Pre-Bo¨tzinger Complex Functions as a Central Hypoxia Chemosensor for Respiration In Vivo

IRENE C. SOLOMON,¹,² NORMAN H. EDELMAN,¹,² AND JUDITH A. NEUBAUER¹
¹Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903-0019; and ²Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York 11794-8661

Solomon, Irene C., Norman H. Edelman, and Judith A. Neubauer. Pre-Bo¨tzinger complex functions as a central hypoxia chemosensor for respiration in vivo. J. Neurophysiol. 83: 2854–2868, 2000. Recently, we identified a region located in the pre-Bo¨tzinger complex (pre-Bo¨tC; the proposed locus of respiratory rhythm generation) in which activation of ionotropic excitatory amino acid receptors using ßt-homocysteic acid (DLH) elicits a variety of excitatory responses in the phrenic neurogram, ranging from tonic firing to a rapid series of high-amplitude, rapid rate of rise, short-duration inspiratory bursts that are indistinguishable from gasps produced by severe systemic hypoxia. Therefore we hypothesized that this unique region is chemosensitive to hypoxia. To test this hypothesis, we examined the response to unilateral microinjection of sodium cyanide (NaCN) into the pre-Bo¨tC in chloralose- or chloralose/urethane-anesthetized vagotomized, paralyzed, mechanically ventilated cats. In all experiments, sites in the pre-Bo¨tC were functionally identified using DLH (10 mM, 21 nl) as we have previously described. All sites were histologically confirmed to be in the pre-Bo¨tC after completion of the experiment. Unilateral microinjection of NaCN (1 mM, 21 nl) into the pre-Bo¨tC produced excitation of phrenic nerve discharge in 49 of the 81 sites examined. This augmentation of inspiratory output exhibited one of the following changes in cycle timing and/or pattern: 1) a series of high-amplitude, short-duration bursts in the phrenic neurogram (a discharge similar to a gasp), 2) a tonic excitation of phrenic neurogram output, 3) augmented bursts in the phrenic neurogram (i.e., eupneic breath ending with a gasplike burst), or 4) an increase in frequency of phrenic bursts accompanied by small increases or decreases in the amplitude of integrated phrenic nerve discharge. Our findings identify a locus in the brain stem in which focal hypoxia augments respiratory output. We propose that the respiratory rhythm generator in the pre-Bo¨tC has intrinsic hypoxic chemosensitivity that may play a role in hypoxia-induced gasping.

INTRODUCTION

Pflüger (1868) established that mammalian ventilation is stimulated by hypoxia. A CNS site for chemosensitivity to the lack of oxygen was assumed, but only by inference from indirect data. Accordingly, the discovery of the hypoxia sensing function of the carotid bodies by Heymans and colleagues (1930) focused full attention on arterial chemoreceptors as the only hypoxia sensors involved in ventilatory control. Over the last 15 to 20 yr, however, many observations have been made that are not wholly consistent with this idea. Among them is the stimulation of breathing in unanesthetized (i.e., awake or decerebrate) deafferented animals (Gautier and Bonora 1980; Miller and Tenney 1975; Tenney and Ou 1976), which is thought to be supramedullary in origin and the demonstration of gasping in response to severe hypoxia in anesthetized deafferented animals (Guntheroth and Kawabori 1975; Oyer-Chae et al. 1993; St. John and Knuth 1981), which is likely to originate in the medulla. Further, some studies in anesthetized deafferented cats have demonstrated a biphasic respiratory response that consists of an initial increase in the frequency and amplitude of ventilation (augmentation) followed by a decrease (depression) that can lead to cessation of breathing (apnea) (Richter et al. 1991).

The sites and mechanisms responsible for centrally mediated hypoxic respiratory excitation (i.e., augmentation and gasping) remain unclear. Recent work from our laboratory (Solomon et al. 1999) investigating the role of the pre-Bo¨tzinger complex [pre-Bo¨tC; which is the proposed origin of respiratory rhythmogenesis (Smith et al. 1991)] in the generation and modulation of inspiratory motor output in vivo has demonstrated that activation of ionotropic excitatory amino acid (EAA) receptors elicits a variety of excitatory responses in the phrenic neurogram, ranging from tonic firing to a rapid series of high-amplitude, rapid rate of rise, short-duration inspiratory bursts that are indistinguishable from gasps produced by severe brain hypoxia. Additionally, studies by Ramirez et al. (1997a, 1998a) from the in vitro transverse brain stem slice preparation that contains the pre-Bo¨tC demonstrate a biphasic response (i.e., augmentation followed by depression) of rhythmic discharges in both pre-Bo¨tC neurons and the hypoglossal nerve, suggesting that the medulla contains the necessary elements required for the production of centrally mediated hypoxic respiratory excitation.

Previous studies have established that a unique population of neurons located in the rostral ventrolateral medulla (RVLM), namely the reticulospinal sympathoexcitatory neurons, many of which exhibit pacemaker-like activity, are intrinsically hypoxia chemosensitive (Sun and Reis 1994). Because these neurons have been previously shown to be essential for the tonic generation of vasomotor tone and the reflex regulation of arterial blood pressure (Ross et al. 1984), it is tempting to speculate that a similar population of respiratory rhythm-generating neurons may be hypoxia chemosensitive as well.

Thus the current studies were designed to test the hypothesis...
that the pre-Bötzinger complex (pre-BöTC) is hypoxia chemosensitive and may serve as a locus for CNS hypoxic modulation of respiratory output.

**METHODS**

**General**

Experiments were conducted in 41 adult cats, weighing 2.8–5.0 kg. Twenty cats were anesthetized initially with ketamine hydrochloride (15 mg/kg im) and acepromazine maleate (1.1 mg/kg im). The right brachial vein was cannulated and α-chloralose (10–20 mg/kg iv) was administered intravenously. Supplemental α-chloralose was given as needed (3–5 mg/kg iv). The remaining 21 cats were anesthetized initially by placing them inside a sealed, plastic induction chamber into which a gaseous mixture of halothane (5%) and oxygen was introduced. After the cats were anesthetized, they were removed from the chamber, and anesthesia was maintained by delivering halothane (1.5–3%) and oxygen through a face mask placed over the cat’s nose and mouth. The right brachial vein was cannulated, and α-chloralose and urethan (35 and 210 mg/kg iv, respectively) were administered. The gaseous anesthesia was then removed. Supplemental α-chloralose and urethan (5 and 10 mg/kg iv, respectively) were given as needed. The adequacy of anesthesia was regularly verified by firmly pinching a toe. If an increase in blood pressure was evoked or, during the absence of paralysis (see Experimental protocol), if the cat withdrew its limb, additional anesthesia was given. Dexamethasone (2 mg iv) was administered to minimize brain swelling. In all cats, both brachial arteries were cannulated. The left brachial cannula was connected to a Statham transducer (P23Db) for measurement of arterial blood pressure; the right brachial cannula was used for sampling arterial blood.

The trachea was cannulated low in the neck and the lungs ventilated with room air enriched with O2. The chest was opened bilaterally through the sixth intercostal spaces, and the expiratory outlet of the lungs during expiration. End-tidal CO2 was monitored continuously with a 100-ms time constant. Both the raw and averaged nerve outputs were recorded on digital tape (A. R. Vetter, Model 4000A) and on a chart recorder.

**Experimental protocol**

We examined the effects of focal hypoxia in the pre-BöTC on phrenic nerve discharge and arterial blood pressure. Focal hypoxia was produced using sodium cyanide (NaCN) which is a powerful stimulant of chemoreceptors (Choi 1988; Eyzaguirre and Zapata 1984) and is often used as a model for producing tissue hypoxia (DiRodriguez and Bazan 1983; Goldberg et al. 1987). Responses from a total of 81 sites in the pre-BöTC were recorded. Responses from an additional 23 sites adjacent (within 200–500 µm) to the pre-BöTC, including the rVRG and the Bötl complex, were also recorded. Sites in the pre-BöTC were initially localized using predetermined stereotaxic coordinates relative to the calamus scriptorius (3.4–4.0 mm rostral), midline (3.8–4.2 mm lateral), and dorsal surface (4.2–4.5 mm ventral). The range in values for the coordinates used to find the pre-BöTC reflects the variability in the dimensions of the brain stems in cats of different sizes. All sites were functionally verified to be in the pre-BöTC as previously described (Solomon et al. 1999) using the glutamate analogue N-homocysteic acid (DLH) and histologically confirmed (see Location of responsive and nonresponsive sites). For functional identification, unilateral microinjection of DLH produced either I) a series of high-amplitude, short inspiratory duration (i.e., gasplike) bursts, 2) tonic excitation of phrenic nerve discharge, or 3) augmented bursts (i.e., eupneic breath ending with a high-amplitude, short-duration burst). Many of these DLH data have been reported previously (Solomon et al. 1999).

All microinjections into the medulla were made with the use of a multibarreled glass pipette (20–40 µm tip diameter) attached to a pressure injection device (General Valve Picospritzer II). One barrel of the pipette contained NaCN (1 mM). The remaining barrels contained DLH (10 mM), saline, and Fast Green dye (2%) which was used to mark the injection sites (≤105 nl). All microinjected chemicals were dissolved in a saline solution, and the pH was adjusted to 7.36–7.44. Microinjection volumes of NaCN, DLH, and saline were 21 nl, and microinjection typically required 1–2 s to complete. The volume of each injection was monitored by observing the displacement of the fluid meniscus using a microscope equipped with an eyepiece reticule. To control for nonspecific effects, equivalent volumes of saline were microinjected into all sites in which NaCN elicited changes in phrenic nerve discharge and/or arterial blood pressure. In all experiments, the order of microinjection of NaCN and DLH into the pre-BöTC was random, with the remaining chemical being microinjected after a 10–15 min recovery period.

In 38 of the 41 cats, responses to microinjection of NaCN into 1–3 sites in the pre-BöTC were examined; 4 sites each were examined in the remaining cats. Our first site was identified using the stereotaxic coordinates defined above, and subsequent microinjections were made after movement of the pipette in 100- to 300-µm increments in the rostral-caudal, mediolateral, or dorsal-ventral direction. Most often, the pipette was moved in a rostral-caudal direction. In 11 of the 41 cats, we also examined the response to microinjection of NaCN into 1–3 sites adjacent (within 200–500 µm) to the pre-BöTC. No more than 4 microinjection sites (including adjacent sites) were examined on one side of the brain stem, and bilateral microinjections were made in 13 animals.

Baseline phrenic neurogram amplitude was initially set at 40–60% of the maximum amplitude evoked in response to ventilating the lungs, through a rebreathing circuit, with 95% O2–5% CO2 until end-tidal CO2 was increased to 8.0%. By setting the baseline amplitude this way, we were able to record increases or decreases in phrenic neurogram amplitude evoked by microinjection of NaCN into the medulla. Before stimulation of sites in the medulla, the cats were paralyzed with gallamine triethiodide (2 mg/kg iv). The effect of the paralytic agent was always allowed to dissipate so that the level of anesthesia could be assessed. Supplemental doses of the paralytic agent were administered as needed.
Histology

At the conclusion of the experiment, the cat was killed under deep anesthesia by an injection of saturated KCl solution. The brain stem was removed and placed in 4% Formalin for at least 48 h. The brain stem was then frozen, sectioned coronally (40 μm), mounted on slides, and stained for cell bodies using 1% Neutral Red dye. With the use of a microprojector, we made drawings of tissue sections containing sites marked with Fast Green dye.

Data analysis

Amplitude of integrated phrenic nerve discharge, inspiratory time (Ti), expiratory time (Te), frequency of phrenic bursts, and rate of rise were determined from the phrenic neurogram off-line (AT CODAS; DATAQ Instruments). Amplitude of integrated phrenic nerve discharge is reported as a percentage of the maximal amplitude recorded in each cat. This maximal amplitude occurred either in response to ventilating the lungs with CO₂ (see Experimental protocol) or in response to microinjection of NaCN or DLH into the pre-BötC. Rate of rise was determined over the linear phase of activity and is reported as a percentage of the maximal rate of rise recorded in each cat. Baseline values for all of these variables were determined by averaging the values obtained for the 60-s period immediately preceding microinjection of NaCN into the pre-BötC. Peak response values for these variables were determined by averaging the first five consecutive breathing cycles that displayed the greatest change from baseline values. Baseline values for mean arterial pressure were taken as the steady-state values. Peak responses were taken as the highest or lowest value reached after microinjection of NaCN.

All values are reported as means ± SE. Responses before and after stimulation are presented as paired data. A Student’s paired t-test or ANOVA was used to determine statistical significance, for which the criterion level was set at P < 0.05.

Results

General effects of microinjection of NaCN into the pre-BötC

We identified 81 histologically confirmed sites in the pre-BötC in which unilateral microinjection of DLH (10 mM, 21 nl) produced either a series of high-amplitude, rapid rate of rise, short inspiratory duration bursts, a tonic excitatory discharge, or an augmented pattern (i.e., eupneic breath ending with a high-amplitude, short-duration burst) of phrenic nerve discharge. Unilateral microinjection of NaCN (1 mM, 21 nl) into these sites produced excitation of phrenic nerve discharge in 49 of the 81 sites examined. This augmentation of inspiratory motor output exhibited one of the following changes in cycle timing and/or pattern: 1) a rapid series of high-amplitude, short-duration bursts in the phrenic neurogram (a discharge similar to a gasp), 2) a tonic excitation of phrenic neurogram output (with or without respiratory oscillations), 3) augmented bursts in the phrenic neurogram (i.e., eupneic breath ending with a high-amplitude, short-duration burst), or 4) an increase in frequency of phrenic bursts accompanied by small increases or decreases in the amplitude of integrated phrenic nerve discharge. These responses will be described in more detail below. In 18 sites, the phrenic neurogram response was accompanied by a marked (≥25 mmHg) increase or decrease in arterial blood pressure; however, changes in arterial blood pressure (≥10 mmHg) accompanied phrenic neurogram responses in 30 of the sites examined. In general, the onset of the respiratory effects preceded the blood pressure response.

Responses to microinjection of NaCN were rapid and reproducible. In each of the sites examined, repeated microinjection of NaCN produced a repeatable and consistent response. In general, we waited at least 10 min before attempting to demonstrate repeatability of the phrenic neurogram and blood pressure responses to microinjection of NaCN. Movement of the pipette tip by as little as 100–200 μm, however, resulted in marked differences in the phrenic neurogram response evoked by unilateral microinjection of NaCN. In 12 cats in which multiple microinjection sites in the pre-BötC were examined on one side of the brain stem, we were able to elicit at least 2 of the different patterns of augmentation of inspiratory output described above. All responses to microinjection of NaCN were completely reversible. Microinjections of equivalent volumes of saline into all responsive sites were ineffective in producing any changes in phrenic nerve discharge or blood pressure.

Similar phrenic neurogram and blood pressure responses were observed in cats under both anesthetic paradigms. Additionally, similar phrenic neurogram and blood pressure responses were observed in cats with carotid sinus nerves intact or cut. Therefore these data will not be considered separately.

Rapid series of high-amplitude, short-duration bursts

Unilateral microinjection of NaCN into nine sites in the pre-BötC produced a rapid series of high-amplitude, short-duration bursts in the phrenic neurogram (Fig. 1). This response typically occurred within 1–2 s from the beginning of microinjection of NaCN and had durations ranging from 27 to 248 s. Bilateral recordings were obtained in response to microinjection of NaCN into five of these nine sites in the pre-BötC, and the effects on phrenic nerve discharge were bilaterally symmetrical in those animals with bilateral recordings. On average, microinjection of NaCN into these sites decreased Ti from 1.75 ± 0.18 to 0.31 ± 0.02 s (mean ± SE, P < 0.01), decreased Te from 2.43 ± 0.27 to 0.80 ± 0.41 s (P < 0.01), increased the amplitude of integrated phrenic nerve discharge from 30.5 ± 7.6 to 79.6 ± 2.3% (P < 0.01) of maximal amplitude, and increased the rate of rise of phrenic nerve activity from 4.5 ± 0.8 to 81.9 ± 18% (P < 0.01) of the maximal rate of rise (Fig. 2). In addition, microinjection of NaCN increased the frequency of phrenic bursts from 14 ± 2 to 45 ± 5 bursts/min (P < 0.01).

In five of these sites, the rapid series of high-amplitude, short-duration bursts in the phrenic neurogram was accompanied by an increase in mean arterial pressure from 109 ± 11 to 134 ± 8 mmHg (P < 0.05). In the remaining sites, the rapid series of bursts was accompanied by either a decrease (n = 2) or no change (n = 2) in mean arterial pressure.

Tonic excitation

Unilateral microinjection of NaCN into 21 sites in the pre-BötC produced a tonic excitation of phrenic nerve discharge. Bilateral recordings were obtained in response to microinjection of NaCN into 12 of these 21 sites in the pre-BötC, and the effects on phrenic nerve discharge were bilaterally symmetrical in those animals with bilateral recordings. The tonic excitation was characterized by either an abrupt rise in phrenic nerve discharge, or an augmented pattern (i.e., eupneic breath ending with a high-amplitude, short-duration burst), or 2) an increase in frequency of phrenic bursts accompanied by small increases or decreases in the amplitude of integrated phrenic nerve discharge.
discharge to a plateau level (nonrhythmic; Fig. 3) or respiratory rhythmic oscillations superimposed on varying levels of tonic discharge (rhythmic). In general, the peak amplitude of integrated phrenic nerve discharge at the onset of the response was higher or the same as that seen during baseline breaths; however, in a few cases, peak amplitude was slightly reduced. Despite the variable effects on the peak amplitude of integrated phrenic nerve discharge, at the onset of the response an increase in the rate of rise of phrenic activity was usually present. The response occurred within 1–3 s from the beginning of microinjection of NaCN and had durations ranging from 36 to 324 s. In addition, this type of tonic excitation exhibited a gradual recovery (n = 8 sites) or an increase in the frequency of phrenic bursts as the tonic discharge declined (n = 5 sites; Fig. 2).

**FIG. 1.** Example of a rapid series of high-amplitude, rapid rate of rise, short-duration bursts in the phrenic neurogram evoked by microinjection of NaCN (1 mM; 21 nl) into the pre-Bötzinger complex (pre-BöC). *Traces from top to bottom:* blood pressure (BP), integrated phrenic nerve activity (ipsilateral), and raw phrenic nerve activity (ipsilateral). Note also that microinjection of NaCN into this site evoked a small increase in arterial blood pressure.

**FIG. 2.** Summary data showing effects on phrenic neurogram timing, amplitude, and rate of rise during high-amplitude, short-duration burst activity evoked by microinjection of NaCN into the pre-BöC. Microinjection of NaCN significantly reduced inspiratory time (Ti) and expiratory time (Te) and significantly increased phrenic neurogram amplitude and rate of rise. Ti and Te are shown in seconds. Phrenic neurogram amplitude and rate of rise are expressed as a percentage of the maximal amplitude and rate of rise recorded in each cat, respectively. Asterisks represent a significant difference (P < 0.01) from preinjection baseline (eupnea).
Fig. 3), or was followed by a transient postexcitatory depres-
sion of phrenic nerve discharge that lasted 90–310 s (n = 8
sites).

In 12 of these sites, the tonic excitation of phrenic nerve
discharge was accompanied by a marked increase in mean
arterial pressure from 103 ± 6 to 141 ± 14 mmHg (P < 0.01).
In five of these sites, the tonic excitation of phrenic nerve
discharge was accompanied by a marked decrease in mean
arterial pressure from 102 ± 6 to 60 ± 8 mmHg (P < 0.01).
No change in mean arterial pressure was seen in the remaining
four sites.

Augmented bursts

Unilateral microinjection of NaCN into nine sites in the
pre-BötC produced augmented bursts in the phrenic neurogram
(i.e., eupneic breath ending with a high-amplitude, short-dura-
tion burst; Fig. 4). Bilateral recordings were obtained in re-
sponse to microinjection of NaCN into eight of these nine sites
in the pre-BötC, and the effects on phrenic nerve discharge
were bilaterally symmetrical in those animals with bilateral
recordings. These augmented bursts appeared as either a series
of augmented breaths or were interspersed between eupneic
breaths. In some cases, the onset of these augmented bursts was
preceded by either an apneustic burst or a high-amplitude,
short-duration burst of phrenic nerve discharge. In addition,
the peak amplitude of integrated phrenic nerve discharge of the
eupneic portion of the augmented burst was often increased
above that seen during baseline breaths. The onset of aug-
mented bursts occurred within 1–3 s from the beginning of
microinjection of NaCN unless preceded by an apneustic burst.
In all cases, the duration of the response with augmented bursts
ranged from 40 to 472 s.

In six of these sites, no change in mean arterial pressure
accompanied the phrenic neurogram response. A marked in-
crease in mean arterial pressure was seen in one of these sites,
and a decrease in mean arterial pressure was seen in the
remaining site.

Increased frequency of phrenic bursts

Unilateral microinjection of NaCN into 10 sites in the pre-
BötC produced an increase in frequency of phrenic bursts
accompanied by small increases or decreases in the amplitude
of integrated phrenic nerve discharge (Fig. 5). Bilateral record-
ings were obtained in response to microinjection of NaCN into
3 of these 10 sites in the pre-BötC, and the effects on phrenic
nerve discharge were bilaterally symmetrical in those animals
with bilateral recordings. In some cases, this increase in the
frequency of phrenic bursts was superimposed on a small tonic
discharge (n = 3). In addition, in two sites, the appearance of
augmented bursts occurred on some breaths. The onset of this
frequency response occurred within 1–4 s from the beginning
of microinjection of NaCN and had durations ranging from 18
to 118 s. On average, microinjection of NaCN into these sites
increased the frequency of phrenic bursts by 29.5 ± 6.0% (P <
0.05) above baseline frequency. In nine of these sites, the
increase in frequency resulted from a decrease in Te (from
3.0 ± 0.2 s to 2.1 ± 0.1 s; P < 0.05), whereas Ti remained
constant or increased slightly. In addition to the increase in
frequency of phrenic bursts, there was a small but significant increase ($P < 0.05$) in the rate of rise of phrenic nerve activity in 6 of the 10 sites examined (not shown).

In two of these sites, the phrenic neurogram response was accompanied by an increase (15–20 mmHg) in mean arterial pressure. In the remaining eight of these sites, the phrenic neurogram response was accompanied by small ($\leq 10$ mmHg) increases or decreases or no change in mean arterial pressure.

**FIG. 4.** Example of augmented bursts in the phrenic neurogram evoked by microinjection of NaCN (1 mM; 21 nl) into the pre-BötC. In this site, microinjection of NaCN elicited a small apneustic burst that preceded the onset of augmented bursts. Note that 1 or 2 augmented bursts were interspersed between eupneic breaths. *Traces from top to bottom:* BP, integrated phrenic nerve activity (ipsilateral), and raw phrenic nerve activity (ipsilateral). Note also that microinjection of NaCN into this site evoked an increase in arterial blood pressure.

**FIG. 5.** Example of an increase in frequency of phrenic bursts in the phrenic neurogram evoked by microinjection of NaCN (1 mM; 21 nl) into the pre-BötC. This increase in frequency of phrenic bursts is accompanied by an increase in the amplitude of integrated phrenic nerve discharge. *Traces from top to bottom:* BP, integrated phrenic nerve activity (ipsilateral), and raw phrenic nerve activity (ipsilateral), integrated phrenic nerve activity (contralateral), and raw phrenic nerve activity (contralateral). Note also that microinjection of NaCN into this site evoked a small decrease in arterial blood pressure.
of the 81 sites examined. The responses evoked by NaCN and DLH into 27 of these sites in the pre-Bo"tC were similar. In the remaining 22 sites, however, the responses evoked by NaCN and DLH were not always identical. The distribution of these inspiratory motor output excitations based on response types to unilateral microinjection of both NaCN and DLH are described in Table 1.

In the nine sites in which unilateral microinjection of NaCN into the pre-Bo"tC produced a series of high-amplitude, short-duration bursts, unilateral microinjection of DLH produced either a rapid series of high-amplitude, short-duration bursts ($n = 5$), a tonic excitatory response ($n = 1$), or a series of high-amplitude, short-duration bursts superimposed on a tonic excitatory response ($n = 3$). Further, unilateral microinjection of DLH increased mean arterial pressure in seven of these sites and decreased it in the remaining two sites (not shown).

In all sites in which microinjection of NaCN into the pre-Bo"tC evoked a tonic excitatory response in the phrenic neurogram, unilateral microinjection of DLH similarly produced a tonic excitatory response. Further, unilateral microinjection of DLH increased mean arterial pressure in 13 of these sites and decreased it in the remaining 8 sites (not shown).

In the nine sites in which microinjection of NaCN into the pre-Bo"tC evoked augmented bursts in the phrenic neurogram, unilateral microinjection of DLH into these sites produced either a rapid series of high-amplitude, short-duration bursts ($n = 2$), a tonic excitatory response ($n = 6$), or augmented bursts ($n = 1$). In addition, unilateral microinjection of DLH increased mean arterial pressure in four of these sites, decreased mean arterial pressure in four sites, and was ineffective in altering mean arterial pressure in the remaining two sites (not shown).

In 32 sites in the pre-Bo"tC in which unilateral microinjection of NaCN was ineffective in eliciting a change in phrenic nerve discharge or mean arterial pressure, unilateral microinjection of DLH produced either a rapid series of high-amplitude, short-duration bursts ($n = 5$), a tonic excitatory response ($n = 22$), a series of high-amplitude, short-duration bursts superimposed on a tonic excitatory response ($n = 3$), or augmented bursts ($n = 2$) in the phrenic neurogram. Further, unilateral microinjection of DLH increased mean arterial pressure in 13 of these sites and decreased it in the remaining 19 sites (not shown).

### Microinjection of NaCN and DLH into sites adjacent to the pre-Bo"tC

We also examined the effects of unilateral microinjection of NaCN and DLH into 23 sites adjacent to the pre-Bo"tC as a control for the spread of injectate. These microinjections were made into sites in the rVRG, Bo"t complex, and 200–500 µm dorsomedial, dorsolateral, or lateral to the pre-Bo"tC.

Unilateral microinjection of DLH into the rVRG ($n = 10$) produced site-specific transient increases or decreases in the peak amplitude of integrated phrenic nerve discharge. In eight of these sites, unilateral microinjection of NaCN was ineffective in producing any changes in phrenic nerve discharge or blood pressure. In the remaining two sites, unilateral microinjection of NaCN produced a small transient decrease in the peak amplitude of integrated phrenic nerve discharge; the response to DLH in these sites was excitatory on phrenic motor output.

Unilateral microinjection of DLH into the Bo"t complex ($n = 3$) produced apnea. In these sites, unilateral microinjection of NaCN was ineffective in producing any changes in phrenic nerve discharge; however, a pressor response was seen in one of these sites.

In the 10 sites located dorsomedial, dorsolateral, or lateral to the pre-Bo"tC, unilateral microinjection of both NaCN and DLH elicited no effect on phrenic nerve discharge or blood pressure.

### Location of responsive and nonresponsive sites

The distribution of sites in which NaCN was microinjected into the medulla is shown in Fig. 6. As landmarks for identifying the rostrocaudal level of the pre-Bo"tC, we identified the caudal pole of the retrofacial nucleus, nucleus ambiguus, the rostral pole of the lateral reticular nucleus, and the rostral pole of the hypoglossal nucleus. Examining our data from the level of obex produced considerable overlap in the types of responses seen, and placed over 90% of our sites in the same coronal section. Therefore in our analyses, all sites in the pre-Bo"tC were identified with reference to the caudal pole of the retrofacial nucleus, not the obex. We found that mapping our sites in this way was consistent with the difference in the types of responses obtained based on movement of the pipette using stereotaxic coordinates in our experiments.

We often found that movement of the pipette tip, using...
stereotaxic coordinates, by as little as 100–200 μm resulted in marked differences in the phrenic neurogram and blood pressure responses evoked by unilateral microinjection of NaCN. An example may be seen in Fig. 7. In this example (Fig. 7A), unilateral microinjection of NaCN into the pre-BötC initially produced a transient rapid series of high-amplitude, rapid rate of rise, short-duration inspiratory bursts superimposed on a small tonic discharge. This series of bursts was followed by three eupneic-like breaths that were coupled to a rapid rate of rise, short-duration burst, which then recovered to a eupneic pattern. In addition, no change in blood pressure was noted in response to microinjection of NaCN into this site. Movement of the pipette tip stereotaxically (and histologically confirmed) 200 μm rostral to the initial site elicited a response that consisted predominantly of an increase in frequency, although some small augmented bursts were seen (Fig. 7B). In this site, microinjection of NaCN also elicited a decrease in blood pressure. Although a frequency response was evoked from both regions, the effects on timing and patterning of phrenic nerve discharge are quite different. With reference to histological identification (see Fig. 6 and explanation below), both injection sites were identified in the coronal section labeled −0.4 mm.

Our histological analyses (Fig. 6) revealed that all microinjection sites identified functionally as pre-BötC were located within the anatomic boundaries described for the pre-BötC in adult cat (Connelly et al. 1992; Ramirez et al. 1998; Schwarzacher et al. 1995; Solomon et al. 1999). As can be noted from our histological analyses, microinjection of NaCN into the pre-BötC produced a series of high-amplitude, short inspira-
Within this region are the sites in which approximate bursts of NaCN produced augmented bursts (i.e., eupneic breath ending with a high-amplitude, short-duration burst). In fact, all but 2 of the 18 sites that included high-amplitude, short inspiratory duration bursts were located in this region of the pre-BöC. Additionally, the majority (~90%) of sites in which microinjection of NaCN elicited a modulation of phrenic burst frequency (i.e., rapid series of high-amplitude, short inspiratory duration bursts; increased frequency of phrenic bursts) are located within this region of the pre-BöC. Encompassing a much larger area of the pre-BöC are the sites in which microinjection of NaCN produced a tonic excitation of phrenic neurogram output. In these sites, we could not distinguish nonrhythmic from rhythmic tonic excitatory responses based on their histological distribution.

We encountered 32 sites in the pre-BöC in which DLH but not NaCN elicited an excitatory pattern of inspiratory output. We could not differentiate these NaCN-responsive (Fig. 6, A–C) from NaCN-unresponsive (Fig. 6D) sites in the pre-BöC based on our histological analyses. In addition, we could not distinguish pressor and depressor responses based on their histological distribution.

**DISCUSSION**

In the present study, we have demonstrated that NaCN-induced focal hypoxia in discrete regions of the pre-BöC alters both respiratory rhythm and pattern in an excitatory manner.
This excitation of inspiratory motor output appears to be specific to the pre-Bötzinger complex because focal hypoxia in the rVRG and Bötz complex was ineffective in producing excitation of inspiratory motor output. Previous studies using NaNCN have shown depression, but not excitation of respiratory output. For example, in cats, perfusion of the fourth ventricle with NaNCN decreases tidal volume or produces apnea (Loeschcke and Koepchen 1958), and topical application of NaNCN to the intermediate area of the ventral medullary surface (VMS) rapidly and reversibly decreases both inspiratory and expiratory related respiratory activities (Haxhiu et al. 1993). Additionally, microinjection of NaNCN into the RVLM of cats and rats, including the intermediate area of the VMS, depresses phrenic nerve activity (Mitra et al. 1993; Sun et al. 1992). Thus this study shows for the first time that focal brain stem hypoxia produced by NaNCN acting in a known respiratory modulatory region augments inspiratory motor output. Based on these findings, we suggest that the pre-Bötzinger complex contains a hypoxia chemosensitive element that when stimulated is capable of producing excitation of inspiratory motor output.

Limitations of pressure injection

The response to NaNCN was induced rapidly on microinjection locally in the region of the pre-Bötzinger complex. Although the neural substrate responsible for initiating this response is unknown, it is most likely to be neurons located in this region or neuronal structures close to pre-Bötz complex neurons. We believe that the transient bilateral increases in phrenic nerve discharge evoked by unilateral microinjection of NaNCN into the pre-Bötz complex resulted from hypoxic excitation of neurons in the immediate vicinity of the pipette tip. It is unlikely that microinjection of NaNCN elicited its effects by inhibiting neurons in this region because microinjection of the excitatory amino acid DLH into the same sites produced similar types of responses in phrenic nerve discharge and arterial blood pressure. Further, it is unlikely that these responses resulted from nonspecific effects of pressure or volume of the injectate, because they were not produced by equal volumes of saline or larger volumes of Fast Green dye from adjacent barrels of the same pipette.

The effective spread of injectate is difficult to gauge without direct measurement. Nevertheless, the dose of NaNCN used in our experiments was relatively small and probably did not spread far from the injection site. Theoretical calculations by Nicholson (1985) demonstrate that based on a 10-nl injection into brain tissue, the concentration of injectate 300 μm from the injection site never exceeds 20% of the initial injectate concentration. Additionally, Lipski et al. (1988) estimated that microinjection of a 30-nl volume had a radius spread of 325 μm. In contrast, Mitra et al. (1993) reported that the distribution of [14C]cyanide based on a 100-nl (20 mM) microinjection was larger than those estimated by Nicholson (1985) and Lipski et al. (1988); however, they suggest that the physiological response to their injections was restricted to a spread within 1 mm from the injection site. The volume and concentration used by Mitra et al. (1993) is much larger than that used in our experiments. Our findings, however, appear to be consistent with the estimations by Nicholson (1985) and Lipski et al. (1988) in that in our experiments, movement of the pipette tip by as little as 100–200 μm produced marked differences in the phrenic neurogram and blood pressure responses to microinjection of NaNCN (see Fig. 7). Additionally, we often found that movement of the pipette tip by ≤500 μm distinguished responsive from unresponsive sites. We cannot exclude the possibility, however, that microinjection of NaNCN had an effect on dendrites whose cell bodies were distant from the site of injection.

Effects of brain hypoxia on respiratory and sympathetic output

To our knowledge, our data are the first to demonstrate in vivo a hypoxia chemosensitive region in the medulla that plays a role in the generation and modulation of respiratory rhythm and pattern. In our experiments, we found that unilateral microinjection of NaNCN into the pre-Bötz elicited a variety of excitatory responses in the phrenic nerve, ranging from a rapid series of high-amplitude, rapid rate of rise, short-duration bursts to tonic firing.

Previous studies have reported that the generalized effect of brain stem hypoxia is primarily a depression of respiratory output that is accompanied by an increase in sympathetic output. In anesthetized, peripherally chemodenervated animals, mild to moderate brain hypoxia produces a depression of respiratory output and an increase in tonic sympathetic nerve activity (Wasicko et al. 1990). Similarly, intraventricular injection of hypoxic saline or NaNCN results in depression of phrenic nerve activity and an increase in sympathetic nerve activity and arterial blood pressure (Arita et al. 1989; Mitra et al. 1992).

The increase in sympathetic nerve activity in response to CNS hypoxic hypoxia or cyanide appears to be mediated by direct hypoxic stimulation of reticulospinal sympathoexcitatory neurons located in the RVLM (Sun and Reis 1994; Sun et al. 1992). These neurons have been previously shown to be essential for the tonic generation of vasomotor tone and the reflex regulation of arterial blood pressure (Ross et al. 1984). The most convincing evidence for direct hypoxic excitation comes from the work of Sun et al. (1992) in which they have demonstrated that microinjection of cyanide into the RVLM of rats evokes a pressor response and a transient depression of phrenic nerve discharge. Additionally, they have shown that iontophoretic application of NaNCN into the RVLM selectively and reversibly excites reticulospinal sympathoexcitatory neurons, many of which exhibit pacemaker-like activity.

Centrally mediated hypoxic respiratory excitation: augmentation and gasping

Although hypoxic respiratory depression has been reported in both anesthetized and awake peripherally chemodenervated animals, stimulation of breathing in these animal preparations has also been demonstrated (Gautier and Bonora 1980; Miller and Tenney 1975; Richter et al. 1991; Tenney and Ou 1976). For example, in anesthetized deafferented cats, Richter et al. (1991) have reported a biphasic respiratory response that consists of an initial increase in the frequency and amplitude of ventilation (augmentation) followed by a decrease (depression) that can lead to cessation of breathing (apnea). Further, in unanesthetized (i.e., awake or decerebrate) deafferented cats,
Tenney and colleagues have reported an increase in respiratory frequency (i.e., tachypnea) (Miller and Tenney 1975; Tenney and Ou 1976). It has been suggested that the stimulation of respiratory frequency in response to global CNS hypoxia originates from suprapontine regions (Tenney and Ou 1976).

In addition to these initial stimulatory effects on breathing, severe brain hypoxia results in a shift from respiratory depression to respiratory excitation. In this case, respiratory excitation takes the form of gasping that is characterized by an abrupt onset (i.e., rapid rate of rise), short-duration burst of inspiratory activity (Fung et al. 1994; St. John and Knuth 1981; Zhou et al. 1991). Further, in the cat, gasping is also characterized by a significant increase in the peak amplitude of integrated phrenic nerve discharge (St. John and Knuth 1981). This form of respiratory excitation (i.e., gasping) in response to severe brain hypoxia has been suggested to originate in the medulla (this study; Solomon et al. 1996; St. John 1996; St. John et al. 1984, 1985); however, the specific medullary sites and mechanisms have been unclear.

Previous work from St. John and colleagues in cats (St. John et al. 1984, 1985) and rats (Fung et al. 1994) has identified a region in the lateral tegmental field (LTF) of the medulla that they propose is critical for the expression of gasping. They have shown that electrical stimulation in this region during gasping evokes premature gasps and that lesioning this region eliminates gasping, although neither maneuver alters the eupneic breathing pattern. Two recent studies have examined the role of the pre-BötC in the production and/or expression of both eupnea and gasping in vivo (Huang et al. 1997; Ramirez et al. 1998b). Huang et al. (1997) have demonstrated that lesioning a region of the ventrolateral medulla that includes the pre-BötC and pre-I region (which is located rostral to the pre-BötC) in decerebrate neonatal rats using kainic acid abolished gasping in response to severe hypoxia but was ineffective in altering the eupneic pattern of breathing. In contrast, Ramirez et al. (1998b) have demonstrated that bilateral synaptic blockade of the pre-BötC in anesthetized cats using tetrodotoxin (TTX) abolished the eupneic pattern of breathing without eliminating gasping in response to asphyxia or severe hypoxia. It is possible, however, that gasping may result from TTX-insensitive mechanisms and may be independent of synaptic transmission [i.e., similar to the hypoxic excitation of reticulospinal sympathoexcitatory neurons of the RVLM (Sun and Reis 1993, 1994a,b)]

In our experiments, we found that both types of respiratory stimulation (i.e., augmentation of frequency and amplitude of eupneic bursts and production of gasplike bursts) could be produced by unilateral microinjection of NaCN into the pre-BötC. Our current findings suggest that direct hypoxic stimulation of a discrete region located in the pre-BötC can modify both inspiratory timing and patterning to produce either an increase in frequency of phrenic bursts or a gasplike inspiratory output. It should be noted, however, that these high-amplitude, short-duration (gasplike) bursts produced by focal hypoxia in the pre-BötC, were rapid with a short Te, whereas gasps produced by severe brain hypoxia usually exhibit a prolonged Te. However, in our experiments, our cats were maintained hyperoxic (arterial \( P_{O2} \geq 200 \) mmHg) throughout the experimental protocol. Thus the typical low frequency of gasps with global brain hypoxia may reflect the depressant effect of hypoxia at sites other than the pre-BötC, whereas the response manifest by focal NaCN may reflect the intrinsic response of the neurons that initiate the gasp.

In our experiments, unilateral microinjection of NaCN into the pre-BötC produces phase resetting of the respiratory rhythm (indicated by our increases in respiratory burst frequency). Thus our findings do not drive the conclusion that the pre-BötC is a pattern generator for gasping and not eupnea because a variety of responses, including increased frequency of eupneic bursts, were observed. Because this region is proposed to represent the primary locus of respiratory rhythm generation (Smith et al. 1991; for a recent review see Rekling and Feldman 1998), our findings are consistent with the idea that the principal respiratory rhythm generator or a subset of neurons embedded within are hypoxia chemosensitive albeit, the physiological range of hypoxic chemosensitivity remains to be determined.

**Hypoxic chemosensitivity in the pre-BötC**

Recently, Ramirez et al. (1998a) examined the hypoxic responses of pre-BötC neurons from in vitro transverse brain stem slices obtained from neonatal mice (postnatal days 0–22). Thirty minutes of exposure to hypoxic artificial cerebrospinal fluid (95% \( N_2 \) and 5% \( CO_2 \)) elicited a biphasic response of inspiratory motor output (i.e., augmentation followed by depression) as previously reported (Ramirez et al. 1997a). During the initial phase of the hypoxic exposure, augmentation of inspiratory motor output was characterized by an increase in the frequency of rhythmic discharges in pre-BötC inspiratory neurons and an increase in frequency of burst discharges on the hypoglossal nerve. In addition, in ~50% of pre-BötC inspiratory neurons examined, the initial augmentation was accompanied by an increase in the amplitude of excitatory synaptic drive potentials.

In our experiments, we found that unilateral microinjection of NaCN elicited responses in ~60% of the sites examined in the pre-BötC. There are at least two possible explanations for this finding that are not mutually exclusive. First, the dose of NaCN used in our experiments was low and therefore may have been inadequate to elicit effects in all of the sites examined. Consistent with this idea are the findings of Mitra et al. (1992) and Sun et al. (1992), who have demonstrated that cyanide increases sympathetic nerve discharge and reticulospinal sympathoexcitatory neuronal activity in a dose-dependent manner. We only used one dose of NaCN in the pre-BötC, based on our pilot experiments conducted in other regions of the medullary respiratory network (Solomon et al. 1995). In these pilot experiments, we found that this 1-mM dose produced transient, repeatable responses in phrenic nerve discharge and that lower doses ranging from 200 to 500 \( \mu M \) were ineffective in consistently altering respiratory motor output, whereas the higher doses ranging from 2 to 20 \( \mu M \) were not repeatable and resulted in irreversible apnea.

Alternatively, only a subpopulation of neurons located in the pre-BötC may be selectively hypoxia chemosensitive. In support of this idea, previous studies have demonstrated that reticulospinal sympathoexcitatory neurons of the RVLM, many of which exhibit pacemaker-like activity, are intrinsically hypoxia chemosensitive (Sun and Reis 1993, 1994a;
Sun et al. 1992). These neurons are directly and reversibly excited by hypoxia, whereas adjacent neurons in this region are inhibited or unaffected (Sun and Reis 1993, 1994b; Sun et al. 1992). We suggest that the pre-BötzC contains a hypoxia chemosensitive element that is similarly selectively and directly excited by hypoxia, and that this chemosensitive element is part of the respiratory rhythm-generating network located in this region. From our experiments, we cannot determine which neuronal elements were responsible for the excitation of inspiratory motor output. We speculate that the hypoxia chemosensitive element contains, but is not limited to, the voltage-dependent pacemaker cells that are believed, at least in the neonate, to provide rhythmic drive to the rest of the respiratory network during the inspiratory phase of network activity (Butera et al. 1999b; Feldman and Smith 1995; Koshiya and Smith 1999; Rekling and Feldman 1998; Smith et al. 1991, 1995). Additionally, the preinspiratory (pre-I) (Connelly et al. 1992; Schwarzacher et al. 1995; Smith et al. 1990) or inspiratory-driver (I-driver) (Segers et al. 1987) neurons proposed to be key elements in respiratory phase transition (Smith et al. 1990, 1995) are likely candidates to be hypoxia chemosensitive.

It should also be noted that unilateral microinjection of NaCN into the pre-BötzC produced modulation of respiratory rhythm and pattern that exhibited different forms of augmentation of inspiratory motor output (i.e., rapid series of high-amplitude, rapid rate of rise, short-duration bursts; tonic firing; increased frequency of eupneic bursts). These differences in the cycle timing (i.e., rhythm) and pattern may reflect a continuum of excitatory responses evoked by stimulation of the respiratory rhythm generator located in the pre-BötzC. As such, our findings could be explained by either the hybrid model or network models of respiratory rhythm generation that do not include conditional bursting pacemaker neurons (Balis et al. 1994; Ogilvie et al. 1992; Ramirez et al. 1997b; Richter et al. 1986). These models postulate that respiratory rhythm generation arises from the interaction of inhibitory synaptic influences with intrinsic membrane properties of specific classes of respiratory-modulated neurons (for a review see Ramirez et al. 1997b). Because network models rely on respiratory phase-dependent synaptic interactions among the various types of respiratory-modulated neurons (Balis et al. 1994; Ogilvie et al. 1992; Ramirez et al. 1997b; Richter et al. 1986), changes in the strength of these synaptic interactions and/or changes in activity of a population of respiratory neurons can modify respiratory rhythm and pattern (Balis et al. 1994; Ogilvie et al. 1992). Previous studies have demonstrated that the pre-BötzC contains a high concentration of pre-I neurons (Connelly et al. 1992; Schwarzacher et al. 1995) that appear to be necessary for phase transition (Schwarzacher et al. 1995; Smith et al. 1990). As such, the modulations of respiratory rhythm and pattern reported in our study could have resulted from hypoxic stimulation of pre-I (I-driver) neurons, which are known to provide excitatory drive to inspiratory neurons exhibiting augmenting (I-AUG) and decrementing (I-DEC) patterns of discharge (Segers et al. 1987). In computer simulations inferred from spike train cross-correlation data (Balis et al. 1994) and the mathematical model of a network oscillator by Ogilvie et al. (1992), increasing excitation of I-AUG neurons decreases Ti and increases the slope of the inspiratory ramp (i.e., produces a “square wave” discharge), whereas increasing excitation of I-DEC neurons lengthens Ti (i.e., produces apneusis). Thus it is possible that focal hypoxia in the pre-BötzC stimulated this population of pre-I (I-driver) neurons that in turn synaptically altered the activity of other inspiratory-modulated neurons (i.e., I-AUG and/or I-DEC) to produce the differences in the cycle timing (i.e., rhythm) and pattern seen in our experiments.
Mechanisms of hypoxic excitation

The cellular mechanisms by which focal hypoxia in the pre-Bo\"tC produced excitation of inspiratory motor output in our current study are not known. It is unlikely that the excitation resulted from pathological consequences of O\textsubscript{2} lack associated with impairment in cell functions because the responses to microinjection were rapid, completely reversible, and reproducible over many trials (even before recovery to eupnea). More likely, the excitation represents a direct cellular signaling process comparable perhaps to those of the carotid bodies, in which application of cyanide in doses comparable with those used here similarly results in rapid vigorous stimulation (Biscoe et al. 1988).

Within a neural network, however, other possibilities must be considered. For example, hypoxia may elicit excitatory transynaptic mechanisms via activation of glutamate receptors or indirect facilitation mediated by withdrawal of a tonic GABAergic input. Hypoxia has been shown to increase release of glutamate in some regions of the brain (Hagberg et al. 1985; Rothman and Olney 1986), which may potently excite N-methyl-D-aspartate receptors (Goldberg et al. 1987; Rothman 1984). It is unlikely that the excitation of phrenic motor output observed in our study resulted simply from hypoxic release of glutamate because hypoxic excitation of phrenic nerve discharge was only seen in a portion of the sites in which the glutamate analogue DLH elicited excitatory effects. In addition, preliminary evidence from our laboratory suggests that microinjection of NaCN into the pre-Bo\"tC following focal administration of the broad spectrum ionotropic EAA receptor antagonist kynurenic acid (Collingridge and Lester 1989), in a dose that blocks the DLH-induced excitation, is ineffective in abolishing the NaCN-induced response (unpublished observation).

Activation of GABA\textsubscript{A} receptors requires ATP-dependent phosphorylation to maintain functional integrity (Chen et al. 1990; Shirasaki et al. 1992; Stelzer et al. 1988). During severe hypoxia, reduced ATP availability may limit phosphorylation, resulting in disinhibition. In our experiments, the responses to NaCN occurred within seconds, so it is unlikely that ATP levels would have decreased significantly. In fact, previous studies have shown that ATP levels change very little in carotid body after brief periods of hypoxia (Verna et al. 1990) or exposure to cyanide (Obes\text{\'} et al. 1989), and that the responses to cyanide occur in both the presence and absence of intracellular ATP (Biscoe and Duchen 1989).

Effects of focal hypoxia in the pre-Bo\"t complex on arterial blood pressure

In most of our sites, the augmentation of inspiratory output was accompanied by a pressor or depressor response; however, we did encounter sites in which no blood pressure changes were observed. Although focal hypoxia in the RVLM is known to increase sympathetic nerve discharge and arterial blood pressure (Mitra et al. 1993; Sun et al. 1992), it is unlikely that the increases in blood pressure reported here resulted from direct hypoxic stimulation of sympathoexcitatory vasomotor neurons located in the RVLM. In this region of the RVLM, focal hypoxia has been shown to not only increase arterial blood pressure but also depress phrenic nerve activity (Mitra et al. 1993; Sun et al. 1992). In addition, the RVLM region containing the C1 neuronal population (McAllen and Dampney 1989) is located rostral and ventral to the pre-Bo\"tC. It seems more likely that the increases in blood pressure observed in our study resulted from enhanced respiratory modulation of sympathetic activity (Bachoo and Polosa 1987; Darnall and Guyenet 1990; Solomon et al. 1997). This proposal is supported by neuroanatomic studies using intracellular biocytin injections demonstrating that inspiratory neurons originating in the rostral rVRG (some in the vicinity of the pre-Bo\"tC) project directly onto tyrosine hydroxylase immunoreactive neurons located in the RVLM (Pilowsky et al. 1994), suggesting that these respiratory neurons may provide direct synaptic input to RVLM sympathoexcitatory neurons.

Our data provide no insight into the mechanism responsible for the production of the depressor responses reported here. Whether respiratory neurons originating in this region provide inhibitory synaptic inputs to sympathoexcitatory neurons of the RVLM or project directly to depressor regions of the caudal ventrolateral medulla remains to be determined.

Conclusion

Our experiments have identified a locus in the brain stem in which focal hypoxia augments respiratory output. Using discrete focal hypoxic stimulation, our data provide strong evidence to suggest that the respiratory rhythm generator in the pre-Bo\"tC has intrinsic hypoxic chemosensitivity resulting in modulation of phrenic motor output. It should be noted that in the in vitro transverse medullary slice model, rendering the entire slice hypoxic has been shown to result in excitation of pre-Bo\"tC neurons and hypoglossal nerve activity. In our experiments, we have demonstrated that focal hypoxia in discrete regions of the pre-Bo\"tC produces increases in respiratory burst frequency and tonic discharge of inspiratory output, as well as modulation of burst pattern in vivo. This hypoxic excitation of inspiratory motor output appears at this time to be specific to the pre-Bo\"tC. The findings establish the potential for hypoxic chemosensitivity in the brain stem for the respiratory control system.

The authors thank T. Le Hoang and J. Cooper for technical assistance. This work was supported by a grant from the National Heart, Lung, and Blood Institute (HL-16022). I. C. Solomon was supported by a postdoctoral fellowship from the American Heart Association, New Jersey Affiliate.

Address for reprint requests: I. C. Solomon, Dept. of Physiology and Biophysics, Health Sciences Center, Basic Science Tower, Level 6, Rm. 140, State University of New York at Stony Brook, Stony Brook, NY 11794-8661.

Received 27 August 1999; accepted in final form 26 January 2000.

REFERENCES


