorded in hypothalamic tissue slices. In each case, the left records of Fig. 2 show integrated firing rate (imp/s) during changes in hypothalamic tissue temperature. The graphs on the right of Fig. 2 plot firing rate as a function of temperature, and the slope (m) is the neuronal thermosensitivity or thermal coefficient (imp·s⁻¹·°C⁻¹). As in previous studies (4, 5, 17, 18), neurons were classified as warm sensitive if they had a positive thermal coefficient of at least 0.8 imp·s⁻¹·°C⁻¹ (Fig. 2A) or cold sensitive if they had a negative thermal coefficient of at least −0.6 imp·s⁻¹·°C⁻¹ (Fig. 2D). Using these same criteria, an in vivo study has shown that, unlike temperature-insensitive neurons, most warm- and cold-sensitive neurons receiveafferent inputs from skin and spinal thermal receptors (5).

Moreover, a recent study has demonstrated morphological distinctions in dendritic orientations between warm-sensitive neurons having thermal coefficients greater than +0.8 imp·s⁻¹·°C⁻¹ compared with temperature-insensitive neurons having lower thermal coefficients (18). In the present study, neurons with thermal coefficients less than +0.8 imp·s⁻¹·°C⁻¹ were considered to be temperature-insensitive. Like previous studies (4, 17, 18), temperature-insensitive neurons were further subdivided into two classes: low-slope and moderate-slope temperature-insensitive neurons. Neurons having essentially no sensitivity to temperature were defined as low-slope temperature-insensitive neurons, and their thermal coefficients ranged between +0.2 and −0.2 imp·s⁻¹·°C⁻¹ (Fig. 2C). Moderate-slope temperature-insensitive neurons, however, exhibited some slight thermosensitivity, and their thermal coefficients were less than +0.8 imp·s⁻¹·°C⁻¹ but greater than +0.2 imp·s⁻¹·°C⁻¹ (Fig. 2B). Previous studies indicate that these two types of temperature-insensitive neurons display different responses to drugs that affect the Na-K pump and second messengers, suggesting that there are functional reasons to make distinctions between these subpopulations of temperature-insensitive neurons (4).

Neurons were exposed to experimental conditions either by changing the gas mixtures aerating the recording chamber or by changing the gas/chemical composition of the aCSF perfusion solution. Neuronal responses to hypercapnia were tested by switching the tissue chamber aeration from a control gas mixture (5% CO₂-95% O₂) to a hypercapnic gas mixture (10% CO₂-90% O₂). During this hypercapnia, however, the aCSF flowing into the recording chamber remained “normal” with 26 mM NaHCO₃ (as pH buffer) and bubbled with 5% CO₂-95% O₂. The hypercapnic tests (with 10% CO₂ chamber aeration) typically lasted for 2–6 min before switching the chamber aeration back to the control (5% CO₂-95% O₂) gas mixture. Some neurons were also tested for their responses to different CO₂ and pH conditions. These additional experimental conditions consisted of (1) normal 5% CO₂ but decreased pH (i.e., isocapnic acidosis) and 2) elevated 10% CO₂ but normal pH (i.e., isohydric hypercapnia). For isocapnic acidosis, the perfusion solution was similar to the normal aCSF except 13 mM NaHCO₃ was replaced with equimolar NaCl and gas saturated with 5% CO₂ (O₂, 300 mmol/kgH₂O ± 1%, 13 mM total HCO₃, pH = 7.18–7.19 at 36–37°C). Isocapnic acidosis produced an equivalent acidosis when the pH was compared with control aCSF aerated with 10% CO₂. For isohydric hypercapnia, 19 mM NaCl was replaced with equimolar NaHCO₃ and the solution was gas saturated with 10% CO₂-90% O₂ (300 mmol/kgH₂O ± 1%, 154 mM total HCO₃, pH = 7.50–7.51 at 36–37°C). Experimental perfusions typically lasted 10–15 min before switching back to the control aCSF. The time course for changes in the perfusion media was estimated in initial experiments that measured the pH of the perfused tissue chamber. After switching from control aCSF to isocapnic acidic aCSF, there were no changes in the chamber pH for 50–60 s, which represents the transit time of the dead space volume in the tubing to the chamber. Following this, the chamber pH rapidly decreased and gradually reached steady state in ~7 min.

**Fig. 1.** Example of extracellular single-unit activity recorded in a rat hypothalamic tissue slice during aeration with 95% O₂-5% CO₂. This 3-s record shows action potentials in a preoptic neuron having a firing rate of 4–5 impulses per second (imp/s) at 37°C.
Following each recording, tissue slice coordinates corresponding to the location of the recording electrode (i.e., the location of the recorded neuron) were taken using a dissecting microscope mounted above the recording chamber. These locations were plotted on standardized horizontal slice maps (8), and the corresponding hypothalamic structure was noted.

Data analysis. Unless otherwise indicated, values are expressed as means ± SE. In all comparisons of firing rate, statistical differences were determined by either Student’s t-tests or ANOVA. When appropriate, post hoc Fisher’s least significant difference determined which comparisons were statistically different. Pearson’s χ² analyses were used to compare the proportions of firing rate responses during hypercapnia, isocapnic acidosis, and isohydric hypercapnia. Statistical significance was defined as P < 0.05, and all statistical tests were performed using JMP IN software (SAS Institute).

Mean firing rates (imp/s) were determined over a 1- to 2-min period at 36–37°C and compared to determine the effects of the experimental conditions; i.e., hypercapnia, isocapnic acidosis, or isohydric hypercapnia. The criteria for a change in firing rate were 1) a 10% change in firing rate that was at least 1 imp/s during the experimental conditions and 2) a firing rate response that showed at least a partial return to the baseline firing rate during the control conditions that followed the experimental period. The criteria for firing rate changes minimized sampling biases when neurons with high and low spontaneous firing rates were compared. Neurons with high spontaneous firing rates are much more likely to change their firing rates by 1 imp/s than neurons with low spontaneous firing rates. Alternatively, other laboratories have used a fixed percent change in mean firing rate as the criterion to classify firing rate responses (30, 31). However, there is a sampling bias with this criterion, as well, since slow firing neurons are
much more likely to exhibit a fixed percent change (e.g., 10–20%) in firing rate. In the present study, firing rate responses were considered significant with a minimum 10% change in the firing rate that was also at least 1 imp/s. This criterion was used because 1) the changes in the mean firing rate were statistically significant (Student’s t-test, \( P < 0.05 \)), 2) it minimized any potential bias associated with only absolute changes in firing rate, and 3) it normalized the responses for neurons with both low and high firing rates.

**RESULTS**

Figure 2 shows examples of the different types of neurons recorded, and Table 1 provides the number of neurons in each population, along with their mean spontaneous firing rates at 36–37°C. A total of 121 neurons was recorded in hypothalamic tissue slices obtained from 89 rats. In most experiments, only one neuron was recorded in slices obtained from an individual rat. In those experiments where recordings were made from more than one tissue slice, no more than three neurons were recorded in slices obtained from the same rat. Table 1 indicates that the majority (71%) of neurons was temperature insensitive. This included 45 low-slope temperature-insensitive neurons (37%) and 41 moderate-slope temperature-insensitive neurons (34%). The remaining neurons were considered thermosensitive and included 31 warm-sensitive neurons (26%) and only 4 cold-sensitive neurons (3%). Most neurons were recorded in the POAH nuclei (71%), while the remainder were recorded in other nearby hypothalamic nuclei (i.e., lateral hypothalamus, dorsomedial hypothalamus, ventromedial hypothalamus, and posterior hypothalamus). There were no regional differences in the types of neurons recorded. The mean firing rate of the 121 neurons in this study was 7.1 ± 0.7 (SE) imp/s, ranging from 0.1 to 57 imp/s. Warm-sensitive neurons had statistically higher firing rates (12.0 ± 1.5 imp/s, \( P < 0.05 \)) than either moderate-slope temperature-insensitive (6.3 ± 1.3 imp/s) or low-slope temperature-insensitive (4.4 ± 1.2 imp/s) neurons.

**Effect of hypercapnia on the firing rate in hypothalamic neurons.** The responses of 112 neurons were recorded during two exposures to 10% CO2, and Fig. 3 shows examples of different firing rate responses to hypercapnia in three POAH neurons. The temperature-insensitive neuron in Fig. 3A is typical of the majority of neurons and showed no change in its firing rate during exposure to 10% CO2. Figure 3B shows a warm-sensitive neuron whose firing rate decreased during 10% CO2, and Fig. 3C shows a warm-sensitive neuron whose firing rate increased during two different exposures to 10% CO2.

<table>
<thead>
<tr>
<th>Neuronal Type</th>
<th>Firing Rate, imp/s</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm sensitive*†</td>
<td>12.0 ± 1.5</td>
<td>31</td>
</tr>
<tr>
<td>Moderate-slope, temp.</td>
<td>6.3 ± 1.3</td>
<td>44</td>
</tr>
<tr>
<td>Low-slope, temp.</td>
<td>4.4 ± 1.2</td>
<td>45</td>
</tr>
<tr>
<td>Cold sensitive</td>
<td>6.9 ± 4.1</td>
<td>4</td>
</tr>
<tr>
<td>All neurons</td>
<td>7.1 ± 0.8</td>
<td>121</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of cells recorded. imp. Impulses. *Firing rate for warm-sensitive neurons is significantly higher than low-slope temperature-insensitive neurons (\( P < 0.05 \)). †Firing rate for warm-sensitive neurons is significantly higher than moderate-slope temperature-insensitive neurons (\( P < 0.05 \)).

Table 2 summarizes the neuronal responses to hypercapnia. While 59% of all neurons were insensitive to elevated CO2, 32% decreased their firing rates and 9% increased their firing rates during 10% CO2 exposures. Firing rate responses to hypercapnia varied in magnitude and onset. Some neurons exhibited rapid changes, while others responded more slowly. Typically, most neurons responded to 10% CO2 within 1–3 min, and their firing rates quickly recovered on return to control 5% CO2.

Table 2 also summarizes the effect of hypercapnia on the different neuronal types. The warm-sensitive neurons had a significantly higher proportion of hypercapnia-inhibited neurons (62%) compared with low-slope and moderate-slope temperature-insensitive neurons (18% and 23%, respectively, \( \chi^2 \), \( P < 0.001 \)). The proportions of hypercapnia-excited neurons were small but very similar for low-slope temperature-insensitive (7%), moderate-slope temperature-insensitive (8%), and warm-sensitive neurons (10%). Temperature-insensitive neurons were least sensitive to hypercapnia; i.e., 75% of low-slope temperature-insensitive and 69% of moderate-slope temperature-insensitive neurons were characterized as CO2 insensitive. In contrast, only 28% of warm-sensitive neurons did not respond to elevated CO2. In this study, four cold-sensitive neurons were recorded and treated with 10% CO2. Of these, two neurons decreased their firing rate, one neuron increased firing rate, and one exhibited no change in firing rate. Because of the small number of cold-sensitive neurons recorded, these four neurons were excluded from subsequent analyses.

No regional differences in CO2 sensitivity in hypothalamic neurons. Hypercapnia produced neuronal responses not only in the POAH but also in nearby hypothalamic regions. Figure 4 gives examples of two CO2-inhibited, warm-sensitive neurons that were recorded outside the POAH. Figure 4A shows a posterior hypothalamic neuron exhibiting decreased firing rates during two exposures to 10% CO2, and Fig. 4B shows a lateral hypothalamic neuron with decreased firing rate during three separate exposures to 10% CO2. For comparisons, all of the recorded neurons were divided into two regional populations: 1) neurons recorded within the POAH (\( n = 86 \)) and 2) neurons recorded ventral, dorsal, posterior, and lateral to the POAH (non-POAH, \( n = 22 \)). Non-POAH neurons were recorded in the posterior hypothalamus (\( n = 8 \)), lateral hypothalamus (\( n = 6 \)), dorsomedial hypothalamus (\( n = 5 \)), and ventromedial hypothalamus (\( n = 3 \)).

Table 3 summarizes the regional effects of hypercapnia on the firing rate responses in temperature-insensitive and warm-sensitive neurons. Regardless of the hypothalamic region, hypercapnia tended to decrease the firing rates of warm-sensitive neurons and had little effect on the firing rates of temperature-insensitive neurons. For warm-sensitive neurons, 59% of POAH neurons and 67% of non-POAH neurons decreased their firing rates during hypercapnia. In contrast, for the temperature-insensitive neurons, only 22% of POAH neurons and 10% of non-POAH neurons decreased their firing rates during hypercapnia. While there were no statistical differences between POAH and non-POAH neurons, it is interesting to note that only POAH neurons showed increased firing rates during hypercapnia. Neurons excited by 10% CO2 were rare. In the POAH, only 9% of temperature-insensitive and 17% of warm-sensitive neurons were excited by 10% CO2, and hypercapnia-excited neurons were not recorded in the non-POAH population.
Hypercapnic responses are mimicked by isocapnic acidosis but not by isohydric hypercapnia. One purpose of this study was to determine whether hypercapnia directly or indirectly contributes to the neuronal firing rate responses, particularly the hypercapnic inhibition observed in many neurons. To differentiate between firing rate responses mediated by hypercapnia compared with hypercapnia-induced acidosis (i.e., acidic pH due to elevated CO₂), 17 neurons were exposed to normal 5% CO₂ but acidic pH (i.e., isocapnic acidosis). Of these neurons, five were low-slope temperature insensitive, five were moderate-slope temperature insensitive, and seven were warm sensitive. Figure 5A shows a medial preoptic, warm-sensitive neuron treated with 10% CO₂ and isocapnic acidosis. The neuron’s firing rate decreased quickly during the 10% CO₂, and it quickly returned a higher firing rate when aeration was returned to the control 5% CO₂. The perfusion was then

Table 2. Effect of hypercapnia on firing rate in hypothalamic neurons

<table>
<thead>
<tr>
<th>Neuronal Type</th>
<th>Firing Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dec</td>
</tr>
<tr>
<td>Warm sensitive*</td>
<td>62%</td>
</tr>
<tr>
<td>Moderate-slope, temperature insensitive</td>
<td>23%</td>
</tr>
<tr>
<td>Low-slope, temperature insensitive</td>
<td>18%</td>
</tr>
<tr>
<td>Cold sensitive</td>
<td>50%</td>
</tr>
<tr>
<td>All neurons</td>
<td>32%</td>
</tr>
</tbody>
</table>

n = no. of neurons recorded. The criterion for change in firing rate was a 10% change that was at least 1 imp/s. Dec, decreased; Inc, increased; NC, no change. *Warm-sensitive neurons had a significantly higher proportion of hypercapnia-inhibited neurons (62%) compared with low-slope and moderate-slope temperature-insensitive neurons (18% and 23%, respectively); χ², P < 0.001.

Fig. 3. Examples of POAH neuronal firing rate responses to temperature and hypercapnia. Thermal coefficient (m) expressed as imp·s⁻¹·°C⁻¹. A: anterior hypothalamic, low-slope temperature-insensitive neuron whose activity did not change during hypercapnia. B: lateral preoptic, warm-sensitive neuron that decreased firing rate during hypercapnia. C: medial preoptic, warm-sensitive neuron that increased firing rate during hypercapnia.
switched to the isocapnic acidosis medium so that the pH was lowered to 7.17–7.19, but the tissue chamber and aCSF medium aeration were maintained at control 5% CO$_2$. After a delay (due to the washout of dead space containing normal aCSF), the neuron decreased its firing rate. Following this, the perfusion medium was switched to normal aCSF, and (again, after a washout delay) the firing rate increased. Table 4 summarizes the effects of isocapnic acidosis on the firing rates of hypothalamic neurons. During isocapnic acidosis, 59% of neurons decreased their firing rates, 12% increased their firing rates, and 29% showed no change in their firing rates. Thus isocapnic acidosis tends to mimic hypercapnia by decreasing spontaneous firing rates in many hypothalamic neurons. This suggests that acidosis mediates the decreased firing rates observed during hypercapnia.

To further distinguish between firing rate responses mediated by hypercapnia or acidosis, some neurons were exposed to elevated CO$_2$ but normal pH (i.e., isohydric hypercapnia). Figure 5B shows a medial preoptic warm-sensitive neuron treated with 10% CO$_2$ and isohydric hypercapnia. This neuron decreased its firing rate during two different exposures to 10% CO$_2$. On the other hand, the same neuron did not decrease its firing rate during isohydric hypercapnia when the perfusing aCSF was gas saturated with 10% CO$_2$, but the pH was maintained at a normal level (i.e., pH = 7.5) due to elevated NaHCO$_3$. Table 4 shows the responses of 17 neurons tested with isohydric hypercapnia. This included nine low-slope temperature-insensitive, five moderate-slope temperature-insensitive, and three warm-sensitive neurons. Most of these neurons were unaffected by isohydric hypercapnia, and while five temperature-insensitive neurons were excited, no neurons were

### Table 3. Regional effects of hypercapnia on firing rate responses in temperature-insensitive and warm-sensitive hypothalamic neurons

<table>
<thead>
<tr>
<th>Neuronal Type</th>
<th>Dec</th>
<th>Inc</th>
<th>NC</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>POAH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm sensitive</td>
<td>59%</td>
<td>18%</td>
<td>23%</td>
<td>17</td>
</tr>
<tr>
<td>Temperature insensitive</td>
<td>22%</td>
<td>9%</td>
<td>69%</td>
<td>69</td>
</tr>
<tr>
<td>Non-POAH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm sensitive</td>
<td>67%</td>
<td>0%</td>
<td>33%</td>
<td>12</td>
</tr>
<tr>
<td>Temperature insensitive</td>
<td>10%</td>
<td>0%</td>
<td>90%</td>
<td>10</td>
</tr>
</tbody>
</table>

$n$ = no. of cells recorded. All neurons were divided into 2 regional populations: neurons recorded in the preoptic-anterior hypothalamus (POAH) and those neurons recorded ventral, dorsal, posterior and lateral to the POAH (i.e., non-POAH). Each regional population was divided into temperature-insensitive and warm-sensitive subpopulations. The 4 cold-sensitive neurons in Table 2 were excluded. There were no differences in the proportions of hypercapnia-responsive neurons between POAH and non-POAH populations. Regardless of the hypothalamic region, warm-sensitive neurons have a higher proportion of hypercapnia-inhibited neurons than the temperature insensitive neurons, and most temperature-insensitive neurons were unaffected by hypercapnia.

Fig. 4. Decreased firing rate responses in non-POAH hypothalamic neurons during hypercapnia. Experimental records show spontaneous firing rate and tissue slice temperature. Thermal coefficient ($m$) expressed as imp·s$^{-1}$·°C$^{-1}$. A: posterior hypothalamic warm-sensitive neuron that decreased firing rate during hypercapnia. B: lateral hypothalamic warm-sensitive neuron that decreased firing rate during hypercapnia.
inhibited by isohydric hypercapnia. This indicates that the decreased neuronal firing rate observed in many neurons during hypercapnia is due to acidosis and not the direct effect of CO₂.

When the firing rate responses to hypercapnia, isocapnic acidosis, and isohydric hypercapnia are compared, the isocapnic acidosis-treated population did not significantly differ from the hypercapnia-treated population; i.e., 59% of the neurons treated with isocapnic acidosis decreased their firing rate, and 32% of neurons decreased their firing rate during hypercapnia. On the other hand, the isohydric hypercapnia-treated population was significantly different from the hypercapnia-treated population (χ², P < 0.01). None of the neurons treated with isohydric hypercapnia showed a decreased firing rate. Both the isocapnic acidosis and isohydric hypercapnia experiments suggest that the hypercapnia-induced inhibition of firing rate is due to acidosis and not the direct effect of CO₂.

**DISCUSSION**

The present study tested the responses of POAH and non-POAH neurons to changes in CO₂ and pH. In both populations, warm-sensitive neurons were most likely to decrease their firing rates during hypercapnia, whereas the low- and moderate-slope temperature-insensitive neurons were least likely to change their firing rates. In addition, hypothalamic neurons, especially warm-sensitive neurons, were very sensitive to changes in pH. The decreased firing rates observed during hypercapnia could be mimicked by isocapnic acidosis (i.e., normal 5% CO₂ but acidic pH), but isohydric hypercapnia (i.e., elevated CO₂ but normal pH) failed to decrease neuronal firing rates. This indicates that the decreased firing rate during hypercapnia is due to acidosis rather than a direct effect of CO₂. Also, compared with hypercapnia, isocapnic acidosis appears to be more potent in decreasing neuronal firing rates.

**Table 4. Effects of CO₂ and pH on the firing rate responses of warm-sensitive and temperature-insensitive hypothalamic neurons**

<table>
<thead>
<tr>
<th>Neuronal Type</th>
<th>Isocapnic Acidosis</th>
<th>Isohydric Hypercapnia*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dec</td>
<td>Inc</td>
</tr>
<tr>
<td>Warm sensitive</td>
<td>86%</td>
<td>0%</td>
</tr>
<tr>
<td>Temperature insensitive</td>
<td>40%</td>
<td>20%</td>
</tr>
<tr>
<td>All neurons</td>
<td>59%</td>
<td>12%</td>
</tr>
</tbody>
</table>

n = no. of cells recorded. Dec, decreased firing rate; Inc, increased firing rate; NC, no change in firing rate; isocapnic acidosis, normal CO₂ and decreased pH; isohydric hypercapnia, elevated CO₂ and normal pH. *When compared with firing rate responses during hypercapnia, the firing rate responses during isohydric hypercapnia were significantly different (χ², P < 0.01). None of the neurons treated with isohydric hypercapnia decreased their firing rates, whereas 32% of neurons decreased their firing rates during hypercapnia (see Table 2).
In the present study, most warm-sensitive neurons were also CO₂ sensitive, whereas most temperature-insensitive neurons were not CO₂ sensitive. This suggests that future experiments should explore the relationship between neuronal thermosensitivity and neuronal CO₂ and pH sensitivity. Do changes in temperature, for example, affect neuronal responses to CO₂ and pH? Conversely, do pH changes alter neuronal thermosensitivity or the range of thermosensitivity (i.e., firing rate changes at hypothermic vs. hyperthermic temperatures)? In addition, the cellular mechanisms underlying POAH neuronal responses to CO₂ and pH remain to be determined. In the present study, Table 2 hints that there is a relationship between neuronal sensitivity to temperature and neuronal sensitivity to CO₂ and pH. Table 2 indicates that hypercapnia-inhibited neurons accounted for 62% of the most thermosensitive population (i.e., the warm-sensitive neurons). In contrast, hypercapnia-inhibited neurons represented 23% of the moderate-slope temperature-insensitive neurons and only 17% of the least thermosensitive population (i.e., the low-slope temperature-insensitive neurons). Thus the membrane channels that determine neuronal thermosensitivity may also play a role in pH sensitivity.

The present study did not employ perfusion media that block synaptic activity. Therefore, in addition to the CO₂ and pH effects on neuronal cell bodies, it is possible that these factors may affect synaptic transmission. It should be noted, however, that this is not a likely explanation for the different responses seen between warm-sensitive and temperature-insensitive neurons, since there are no major differences between these two neuronal populations in terms of neurotransmitters and the frequencies of their excitatory and inhibitory postsynaptic potentials (18).

As indicated in Table 2, the present study found no differences between the POAH and non-POAH populations in terms of neuronal responses to hypercapnia. It is noteworthy, however, that none of the 22 non-POAH neurons (including 8 posterior hypothalamic neurons) were excited by exposure to 10% CO₂. In contrast, a rat tissue slice study by Dilllon and Waldrop (10) recorded neurons in the caudal hypothalamus during exposure to 7% CO₂. Of the 54 neurons recorded in this previous study, hypercapnia excited 22%, inhibited 11%, and had no effect on 67% (10).

Mechanisms of CO₂ sensitivity have been studied in other parts of the nervous system, and the neuronal responses to CO₂ have been attributed to changes in intracellular or extracellular pH (14). During hypercapnia, the intracellular and extracellular environments become more acidic, and many of the CO₂-induced neuronal effects are believed to be mediated by changes in H⁺ concentration or pH (6). Although extracellular recordings do not fully elucidate the precise cellular mechanisms for CO₂ sensitivity in hypothalamic neurons, the present study has identified that acidic pH (whether produced by elevated CO₂ or decreased HCO₃⁻) decreases the spontaneous firing rates primarily in warm-sensitive neurons.

Hypercapnia and acidosis may decrease neuronal firing rates either by hyperpolarizing resting membrane potentials or by altering transient potentials (i.e., hyperpolarizing afterpotentials and depolarizing prepotentials) that influence the intervals between successive action potentials. Hypercapnia has been reported to hyperpolarize the membrane potential of bulbar respiratory neurons (37), inspiratory phrenic motoneurons (22), spinal motoneurons and cortical neurons (6), neurons of the nucleus tractus solitarii (7, 9), and ventral medullary neurons (24). Transient potentials are also affected by hypercapnia. CO₂-induced changes in the rate of rise of the depolarizing prepotential (i.e., pacemaker potential) have been described in CO₂-sensitive neurons in the ventrolateral medulla (33). Hypercapnia, extracellular acidosis, and intracellular acidosis have also been shown to inhibit the depolarizing pacemaker currents mediated by hyperpolarization-activated cyclic nucleotide-gated cation channels (12, 32, 43), which contribute to the depolarizing prepotential in a number of spontaneously active neurons. Therefore, hypercapnia and acidosis can decrease the rate of depolarization of the prepotential in spontaneously active neurons, thus prolonging the interspike interval and decreasing the firing rate. This may be an important link between a neuron’s sensitivity to CO₂ and pH and its sensitivity to temperature. Our previous studies have shown that the depolarizing prepotential is the primary mechanism for warm sensitivity in POAH neurons (4, 17). Unlike temperature-insensitive neurons, the depolarizing prepotential of warm-sensitive neurons is strongly dependent on temperature. Warming increases the prepotential’s rate of depolarization, and since threshold is reached sooner, the interspike interval shortens and firing rate increases. Thus the ionic channels that determine the temperature sensitivity of the depolarizing prepotential may be the same channels that account for acidic-induced changes in firing rate.

Although hypercapnia and isocapnic acidosis both tended to decrease firing rate in hypothalamic neurons, isohydric hypercapnia did not decrease firing rates and, instead, often increased firing rates. During isohydric hypercapnia, 29% of neurons increased firing rate as opposed to 12% during isocapnic acidosis and 9% during hypercapnia. It may be proposed that this increased firing rate is due to either the hypercapnia (i.e., from 5% to 10% CO₂) or an increased extracellular HCO₃⁻ concentration (i.e., from 26 to 45 mM). Given that hypercapnia (i.e., 10% CO₂) only increased firing rate in 9% of neurons, it seems most likely that the increased HCO₃⁻ concentration during isohydric hypercapnia contributed to the increased firing rate. It is possible that hypothalamic neurons possess HCO₃⁻ transport mechanisms that become active and increase the intracellular HCO₃⁻ concentration during isohydric hypercapnia. Increasing the intracellular HCO₃⁻ concentration could offset any intracellular acidification caused by the elevated 10% CO₂ and may even contribute to an intracellular alkalization. Since acidosis tends to decrease firing rates, it is possible that alkalosis increases firing rates.

Unlike temperature-insensitive neurons, POAH warm-sensitive neurons not only sense their own temperature, but in addition, they receive synaptic input fromafferent pathways of skin and spinal thermoreceptors (5). Because of this integrative role, it is likely that POAH warm-sensitive neurons play important roles in thermoregulation. Various neuronal models have been proposed to explain hypothalamic synaptic networks controlling thermoregulatory responses (3, 4, 19). In these networks, warm-sensitive neurons excite effector neurons that control species-dependent heat-loss responses (e.g., skin wetting, panting, sweating, cutaneous vasodilation), and the models predict that any condition that decreases the firing rate of the warm-sensitive neurons would suppress heat-loss responses and, thereby, raise body temperature. The present study indi-
cates that many of these warm-sensitive neurons are inhibited by decreased extracellular pH, which would occur during hypercapnia. This could explain why acid-base changes have been associated with hyperthermia and heat-related illnesses.

Stupfel (36) has shown that hypercapnia produces hyperthermia in 40°C heat-exposed rats. In this previous study, when compared with control (normocapnic) rats, the hypercapnic rats had significant increases in both core temperatures and mortality rates (36). Since a critical factor appears to be changes in pH (rather than CO2), this could also explain why hyperthermia in humans (2, 11, 13, 35, 42) and animals (27, 45) is often associated with conditions such as metabolic acidosis, lactic acidosis, and respiratory acidosis. Thus the acidic conditions seen in some heat-related illnesses may be the same conditions that preferentially inhibit hypothalamic warm-sensitive neurons controlling heat-loss responses.

Heat stroke in humans is defined by body temperature above 40.6°C (105°F), and this is associated with impaired heat-loss responses, including reduced skin blood flow and anhidrosis (i.e., absence of sweating) (20, 34). There are various forms of heat stroke, and the underlying causes of each form are complex. Heat stroke is often accompanied by acid-base changes, including either lowered pH during metabolic acidosis and lactic acidosis or elevated pH during respiratory alkalosis associated with thermal hyperventilation (2, 13, 20, 21, 35, 41, 44). Metabolic acidosis and lactic acid accumulation are closely associated with increased body temperature in human heat stroke and in animal models of heat stress (27, 45). The present study, therefore, offers a mechanism for one form of heat-related illness in which acidosis affects hypothalamic warm-sensitive neurons in such a way as to impair heat-loss responses, thus producing hyperthermia.

Finally, in addition to CO2 effects on thermoregulation, there is evidence that temperature modifies CO2-associated respiratory responses (1, 44). Since there appears to be link between neuronal sensitivity to temperature and CO2, it is possible that this link may be evident in a hypothalamic role in the neural control of breathing. While breathing responses to POAH changes in pH and CO2 are not known, a previous study suggests that CO2-sensitive neurons in the posterior hypothalamus have a respiratory role (10). In terms of breathing, thermoregulatory models suggest that some POAH warm-sensitive neurons control panting and associated responses that increase respiratory heat loss (3, 4, 19). Numerous studies in panting animals (e.g., dogs, cats, rabbits) show that localized POAH warming causes profound increases in breathing frequency (reviewed in Ref. 3), and there is evidence that similar increases in breathing frequency may be elicited by hypothalamic warming in nonpanting mammals, including rats (16) and humans (44). In humans, for example, respiratory responses to CO2 and pH are enhanced by increases in central temperature (44), and in rats, localized POAH warming provides an additional respiratory drive to CO2-modified breathing responses (1). In the present study, therefore, the neurons excited by both warming and CO2 may represent a basis for this interaction between temperature and CO2 in the control of breathing.

ACKNOWLEDGMENTS

The authors express gratitude to Lorry Kaple for valued assistance and to Drs. J. B. Dean, R. W. Putnam, and P. W. Burgoon for helpful advice.


