Hyperthermia Modulates Respiratory Pacemaker Bursting Properties

Andrew K. Tryba and Jan-Marino Ramirez
Department of Organismal Biology and Anatomy, The University of Chicago, Chicago, Illinois 60637-1508
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Tryba, Andrew K. and Jan-Marino Ramirez. Hyperthermia modulates respiratory pacemaker bursting properties. J Neurophysiol 92: 2844–2852, 2004. First published June 9, 2004; 10.1152/jn.00752.2003. Most mammals modulate respiratory frequency (RF) to dissipate heat (e.g., panting) and avoid heat stroke during hyperthermic conditions. Respiratory neural network activity recorded in an isolated brain stem-slice preparation of mice exhibits a similar RF modulation in response to hyperthermia; fictive eupneic frequency increases while inspiratory network activity amplitude and duration are significantly reduced. Here, we study the effects of hyperthermia on the activity of synaptically isolated respiratory pacemakers to examine the possibility that these changes may account for the hyperthermic RF modulation of the respiratory network. During heating, modulation of the bursting frequency of synaptically isolated pacemakers paralleled that of population bursting recorded from the intact network, whereas nonpacemaker neurons were unaffected, suggesting that pacemaker bursting may account for the temperature-enhanced RF observed at the network level. Some respiratory neurons that were tonically active at hypothermic conditions exhibited pacemaker properties at approximately the normal body temperature of eutherian mammals (36.81 ± 1.7°C; mean ± SD) and continued to burst at 40°C. At elevated temperatures (40°C), there was an enhancement of the depolarizing drive potential in synaptically isolated pacemakers, while the amplitude of integrated population activity declined. Isolated pacemaker bursting ceased at 41–42°C (n = 5), which corresponds to temperatures at which hyperthermic-apnea typically occurs in vivo. We conclude that pacemaker properties may play an important role in the hyperthermic frequency modulation and apnea, while network effects may play important roles in generating other aspects of the hyperthermic response, such as the decreased amplitude of ventral respiratory group activity during hyperthermia.

INTRODUCTION

For many animals, an elevation in body temperature enhances both heart rate and respiration frequency. The increase in cardiac output and respiration help cool the body and restore its temperature to a normal range. In fact, the temperature-dependent modulation of respiratory frequency (RF) is a major mechanism to dissipate heat (e.g., panting) and avoid heat stroke. If respiratory modulation fails to lower the body temperature, hyperthermia associated with conditions such as fever and heat stroke may lead to apnea and death (Eshel et al. 1990). Hyperthermia has also been implicated in sudden infant death syndrome (SIDS) (Fleming 1990; Harper et al. 2000; Poets et al. 1999; Russell and Vink 2001) as the body temperature of SIDS victims is typically markedly elevated on death (Fleming 1990). Here we examine mechanisms that may account for hyperthermic apnea.

The hypothalamus can clearly play a role in modulating RF with changes in body temperature (Boden et al. 2000; Inomoto et al. 1983; Ni et al. 1996). However, temperature can also directly affect the activity of the respiratory neural network located in the medulla. One area that seems to play a critical role in the generation of inspiratory activity is the pre-Bötzinger complex (PBC). This area is both necessary for the generation of normal respiratory activity (Gray et al. 2001; Ramirez et al. 1998) and sufficient to generate fictive respiratory activity when isolated in the brain stem slice preparation (Lieske et al. 2000; Smith et al. 1991). This slice preparation also contains the hypoglossal nucleus (XII), a motor output that is modulated in-phase with inspiratory activity. Brain stem-slice preparations containing the PBC and XII nucleus revealed that the PBC may modulate RF during brain stem temperature changes independently of influences from higher brain structures, such as the hypothalamus (Peever et al. 1999; Tryba and Ramirez 2003). Elevated temperatures increase fictive RF while inspiratory burst amplitude and duration decline in this in vitro preparation as similarly described in vivo (Galland et al. 1993; Tryba and Ramirez 2003). How is fictive respiratory activity modulated with temperature in the slice preparation? To begin to understand the mechanisms underlying RF modulation during hyperthermia, we compared the hyperthermic response of respiratory pacemaker neurons before and after they were synaptically isolated from respiratory network activity. Respiratory pacemaker neurons are thought to play a critical role in the generation of the respiratory rhythm in neonatal mammals (Rekling and Feldman 1998; Smith et al. 1991; Thoby-Brisson and Ramirez 2001).

METHODS

All experiments conformed to the guiding principles for the care and use of animals approved by the National Institutes of Health and the Institutional Animal Care and Use Committee at The University of Chicago.

Medullary brain slice preparation

All experiments used the transverse, rhythmic medullary brain-slice preparation (Funk et al. 1994; Ramirez et al. 1996). Mice (0–7 day old CD-1 outbred mice, Charles River Laboratories, Wilmington, MA) were deeply anesthetized with ethyl ether (Sigma; delivered by inhalation). On cessation of reflex activity, animals were quickly decapitated at the C3/C4 spinal level (Ramirez et al. 1996). The brain stem was dissected-out in ice-cold artificial cerebral spinal fluid (ACSF) that was equilibrated with carbogen (95% O2:5% CO2). Rhythmic 650-μm-thick slices containing the ventral respiratory group (VRG) (Ramirez et al. 1996) were obtained by slicing the medulla using a
microslicer (Leica, VT1000S, Nussloch, Germany). Slices were submerged under circulating artificial cerebrospinal fluid (ACSF; 30°C; flow rate 15 ml/min) containing (in mM) 118 NaCl, 3 KCl, 1.5 CaCl₂, 1 MgCl₂·6H₂O, 25 NaHCO₃, 1 NaH₂PO₄, and 30 d-glucose, equilibrated with carbogen (95% O₂-5% CO₂, pH = 7.4 at room temperature, ~22°C). KCl was elevated from 3 to 8 mM over a span of 30 min before commencing recordings (Tryba et al. 2003). All chemicals were obtained from Sigma (St. Louis, MO). The pH of four different ACSF batches had a mean pH of 7.43 ± 0.01 (mean ± SD) at 30°C and a pH of 7.46 ± 0.02 at 40°C (n = 4; P = 0.034, paired t-test). The mean difference between the ACSF pH at 30 and 40°C was 0.04 ± 0.02 pH units.

Bath temperature was monitored and automatically adjusted to within ±0.7°C (range) of the set temperature using a Warner Instrument (Hamden, CT) TC-344B temperature regulator with an in-line solution heater (SH-27B). Hyperthermia was achieved by warming the ACSF bathing the brain-slice preparation. Reported temperatures refer to preparation bath ACSF temperature. Unless otherwise noted, the ACSF temperature in the preparation bath was actively heated from 30 to 40°C and then allowed to passively cool to 30°C at which point the bath temperature was actively maintained at 30°C. ACSF temperature at various locations within the bath were routinely uniform. In these experiments, we raised the bath temperature from 30 to 40°C at a rate of ~7°C/min, which is much more rapid than during heat stroke. The rapid heating protocol was chosen because we used whole cell current clamping to record from PBC neurons during heating and our success in maintaining seals increased using a protocol having shorter recording times. Because warmer saline has a lower oxygen partial pressure, we used slices from young (P0- to P7-day old) mice that do not have a marked response to hypoxia but have a hyperthermic response (Tryba and Ramirez 2003).

![Diagram](http://example.com/diagram.png)
**Electrophysiological recordings**

**INTEGRATED VRG POPULATION ACTIVITY.** Extracellular recordings were obtained with electrodes positioned on the slice surface of the VRG. The VRG signal collected was amplified and filtered (low-pass: 1.5 kHz, high-pass: 250 Hz), rectified and integrated using an electronic filter (time constant: 60 ms, Figs. 1, A and B) (Ramirez et al. 1996). The integrated VRG population activity is in-phase with that of the hypoglossal motor nucleus (i.e., 1:1 coupling), thus the VRG population bursts can serve as a marker of fictive inspiratory bursts. The inspiration (Telgkamp and Ramirez 1999; Tryba and Ramirez 2003). The frequency of VRG integrated inspiratory bursts during fictive eupnea was used to define RF.

**WHOLE CELL PATCH RECORDINGS.** Intracellular patch-clamp recordings were obtained from VRG neurons using the blind-patch technique (Tryba et al. 2003) (Figs. 1, A and B). Patch electrodes were manufactured from filamented borosilicate glass tubes (Clark G150F-4; Warner Instruments, Hamden, CT) filled with a solution containing (in mM) 140 K-gluconic acid, 1 CaCl$_2$·6H$_2$O, 10 EGTA, 2 MgCl$_2$·6H2O, 4 Na$_2$ATP and 10 HEPES. Only inspiratory neurons active in-phase with population activity were recorded in this study (the technique is described in Tryba et al. 2003). The discharge pattern of each cell type was first identified in the cell-attached mode prior to breaking the seal. Experiments were then performed in the whole cell patch-clamp mode and only inspiratory neurons were considered in this study. Inspiratory neurons were isolated and identified as pacemakers or nonpacemakers based on previously described criteria (Thoby-Brisson and Ramirez 2001; Tryba et al. 2003); pacemaker neurons are located in the PBC (Rekling and Feldman 1998; Smith et al. 1998; Thoby-Brisson and Ramirez 2001). The $V_m$ values were corrected for liquid junction potentials as described by Neher (1992). In current-clamp, neurons were isolated from chemical synaptic input using a mixture of glutamatergic, GABAAergic or glycinergic antagonists. These drugs were bath applied at the final concentrations of: 40 mM 6-cyano-7-nitroquinoline-2,3-dione (CNQX; Tocris, Ellisville, MO), 10 mM (RS)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid [(RS)-CPP Tocris], 1 mM strychnine (Sigma), and 20 mM bicuculline Free-Base (Sigma). CNQX and CPP are AMPA and N-methyl-d-aspartate (NMDA) receptor antagonists, whereas bicuculline free base and strychnine are GABA and glycine receptor antagonists, respectively; bath application at these concentrations have been shown to be effective in the PBC slice preparation in other studies (Thoby-Brisson and Ramirez 2001; Thoby-Brisson et al. 2000; Tryba et al. 2003). Note that unlike bicuculline methiodide, the bicuculline free-base derivative is a specific GABA receptor antagonist that does not block apamin-sensitive Ca$^{2+}$-activated K$^+$ currents (Debarboux et al. 1998; Johnson and Seutin 1997). Neurons shown in Figs. 4B (n = 4) and 5, A and B (n = 7), were isolated from chemical synaptic transmission using 200 mM Cd$^{2+}$ (Elsen and Ramirez 1998). In cases where cadmium was used, it was applied as it rapidly blocks chemical synaptic transmission by blocking calcium currents (Elsen and Ramirez 1998). Cadmium is also used as a tool to discriminate between different types of pacemaker neurons as it indicates that bursting in these pacemakers persists in the presence of voltage-gated calcium channel blockade. Neurons that continue to burst in Cd$^{2+}$ are cadmium-insensitive pacemaker neurons as previously described by Thoby-Brisson and Ramirez (2001). The remaining pacemakers studied here (n = 5) were isolated from chemical synaptic transmission using CNQX, CPP, bicuculline and strychnine in the absence of Cd$^{2+}$; these data may include both cadmium-sensitive and -insensitive types of pacemakers as described by Thoby-Brisson and Ramirez (2001). In all, this study encompasses n = 16 total pacemakers (n = 11 isolated in Cd$^{2+}$ and n = 5 isolated in a mixture of synaptic antagonists).

**DATA ANALYSIS.** All recordings were transferred to a personal computer (PC) using a Digidata (Axon Instruments) A/D conversion board. Data were stored using Axotape (version 2.0; Axon Instruments) and analyzed off-line with software programs written in Igor Pro (Wavemetrics, Lake Oswego, OR). Only signals with good signal-to-noise ratios were quantitatively analyzed.

**QUANTITATIVE METHODS.** Student’s t-test were used to test for significant differences between mean pacemaker burst frequency (paired t-test), burst amplitude (% control, 1-sample t-test) and duration data (paired t-test) when the bath temperature was maintained at 30 and 40°C temperatures; comparisons were made for data collected over a 40-s duration (n = 5). Burst duration was defined as the time from first to last action potential within a burst. Burst amplitude was measured as the absolute value of the depolarization burst peak height from baseline and normalized as percent of control. Values were assumed to be significant at $P < 0.05$. Comparison of pacemaker burst frequency while embedded in the respiratory network and after isolation from chemical synaptic transmission was made using ANOVA analysis. As the pacemakers are temperature-sensitive, pacemaker data included in this analysis had heating profiles with similar time courses. For this reason, only a limited number of pacemaker neurons could be combined in the analysis. We also only included data from neurons the membrane potential of which returned to baseline after heating. Several neural recordings had to be excluded from data analysis because the recording became unstable at elevated temperatures. Mean values are followed by ± SD except where noted.

**R E S U L T S**

**Hyperthermic response**

During hyperthermia, both panting and nonpanting mammals modify their breathing by increasing RF and decreasing both inspiratory amplitude and duration. At elevated temperatures, fictive respiration generated in the VRG also includes an increase in RF and decline in amplitude and duration of inspiratory bursts (Tryba and Ramirez 2003). These changes in population activity may be due to network interactions and/or pacemaker properties. To examine this issue, we recorded from...
inspiratory neurons in control ACSF at 30°C (Fig. 1, A and B) while we transiently increased the bath temperature to 40°C and allowed it to cool to the control temperature (from 40 to 30°C).

Is the hyperthermic-enhancement of fictive respiratory frequency and reduction in population burst amplitude and burst duration correlated with similar changes in pacemaker bursting properties at elevated temperatures? In the absence of VRG network activity, synaptically isolated PBC pacemaker neurons increased their bursting frequency when the bath temperature was increased from 30°C (0.34 ± 0.12 Hz) to 40°C (1.3 ± 0.38 Hz; P < 0.001) (Figs. 2, A and B, and 3, A and B, n = 5). The mean frequency increased throughout heating (P < 0.001; ANOVA, Fig. 3A). The increase in frequency with temperature was fit by the equation, \( \ln(F) = (T - T_o)/A \), where \( \ln = \) natural logarithm, \( F = \) the isolated pacemaker frequency, \( T_o = 36.47°C, A (1/slope) = 6.609 \) and \( T = \) temperature (Fig. 3B; \( r^2 = 0.59; \) slope is non-zero \( P < 0.0001 \)). Thus the equation predicts a 2.13-fold increase in pacemaker bursting frequency per 5°C increase in temperature for heating over this temperature range. In addition to the increase in isolated pacemaker frequency, the amplitude of the depolarizing drive potential underlyng a burst (i.e., burst amplitude) increased at 40°C (169.7 ± 45.5%; n = 5, P = 0.026; Figs. 2, A and B, and 3C), while the burst duration at 30°C (1.44 ± 0.80 s) was reduced at 40°C (0.39 ± 0.21 s; Fig. 3D, n = 5; P = 0.019). It is possible that the enhanced depolarizing drive potential underlying a burst observed at 40°C could reach inactivation, and in

**FIG. 3.** Quantification of the changes in synaptically isolated pacemaker bursting properties change when the temperature is raised. A: mean pacemaker burst frequency (±SE, n = 5) increased with temperature (P < 0.001), but the incremental increase in frequency was relatively constant; ○, the mean (±SE) ratio of Freq. T /Freq. T-1 (i.e., the factor by which frequency increased for each degree increase in temperature). B: the increase in frequency with increase temperature (shown in A, n = 5) can be fitted to a logarithmic equation that predicts a 1.16-fold increase in pacemaker bursting frequency for a 1°C increase in temperature during heating between 30 and 40°C; for reference, this prediction is plotted in A. C: the amplitude of the depolarizing drive potential underlying bursts increased. D: the burst duration was reduced when the temperature was raised from 30 to 40°C.
some cases, it did with heating (Fig. 2B), potentially reducing the number of action potentials/burst. The enhanced depolarization makes it difficult to precisely count action potentials within bursts as the spike amplitude can be truncated due to inactivation. With that caveat stated, we did not find significant differences in the mean number of action potentials/burst for 10 bursts taken from isolated pacemaker neurons at 30°C (22.02 ± 9.8; mean ± SE) versus 40°C (12.7 ± 4.1; n = 5, P = 0.204).

To further examine whether temperature enhancement of pacemaker frequency underlies the increase in fictive RF recorded at the network level, we examined the temperature-induced FM of pacemakers within the intact network as well as after isolation from synaptic transmission. As the respiratory network is very sensitive to temperature changes (Tryba and Ramirez 2003), we analyzed data from experiments with overlapping temperature profiles during heating, but note that during passive cooling of the bath to 30°C, the SD of the bath temperature increased (Fig. 4A). When the bath temperature was transiently increased to 40°C and returned to the control temperature (30°C), pacemaker burst frequency increased and decreased in parallel with that of the intact network (Fig. 4A). After isolation from chemical synaptic transmission with 200 μM Cd²⁺ (n = 4), pacemaker burst frequency paralleled that observed in the intact network at control temperatures, during heating and during hyperthermic-augmentation of fictive respiration (Fig. 4B, bins 1–9, P > 0.05). Cooling was initiated at approximately the same time in each of the experiments, yet during initial cooling of the bath from 40°C, the burst frequency of the isolated pacemakers was less than when it was embedded in the intact network (Fig. 4B; bins 10 and 11: P < 0.001 and P < 0.008, respectively; n = 4). This deviation may reflect the increase in bath-temperature SD during passive cooling from 40 to 30°C (i.e., cooling at different rates; Fig. 4B). However, as the temperature was further decreased to ~30°C, the bursting frequency of these neurons was similar to when they were embedded in the network or isolated from synaptic input (bins 12–20, P > 0.05, Fig. 4B; n = 4).

Conditional bursting of inspiratory pacemaker neurons

Not all neurons continued to burst at 30°C after isolation from chemical synaptic transmission, but some neurons (n = 7) became rhythmic after heating and burst robustly at 40°C. In those cases, when the bath temperature was 30°C, the isolated neurons were either silent (n = 3) or fired action potentials in clusters that did not resemble bursts (n = 4; Fig. 5A). However, raising the bath temperature induced bursting with the first distinct burst occurring at a mean temperature of 36.8 ± 1.2°C (n = 7) and continuing when the temperature was elevated to ~40°C (Fig. 5B; n = 7) and (as in Figs. 2, A and B, and 3C) bursts including an enhanced underlying depolarization (Fig. 5, A and B). Qualitatively similar changes were observed when the cell was embedded in the network (Figs. 5, C and D).

Hyperthermic fictive apnea

The respiratory network (Tryba and Ramirez 2003) and isolated pacemakers (Figs. 6, A and B) can generate stable fictive eupneic activity at temperatures of 30 and 40°C. How-
ever, we found that there is an upper critical temperature range at which pacemaker bursting mechanisms failed to generate endogenous rhythmic activity when the bath temperature was raised >40°C (Fig. 6C). The mean temperature at which synaptically isolated pacemakers failed to continue to generate endogenous bursting was 41.8 ± 0.9 (n = 5; Fig. 6, C and D). In all cases examined, bursting properties recovered after cooling (Fig. 6C).

**Effect of elevated temperature on the activity of nonpacemaker neurons**

Embedded in the respiratory network, nonpacemaker neurons increase their frequency of rhythmic activity when the bath temperature is elevated to 40°C (Fig. 7, A and B, n = 8). However, at the control temperature (30°C), nonpacemakers became either silent (n = 5) or tonically active (n = 3) after isolation from chemical synaptic input. These neurons remained either tonically active or silent after raising the bath temperature to 40°C (Fig. 7, A and B) and did not show a significant difference in $V_m$ at 30°C (−54.5 ± 6.8 mV) versus 40°C (−54.7 ± 6.4 mV; $P = 0.803, n = 8$).

**DISCUSSION**

Our data indicate that the bursting properties of inspiratory pacemaker neurons cease when exposed to temperatures between 41 and 42°C; the data from neonates further suggest the possibility that respiratory failure during heat shock may result from cessation of respiratory pacemaker activity. Conversely, hypothermic-apnea may also result from a cessation of the central rhythm generator activity as hypothesized by Mellen et al. 2002. Although caution should be used when extrapolating in vitro findings to the situation in vivo, these data are consistent with the findings that there are upper and lower critical body temperatures for sustaining respiration. In rats, a body temperature of 41.5°C is lethal to half of a population primarily due to respiratory as well as cardiac autonomic failure (Hubbard et al. 1976) and an average lethal core temperature for eutherian mammals is between 42 and 44°C (Adolf 1947). In fact, in most mammals, a body temperature >40°C severely compromises physical performance (Fuller et al. 1998).

Modulation of RF is a major mechanism by which most mammals dissipate heat (Altman and Dittmaer 1966; Saiki and Mortola 1996). At hyperthermic temperatures, most mammals (including nonpanting species) increase RF but decrease inspiratory amplitude and duration. This increases ventilation to dissipate heat. As in vivo, fictive eupneic activity at the population level includes an increase in RF accompanied by a reduction in inspiratory activity burst amplitude and duration (Tryba and Ramirez 2003). Our data indicate that elevated temperatures directly modulate respiratory pacemaker bursting properties, by increasing the bursting frequency of isolated pacemakers (Fig. 3, A and B).

![FIG. 5. Respiratory pacemaker burst generation can be conditionally dependent on temperature. A: some inspiratory neurons did not continue to burst rhythmically in absence of integrated VRG activity (\textit{fVRG}, top) after blockade of chemical transmission at 30°C. B: however, at 40°C, some of these neurons generated robust rhythmic pacemaker activity. Qualitatively similar changes in bursting were observed when the cell was heated while it was synaptically embedded in the network at 30°C (C) and at 40°C (D). Note the x-axis time calibration in D is different from that in A–C as it was expanded for clarity.](http://jn.physiology.org/doi/10.1152/jn.00287.2004)
Fig. 6. Respiratory pacemaker bursting can cease at elevated temperatures. Synaptically isolated PBC pacemakers that burst at both 30°C (A) and 40°C (B) ceased generating endogenous bursting when the temperature (temp., C, top) was increased further to between 40 and 44°C (C); an expansion of the trace outlined by the dotted box in C is shown in D, which shows the cell is essentially in an inactivated state. The mean temperature at which synaptically isolated pacemakers stopped bursting when the temperature was raised >40°C, was 41.8 ± 0.85°C (n = 5). Note in C that pacemaker activity and baseline membrane potential is restored after cooling from 44°C. Note that the voltage calibration bar in A applies to A–D and the time scale in A also applies to Fig. 6B.

Fig. 7. Nonpacemaker respiratory neuron activity is enhanced at higher temperatures. In the intact network, the frequency of nonpacemaker inspiratory neuron bursting increases when the bath temperature is raised from 30°C (A) to 40°C (B). However, they are either tonically active or silent after isolation from chemical transmission with bath-applied 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (RS)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), bicuculline and strychnine for both bath temperatures of 30°C (C) and 40°C (D).

B), enhancing the depolarization underlying their bursts (Fig. 3C), reducing burst duration (Fig. 3D), and additionally, inducing bursting properties in some inspiratory neurons that did not burst at 30°C (Fig. 5, A and B). Interestingly, during heating, the bursting frequency of PBC pacemakers directly paralleled that of the intact network, both before and after synaptic isolation (Fig. 4, A and B). Additionally, the approximate twofold/5°C increase in isolated pacemaker frequency we observed is similar to the rate of increase in respiratory network output (fictive RF) that occurs for temperatures between 25 and 35°C in vitro (Peever et al. 1999). In contrast to pacemakers, the activity of isolated nonpacemaker neurons was not significantly altered during hyperthermia. These data suggest that pacemakers rather than nonpacemaker neurons may be responsible for FM of fictive respiration during an increase in temperature. Pacemakers are also thought to underlie hypoxic modulation of RF (Thoby-Brisson and Ramirez 2000). However, we cannot rule out the possibility that synaptic interactions may also play an important role in modulating the response of the respiratory network to temperature changes. Indeed, while changes in the frequency (Fig. 3, A and B) and duration (Fig. 3D) of synaptically isolated pacemaker bursts were similar to those of respiratory network population activity during heating (Fig. 4, A and B) (Tryba and Ramirez 2003), fictive inspiratory population activity amplitude declined (Tryba and Ramirez 2003). This effect on the population amplitude is opposite to the hyperthermic effect on the amplitude of individual, synaptically isolated pacemaker in which burst amplitude increased (Fig. 3C).
This discrepancy is not too surprising, because the integrated population amplitude is not only determined by the amplitude of the depolarizing drive potential in individual neurons. Integrated population amplitude depends on various factors including the number of neurons active during inspiration and the intraburst spike frequency of inspiratory pacemaker and non-pacemaker neurons as well as the synchrony between neurons. A reduced population amplitude could also be due to changes in synaptic interactions among network members (Kelty et al. 2002). At hyperthermic temperatures, there is enhancement of both glycnergic and glutamatergic spontaneous miniature postsynaptic potentials with inhibition being enhanced to a greater extent than excitation (Kelty et al. 2002). This may not only reduce the number of neurons active during each fictive inspiratory burst but may also modulate pacemaker activity as inhibition can suppress pacemaker bursting (Tryba et al. 2003). We therefore hypothesize that pacemaker properties play an important role in determining the frequency response and possibly the burst duration of the respiratory network activity, but that the hyperthermic response of the respiratory network, which includes also a modulation in amplitude of population activity, will be the result of a complex interaction between cellular and synaptic properties.

At the population level, the hyperthermic response is consistent with fictive eupneic activity (Tryba and Ramirez 2003) that includes both caudum-sensitive and -insensitive pacemakers (Thoby-Brisson and Ramirez 2001). Although not specifically tested here, note that heating may differentially modulate these different types of pacemakers.

In this study, the pH of the ACSF increased (on average) by 0.04 ± 0.02 pH units when the temperature was raised from 30 to 40°C. Both VRG population and PBC pacemaker bursting frequency increase with a decrease in pH (Johnson et al. 1998). Thus the increase in VRG population (Tryba and Ramirez 2003) and PBC pacemaker bursting frequency at elevated temperatures that we demonstrated is opposite to what one would expect if an elevation in pH played a significant role in determining the fictive RF in our studies. Peever et al. (1999) also examined the issue of how the frequency of respiration (hypoglossal activity) is influenced by temperature and pH in transverse brain stem slice preparations. While motor output (hypoglossal bursting) RF was modulated by temperature, at any given temperature, large pH changes did not significantly modify the fictive RF. Thus although the ACSF pH changes with changes in temperature, the principal effect on RF observed here is likely due to temperature rather than pH changes.

An important observation was that bursting properties of respiratory pacemakers can be conditionally dependent on temperature; that is, some synaptically isolated pacemakers failed to generate endogenous rhythmic bursting at both hypothermic (30°C; Fig. 5A) and hyperthermic temperatures (>40°C; Fig. 6, C and D). To our knowledge, there are few other reports of modulation of pacemaker properties at elevated temperatures (e.g., lobster stomatogastric system: Johnson et al. 1992; mollusks:Gola 1976; Thompson et al. 1986; Treistman and Bablanian 1985). Interestingly, some pacemakers initiated bursting activity within a normothermic range (36.8 ± 1.2°C), suggesting that expression of pacemaker properties is temperature-sensitive. Note that the mouse mean basal temperature is 36.5 ± 0.1°C (Wikström et al. 1998) and normothermia for eutherian species is between 36 and 38°C (Morrison and Ryser 1952). More importantly, because many in vitro respiration studies—including our own—were performed at hypothermic temperatures (to preserve preparation viability), it should be considered that the full repertoire of respiratory neurons may not be appreciated under these conditions. Our finding may have important implications for various modeling studies that address the role and significance of pacemaker neurons in respiratory rhythm generation (Butera et al. 1999; Rybak et al. 1997).

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REFERENCES


