INTRODUCTION

It has been proposed that rhythmic activity in central neural networks can arise from both interneuronal gap-junction-mediated electrical communication and inhibitory synaptic transmission (Tamas et al. 2000; Zhang et al. 1998). One of the most fundamental biological rhythms is the respiratory rhythm. In the rodent, the pre-Bötzinger complex (PBC) located in the ventrolateral medulla is now generally accepted as the site where respiratory rhythm is generated (Ballanyi et al. 1999; Koshiya and Smith 1999; Rekling and Feldman 1998). This structure is likely responsible for the long-time-scale features of respiratory cycle timing, i.e., respiratory frequency. Yet inspiratory motor activity is also associated with short-time-scale neuronal synchrony that occurs within an inspiratory burst (Cohen et al. 1987; Sica et al. 1991). Little is known about mechanisms that modulate this short-time-scale synchrony and how such modulation might alter respiratory cycle timing.

There now exist two very powerful in vitro mammalian preparations for studying the properties of respiratory rhythm and neural activity. One preparation, originally developed by Suzue (Suzue et al. 1983), uses the brain stem-spinal cord from the neonatal rodent, termed the en bloc preparation. Using the en bloc preparation, rhythmic inspiratory phase activity can be recorded from numerous motor nerves including the phrenic and hypoglossal nerves. The second preparation is the medullary slice preparation developed by Smith and colleagues (1991), where rhythmic inspiratory activity can readily be recorded from the hypoglossal nerve roots. We used both of these preparations to investigate the role played by gap junctions and inhibitory synaptic transmission in the modulation of inspiratory phase activity.

Gap-junction communication has been shown to be important in a number of neuronal systems, including spontaneous bursting in the embryonic retina (Wong et al. 1998), synchronization within the neocortex (Galarreta and Hestrin 1999; Gibson et al. 1999), and motor pattern coordination within the neonatal spinal cord (Tresch and Kiehn 2000). A preliminary report (O’Neal et al. 1999) showed the presence of gap-junction-related proteins (connexins) in neurons of the PBC, and thus gap-junction communication could potentially be important for their function. We thus decided to investigate whether blocking of gap junctions has effects on respiratory rhythm generation and the synchronous activity observed within inspiratory motor bursts. In recent years, a number of relatively specific gap-junction blockers have been developed. These include glycyrrhetinic acid derivatives and related compounds including: 18α-glycyrrhetinic acid (18α-GA), 18β-glycyrrhetinic acid (18β-GA), and carbenoxolone (CBX) (Davidson and Baumgarten 1988; Davidson et al. 1986; Goldberg et al. 1996). In the experiments described, we tested the effects of these gap-junction blockers on inspiratory neural activity.

A number of studies have shown that inhibitory interneurons are also involved in the synchronization of neuronal systems
(Cobb et al. 1995; Tamas et al. 2000; Zhang et al. 1998). Yet the role of GABA<sub>A</sub> and glycine receptor-mediated synaptic transmission in the generation of rhythmic respiratory activity and synchronization of inspiratory motor activity is not fully understood (Ballanyi et al. 1999; Ramirez and Richter 1996; Rekling and Feldman 1998). Depending on the type of preparation and its age, blockade of GABAergic and glycinergetic synaptic transmission can produce differing effects on respiratory motor outflow (Pierrefiche et al. 1998; Ramirez et al. 1996). Also, in vivo experiments have shown that intraventricular administration of bicuculline to block GABA<sub>A</sub> receptors and strychnine to block glycine receptors altered the degree of synchrony in phrenic nerve activity (Schmid and Böhmér 1989). Thus in this study, we tested the effects of blockade of either or both GABA<sub>A</sub> receptors and glycine receptors on respiratory rhythm and synchronous activity within an inspiratory burst.

**METHODS**

**Preparations**

In vitro experiments were performed on either rhythmically active brainstem-spinal cord (en bloc) or medullary slice preparations from Swiss Webster mice (P1–5).

The methods used are fully described elsewhere (Bou-Flores et al. 2000; Gibson and Berger 2000; Hilaire et al. 1997a,b). In brief, for both preparations, mice were anesthetized deeply with halothane. For the en bloc preparation, the medulla and the cervical spinal cord were isolated and removed from the animal and then superfused at room temperature with an artificial cerebrospinal fluid (ACSF; see following text for composition). For slice studies, the medulla was first isolated, and then a transverse slice (500- to 700-μm thick) was cut at the level of nucleus ambiguus with a vibratome. This slice, including the most rostral hypoglossal nerve rootlets, was then placed into a recording chamber and superfused for at least 30 min with a high K<sup>+</sup> ACSF solution (see following text for composition) before making recordings.

**Recording**

For both preparations, the temperature of the recording chamber was maintained between 27 and 28°C. Glass suction electrodes, filled with ACSF, were used to record from the cut ends of the C<sub>1</sub> phrenic rootlets (en bloc preparation) and the cut ends of the hypoglossal rootlets (medullary slice and en bloc preparations). Raw nerve signals were amplified using a CyberAmp 320 (Axon Instruments) and bandpass filtered from 10 Hz to 10 kHz. In addition, the filtered signal was further band-pass filtered from 1 to 200 Hz, and using a sampling rate of 2,000 Hz, a power spectrum was computed based on 512 data points. The spectral resolution was therefore 3.91 Hz/bin. The sampling period for the power spectral analysis commenced at the start of each inspiratory burst. The average power spectrum was computed in one of two ways. First, and in all cases, we computed an average power spectral density (PSD) based on the absolute power (μV<sup>2</sup>) of the signal in each frequency bin. Second, when comparing the PSDs between nerves (see Fig. 6 for example), we computed the relative power (absolute power in each bin/total power) where the total power is the absolute power in each bin summed over all frequencies in the power spectrum.

All the results are expressed as percentage of the control values. All values are given as means ± SE. Statistical analysis was performed with a two-tailed Student’s t-test, and significance was assumed if P < 0.05.

**RESULTS**

In both neonatal mouse en bloc and medullary slice preparations, the central respiratory network continues to produce rhythmic inspiratory bursts as observed in the phrenic (en bloc) and hypoglossal (slice) nerve roots. These exhibit little variability in burst frequency and amplitude. In the en bloc preparations (n = 30), we found the mean control frequency and mean duration of the inspiratory bursts (TI) to be 5.0 ± 0.5 min<sup>-1</sup> and 867 ± 44 ms, respectively. In the medullary slice preparations (n = 20), we found the mean control frequency and mean TI to be 5.4 ± 0.4 min<sup>-1</sup> and 858 ± 40 ms, respectively. The mean control frequency and mean TI were not significantly different between the two preparations. There-

Data analysis

In both preparations, the number of integrated bursts of activity was measured every minute during a period of at least 5 min prior to any drug application to estimate the mean control respiratory frequency. Drugs were then applied and the resulting changes were expressed as percentage of the control values.

For analysis of the “leaky” integrated nerve activity, we measured its average peak amplitude and integrated area based on averaging approximately 30 rectified-integrated bursts of activity. During the control period, the data were taken just prior to drug application. During drug application, the data were derived from the period that began after the onset of the effect on respiratory frequency and always ended at the point where drug application stopped. During wash periods, data were taken after 30 min of washing.

To analyze synchronous activity during an inspiratory burst, we computed an average power spectrum (Clampfit Version 8.0, Axon Instruments) that was derived from an analysis of 10 inspiratory bursts. The data during control were derived from the period just prior to drug application. During drug application, the 10 inspiratory bursts were from the period just prior to the point where drug application stopped. This period typically was 18–20 min after the start of drug application. During wash periods, data were taken after 30 min of washing. For the power spectrum analysis, the filtered nerve signal was further band-pass filtered from 1 to 200 Hz, and using a sampling rate of 2,000 Hz, a power spectrum was computed based on 512 data points. The spectral resolution was therefore 3.91 Hz/bin. The sampling period for the power spectral analysis commenced at the start of each inspiratory burst. The average power spectrum was computed in one of two ways. First, and in all cases, we computed an average power spectral density (PSD) based on the absolute power (μV<sup>2</sup>) of the signal in each frequency bin. Second, when comparing the PSDs between nerves (see Fig. 6 for example), we computed the relative power (absolute power in each bin/total power) where the total power is the absolute power in each bin summed over all frequencies in the power spectrum.

All the results are expressed as percentage of the control values. All values are given as means ± SE. Statistical analysis was performed with a two-tailed Student’s t-test, and significance was assumed if P < 0.05.
fore we combined the data for both preparations and found the mean control frequency and mean TI to be $5.2 \pm 0.3$ min$^{-1}$ and $862 \pm 31$ ms, respectively. These values are similar to those published elsewhere (Hilaire et al. 1997a,b; Ramirez et al. 1996).

Effect of gap-junction blockade on respiratory rhythm

To assess the involvement of gap junctions in modulation of respiratory rhythm, we added gap-junction blockers to the ACSF superfusing both en bloc and medullary slice preparations. We investigated the effects of various glycyrrhetinic acid compounds that have been shown to block gap-junction communication by a mechanism that may involve conformational changes in connexin structure (Goldberg et al. 1996).

As shown on Fig. 1A, replacing normal ACSF with ACSF containing CBX dramatically decreased respiratory frequency as indicated from recording of phrenic nerve root activity in an en bloc preparation. The effect of CBX was reversible and respiratory frequency recovered within 10–15 min of resuming normal ACSF superfusate. The decrease in respiratory frequency was primarily due to a major increase in expiratory time (TE) rather than a large increase in TI (Fig. 1A).

We tested the effects of three different gap-junction blockers on respiratory frequency in the en bloc preparation. To accomplish this, each blocker was applied only once to any one preparation, and five tests were performed with each blocker. We found on average that CBX, 18α-GA, and 18β-GA all significantly decreased respiratory frequency by $82 \pm 3$, $64 \pm 8$, and $73 \pm 8\%$, respectively (Fig. 2A). For all gap-junction blockers, respiratory frequency recovered 10–15 min after removal of these blockers from the superfusate.

We next tested the effect of CBX in the medullary slice preparation since in the en bloc experiments this compound had the greatest effect. In medullary slice preparations CBX ($n = 5$) also significantly reduced the respiratory frequency by $50 \pm 8\%$ (Fig. 3A). Recovery was observed when CBX was removed from the superfusate in a similar time course as that observed with the en bloc preparations. Structural differences between en bloc and medullary slice preparations may be responsible for the quantitative difference in the effect of CBX on respiratory frequency.

Effect of gap-junction blockade on inspiratory activity

Next we studied the effects of gap-junction blockers on inspiratory burst activities in the en bloc preparation by measuring both the area and peak amplitude of the integrated phrenic activity. In the en bloc preparation, the gap-junction blockers 18α-GA and 18β-GA did not significantly affect the area and peak amplitude of the phrenic burst activity (Fig. 2B and C). CBX did not significantly alter the peak amplitude of the integrated phrenic burst but significantly increased the area (by $150 \pm 17\%$, Fig. 2B). Next we tested the effect of CBX on hypoglossal activity in the slice. We found that CBX ($n = 5$) significantly decreased by $36.9 \pm 13\%$ the area of integrated hypoglossal bursts (Fig. 3B) but had no significant effect on the peak amplitude of integrated hypoglossal inspiratory bursts (Fig. 3C).

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**FIG. 1.** Gap-junction blockade with carbenoxolone (CBX) caused changes in respiratory activity in the in vitro en bloc preparation from a neonatal mouse. CBX (100 μM, 20 min) was added to the ACSF superfusing the preparation. A: CBX decreased the respiratory frequency (estimated from the frequency of occurrence of the phrenic bursts). B: CBX increased the expiratory time (TE). Data shown are the inter-burst intervals on a burst-by-burst basis. A and B are from the same experiment and have the same time base. C: CBX caused an increase in the area of the integrated phrenic burst without a large effect on peak integrated activity. Shown are the average of 5 integrated inspiratory phrenic bursts during normal ACSF (a), ACSF containing CBX (b), and return to normal ACSF (c). a–c are derived from the periods marked in A and are presented for illustrative purposes, therefore not reflecting the averaging of at least 30 bursts that was used in the subsequent quantitative analyses.

**FIG. 2.** Summary of the effects of various gap-junction blockers on phrenic nerve inspiratory activity in the in vitro en bloc preparation. A: application of all gap-junction blockers caused a significant reduction in respiratory frequency. B: except for CBX, none of the gap-junction blockers caused a significant increase in the area of the integrated phrenic bursts. C: none of the gap-junction blockers caused a change in the peak amplitude of the integrated phrenic burst. In this and all subsequent figures, results are expressed as means ± SE of control. Asterisks and ns indicate significant and nonsignificant differences from control, respectively (P < 0.05).
Effect of gap-junction blockade on synchronous activity within an inspiratory burst

We analyzed short-time-scale synchronization of inspiratory-phase activity in both phrenic and hypoglossal nerves by performing power spectral analysis of inspiratory activity recorded in each nerve. In en bloc preparations, application of CBX markedly enhanced the degree of phrenic nerve synchrony as can be seen in the raw filtered neurogram shown in Fig. 4A. Power spectral analysis of this activity revealed that in control conditions there was a peak in the power spectrum between 30 and 40 Hz and that application of CBX caused a large increase in the amplitude of this spectral peak (Fig. 4B). Removal of CBX from the superfusate resulted in a partial recovery of the power spectrum (Fig. 4B). We tested the effects of CBX and 18α-GA on phrenic nerve synchrony in each of five en bloc preparations. We found that these gap-junction blockers caused significant increases (by 112 ± 29 and 110 ± 25%, respectively) in the peak amplitude of the 30- to 40-Hz spectral peak compared with that observed in control solutions (Fig. 5A).

In five en bloc preparations, we performed simultaneous recordings from phrenic and hypoglossal nerve rootlets to determine whether or not inspiratory-phase synchronization was similar in these two nerve recordings. While both nerves showed synchronized activity, the resultant power spectrum for each nerve was different. As shown in Fig. 6A, the peak in the power spectrum occurred at different frequencies in each nerve. In the en bloc preparation, we consistently found that the peak of the power spectrum for the hypoglossal nerve occurred at 10–20 Hz in contrast to the peak for the phrenic nerve, which occurred at 30–40 Hz. These results suggest that the two different frequencies of synchronization may arise from two separate premotor respiratory central pattern generators that project to the respective motor output systems (Peever and Duffin 2000).

In contrast to the preceding results in the en bloc preparation, in the medullary slice preparation, where only hypoglossal nerve activity is present, the power spectrum of hypoglossal inspiratory bursts revealed that two distinct spectral peaks were present, one in the 30- to 40-Hz range and one in the 10- to 20-Hz range (Fig. 6B). Thus important differences exist in these two preparations with respect to synchronized hypoglossal activity that occurs during the inspiratory burst. Our observation that in the en bloc preparation the hypoglossal has greater power at lower frequencies compared with the phrenic spectrum has previously been seen in intact in vivo systems (Cohen et al. 1987; Sica et al. 1991).

In five medullary slice preparations, we observed that application of CBX to the superfusion fluid caused the hypoglossal spectral peak at 10–20 Hz to be enhanced by on average 116 ± 18% (Fig. 5B). In contrast, we observed that the spectral peak at 30–40 Hz was not significantly affected by CBX.

Effect of GABA_A and glycine receptor antagonists on respiratory rhythm

We next investigated the effect of blocking GABAergic and glycineergic synaptic transmission on respiratory rhythm and on synchronous oscillations of phrenic and hypoglossal nerve...
activities. The protocol of the experiments performed was similar to that used in the gap-junction blocker experiments described in the preceding text. In the following experiments, we determined the effects of adding bicuculline (5 μM, 20 min) and strychnine (1 μM, 20 min) to the superfusate bathing en bloc and medullary slice preparations.

In the en bloc preparation, application of bicuculline (n = 5), strychnine (n = 5), or both (n = 5) caused a significant enhancement by 78 ± 10, 60 ± 6, and 45 ± 3% of respiratory frequency, respectively (Fig. 7A). In the medullary slice preparation (Fig. 7A), similar effects were observed; respiratory frequency rose significantly by 79 ± 14, 34 ± 7 and 69 ± 15% when the superfusate contained bicuculline (n = 5), strychnine (n = 5), or both (n = 5), respectively. These results in the mouse on the effect of bicuculline and strychnine on respiratory frequency are in general agreement with a previous study that used the rat medullary slice preparation (Shao and Feldman 1997).

**Effect of GABA A and glycine receptor antagonists on inspiratory activity**

In the en bloc preparation, the integrated area of the phrenic burst (Fig. 7B) was enhanced significantly by 30 ± 5, 31 ± 14, and 25 ± 4% if bicuculline (n = 5), strychnine (n = 5), or both (n = 5) was added to the superfusate, respectively. In contrast, in the medullary slice preparation, application of strychnine (n = 5) or bicuculline (n = 5) alone did not significantly change the integrated hypoglossal area, but application of both antagonists together significantly increased the area by 101 ± 11% (n = 5, Fig. 7B). The latter results are consistent with a previous study in the rat from our laboratory (Gibson and Berger 2000). We observed that application of both antagonists together significantly decreased by 20 ± 5% the peak integrated phrenic and hypoglossal activities (Fig. 7C). In contrast, application of strychnine alone significantly increased by 27 ± 5% peak integrated phrenic activity (Fig. 7C). Peak integrated hypoglossal nerve activity was not altered by application of either antagonist alone (Fig. 7C).

Since application of bicuculline (n = 5) did not cause any variation in peak phrenic activity (Fig. 7C) but increased the integrated area of the phrenic nerve burst (Fig. 7B), we determined the effect of bicuculline on the TI of the phrenic burst and found an enhancement of 44 ± 9% (data not shown). As expected, since co-application of both antagonists caused an enhancement of the integrated area of activity for both nerves with a reduction in peak activity, we observed that bicuculline enhanced TI of phrenic and hypoglossal activity by 44 ± 9 and 46 ± 8%, respectively (data not shown).

**Effect of GABA A and glycine receptor antagonists on synchronous activity within an inspiratory burst**

In en bloc preparations, application of bicuculline markedly reduced the degree of phrenic nerve synchrony. Power spectral analysis ofnerve activity showed that the 30–40-Hz peak in
both the 10- to 20-Hz and the 30- to 40-Hz peaks by 60% slice preparation (Fig. 9). Bicuculline reduced in amplitude power spectrum of hypoglossal nerve activity in the medullary mice studied was separated into two age groups, P0 –1 and power spectra. The variability was reduced if the population of either Stry or Bic did not significantly affect the area of the integrated hypoglossal inspiratory burst, but a co-application of Stry and Bic significantly enhanced the integrated area. C: application of Stry but not Bic caused a significant increase in the peak amplitude of the integrated phrenic burst. Application of Bic or Stry had no effect on the peak amplitude of the integrated hypoglossal burst. Co-application of Stry and Bic caused a significant reduction in the phrenic and hypoglossal integrated peak activity.

the power spectrum was reduced in amplitude on average by 87 ± 7% (n = 5, Fig. 8). Bicuculline had similar effects on the power spectrum of hypoglossal nerve activity in the medullary slice preparation (Fig. 9). Bicuculline reduced in amplitude both the 10- to 20-Hz and the 30- to 40-Hz peaks by 60 ± 6 and 60 ± 8%, respectively. Application of strychnine produced variable effects on the power spectra. The variability was reduced if the population of mice studied was separated into two age groups, P0–1 and P2–4. In the youngest age group, we did not observe a significant effect of strychnine on the power spectrum of the phrenic nerve activity (Fig. 8). In contrast, for the older age group, we observed an 88 ± 3% reduction in the amplitude of the 30- to 40-Hz peak in the phrenic power spectrum (Fig. 8). In contrast to the effects of strychnine on synchronization of phrenic activity in the en bloc preparation, we observed in the medullary slice preparation that in both age groups strychnine application to the superfuse caused an enhancement of the 10- to 20-Hz peak in the power spectrum of hypoglossal nerve activity (Fig. 9). Specifically, strychnine enhanced the amplitude of this peak by 158 ± 64 and 123 ± 23%. Only in the older preparation (P2–4) did we observe that strychnine reduced by 60 ± 5% the 30- to 40-Hz peak in the hypoglossal power spectrum (Fig. 9).

Co-application of both antagonists gave more consistent results. We observed that this caused a decrease by 80 ± 4% in the 30- to 40-Hz spectral peak of phrenic activity (Fig. 8) and by 90 ± 4 and 85 ± 0.5% in the 10- to 20-Hz and 30- to 40-Hz spectral peaks of hypoglossal activity, respectively (Fig. 9).

**DISCUSSION**

These experiments have focused on modulation of two different timing characteristics of respiratory motor outflow. One, on a longer time scale associated with respiratory frequency, and the other, on a shorter time scale associated with synchronization of activity within the inspiratory motor burst. The result that gap-junction blockade and inhibitory synaptic transmission antagonism have opposite effects on each of these timing signals provides new information on mechanisms by which these signals can be modulated.

Our primary observations include the findings that gap-junction blockade consistently resulted in a reduction in respiratory frequency, and this occurred in both en bloc and medullary slice preparations. These results are consistent with a role of gap junctions in the generation of respiratory cycle timing. Further, in most cases, gap-junction blockade also caused a marked increase in short-time-scale synchronized
activity in both phrenic and hypoglossal inspiratory bursts. This occurred in the absence of a shift in the predominant frequencies in the power spectra of this synchronized activity. In addition, we observed that gap-junction blockade caused minimal or mixed effects on the two measures we used to quantitate amplitude of inspiratory phase motoneuron activity. In contrast to the results with the gap-junction blockers, blockade of GABA_A and glycine receptors caused an increase in respiratory frequency. We also found that simultaneous blockade of both of these receptors consistently resulted in a reduction in short-time-scale synchronized activity in both phrenic and hypoglossal inspiratory bursts.

The timing of respiratory activity is thought to arise from the most upstream element in the respiratory rhythm-generating mechanism, perhaps involving a network of coupled pacemakers (Ballanyi et al. 1999; Koshiya and Smith 1999; Rekling and Feldman 1998). The location of this rhythm-generating structure in the rodent brain stem is in the ventrolateral medulla and within the PBC. Conceptually it is thought that the respiratory rhythm-generating system is responsible for the generation of respiratory frequency. An inspiratory pattern-generating system responsible for shaping the temporal form of the inspiratory burst is thought to be downstream of the neural elements responsible for respiratory rhythm generation (Ballanyi et al. 1999; Feldman et al. 1990). How a unique temporal pattern of inspiratory drive is directed to each of the various classes of inspiratory motoneurons (e.g., phrenic, inspiratory hypoglossal and external intercostal motoneurons) is not known, but may involve a combination of unique neural elements, circuit connections, and neuronal properties. Further processing of inspiratory drive may occur among a set of premotor neurons that then project directly to motoneurons. Thus electrical and inhibitory chemical synaptic connections may occur at a number of points in the circuit from the respiratory rhythm-generating mechanism in the PBC to the inspiratory motoneurons themselves. It is currently unknown which structures in the brain stem are responsible for the generation of inspiratory-phase short time scale synchronization. Since short-time-scale synchronization has been shown to be present upstream of respiratory motoneurons (Mitchell and Herbert 1974; O’Neal et al. 1999), it is reasonable to conclude that the respiratory motoneurons themselves are not the sole source of such synchronization.

Role of gap junctions in inspiratory neural activity

Several studies in neonatal rat and mouse have reported the presence of electrical coupling in postnatal hypoglossal and other inspiratory brain stem and phrenic motoneurons (Martin-Caraballo and Greer 1999; Mazza et al. 1992; Rekling and Feldman 1997). Electrical coupling is lost in older motoneurons (Chang et al. 1999; Mazza et al. 1992). It has been suggested that such transient gap-junction communication between motoneurons enhances motoneuron synchronous activity and may function to preserve multiple innervation of single muscle fibers (Balice-Gordon and Lichtman 1994). We believe it unlikely that our observations of the effect of gap-junction blockade on respiratory frequency and short time scale synchronization involve these inter-motoneuronal electrical communications. Preliminary anatomical data has indicated that connexins are present in the neonatal and adult rodent PBC (O’Neal et al. 1999). Thus the anatomical substrate for our observed effects of blocking gap junctions, leading to a marked reduction in respiratory frequency, is likely within the PBC. In light of these results, it is interesting that models of electrically coupled oscillatory neurons have shown that a reduction in electrical coupling can lead to either a decrease or increase in the frequency of slow oscillations observed in coupled neuronal networks depending on the set of underlying neuronal voltage- and time-dependent conductances (Kepler et al. 1990).

It is our view that short-time-scale synchrony arises from a mechanism that is different from the one that generates the basic respiratory cycle frequency, perhaps involving the inspiratory pattern-generator circuit, premotor neurons, or even electrically coupled neonatal respiratory motoneurons. Previous studies in the en bloc preparation using power spectral analysis of both phrenic and cranial nerve inspiratory phase discharge, as well as inspiratory phase synaptic current recorded in single phrenic motoneurons, revealed a dominant spectral peak in a frequency range close to those observed in the present experiments (Liu et al. 1990; Smith et al. 1990). Our results showed that blockade of gap junctions resulted in an increase in the short-time-scale synchrony observed during phrenic and hypoglossal motoneuron inspiratory bursts. This observation may seem counterintuitive because electrical coupling between neurons is thought to promote not reduce neuronal synchrony. Yet studies have shown that synchrony in neuronal networks can be either increased or decreased by electrical coupling (Marder 1998). For example, Traub and Wong (1983) found that in hippocampal network simulations electrical coupling between neurons can either increase or decrease neuronal synchronization. Thus if the electrically coupled neurons act as an electrical load on a network that is producing synchronized activity, then the magnitude of synchronization can be reduced. This idea is supported by our observations that application of gap-junction blockers to block electrical coupling did not shift the frequency of the peak in the power spectrum, but increased its amplitude.

Role of GABA_A and glycine receptor-mediated synaptic transmission in inspiratory neural activity

There remains considerable controversy regarding the role of GABA_A and glycine receptor-mediated synaptic transmission in respiratory rhythm generation (Ballanyi et al. 1999; Ramirez and Richter 1996; Rekling and Feldman 1998). The presence of a GABAergic hindbrain rhythm-generating mechanism during vertebrate embryonic development has recently been shown (Fortin et al. 1999). Blockade of GABA_A and glycine receptor-mediated synaptic transmission results in inconsistent alteration in respiratory motor outflow that appears to be dependent on the preparation, including the age of the experimental animal (Pierrefiche et al. 1998; Ramirez et al. 1996). This inconsistency was clearly seen in our results, as the effects of glycine receptor blockade on the degree of synchrony were dependent on the postnatal age of the preparation. Different effects on synchrony also depended on whether this was studied in the en bloc or medullary slice preparations. In contrast, we observed consistent results with GABA_A receptor blockade or simultaneous blockade of GABA_A and glycine receptor-mediated synaptic transmission, causing increased respiratory frequency and decreased synchrony in motor outflow.
We observed that GABA_A receptor blockade with bicuculline consistently reduced the degree of phrenic nerve synchrony recorded in the en bloc preparation and also both the 10- to 20-Hz and the 30- to 40-Hz peaks observed in hypoglossal activity in the medullary slice preparation. These results are consistent with computer simulations of neocortical and hippocampal gamma oscillations (20–80 Hz) that demonstrated that GABA_A-mediated synaptic transmission was critical to network synchronization (Bush and Sejnowski 1996; Wang and Buzsáki 1996).

It is interesting that in vertebrates during the embryonic period, the hindbrain shows the development of two types of rhythmic activity that can be observed in medullary cranial motoneurons. These two rhythmic activities are characterized by a low and a high frequency, respectively. It is thought that such primordial rhythm-generating circuits may evolve into the mature respiratory rhythm-generating mechanism (Fortin et al. 1999). GABA_A receptor-mediated events have a role in the high-frequency embryonic rhythms. Blockade of GABA_A receptors with bicuculline abolished the high-frequency events but did not affect the low-frequency rhythmic activity (Fortin et al. 1999). Our results showing that blockade of GABA_A receptor-mediated events with bicuculline dramatically reduced the power of the synchronous activity observed during rhythmic inspiratory bursts of both hypoglossal and phrenic nerve activities appear to extend these previous results from the embryological period into the postnatal period. Thus the mechanism responsible for synchronous activity has a common GABAergic component at all stages of development.

In some neural systems during the postnatal period, activation of synaptic GABA_A and glycine receptors results in neuronal depolarization. In hypoglossal motoneurons, glycineergic depolarization is converted to glycinegenic hyperpolarization over the first two weeks of postnatal life (Singer et al. 1998). This appears to be due to a reduction in the intracellular Cl⁻ concentration with postnatal development. In contrast, some receptor-mediated events with bicuculline dramatically reduced the power of the synchronous activity observed during rhythmic inspiratory bursts of both hypoglossal and phrenic nerve activities appear to extend these previous results from the embryological period into the postnatal period. Thus the mechanism responsible for synchronous activity has a common GABAergic component at all stages of development.

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Function of short-time-scale synchronization in inspiratory neural activity

An important issue is what might be the functional role of short-time-scale synchronization of motoneuronal activity during the inspiratory burst. One possibility is that the firing rate of motoneurons may be increased for the same average excitatory presynaptic activity if this presynaptic activity occurs synchronously rather than asynchronously (Murthy and Fetz 1994). A recent computer modeling study simulating a motor unit pool was used to compute the muscle force output as a function of mean motoneuron input firing rate under conditions of differing degrees of input synchrony. It was found that for the same mean input firing rate that muscle force output rose with increased input synchrony (Baker et al. 1999). Thus short-time-scale synchronization during the inspiratory motor burst could function to facilitate premotor and motoneuron activity as well as increase inspiratory muscle force output. The ability to modulate the degree of synchronization through modulation of neuronal electrical coupling and inhibitory synaptic communication could enhance or reduce motoneuronal activity and thereby affect inspiratory muscle force.

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REFERENCES


