Differential Responses of Respiratory Nuclei to Anoxia in Rhythmic Brain Stem Slices of Mice

PETRA TELGKAMP AND JAN-MARINO RAMIREZ
Department of Organismal Biology and Anatomy, The University of Chicago, Chicago, Illinois 60637

Telgkamp, Petra and Jan-Marino Ramirez. Differential responses of respiratory nuclei to anoxia in rhythmic brain stem slices of mice. J. Neurophysiol. 82: 2163–2170, 1999. The response of the neonatal respiratory system to hypoxia is characterized by an initial increase in ventilation, which is followed within a few minutes by a depression of ventilation below baseline levels. We used the transverse medullary slice of newborn mice as a model system for central respiratory control to investigate the effects of short-lasting periods of anoxia. Extracellular population activity was simultaneously recorded from the ventral respiratory group (VRG) and the hypoglossus (XII) nucleus (a respiration-related motor output nucleus). During anoxia, respiratory frequency was modulated in a biphasic manner and phase-locked in both the VRG and the XII. The amplitude of phasic respiratory bursts was increased only in the XII and not in the VRG. This increase in XII burst amplitude commenced ~1 min after the anoxic onset concomitant with a transient increase in tonic activity. The burst amplitude remained elevated throughout the entire 5 min of anoxia. Inspiratory burst amplitude in the VRG, in contrary, remained constant or even decreased during anoxia. These findings represent the first simultaneous extracellular cell population recordings of two respiratory nuclei. They provide important data indicating that rhythm generation is altered in the VRG without a concomitant alteration in the XII burst amplitude, whereas the burst amplitude is modulated only in the XII nucleus. This has important implications because it suggests that rhythm generation and motor pattern generation are regulated separately within the respiratory network.

INTRODUCTION

The neuronal network controlling mammalian breathing responds to hypoxia in a biphasic manner (Bureau et al. 1984; Haddad and Jiang 1993). Initially, ventilation is increased (augmentation), but during prolonged hypoxia the augmentation is followed by a depression and cessation of respiratory activity (apnea) (Lawson and Long 1983; Neubauer et al. 1990). However, this is a simplified description of the events that characterize the hypoxic response of the respiratory system. Recordings from respiration-related areas of the brain reveal that severe hypoxia or anoxia affects central respiratory activity in a differential manner. The augmentation in one respiration-related area does not necessarily correspond to the activation of other areas. For example, activities of phrenic and intercostal (Sears 1964; St. John and Bartlett 1979) as well as hypoglossal motoneurons (XII) (Hwang et al. 1983) increase significantly during the hypoxic augmentation. At the same time a significant proportion of bulbospinal neurons in the ventral respiratory group (VRG) and the dorsal respiratory groups (DRG) show no change or exhibit a depression in discharge frequency, whereas some neurons in the VRG increased their activity (St. John and Bianchi 1985; St. John and Wang 1977). Thus it remains unclear whether the population of respiratory neurons in the VRG increase their activity during hypoxia like the neurons in the motor nuclei or whether the population activity decreases in the VRG. An overall decrease in the VRG population activity would be surprising, because it is generally assumed that one particular region of the VRG, the pre-Bötzing complex, is essential for respiratory rhythm generation (Ramirez and Richter 1996; Rekling and Feldman 1998; Smith et al. 1991). Conceptually this issue is of great interest because it remains unknown whether the initial augmentation results in a general excitation of the respiratory network including the VRG and its motor output or whether alternatively the anoxic response is the result of a complex modulatory process that regulates different regions of the respiratory control system in a differential manner. To better characterize the anoxic response of the respiratory system, we used the medullary slice preparation from neonatal mice postnatal days 1–7. The effect of anoxia on the respiratory network was assessed by recording simultaneously extracellular population activities from the hypoglossus (XII) nucleus and the VRG (Fig. 1). This experimental approach allowed us for the first time to directly compare the anoxic effects on two different respiratory neuron population activities. This is new because previous studies focused either on a characterization of only the XII activity during hypoxia (Ramirez et al. 1997), or characterized the hypoxic response of only single VRG neurons (Ramirez et al. 1998). Like the analysis of single neuronal activity in other in vitro and in vivo studies (Ballanyi et al. 1994; England et al. 1995; Richter et al. 1993), the previous studies demonstrated important properties of single cell types within the VRG but provided no direct insight into the population response of the VRG. In the simultaneous population recordings from the XII and the VRG, as performed here, we describe not only in more detail the sequence of events that characterize the anoxic augmentation in different cell populations, but we will also demonstrate that respiratory burst amplitude and frequency are altered independently in the VRG and XII. These findings have important implications for the underlying mechanisms of anoxic augmentation.

METHODS

Preparation

Male and female mice (CD-1: Charles River Laboratories, see www.criver.com/1999rm/htdocs/cdmice_swiss.html) of postnatal age...
the ACSF with 95% N2-5% CO2 (pH 7.4). Exposure to anoxia was regular for up to 13 h. Anoxia was induced by bubbling with carbogen (95% O2-5% CO2, pH 7.4). The brain stem was fixed previously in detail (Ramirez et al. 1996), thus we will summarize only the most important steps. The brain was removed from the skull and immediately transferred into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 118 NaCl, 3 KCl, 1.5 CaCl2, 1 MgCl2 *, 6 H2O, 25 NaHCO3, 1 Na2HPO4, and 30 d-glucose and equilibrated with carbogen (95% O2-5% CO2, pH 7.4). The brain stem was fixed on an agar block and secured in a vibratome with the rostral end up. Thin slices were sectioned serially from rostral to caudal until reaching the rostral boundary of the pre-Bötzinger complex. The level of the pre-Bötzinger complex was recognized by cytoarchitectonic landmarks, such as the absence of the facial nucleus and the presence of inferior olive (IO), nucleus of the solitary tract (NTS), hypoglossal nucleus (XII), and nucleus ambiguus (NA). The rostrocaudal distance between the caudal end of the facial nucleus and the obex was ~700 μm in newborn mice. Portions of the VRG and XII were isolated in a 500- to 600-μm slice that was obtained ~200 μm caudal to the caudal end of the facial nucleus. The slice was immediately transferred into a recording chamber.

Submerged under a stream of ACSF (temperature, 29°C; flow rate 11 ml/min), the preparation was stabilized for 30 min in ACSF. The potassium concentration in the ACSF was raised to 8 mM over a period of 30 min and maintained at this concentration to keep rhythmic activity regular for up to 13 h. Anoxia was induced by bubbling the ACSF with 95% N2-5% CO2 (pH 7.4). Exposure to anoxia was restricted to a period of 5 min. Although the anoxic stimulus in the in vitro situation is different from the hypoxic stimulus in vivo in that the oxygen concentration switches from normo- to even hyperoxic to anoxic conditions, the response patterns are similar to those described for the in vivo and chemo-deafferented animal.

Recording and data evaluation

Extracellular population activities of neurons in the hypoglossal nucleus and VRG were recorded with electrodes that had an impedance of 120–150 kΩ, when filled with CSF. The electrodes were positioned with the visual aid of a binocular microscope (Zeiss, Axioskop) and the acoustic aid of a loudspeaker monitoring neuronal activity that was evoked when touching the slice surface with the electrode. Signals were amplified (1,500 times), band-pass filtered (low-pass 1.5 kHz, high-pass 250 Hz), and electronically integrated (Paynter filter, set at a time constant of 40–50 ms; Fig. 1, integrated traces). The data were digitized with a Digidata board (Axon Instruments), stored on a PC (Dell Pentium computer) and analyzed off-line with the software programs Axotape (Axon Instruments) and IGOR (Brain Waves). Inspiratory bursts were detected in IGOR with a manually chosen threshold to determine relative time course of respiratory frequency and tonic and phasic burst amplitude for the different phases of anoxia. In both the XII and the VRG only recordings with good signal-to-noise ratio were analyzed (such recordings showed significantly larger amplitudes of integrated inspiratory activity than the variances of the noise and expiratory activity). A potential problem in analyzing the amplitude of the integrated traces was the superimposition of respiratory burst activity and tonic activity (that resulted in a baseline shift of the integrated activity). Tonic activity and respiratory burst activity did not add up in a strictly linear manner. We therefore obtained two measurements during phases of tonic activation. In the figures, measurements excluding tonic activity are represented as white bars, and those with additional tonic activity are indicated by black bars.

Only one anoxic response per slice was examined and analyzed, because respiratory frequencies often increased after repeated anoxic exposures. The data were statistically analyzed and graphs created in Prism (GraphPad Software). Means are presented as means ± SE. Significance was determined using variance analysis and the paired Student’s t-test comparing means of control conditions with the means of different phases of the anoxic response. Significance was assumed when P < 0.05.

RESULTS

The effects of anoxia on XII and VRG activity were evaluated from the integrated traces of the extracellularly recorded population activity (Fig. 1). In these integrated traces, respiratory bursts were characterized by rapid upward deflections; changes in tonic activity were evident in slow changes in the baseline of integrated activity (Fig. 2). Introduction of anoxia altered 1) frequency and 2) amplitude of phasic-respiratory activity, as well as 3) amplitude of tonic activity in VRG and XII (Fig. 2). These alterations will be described in the next paragraph.

Qualitative description of the anoxic response

FREQUENCY OF RESPIRATORY BURSTS. The frequency of respiratory bursts was transiently increased in the VRG and the XII...
nucleus upon introducing anoxic conditions. The rhythmic burst activity occurred phase-locked in the VRG and XII (Fig. 2B). Thus the time courses of frequency alteration were the same in both areas. In two of nine preparations, this frequency increase was above control levels for the entire 5 min of the examined anoxic period (Fig. 2A). However, in most preparations (7 of 9 preparations) the frequency increase was followed by a decrease in respiratory frequency below control frequencies as depicted in Fig. 2B. This is illustrated in sequential histograms, in which instantaneous frequency values are plotted against time (Fig. 3). The frequency histograms of seven slices were superimposed in Fig. 3A. Here the frequency values of each slice are represented by a different symbol. Zero on the x-axis indicates the onset of anoxia. Note that there is considerable variability in the occurrence of the maximal frequency and the onset of the frequency depression. Two extreme examples are shown in Fig. 3B. The mean frequency values obtained for these slices at any given time during anoxia are shown in Fig. 3C. These values indicate that on average the frequency value is modulated in a biphasic manner during anoxia.

AMPLITUDE OF RESPIRATORY BURSTS. The amplitude of the respiratory bursts was differentially modulated in the XII and in the VRG. In the examined preparations using CD-1 mice, anoxia enhanced the amplitude of phasic respiratory rhythmic activity in the XII (Fig. 2A, rapid upward deflections, top trace, thin arrows), whereas no or only little alteration occurred in the VRG (Fig. 2A, rapid upward deflections, bottom trace). The modulation of the amplitude is illustrated in sequential histograms in Fig. 4. In these histograms phasic respiratory burst amplitude values (in a period of 30 s) in the VRG and XII were plotted against time (Fig. 4, A and B, left panel). We plotted changes of amplitudes both including (▲) and excluding (■) tonic activity at times when those activities overlapped (Fig. 4, A and B, right panel). Amplitudes were normalized against

---

**FIG. 2.** Differential anoxic response of VRG and XII nucleus mass activity. Integrated extracellular VRG and XII activities are differentially affected by anoxia. An initial increase in respiratory burst frequency and a small increase in tonic activity (upward shift of baseline) are followed by a strong increase in tonic activity (wide arrow). A: in this experiment, the frequency increase preceded the pronounced tonic activation and remained above control levels for the entire 5-min exposure to anoxia. Note the strong amplitude modulation in hypoglossal respiratory activity. During prolonged anoxia, the amplitude of phasic activity decreased in the VRG, while remaining increased in the XII. B: VRG and XII integrated extracellular activities were obtained during control conditions (left panel), during a period of increased frequency at the beginning of an anoxic insult (middle panel) and during a period of frequency depression at the end of a 5-min anoxic insult (right panel).
ANOXIA-INDUCED ALTERATION IN TONIC ACTIVITY. The anoxia-induced alteration in tonic activity was reflected in a slow upward shift in the baseline of integrated traces in the XII and VRG (Fig. 2A, wide arrow). In the XII, tonic activity always returned to baseline levels during the first 3 min of anoxia. In the VRG, tonic activity decreased in most cases (6 of 9) below control levels during the 5 min of anoxia. Thus the VRG showed a biphasic modulation of tonic activity. Because it is difficult to determine the zero of our extracellular recordings because of some tonic intraburst activity even under control conditions, we measured the amplitude of tonic activity (Fig. 6, B and C, black bars) for quantitative comparisons using the following procedure. The baseline in control conditions was set as 0 and the amplitude of control respiratory phasic activity set as 100%. This normalizing procedure eliminated differences between different recordings. The amplitude of tonic activity was given as a percentage relative to the phasic activity at control conditions.

Quantification of the anoxic effect

The sequential histograms in Figs. 3–5 have indicated that the amplitude and frequency were modulated in a transient manner: an initial increase in the frequency was followed by a depression. The burst amplitude in XII was enhanced during the entire 5 min of anoxia, but the average histogram (Fig. 5C) indicates that there was also a transient peak in the AM. Similarly, the increase in tonic activation of the XII and VRG were transient events. This raises the question, whether the peak of the FM occurred at the same time as the peak of the burst AM and the peak of the tonic activation. This issue could not be addressed adequately by comparing the times of maximal frequency, burst amplitude, and tonic activation, because the onset of these modulations varied considerably between different slices (Figs. 3B and 5B). Therefore we addressed this issue by measuring the modulation of the different parameters at different stages of the anoxic response. Measurements were obtained during the following stages of anoxia:

1) First minutes of anoxia before the onset of the pronounced increase in tonic activity.
2) During the maximal frequency augmentation.
3) Following the pronounced tonic augmentation.
4) During maximal frequency depression and tonic amplitude depression in VRG.

During each of these stages, the mean frequency and amplitude values of respiratory rhythmic bursts were determined for each experiment. Despite different time courses of the anoxic response, this procedure allowed a direct comparison of the different parameters during the different anoxic stages.

1) One to two minutes before the onset of tonic activity, the frequency increased on average by $37 \pm 25\%$ (from 0.389 to 0.472 Hz, mean $\pm$ SE, $n = 8$; Fig. 6A1). Overall, however, the mean frequency increase ($n = 8$) was not significant, because 50% of the slices ($n = 4$) did not show a significant frequency increase before the onset of tonic and phasic amplitude activation of the XII. Figure 6A1, right panel, shows a Box-Whisker plot of the FM to illustrate the variabilities between the different preparations. The whiskers show the range of data; they extend from the smallest to the largest values. The box extends from the 25th percentile to the 75th percentile; the horizontal line represents the median (50th percentile).

cut

control respiratory bursts to compensate for different recording qualities. Changes in tonic activity were evaluated by comparing the amplitude values measured during three to five interburst intervals in anoxia with respective amplitude values obtained during three to five interburst intervals in control conditions.

An example of a VRG response is shown as a sequential amplitude histogram in Fig. 4A. Note the absence of a significant amplitude modulation in the VRG response. In contrast to those findings in the VRG, an obvious AM occurred in the XII (Fig. 4B). Note that the phasic burst amplitude in the XII was also enhanced after subtracting the tonic modulation (Fig. 4B, right panel). Although all slices showed a modulation in the XII amplitude, the onset and duration of this modulation varied from preparation to preparation. This is illustrated in Fig. 5A by superimposing the sequential amplitude histograms of five slices. These histograms were obtained after subtracting the tonic modulation. Two extreme examples are shown in Fig. 5B: a slice that responded with a rapid onset is indicated by black squares, a slice with a slow onset of AM is indicated by open circles. The mean AM shown in Fig. 5C indicates that the amplitude in the XII was on average enhanced during the entire duration of the anoxic stimulus.
2) The maximal frequency augmentation of respiratory rhythmic activity (124.5 ± 45.7% increase, n = 9; Fig. 6A2) occurred after the onset of phasic and tonic AM in the XII (see Fig. 2A). At this stage, the frequency was significantly enhanced compared with the control conditions. Similarly, when compared with the control conditions, the phasic and tonic XII amplitude were significantly enhanced during the peak frequency (Fig. 6B2: left panel: mean values, right panel: Box and Whisker plots). The concurrent phasic and tonic activation is illustrated in Fig. 6B2 by superimposing an open (phasic modulation, 104.4% ± 59.62 increase) and a black bar (tonic modulation, 153.5% increase, left panel). In contrast to the AM of the phasic XII activity, there was no significant AM of the phasic VRG bursts (−7.72 ± 12.92%, n = 9), see Fig. 6C2, open bar). Mean maximal tonic VRG activity reached a level of 25.7 ± 10.33% (n = 9; Fig. 6C2). The maximal frequency increase did not precisely coincide with the maximal tonic activation of either the XII or the VRG. In the XII, the maximal increase in tonic activation occurred after the maximal frequency and the tonic activity declined rapidly shortly after. In the VRG, tonic activity increased fast and remained at an increased level for a longer period than the maximal increase in tonic activity and was independent from the FM.

3) After the cessation of the tonic XII activation (see Fig. 2A), respiratory XII burst amplitude was still significantly increased (209.6 ± 73.75%, n = 6; Fig. 6B3). In contrast, the amplitude of respiratory VRG bursts was not significantly altered (−1.03% ± 12.6 of control, n = 9, Fig. 6C3). At this stage of the anoxic response, the frequency of respiratory rhythmic activity was either still enhanced (Fig. 2) or already depressed compared with control conditions (Fig. 3A). On average, it was below the maximal frequency values and was at this stage not significantly different from control conditions (30.6 ± 27.5%; n = 9; Fig. 6A3).

4) The maximal depression occurred toward the end of the 5-min period of anoxia and was characterized by a significant depression in the VRG tonic and phasic activity (−59.9 ± 15.7%, n = 9; Fig. 6C4, black and open bar). At this stage, the frequency of respiratory activity was significantly decreased (34.71 ± 15.03% n = 6; Fig. 6A4) despite a considerable variability between experiments as indicated in the Box-Whisker plot (Fig. 6A4, right panel). The maximal depression in the amplitude of VRG activity occurred at a time when the amplitude of the phasic XII bursts was still augmented in 80% of the slices (see, e.g., Fig. 2B). However, as indicated in the Box-Whisker plot (Fig. 6B4, right panel), there was variability and in two slices the XII amplitude had returned to baseline levels. On average, the amplitude at this stage was not significantly different from control conditions (115.2 ± 65.3%, n = 6, Fig. 6B4). There was also no significant tonic depression in the XII nucleus (−19.8 ± 9.6%, n = 7; Fig. 6B4, black bar, left panel).

**DISCUSSION**

Here we have demonstrated that anoxia affects two respiration-related areas within the brain stem in a differential manner: the VRG and the XII nucleus. The VRG contains the pre-Bötzinger complex, the presumed site for respiratory rhythm generation (Smith et al. 1991), and the XII nucleus represents a respiratory motor output nucleus (e.g., Withington-Wray et al. 1988). The respiratory frequency was phase-
locked between the two areas and showed a similar modulation. This was expected, because the respiratory rhythm presumably originates in this preparation within the VRG and projects from there to the XII nucleus (Funk et al. 1993). However, the following differential effects were observed for other parameters of respiratory activity.

1) The modulation of tonic activity and of respiratory frequency were independently affected. In the VRG, tonic activity decreased sometimes below control levels while the frequency was still enhanced. This suggests that an overall excitatory effect cannot be the only explanation for the frequency increase. Given that the tonic activity in the integrated trace included also expiratory activity, this effect could be explained by an earlier shut down of expiratory compared with inspiratory neurons, as has been discussed earlier (Ballanyi et al. 1994).

2) The frequency modulation and the modulation of the amplitude of phasic XII bursts do not occur simultaneously, because the XII burst amplitude remained augmented during the frequency depression.

3) The respiratory burst amplitude was significantly enhanced only in the XII nucleus and not in the VRG. In most experiments, the burst amplitude commenced to decrease in the VRG while the amplitude of the hypoglossal bursts was still enhanced. This suggests that the modulation of burst amplitude is regulated independently or via different mechanisms in these two areas. The AM in the XII has previously been observed by Ramirez et al. 1997. As shown in Fig. 5C of Ramirez et al. 1997, there were some neonates that had an AM, but the modulation averaged over several animals was not statistically different from the control. This is different from our current finding, where the majority of neonates showed a significant AM (Fig. 6B). This difference may be explained by differences in the metabolic state of the slices. A manuscript by Wilken et al. (1998) indicated that neonatal slices that were treated with creatine exhibit a pronounced and significant augmentation. Therefore it is conceivable that the slices examined in this study had a larger ATP pool to begin with, and even untreated neonates exhibited a pronounced augmentation. This difference in the metabolic state could be due to slight differences in the preparation time. Although we prepare the slices in principal as described before, the slicing technique became much more routine and preparing a slice takes usually <5 min. We also use a different vibratome (FHC), which might result in a slightly different preservation of the slices. Another difference is that the results were obtained with different mouse strains (we used CD-1 mice; see METHODS).

FIG. 5. Amplitude modulation by anoxia. The anoxic AM of respiratory bursts in the hypoglossal nucleus shows different time courses in different experiments. A: superimposed normalized hypoglossal amplitudes for 5 experiments (in 10-s bins). Time = 0 s represents the onset of the anoxic insult. B: 2 extreme responses are plotted. C: despite the different time courses, the mean hypoglossal amplitude (n = 5) is increased throughout a 5-min anoxic period.

FIG. 6. Quantification of the anoxic effect on frequency (A) and burst amplitude in XII (B) and VRG (C). The quantification of frequency and amplitude values is based on mean values that were taken at different phases of the anoxic response. Column 1 depicts the anoxic alterations before the tonic activation. Values of column 2 were obtained during the maximal FM. Column 3 respresents values obtained after the tonic activation, and column 4 indicates values obtained during the maximal frequency depression (n = 9). Changes in the amplitude of integrated tonic activity are indicated as black bars, changes in phasic activity are shown as open bars. Left panels: mean values. Right panels: Box and Whisker Plots. A: frequency was significantly altered during maximal FM as well as during the end of the anoxic insult (frequency depression) compared with control conditions. B: hypoglossal amplitude including tonic activity was significantly altered during phase 2 and 3, whereas hypoglossal activity excluding tonic activity was significantly different from control values only after the disappearance of tonic activity. C: VRG burst amplitude was not significantly altered during anoxia.
4) In the present study we demonstrated that the amplitude of tonic modulation differed between the VRG and XII nucleus. The XII exhibited a pronounced larger tonic activation than the VRG. This tonic activation has been previously mentioned in hypoglossal recordings of the unanesthetized, but not the anesthetized in vivo animal (Weiner et al. 1982). Thus our data are consistent with the unanesthetized in vivo animal. The fact that the tonic activation is stronger in the XII than in the VRG may reflect a higher excitability of XII neurons in response to anoxic insults. A higher anoxic excitability of XII neurons compared with neocortical and hippocampal cells has been described previously (Donnelly et al. 1992; O’Reilly et al. 1995). The authors showed that hypoglossal neurons exhibited stronger depolarizations and a higher depolarization rate. Because this depolarization results in an increase in the frequency of action potentials, we assume that the high tonic activity observed in our experiments probably reflects this depolarization. Although a high excitability has been attributed to brain stem neurons in general (Haddad and Jiang 1993; Jiang and Haddad 1991), our data suggest that this generality may not be justified as the VRG responded with a reduced tonic modulation compared with the XII. However, it must be emphasized that this difference is based on integrated population recordings, which provide no direct insights into the mechanisms that are responsible for this difference. But they are consistent with intracellular recordings from VRG neurons that exhibited only a weak depolarization (Ramirez et al. 1998).

Because the XII and VRG serve different functions in respiratory control, the differential modulation of respiratory parameters has interesting conceptual implications and suggests that the anoxia-induced augmentation and depression cannot be attributed to just a “general” excitation and subsequent shut-down of brain stem neurons. Our experiments show that both excitation and depression exhibit region-specific differences. The different responses of the VRG and XII are of particular interest, because it is generally assumed that the respiratory rhythm is generated within the VRG (Bianchi et al. 1995; Duffin and van Alphen 1995) or more specifically within the pre-Bötzinger complex (Rekling and Feldman 1998; Smith et al. 1991). In the VRG cell population, the increase in the excitability induced by anoxia is less pronounced than in the XII. This finding supports in vivo data that indicate that the activity of some VRG cells declines during anoxia (England et al. 1995; Richter et al. 1991, 1993). It has been shown that specific subpopulations of VRG neurons in neonatal mice in vitro, like, e.g., biphasic expiratory cells and some inspiratory cells, ceased their firing, whereas other subpopulations (50% of the inspiratory cells) remained active even during anoxic conditions (Ballanyi et al. 1994; Ramirez et al. 1998; Völker et al. 1995). This partial inactivation of respiratory neurons could explain the decrease in the integrated inspiratory burst amplitude and the early depression of tonic activity of neurons of the VRG population.

However, it is remarkable that despite this partial inactivation, the reconfigured network is able to generate stable respiratory rhythmic activity. Even more remarkably, the frequency is initially enhanced and hypoglossal burst amplitude increased. The lack of an enhanced phasic amplitude in the VRG has an interesting implication because it suggests that the enhanced amplitude in the XII motor output that is typical for the anoxic augmentation cannot be attributed to an increased direct drive from the VRG to the XII nucleus. It rather seems that the enhanced amplitude of the XII burst has to be attributed to either a neural mechanism localized within the XII nucleus or to a modulation at sites downstream to the VRG/pBC. There are some direct projections from the VRG to the hypoglossus nucleus (Dobbins and Feldman 1995), and therefore one possible mechanism might involve changes in synaptic transmission. However, the evidence for monosynaptic projections from the VRG to the hypoglossus nucleus are minimal (Lipski et al. 1994). Therefore another possible mechanism for the amplification of the inspiratory drive involves paucisynaptic connections or additional inputs from further areas in the slice preparation. Examples for those would be nonrespiratory neurons in the dorsomedial medullary reticular formation that provide input into the XII (Woch et al. 1998) or inspiratory premotor neurons in the tegmental field (Wilson et al. 1998). An independent regulation of respiratory drives and frequency has previously been described also for the ventilation in the in vivo neonatal monkey, where respiratory drives were enhanced during decreased respiratory frequencies and decreased minute ventilation (LaFramboise and Woodrum 1985). Similarly, St. John and Bianchi (1985) attributed the augmentation of the motor output to a modulation of respiratory activity within the XII and phrenic motor nuclei. Possible mechanisms that lead to a differential modulation of the motor output during anoxia are as yet unknown. However, it is well established that these motor nuclei receive various modulatory inputs that affect the respiratory drive (see, e.g., Dong and Feldman 1995; Funk et al. 1994). Because neuromodulators are known to be released during hypoxia (Goiny et al. 1991; Lindefors et al. 1986; Yan et al. 1995a,b), they may be involved in mediating the differential response within the motor nuclei and the VRG. Alternatively, ion channel properties might differ between the VRG and XII motor nuclei, which might result in the differential response of these functionally different areas. The transverse slice preparation appears to be an ideal model system to further study the underlying mechanisms that lead to this differential anoxic response.

Although we did not determine the mechanisms that lead to regional differences in the anoxic response of VRG and XII nucleus, our results indicate that the frequency and amplitude modulation is regulated in a differential manner within the respiratory network. This is an experimental evidence for the hypothesis formulated earlier by Feldman and colleagues, that rhythm- and pattern-generating mechanisms are separate (Feldman et al. 1990). This independent regulation could be interpreted as part of an adaptive process that decreases the activity in the neuronal network that is responsible for respiratory rhythm generation, while it enhances the intensity of the motor output locally within the motor nucleus.

This study was supported by National Heart, Lung, and Blood Institute Grant 60120 to J.-M. Ramirez.

Address for reprint requests: J.-M. Ramirez, Dept. of Organismal Biology and Anatomy, University of Chicago, 1027 E. 57th St., Chicago, IL 60637.

Received 13 October 1998; accepted in final form 15 June 1999.

REFERENCES


