Response of the Respiratory Network of Mice to Hyperthermia

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Tryba, Andrew K. and Jan-Marino Ramirez. Response of the respiratory network of mice to hyperthermia. J Neurophysiol 89: 2975–2983, 2003. First published February 26, 2003; 10.1152/jn.00743.2002. Most mammals modulate respiratory frequency (RF) to dissipate heat (i.e., panting) and avoid heat stroke during hyperthermic conditions. During hyperthermia, the RF of intact mammals increases and then declines or ceases (apnea). It has been proposed that this RF modulation depends on the presence of higher brain structures such as the hypothalamus. However, the direct effects of hyperthermia on the respiratory neural network have not been examined. To address this issue, the respiratory neural network (i.e., ventral respiratory group (VRG)) was isolated in a brain stem preparation taken from the medulla of mice (P0–P6). Integrated population activity, predominated by inspiratory neurons, was recorded extracellularly from VRG neurons. The bath temperature was then heated from 30 to 40°C, resulting in a biphasic frequency response in VRG activity. Following an initial six- to sevenfold increase and subsequent decline, fictive RF was maintained at a frequency that was higher than baseline frequency; at 40°C, the RF was maintained at about two to four times that at 30°C. The inspiratory burst amplitude and duration were significantly reduced during hyperthermic conditions. An increase in RF and decrease in VRG burst amplitude and duration also occurred when heating from 37 to 40°C. Fictive apnea typically occurred during cooling to the control temperature. Furthermore, changes in hypoglossal motor nucleus activity paralleled those of the VRG, suggesting that temperature modulation of the VRG is likely to have a behaviorally relevant impact on respiration. We conclude that the VRG activity itself is modulated during hyperthermia and the respiratory network is particularly sensitive to temperature changes.

INTRODUCTION

For most mammals, an elevated body temperature results in an increase in respiratory frequency (RF) and decrease in tidal volume with a net increase in ventilation (Galland et al. 1993; Ni et al. 1996; Parmeggiani et al. 1998). This respiratory response is adaptive, because it increases heat loss through convection and evaporative cooling. Rapid shallow breathing persists throughout hyperthermia but the respiratory rate declines as the heat-load is dissipated (Galland et al. 1993). If this mechanism fails to decrease the heat-load, hyperthermia associated with conditions such as fever and heat stroke may lead to apnea and death (Eshel et al. 1990). Apnea associated with hyperthermia can also occur during recovery from hyperthermia (i.e., cooling; Galland et al. 1993). Understanding the mechanisms underlying modulation of respiration during hyperthermia may be relevant to several clinical conditions, including fever, heat stroke, and sudden infant death syndrome (SIDS; Fleming et al. 1990; Harper et al. 2000; Poets et al. 1999; Russell and Vink 2001). In the case of SIDS victims, they typically have a markedly elevated body temperature upon death (Fleming et al. 1990).

It is generally believed that the increase in respiratory rate in response to hyperthermia is mediated by the hypothalamus that detects changes in body temperature and modulates respiration (Inomoto et al. 1983; Parmeggiani et al. 1998; Pleschka and Wang 1997). This pathway may not be responsible for hyperthermic apnea, which is thought to be initiated by cardiovascular failure, rather than direct modulation of central respiratory network activity (Eshel et al. 1990). At high temperatures, cardiac output initially increases and then declines to levels that can trigger cardiac arrest. Cerebral failure and apnea may occur secondarily to cardiac arrest (Eshel et al. 1990). However, the response of the central respiratory neural network to a range of temperatures associated with fever (i.e., 38–41°C) has not been examined. In this study, the respiratory neural network (i.e., ventral respiratory group (VRG)) was isolated in transverse brain slice preparations taken from the medulla of mice. We evaluated the effect of hyperthermic temperatures on fictive respiration by measuring population activity in the VRG and hypoglossal (XII) motor nucleus under control conditions (30°C) and at an elevated bath temperature (40°C).

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. All efforts were made to minimize both the number of animals used and their suffering.

Medullary brain slice preparation

All experiments used the transverse, rhythmic medullary brain slice preparation (Funk et al. 1994; Ramirez et al. 1996). Mice (0- to 6-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 C7/C8 spinal level (Ramirez et al. 1996). The brainstem was dissected out in ice cold artificial cerebral spinal fluid (ACSF) that was equilibrated with carbogen (95% O₂-5% CO₂, pH = 7.4). Rhythmic 650 μm thick slices containing the VRG (Ramirez et al. 1996) were obtained by slicing the medulla using a microslicer (VT1000S, Leica, Nussloch, Germany). Slices were submerged in a recording chamber (6 ml) under circulating ACSF (30°C; flow rate 15 ml/min, total volume = 200 ml) containing (in mM) 118 NaCl, 3 KCl, 2.5 CaCl₂, 1 MgCl₂, 25 NaHCO₃, 10 dextrose, and 100 µM kainic acid. Slices were allowed to equilibrate for 90 min before testing.

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were obtained with glass suction electrodes positioned on the slice within a span of 30 min before commencing recordings. All chemicals were obtained from Sigma (St. Louis, MO).

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. Unless otherwise noted, the ACSF temperature in the preparation bath was raised from 30 to 40°C (or 37–41°C), and then allowed to cool to the initial temperature; bath temperature at various locations within the bath were routinely uniform. Data for each heating protocol was collected from preparations that had not undergone prior heat treatment. 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, in another series of experiments (for which the data here provide a basis), we used whole cell current clamping to record from VRG neurons during heating, and our success in maintaining seals increased using a protocol having shorter recording times (Tryba and Ramirez 2001).

**Electrophysiological recordings**

INTEGRATED VRG POPULATION ACTIVITY. Extracellular recordings were obtained with glass suction electrodes positioned on the slice surface in the VRG. In some preparations, the hypoglossal nucleus activity was simultaneously recorded with a separate electrode (Fig. 1A). The 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; Fig. 1B; 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 serve as a marker of fictive inspiration (Telgkamp and Ramirez 1999). The frequency of VRG integrated inspiratory bursts during fictive eupnea was used to define respiratory frequency (RF).

**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.** The average RF around the peak RF during heating was computed for each preparation by averaging 11 data points, consisting of the peak value and 5 values on either side of the peak value. These data were compared by Student’s t-test with 11 points averaged at the start of the control record at 30°C. Student’s t-tests were also used to test for significant differences between RF (paired t-test), inspiratory burst duration (percentage control, 1-sample t-test), and amplitude data (unpaired t-test) when the temperature was maintained at 30 and at 40°C temperatures; comparisons were made for data collected over a 400 s duration (n = 6), or for 20 bursts at 30 versus 40°C after the temperature had been maintained at 40°C for 1 min (n = 24). Similar methods were used to evaluate bursts over

**FIG. 1.** Mouse medullary slice containing the neural network for respiration. A: extracellular recording electrodes were placed on the surface of the brain slice preparation to record population activity from the ventral respiratory group (VRG) and the hypoglossal nucleus (XII). B: raw ventral respiratory group (VRG) and XII population activity was integrated (fXII); integrated activity is predominated by inspiration, giving rise to fictive inspiratory bursts in the integrated traces (fVRG, fXII).

**FIG. 2.** Modulation of the respiratory network (VRG) and hypoglossal activity during heating and anoxia. A: integrated VRG (fVRG) and XII (fXII) population activity during heating the bath artificial cerebrospinal fluid (ACSF) from 30 to 40°C (top). During heating to 40°C, there is a rapid increase in fictive eupnic frequency that declines to a rate that is higher than that at the control temperature (30°C), while the bath temperature is maintained at 40°C. Downward arrow on temperature trace (top) indicates onset of heating the ACSF. Large amplitude bursts represent sighs (Lieske et al. 2000). Dashed line across fXII trace serves as a reference for AM of hypoglossal activity. Note that a hypoglossal nucleus burst follows each burst in VRG activity. B: anoxia also initiates a biphasic increase and decrease in inspiratory frequency, but this increase is not as pronounced as during hyperthermic conditions and the decrease culminates with respiratory frequency (RF) less than control (A). Downward arrow on anoxia stimulus bar (black bar = 10 min), indicates onset of anoxic stimulus application. Hypoglossal burst amplitude increased during hypoxia (dashed line serves as reference; Telgkamp and Ramirez 1999), whereas it declines during hyperthermia (A).
200 s while bath temperatures were maintained at 36.8 versus 41°C (n = 5). Burst durations were calculated as the burst width at half-maximum height. Burst amplitude was measured as burst peak height from baseline and normalized as percent of control; changes in baseline activity due to tonic activation were subtracted as described by Telgkamp and Ramirez (1999). Expiratory duration was calculated as the time between the offset of one inspiratory burst and the onset of the subsequent burst. Values were assumed to be significant at P < 0.05.

RESULTS

Biphasic hyperthermic response

During hyperthermia, both panting and nonpanting mammals modify their breathing by increasing RF and decreasing both inspiratory amplitude and duration. Here we used the medullary brainslice preparation to examine if hyperthermic temperatures directly influence the central respiratory network in the VRG as well as the hypoglossal nuclei (Fig. 1, A and B). To test this, we recorded population activity from both VRG and hypoglossal nuclei and examined how fictive RF, burst amplitude, and duration change when the slice preparation is exposed to ACSF at temperatures between 30 and 40°C.

Increasing the ACSF bath temperature from 30 to 40°C produced a biphasic increase and subsequent decrease in the frequency of integrated VRG (n = 24 preparations) and hypoglossal activity (n = 6 preparations; Fig. 2A). Note that the response to hyperthermia is qualitatively different from that to anoxia (Fig. 2B). Although there was a decline in RF from the peak interburst interval, the RF remained higher at 40°C than that at 30°C (Fig. 2A). The mean inspiratory frequency, burst duration, and amplitude, as well as expiratory time, was determined for 20 VRG bursts at 30°C and compared with averaged values for 20 bursts at 40°C. The values at 40°C were obtained after the bath temperature had been maintained at 40°C for 1 min. At 40°C, the mean inspiratory burst frequency increased, while the burst duration and burst amplitude declined relative to data collected at 30°C (Fig. 3, A–C; Table 1). The mean expiratory duration was also significantly less at 40°C than at 30°C (mean reduction = 76%; P < 0.0001, n = 24 preparations).

While we initially compared data from 24 preparations during steady-state temperature conditions, the VRG response may depend on the time-course of heating the ACSF. In fact, as the bath temperature was raised from 30 to 40°C, there was an initial increase in fictive RF to a maximum value that was higher than the steady-state frequency at 40°C (Fig. 4A). The RF then decreased from this initial peak frequency, even though the bath temperature was maintained at 40°C (Fig. 4A). Since the response to heating changes with time, the response of the VRG and XII nucleus was further quantitatively evaluated for six preparations where heating the bath was achieved over the same time-course (Fig. 4A; n = 6). In each preparation, VRG activity was compared at the same time points over 400 s duration at 30 and 40°C (Fig. 4A, stippled areas; Table 2; n = 6). The rate of temperature increase, between 30 and 37°C, was relatively linear, with a slope of 7.17°C/min, but the rate slowed as the bath was heated between 37 and 40°C (Fig. 4A). As the bath temperature was raised from 30 to 40°C, there was an initial increase in fictive RF to a maximum value that was approximately 6.75 times the control (mean of ratios, P < 0.001, n = 6 slices). Thereafter, the VRG inspiration frequency declined from the peak frequency, but with the temperature maintained at 40°C, the average RF was 2.21 ± 0.44 SE (mean of ratios, n = 6) times higher than that at 30°C (Fig. 4A; Table 2). The VRG burst amplitude and duration declined when the temperature was maintained at 40°C (Fig. 4, B and C; Table 2).

To assess the effect of hyperthermia on a respiratory motor output, we obtained recordings from the hypoglossal nucleus. All examined hypoglossal preparations responded in a manner that was qualitatively and quantitatively similar to the biphasic response described above for the VRG when the bath temper-
temperature was increased from 30 to 40°C (Figs. 2A and 5, A–D; Table 2; n = 4).

To examine the hyperthermic response when the initial bath temperature was closer to normothermia (Wikström et al. 1998), the bath temperature was maintained at 37°C for 10 min and then raised from about 37°C (36.8 ± 0.36°C SD) to 41.022 ± 1.39°C SD (n = 5). In each preparation, VRG activity was compared at the same time points over a 200 s duration at 36.8°C (0–200 s in Fig. 6A) and 41.0°C (600–800 s in Fig. 6A; Table 3; n = 5). As was the case when raising the bath temperature from 30 to 40°C, increasing ACSF temperature from about 37°C to 41°C also increased the average VRG RF by 2.20 ± 0.11 SE times (mean of ratios, n = 5; Fig. 6B). Burst duration and amplitude also declined with this increase in temperature (n = 5; Table 3; Fig. 6C and D).

Cooling from hyperthermic temperatures

In mammals, rapid shallow breathing typically persists throughout hyperthermia, but the respiration rate declines as the heat-load is dissipated (Galland et al. 1993). In our experiments, apnea typically occurred during cooling (Fig. 7A, 7B), which is consistent with previous studies in rat brain stem preparations (Mellen et al. 2002) and in intact piglets (Galland et al. 1993). When bath temperature was increased from 30°C to 40°C, fictive eupnea recovered within the 200 s following the apnea, since the mean eupneic RF during this time was comparable to control RF at 30°C (P = 0.29; n = 6). Fictive apnea is defined here as cessation of fictive eupnea, for a duration of one minute or more. Under control conditions at 30°C, eupnea was continuous, however, fictive apnea occurred in 22 of 24 preparations during cooling to the control temperature (30°C) from 40°C and persisted for an average of 1.9 ± 0.3 min (n = 22 slices). The temperature at which apnea began ranged from 31.1°C to 39.8°C, having a mean temperature of 34.19°C (±2.67 SD, n = 22). Note that while the SD is roughly 3°C about the mean temperature at which apnea occurred, the range over which apnea occurred was large (variance was 3.26 times the SD). In the two preparations where apnea did not occur, fictive respiration still slowed markedly during cooling. Additionally, in preparations where the bath temperature was increased from 36.8 to 41°C, fictive apnea occurred in four of the five preparations during cooling to the initial temperature; fictive eupnea stopped for 23 s in the remaining preparation. In these cases, apnea began at a temperature range between 36.18 and 39.4°C (37.66 ± 1.43°C, n = 4) and lasted an average of 2.73 ± 1.34 min.

Hyperthermic versus hypoxic response

Raising the temperature of the ACSF may reduce the partial pressure of dissolved oxygen (pO$_2$); thus it is possible that the response we observe to hyperthermia represents the combined effects of heating and some degree of hypoxia. A quantitative evaluation of the hypoxic response of the VRG and XII nuclei has been previously described (Ramirez et al. 1997; Telgkamp and Ramirez 1999) and is beyond the

<p>| Table 1. Mean fictive inspiratory frequency, duration, and amplitude for 20 bursts at 30 or 40°C |
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<tr>
<th>Frequency (Hz)</th>
<th>Duration (s)</th>
<th>Amplitude (%)</th>
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<tr>
<td>VRG (n = 24)</td>
<td></td>
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<tr>
<td>30°C</td>
<td>0.28 ± 0.097 SE</td>
<td>0.43 ± 0.089 SE</td>
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<tr>
<td>40°C</td>
<td>1.04 ± 0.38 SE</td>
<td>0.205 ± 0.052 SE</td>
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<tr>
<td>P value</td>
<td>&lt;0.0001</td>
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<td>Mean of ratios</td>
<td>4.34 ± 2.68 SE</td>
<td>0.50 ± 0.142 SE</td>
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FIG. 4. Modulation of fictive eupneic burst frequency, amplitude, and duration during temperature changes. Mean values (±SD bars) in Fig. 4 are given for 20-s bins (n = 6 preparations). Histograms show (A) mean instantaneous frequency, (B) burst amplitude, and (C) burst duration data recorded from the VRG during heating the bath ACSF from 30 to 40°C. Mean temperature (°C, ±SD) is plotted in A–C, and the rate of change of temperature is given in A. Stippled areas in A indicate period of time from which statistical comparison of raw data were made [Table 2; i.e., 30°C (0–400 s) and 40°C (800–1,200 s)]. Note that the largest FM occurs when the bath temperature changes (A). Bath temperature was passively cooled from 40°C down to the control (30°C), which can account for the increase in the SD around the mean temperature shown in A.
scope of this study. Here, we qualitatively compared the hypoxic and hyperthermic response of the VRG ($n = 6$ preparations), and in four preparations, we simultaneously recorded XII population activity. Brain slices from young animals (P0–P6) were exposed to anoxia for 10 min while recording from the respiratory nuclei (Fig. 2B). Following this anoxic challenge and return of the RF to baseline levels (i.e., after 30 min; see Blitz and Ramirez 2002), we exposed the same slice to hyperthermia (5 min; $n = 6$ slice preparations; Fig. 2, A and B). As can be seen in Fig. 7, B and C, while young animal preparations have a limited response to hypoxia ($n = 6$ preparations, Ramirez et al. 1997), the biphasic response to hyperthermia is very pronounced (see also Fig. 2, A and B). Unlike during heating, the response to hypoxia included an augmentation in hypoglossal burst amplitude ($n = 4$ preparations; dashed line in Fig. 2, A and B; Telgkamp and Ramirez 1999). Note that the duration of anoxia application (Fig. 2B) was more than twice that of hyperthermia (Figs. 2, A and B, and 7B). This protocol was used to maximize detection of a potential hypoxic response and to reduce the possibility that the larger response observed during hyperthermia was simply the result of a rapid (temperature-dependent) decline in pO$_2$ within the ACSF and tissue.

**DISCUSSION**

Most mammals increase RF to dissipate heat, and the RF decreases as body temperatures decline (Altman and Dittmaer 1966; Saiki and Mortola 1996). Our data indicate that heating directly modifies respiratory neural activity genera-

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<th>Frequency (Hz)</th>
<th>Duration (s)</th>
<th>Amplitude (%)</th>
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<tr>
<td>VRG ($n = 6$)</td>
<td></td>
<td></td>
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<tr>
<td>30°C</td>
<td>0.19 ± 0.025 SE</td>
<td>0.48 ± 0.022 SE</td>
<td>100</td>
</tr>
<tr>
<td>40°C</td>
<td>0.43 ± 0.11 SE</td>
<td>0.205 ± 0.032 SE</td>
<td>43.28 ± 8.36 SE</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&lt;0.001</td>
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<th>Frequency (Hz)</th>
<th>Duration (s)</th>
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<tr>
<td>Hypoglossal ($n = 4$)</td>
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<tr>
<td>30°C</td>
<td>0.044 ± 0.053 SE</td>
<td>100</td>
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<tr>
<td>40°C</td>
<td>0.17 ± 0.019 SE</td>
<td>26.48</td>
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<tr>
<td>$P$ value</td>
<td>0.007</td>
<td>0.017</td>
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**TABLE 2. Fictive inspiration frequency, duration, and amplitude at 30 or 40°C over 400 s**

**FIG. 5.** The respiratory motor nucleus (hypoglossal) population activity is modified during temperature changes. Fictive hypoglossal burst amplitude (A) and burst duration (B) declined during heating from 30°C (0–500 s) to 40°C (800–1,400 s). Mean burst amplitude (C) and duration (D) were significantly less at 40 than 30°C. Statistical comparison of mean data ($±$SD) in C and D are from raw data collected over the times 0–400 s (30°C) and 800–1,200 s (40°C) that are binned and shown in A and B. Hypoglossal FM is not shown because it follows that of the VRG (Figs. 1 and 2).
ated in the VRG to produce a biphasic increase and decrease in RF (Figs. 2A and 4A). Heating to hyperthermic conditions modifies fictive respiration to include an increase (augmentation) in RF that is typically followed by a decrease in RF (Figs. 2A and 4A). This in vitro response is similar to that from intact piglets where RF is modulated in a biphasic manner. The in vivo response also included an augmentation and decline from the peak frequency during hyperthermia, but it occurs over a much longer time course (Galland et al. 1993). Interestingly, as with intact piglets (Galland et al. 1993), the response to heating may include an apnea that typically occurs during cooling (Fig. 7A). Additionally, our hypoglossal recordings serve to demonstrate that the temperature response of the VRG is likely to have a meaningful impact on motor output (Fig. 2A). Thus the VRG, isolated in a transverse slice preparation, may serve as an important model for investigating the neural mechanisms that lead to the hyperthermic response of the respiratory network.

Is the respiratory network more sensitive to changes in temperature or the absolute magnitude of the temperature? This is an important clinical question since treatment of hyper- and hypothermia and ischemia may involve altering brain temperature to preserve neurological function (Inamasu et al. 2000; Wass et al. 1998). If the absolute temperature is the primary determinant of RF modulation, eupneic frequency would increase with increasing temperature. In fact, in vitro studies by Peever et al. (1999) suggest that RF rises nearly linearly and doubles for every 5°C increase in temperature between 25 and 35°C. In the present study, we demonstrate that there is an initial six to sevenfold increase in RF when the bath temperature was being raised from 30 to 40°C (Fig. 4A). However, the initially elevated RF then decreased to about two to four times that at 30°C (Tables 1 and 2) as the bath temperature was maintained at 40°C. Thus the most dramatic frequency response occurred as the bath temperature increased and not when the temperature was maintained at an elevated steady state level (Fig. 4A). Along these lines, note the hyperthermic response of the respiratory network was not markedly different on elevating the

TABLE 3. Fictive inspiration frequency, duration, and amplitude at 37 or 41°C over 200 s

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<th></th>
<th>37°C</th>
<th>41°C</th>
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<tr>
<td>Frequency (Hz)</td>
<td>0.19 ± 0.021 SE</td>
<td>0.41 ± 0.062 SE</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>1.44 ± 0.18 SE</td>
<td>0.85 ± 0.17 SE</td>
</tr>
<tr>
<td>Amplitude (%)</td>
<td>71.24 ± 3.82 SE</td>
<td>0.17 ± 0.17 SE</td>
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P value | 0.024 | 0.001 | 0.003 |
cooling to control temperature (30°C) and differences in the hyperthermic evoked response. While there was continuous rhythmic burst (fictive eupnea) prior to and during heating, cooling typically (n = 91.6% of 24 preparations) evoked fictive apnea (dashed box labeled apnea). B: cumulative histograms of the response of n = 6, P0–P6 animals on heating the bath to 40°C (open bars ± SD) and during anoxia (filled bars ± SD). The graphs are aligned to the onset of the stimulus indicated by downward arrows above histograms (open arrowhead = temperature onset; closed arrowhead = anoxia onset). C: instantaneous frequency plot of a hyperthermic response (open triangles) to heating to 40°C (line with arrows) and the frequency response (open circles) of the same preparation to anoxia (solid bar with vertical ends).

FIG. 7. Eupneic instantaneous frequency during heating to 40°C and cooling to control temperature (30°C) and differences in the hyperthermic and hypoxic response. A: fictive eupneic frequency (Hz) during heating shows an increase in frequency during heating that declined during cooling. While there was continuous rhythmic burst (fictive eupnea) prior to and during heating, cooling typically (n = 91.6% of 24 preparations) evoked fictive apnea (dashed box labeled apnea). B: cumulative histograms of the response of n = 6, P0–P6 animals on heating the bath to 40°C (open bars ± SD) and during anoxia (filled bars ± SD). The graphs are aligned to the onset of the stimulus indicated by downward arrows above histograms (open arrowhead = temperature onset; closed arrowhead = anoxia onset). C: instantaneous frequency plot of a hyperthermic response (open triangles) to heating to 40°C (line with arrows) and the frequency response (open circles) of the same preparation to anoxia (solid bar with vertical ends).
tions may affect the respiratory network such that the respiratory activity recorded may not be consistent with eupnea but may represent another form of breathing such as gasping. Their in vivo data suggested that one can distinguish between eupnea and gasping because the two patterns behave differently when the body temperature is raised from hypothermic to normothermic temperatures. In intact rats and in a perfused brain stem spinal cord preparation, the eupneic RF approximately doubled when the (body or perfusate) temperature was raised from 32 to 38°C, while gasping frequency did not increase with temperature elevations. Several lines of evidence suggest that our data from the isolated respiratory network is consistent with eupnea rather than gasping. First, the RF nominally doubled for eupnea, but not gasping, both in vivo and in a perfused preparation for temperatures increases from 32 to >35°C (St. Jacques and St. John 2000). This was also the case in vitro when we raised and maintained the ACSF bath temperature from 30 to 40°C (Tables 1 and 2) or from about 37 to 41°C (Table 3). Second, in perfused rat preparations, gasping involves an increase in burst amplitude at elevated temperatures (St. Jacques and St. John 2000). In contrast, we found inspiratory burst amplitude was less at 40°C than at 30°C (Tables 1 and 2; Figs. 2A and 3B), which is consistent with shallow breathing in vivo. Third, in vivo and in perfused rat preparations, inspiratory time decreases with increasing temperature during eupnea, but not during gasping (St. Jacques and St. John 2000). Here we also found that time of expiration decreased significantly with increased temperature (see RESULTS). Taken together, these data strongly suggest that the respiratory activity recorded from the VRG and hypoglossal nucleus throughout this study is consistent with fictive eupnea, but not gasping.

Having found that the respiratory activity of the VRG is sensitive to changes in temperature, an important question is how the activity of individual neurons in the network changes during heating. This is an important question, because the rapid fictive respiration seen under hyperthermic conditions may, for instance, reflect 1) changes in synaptic transmission between network members (Kelty et al. 2001); 2) modulation of pacemaker neurons; or 3) a combination of these factors. Understanding which components of the respiratory network contribute to the hyperthermic response may in the future allow for directed manipulation of the response using therapeutic pharmaceutical agents.

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