Glutamate Neurotransmission Is Not Required for, But May Modulate, Hypoxic Sensitivity of Pre-Bötzing Complex In Vivo

Irene C. Solomon

Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York

Submitted 7 September 2004; accepted in final form 28 October 2004

Solomon, Irene C. Glutamate neurotransmission is not required for, but may modulate, hypoxic sensitivity of pre-Bötzing complex in vivo. J Neurophysiol 93: 1278–1284, 2005. First published November 3, 2004; doi:10.1152/jn.00932.2004. Focal hypoxia in the pre-Bötzing complex (pre-BötC) in vivo elicits excitation of inspiratory motor output by modifying the patterning and timing of phrenic bursts. Hypoxia, however, has been reported to enhance glutamate release in some regions of the brain, including the medullary ventral respiratory column; thus the pre-BötC–mediated hypoxic respiratory excitation may result from, or be influenced by, hypoxia-induced activation of ionotropic glutamate [i.e., excitatory amino acid (EAA)] receptors. To test this possibility, the effects of focal pre-BötC hypoxia [induced by sodium cyanide (NaCN)] were examined before and after blockade of ionotropic EAA receptors [using kynurenic acid (KYN)] in this region in chloralose-anesthetized, vagotomized, mechanically ventilated cats. Before blockade of ionotropic EAA receptors, unilateral microinjection of NaCN (1 mM; 10–20 nl) into the pre-BötC produced either phasic or tonic excitation of phrenic nerve discharge. Unilateral microinjection of KYN (50–100 mM; 40 nl) decreased the amplitude and frequency of basal phrenic nerve discharge; however, subsequent microinjection of NaCN, but not DL-homocysteic acid (DLH, a glutamate analog), still produced excitation of phrenic motor output. Under these conditions, the NaCN-induced excitation included frequency modulation (FM) of phasic phrenic bursts, and in many cases, augmented and/or fractionated phrenic bursts. These findings show that the hypoxia-sensing function of the in vivo pre-BötC, which produces excitation of phrenic nerve discharge, is not dependent on activation of ionotropic glutamate receptors, but ionotopic glutamate receptor activation may modify the expression of the focal hypoxia-induced response. Thus these findings provide additional support to the concept of intrinsic hypoxic sensitivity of the pre-BötC.

INTRODUCTION

The pre-Bötzing complex (pre-BötC), in addition to its role in the generation and modulation of eupneic breathing (Gray et al. 2001; Ramirez et al. 1998b; Rekling and Feldman 1998; Smith et al. 1991, 2000), seems to participate in the production and/or expression of gasping (Huang et al. 1997; Lieske et al. 2000; Solomon 2000, 2002b, 2004; Solomon et al. 1999). The mechanisms responsible for the generation of gasping, which represents a form of respiratory excitation in response to severe brain hypoxia, remain to be elucidated. One possible mechanism that has been proposed to participate in the production of hypoxia-induced gasping, at least in part, is direct hypoxic excitation of neurons located within the pre-BötC (Solomon 2000; Solomon et al. 2000). Support for a hypoxia-sensing function of this region includes the demonstration of 1) excitation of phrenic nerve discharge, which ranges from a gasp-like discharge pattern to tonic phrenic nerve discharge, in response to focal pre-BötC hypoxia in vivo (Solomon et al. 2000); 2) an increase in the frequency of hypoxia-induced gasps by focal pre-BötC hypoxia in vivo (Solomon 2002b); 3) an increase in the frequency of rhythmic discharges in pre-BötC inspiratory neurons and hypoglossal nerve discharge during exposure to hypoxia of the in vitro transverse medullary slice preparation (Ramirez et al. 1998a), and 4) continuous rhythmic bursting in a subset of pre-BötC pacemaker neurons during anoxia in the in vitro transverse medullary slice preparation (Peña et al. 2004; Thoby-Brisson and Ramirez 2000). Although these observations suggest that this region both in vivo and in vitro may be directly excited by hypoxia (i.e., intrinsic hypoxic chemosensitivity), in some regions of the brain, including the ventral respiratory group (VRG) (Richter et al. 1999), hypoxia has been reported to enhance glutamate release (Hagberg et al. 1985; Mizusawa et al. 1994; Richter et al. 1999; Rothman and Olney 1986). Thus the pre-BötC–mediated hypoxic respiratory excitation may alternatively result from transynaptic mechanisms involving excitation via activation of glutamate [i.e., excitatory amino acid (EAA)] receptors. In fact, in the anesthetized adult cat model, activation of ionotropic EAA receptors in the pre-BötC elicits patterning and timing changes in phrenic nerve discharge that are similar to those evoked by focal pre-BötC hypoxia under both hyperoxic (Solomon et al. 1999, 2000) and hypoxic (Solomon 2002b) conditions. Therefore this study was undertaken to determine whether the respiratory excitation elicited by focal pre-BötC hypoxia is mediated by hypoxia-induced glutamate release acting on ionotropic EAA receptors. Since I believe that this region exhibits intrinsic hypoxic chemosensitivity, I hypothesized that excitation of phrenic nerve discharge elicited by focal pre-BötC hypoxia would not require activation of ionotropic EAA receptors in this region. Furthermore, based on recent observations from my laboratory showing that blockade of ionotropic EAA receptors in the pre-BötC may play a modulatory role in the expression hypoxia-induced gasping (Solomon 2004), I also hypothesized that activation of ionotropic EAA receptors in this region would modify the focal pre-BötC hypoxia-induced phrenic motor output response. To test these hypotheses, I examined the effects of focal pre-BötC hypoxia induced by sodium cyanide (NaCN) before and after pharmacological blockade of ionotropic EAA receptors in this region.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

General methods

All experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at the State University of New York at Stony Brook in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals. A detailed description of the general methods has been published previously (Solomon et al. 1999, 2000).

In brief, anesthesia was induced in adult cats (2.8–3.8 kg; n = 10) with halothane (5%) in oxygen and maintained with intravenous α-chloralose (initial 35–50 mg/kg; supplemental 3–5 mg/kg). The adequacy of anesthesia was regularly verified by absence of a withdrawal reflex (in the unparalyzed state) or blood pressure response (during muscular paralysis) to a noxious paw pinch. The right brachial vein and both brachial arteries were cannulated for administration of drugs, measurement of arterial blood pressure (Statham transducer, P23XL), and sampling of arterial blood. The trachea was cannulated, the vagus nerves, and in some experiments, the carotid sinus nerves, were transected bilaterally, and the lungs were mechanically vented (during muscular paralysis) to a noxious paw pinch. The right brachial vein and both brachial arteries were cannulated for administration of drugs, measurement of arterial blood pressure (Statham transducer, P23XL), and sampling of arterial blood. The trachea was cannulated, the vagus nerves, and in some experiments, the carotid sinus nerves, were transected bilaterally, and the lungs were mechanically ventilated with 40% O₂ in a balance of N₂. The cat was paralyzed with vecuronium bromide (0.2–0.4 mg/kg, iv), supplemented as needed. The dorsal surface of the brain stem was exposed, and the C₅ rootlet of the cervical spinal nerve was isolated for recording.

Experimental protocol

I examined the effects of blockade of ionotropic EAA receptors in the pre-BoßC on the patterned, timing, and frequency of phrenic nerve discharge elicited by focal pre-BoßC hypoxia. Blockade of ionotropic EAA receptors was produced by unilateral microinjection of the broad-spectrum ionotropic EAA receptor antagonist kynurenic acid (KYN; 50–100 mM; 40 nl), and focal hypoxia was produced by unilateral microinjection of NaCN (1 mM; 10–20 nl). For all experiments, sites in the pre-BoßC were initially functionally identified using microinjection of DL-homocysteic acid (DLH; 10 mM; 10–20 nl) under hyperoxic conditions, as previously described (Solomon et al. 1999); however, only sites in which microinjection of NaCN elicited either phasic or tonic excitation of phrenic nerve discharge were studied further. Since the effects of KYN were completely reversible, a second site in the pre-BoßC was examined in some experiments. Following recovery from the initial NaCN-induced excitation of phrenic nerve discharge, KYN was microinjected into the pre-BoßC site, ~10–15 min was allowed for the antagonist to exert its effects, and the NaCN microinjection was repeated (n = 14). In a subset of pre-BoßC sites (n = 6), DLH (20 nl) was microinjected into the same site in the pre-BoßC either 2–3 min before or within 5 min following microinjection of NaCN to confirm that KYN blocked ionotropic EAA receptors in this region. In four experiments, at ~60–90 min after microinjection of KYN into the pre-BoßC (when phrenic nerve discharge appeared to have recovered from blockade of ionotropic EAA receptors in this region), an additional NaCN microinjection was performed (i.e., recovery) in the same pre-BoßC site. At the end of each experiment, Fast green dye (2%; ≤120 nl) was microinjected to mark the injection site, the brain stem was removed, and the tissue was processed for histological analysis and verification of the location of the injection sites (Fig. 1B) as previously described (Solomon et al. 1999, 2000).

Data analysis

Peak amplitude of integrated phrenic nerve discharge, inspiratory duration (Tᵢ), expiratory duration (Tₑ), rate of rise, and frequency of phrenic bursts were determined in response to unilateral microinjection of NaCN into the pre-BoßC before and after blockade of ionotropic EAA receptors. Preinjection baseline values were calculated by averaging the values obtained for five consecutive phrenic bursts preceding microinjection, and response values were determined as the peak change from preinjection baseline values for a tonic nonphasic excitation of phrenic nerve discharge or by averaging the values...
obtained for five consecutive breathing cycles displaying the greatest change from preinjection baseline values for phasic phrenic nerve discharge responses. For tonic nonphasic excitation of phrenic nerve discharge, \( T_I \) represents the duration of tonic firing, and \( T_E \) was not determined. Amplitude of integrated phrenic nerve discharge and frequency of phasic phrenic bursts are reported as a percent change from preinjection baseline levels of discharge determined under control conditions (i.e., before microinjection of KYN), which were set at 100% in each cat. The onset latency for NaCN-induced responses was measured from the beginning of microinjection, and the total duration of the NaCN-induced response was considered the time between the onset of the response and return of phrenic nerve discharge to preinjection conditions.

Data are reported as means \( \pm SE \). A Student’s paired \( t \)-test or one-way ANOVA with repeated measures followed by Scheffé post hoc analyses for multiple comparisons, as appropriate, were used to determine statistical significance, for which the criterion level was set at \( P < 0.05 \).

RESULTS

Effects of unilateral KYN on basal and DLH-induced phrenic nerve discharge

Unilateral blockade of ionotropic EAA receptors by microinjection of KYN into the pre-Bo\( \ddot{t} \)C reduced the amplitude and frequency of phasic phrenic nerve discharge. The effects of KYN appeared similar in sites in which focal pre-Bo\( \ddot{t} \)C NaCN elicited phasic versus tonic excitation of phrenic nerve discharge, and examples showing these effects can be seen in the baseline portion of the recordings shown in Figs. 1–3 (i.e., baseline discharge “Before KYN” vs. “After Unilateral KYN”). The effects of unilateral KYN in the pre-Bo\( \ddot{t} \)C were gradual, and the reduction in peak amplitude of integrated phrenic nerve discharge and frequency of phrenic bursts began within 1–2 min, was maximal by \( \sim 9–15 \) min, and lasted \( \sim 40–60 \) min following the end of microinjection. The maximal decrease in peak amplitude of integrated phrenic nerve discharge and frequency of phasic phrenic bursts during this time period were 37.4 \( \pm \) 5.7 and 35.9 \( \pm \) 4.2%, respectively. The reduction in phrenic burst frequency was mediated by a prolongation of \( T_E \) (\( P < 0.05 \)), which was observed in all experiments, and an increase in \( T_I \) (\( P < 0.05 \)), which was seen in most experiments.

Prior to microinjection of KYN, microinjection of DLH into the pre-Bo\( \ddot{t} \)C elicited either phasic (\( n = 2 \)) or tonic (\( n = 4 \)) excitation of phrenic nerve discharge (as previously described, Solomon 2002a; Solomon et al. 1999); following microinjection of KYN, however, similar microinjection of DLH was ineffective in altering the KYN-induced pattern of phrenic nerve activity, confirming that KYN blocked ionotropic EAA receptors in this region. An example showing the effects of KYN on the DLH-induced tonic excitation of phrenic nerve discharge is shown in Fig. 1. In this example, \( \sim 12 \) min after microinjection of KYN, similar microinjection of DLH no longer elicited excitation of phrenic nerve discharge. Also shown in Fig. 1 is the distribution of pre-Bo\( \ddot{t} \)C sites examined in this study. Our histological analyses confirmed that all microinjection sites were located within the anatomical boundaries described for the adult cat pre-Bo\( \ddot{t} \)C (Connelly et al. 1992; Ramírez et al. 1998b; Schwarzacher et al. 1995; Solomon et al. 1999).

Characteristics of NaCN-induced pre-Bo\( \ddot{t} \)C activation before and after KYN

In each of the experiments conducted, unilateral microinjection of NaCN into the pre-Bo\( \ddot{t} \)C elicited a marked excitation of phrenic nerve discharge under both baseline conditions and during blockade of ionotropic EAA receptors in this region. In
sites in which a rapid series of high-amplitude short-duration phrenic bursts was elicited before KYN (n = 5), microinjection of NaCN after KYN elicited similar phasic activity, although additional patterning and timing changes were observed prior to recovery to the preinjection basal level of discharge. An example showing the effects of blockade of ionotropic EAA receptors in the pre-Bo¨tC on the NaCN-induced phasic excitation of phrenic nerve discharge is shown in Fig. 2A. In this example, microinjection of NaCN into this region after KYN still produced a rapid series of high-amplitude short-duration phrenic bursts followed by phrenic bursts, including augmented burst discharges (i.e., eupneic discharge pattern ending with a high-amplitude short-duration burst), exhibiting an increased amplitude and frequency (above baseline) prior to return to the KYN-induced phrenic burst pattern and frequency. The onset latency to excitation of phrenic nerve discharge was unchanged (i.e., 1–2 s under both conditions); however, in three of the five experiments, the induction of the rapid series of high-amplitude short-duration phrenic bursts was delayed by 6.3 ± 2.4 s (range, 2.3–11.4 s). In addition, in three of the five experiments, the number of NaCN-induced high-amplitude short-duration phrenic bursts was reduced (from 66 to 51 bursts) after KYN microinjection into this region; no reduction in the number of NaCN-induced phrenic bursts was observed in the other two experiments (i.e., 31 and 14 before KYN and 34 and 16 after KYN, respectively). Although the number of NaCN-induced high-amplitude short-duration phrenic bursts may not have been identical before and after KYN microinjection, the duration of the total NaCN-induced response was typically longer [by 44.9 ± 15.7% (SE)] following microinjection of KYN due to the additional NaCN-induced modulation of phasic phrenic nerve discharge that preceded return to the KYN-induced phrenic burst pattern and frequency. The patterning and timing characteristics associated with the NaCN-induced high-amplitude short-duration phrenic bursts, however, were unaffected by blockade of ionotropic EAA receptors in the pre-Bo¨tC (Fig. 2B).

In sites in which tonic phrenic nerve discharge was elicited before KYN (n = 9), microinjection of NaCN after KYN still elicited phrenic nerve excitation; however, the magnitude of tonic discharge was markedly reduced or absent under these conditions, and the excitatory response was modified to include phasic phrenic bursts. An example showing the effects of blockade of ionotropic EAA receptors in the pre-Bo¨tC on the NaCN-induced tonic excitatory response is shown in Fig. 3A. In this example, the NaCN-induced tonic excitation that was

FIG. 3. Example showing effects of blockade of ionotropic EAA receptors in the pre-Bo¨tC on NaCN-induced pre-Bo¨tC-mediated tonic excitatory response. A: before unilateral microinjection of KYN (100 mM; 40 nl) into the pre-Bo¨tC, microinjection of NaCN (1 mM; 20 nl) into this region produced tonic excitation of phrenic nerve discharge. After KYN, however, similar microinjection of NaCN elicited FM of phasic phrenic bursts, which exhibited a modified burst pattern consisting of augmented bursts, fractionated bursts, and abrupt onset bursts. Following recovery from KYN, microinjection of NaCN elicited tonic excitation of phrenic nerve discharge (which was only partially recovered in this example). B: examples of augmented and fractionated bursts elicited by microinjection of NaCN into the pre-Bo¨tC after KYN on an expanded time scale. C: summary data showing effects of KYN in the pre-Bo¨tC on phrenic burst amplitude and burst duration (Tt) of the NaCN-induced tonic (before KYN) and phasic (after unilateral KYN) excitatory responses. During blockade of ionotropic EAA receptors in the pre-Bo¨tC, Tt of the NaCN-induced phasic bursts was substantially reduced compared with the NaCN-induced tonic excitation observed before KYN or following recovery (Rec) from blockade. *Significant difference (P < 0.05) between pairs indicated.
observed under baseline conditions was converted to a phasic response after unilateral microinjection of KYN into the pre-Bo¨tC. This NaCN-induced phasic activity was characterized by an increase in the frequency of phasic phrenic bursts, which was mediated by reductions in both $T_I$ ($P < 0.05$) and $T_E$ ($P < 0.05$), and a modified phrenic burst pattern. These NaCN-induced phasic phrenic bursts also exhibited higher amplitudes and shorter durations than the KYN-induced or baseline (i.e., pre-KYN) phrenic bursts, although the amplitude of these bursts was slightly but not significantly reduced compared with the peak amplitude of the NaCN-induced tonic discharge (see Fig. 3C). During blockade, the NaCN-induced phasic activity exhibited predominantly augmented burst discharges, fractionated burst discharges, and in many cases, abrupt onset (i.e., rapid rate of rise) bursts. These burst patterns can be seen in the examples provided in Fig. 3A (“After Unilateral KYN—Blockade”) as well as Fig. 3B, which shows augmented and fractionated bursts on an expanded time scale. Summary data showing the effects of blockade of ionotropic EAA receptors in the pre-Bo¨tC on phrenic burst amplitude and $T_I$ during the NaCN-induced tonic versus phasic phrenic nerve discharge are provided in Fig. 3C. It should be noted that the high-amplitude short-duration burst component of the augmented burst discharges was not included in the burst amplitude measurements. In addition to the marked alteration in the patterning and timing of the NaCN-induced phrenic discharge response during blockade, the duration of the NaCN-induced effect was typically longer (by $61.3 \pm 20.0\%$) following KYN than that observed for NaCN-induced tonic phrenic nerve discharge. In contrast, the onset latency from the beginning of microinjection to excitation of phrenic nerve discharge was unchanged, with the NaCN-induced modulation of phrenic nerve discharge occurring within $1–2$ s both before and after microinjection of KYN.

NaCN-induced pre-Bo¨tC activation following recovery from KYN

Following recovery from KYN ($n = 4$), focal pre-Bo¨tC hypoxia elicited respiratory excitation that was similar to that observed under baseline conditions, although in one experiment, only partial recovery of the NaCN-induced tonic excitatory response was noted. Furthermore, the onset latency to phrenic excitation was similar for the baseline and recovery NaCN microinjection trials ($1.4 \pm 0.7$ vs. $1.5 \pm 0.6$ s, respectively), and the total duration of the NaCN-induced tonic phrenic nerve discharge was almost completely recovered. Summary data showing the characteristics of NaCN-induced pre-Bo¨tC-mediated phrenic nerve excitation for these recovery experiments are included in Fig. 3C.

DISCUSSION

I have shown that focal pre-Bo¨tC hypoxia can elicit excitation of phrenic nerve discharge during blockade of ionotropic EAA receptors in this region, suggesting that hypoxia-induced glutamate release and subsequent activation of ionotropic EAA receptors in this region are not required for the hypoxia sensing function of the pre-Bo¨tC. Although a focal pre-Bo¨tC hypoxia-induced respiratory excitation was observed during blockade, the response was not identical to that evoked before blockade. Prior to blockade, focal pre-Bo¨tC hypoxia (induced by NaCN) produced either phasic or tonic excitation of phrenic nerve discharge, with phasic activity being characterized by a rapid series of high-amplitude short-duration phrenic bursts (as previously described, Solomon et al. 2000). Although this NaCN-induced pattern of phasic activity was also observed during blockade, it was accompanied by a further modulation of phrenic burst pattern, which included augmented and fractionated bursts. The NaCN-induced tonic excitatory response was also modified during blockade, such that the magnitude of tonic discharge was markedly reduced (or absent in some instances) and phasic phrenic bursts were elicited in response focal pre-Bo¨tC hypoxia. Under these conditions, the NaCN-induced phasic excitation exhibited both frequency modulation (FM) as well as modulation of phrenic burst pattern, which consisted of predominantly augmented and/or fractionated phrenic bursts and bursts exhibiting an abrupt onset. Thus based on the above observations, it seems that, although not essential for the focal hypoxia-induced pre-Bo¨tC–mediated excitatory response, activation of ionotropic EAA receptors in the pre-Bo¨tC may play a modulatory role in the expression of the focal pre-Bo¨tC hypoxia-induced response.

I also showed that unilateral blockade of ionotropic EAA receptors in this region reduces both the amplitude and frequency of basal phrenic nerve discharge. Although previous in vivo studies in anesthetized cat have shown that blockade of ionotropic EAA receptors in the region of the rostral ventral respiratory group (rVRG)/pre-Bo¨tC (Abrahams et al. 1991) or pre-Bo¨tC alone (Solomon 2004) produces a progressive reduction in respiratory output leading to apnea, these previous studies investigated the effects of bilateral, not unilateral, blockade of ionotropic EAA receptors. I believe that the microinjections of KYN were effective in producing complete blockade because they were effective in eliminating the excitation of phrenic nerve discharge elicited by microinjection of the ionotropic glutamate agonist DLH into this region. Furthermore, I believe that the microinjections of KYN were sufficient to fully block ionotropic EAA receptors in the unilateral pre-Bo¨tC since the injection volumes were fairly large (i.e., $40$ nl) and similar microinjections bilaterally (as done in a previous study, Solomon 2004) is sufficient to abolish basal phrenic nerve discharge. Thus this observation suggests that unilateral activation of ionotropic EAA receptors in this region, mediated by endogenous release of glutamate, is sufficient to generate phrenic nerve discharge under hypoxic, normocapnic conditions, albeit with a modified rate and pattern of inspiratory motor activity.

Hypoxic sensitivity of the pre-Bo¨tC and respiratory excitation

Previous studies, including work from our laboratory, have suggested that the pre-Bo¨tC exhibits intrinsic hypoxic chemosensitivity (Peña et al. 2004; Ramírez et al. 1998a; Rybak et al. 2003; Solomon 2000, 2002b; Solomon et al. 2000; Telgkamp and Ramirez 1999; Thoby-Brisson and Ramirez 2000). These studies have shown an increase in inspiratory-related discharges in response to hypoxic/anoxic exposure in vitro (Ramírez et al. 1998a; Telgkamp and Ramirez 1999; Thoby-Brisson and Ramirez 2000), global NaCN exposure in vitro (Rybak et al. 2003), and focal pre-Bo¨tC NaCN in vivo (Solomon 2002b; Solomon et al. 2000; this study). The current
observations confirm and extend our previous findings by showing that focal pre-Bo\"etzinger hypoxia (induced by NaCN) elicits excitation of phrenic nerve discharge during blockade of ionotropic EAA receptors in the pre-Bo\"etzinger complex and thus provides additional support for intrinsic hypoxic chemosensitivity of this region. Since my experiments used unilateral, not bilateral, focal pre-Bo\"etzinger perturbations, I cannot exclude a potential contribution from activation of ionotropic EAA receptors in the contralateral pre-Bo\"etzinger complex in the hypoxia-induced respiratory excitation; however, I am confident that the current observations represent the direct excitatory effects of focal pre-Bo\"etzinger hypoxia (i.e., hypoxic stimulation) and not those of hypoxia-induced glutamate release acting on ionotropic EAA receptors in this unilateral region since the focal NaCN-induced excitatory response persisted following microinjection of KYN. These hypoxia-excited pre-Bo\"etzinger neurons, however, may project to the contralateral pre-Bo\"etzinger complex, resulting in glutamate release and subsequent activation of ionotropic EAA receptors in the contralateral pre-Bo\"etzinger complex.

Although this study and our previous studies provide no insight into the specific population(s) of respiratory-modulated pre-Bo\"etzinger neurons that are hypoxia chemosensitive, results from the in vitro transverse medullary slice preparation have shown that a subset of pre-Bo\"etzinger pacemaker neurons, which includes the Cd-insensitive pacemaker cells (Peña et al. 2004), exhibit continuous rhythmic bursting during anoxia (Peña et al. 2004; Thoby-Brisson and Ramírez 2000). It remains to be determined whether a similar population of pacemaker cells are present and play a similar role in hypoxia sensing the adult cat in vivo pre-Bo\"etzinger complex. These findings, however, suggest that the basic rhythm generating circuitry located in the pre-Bo\"etzinger complex (Peña et al. 2004; Rekling and Feldman 1998; Smith et al. 1991, 2000) is responsive to focal hypoxia and that focal pre-Bo\"etzinger hypoxia produces respiratory excitation that is independent of hypoxia-induced glutamate release acting on ionotropic EAA receptors in this region. Furthermore, since focal hypoxia in this region during blockade of ionotropic EAA receptors produces not only FM of phrenic nerve discharge but also modulation of phrenic burst pattern, these findings suggest that hypoxia-induced activation of the presumptive rhythm generating pre-Bo\"etzinger neurons and/or another subset of hypoxia-sensitive pre-Bo\"etzinger neurons participates in shaping the pattern of inspiratory motor discharge.

Although the patterning and timing characteristics of the NaCN-induced high-amplitude short-duration bursts were similar both before and after blockade, all of the NaCN-induced responses during blockade included the production of augmented and/or fractionated phasic phrenic bursts. These discharge patterns are commonly elicited in response to severe brain hypoxia and/or reoxygenation from severe hypoxia (Lieske et al. 2000; Melton et al. 1996; Richardson 1986; St. John 1990, 1996); in addition, augmented bursts have been observed in response to focal pre-Bo\"etzinger hypoxia in the absence of blockade in a subset of pre-Bo\"etzinger sites (Solomon et al. 2000). Furthermore, the NaCN-induced tonic excitation of phrenic nerve discharge that was observed before blockade was converted to or modified to include phasic phrenic discharge during blockade, albeit with an increase in burst frequency and a modified burst pattern. This observation may be interpreted to suggest that NaCN-induced tonic excitation of phrenic nerve discharge is elicited, at least in part, by glutamate release in response to focal pre-Bo\"etzinger hypoxia. It remains to be determined, however, whether this NaCN-induced phasic excitation is present, but masked by the tonic discharge, under baseline conditions.

In summary, the results indicate that activation of ionotropic EAA receptors in the pre-Bo\"etzinger complex is not essential for the focal pre-Bo\"etzinger hypoxia-induced respiratory excitation, although the basal rate and pattern of inspiratory motor output seem to be dependent on such activation. Although the focal hypoxia sensing function of the in vivo pre-Bo\"etzinger complex is not mediated by activation of ionotropic EAA receptors in this region, ionotropic EAA receptor activation seems to play a modulatory role in the expression of the focal hypoxia-induced response by eliciting further modulation of frequency and patterning of NaCN-induced phrenic bursts. Thus these observations provide further support for intrinsic hypoxic chemosensitivity of the in vivo pre-Bo\"etzinger complex.

ACKNOWLEDGMENTS

The author thanks T. J. Halat for excellent technical assistance.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-63175.

REFERENCES

Abrahams TP, Hornby PJ, Walton DP, Taveira DaSilva AM, and Gillis RA. An excitatory amino acid(s) in the ventrolateral medulla is (are) required for breathing to occur in the anesthetized cat. J Pharmacol Exp Ther 259: 1388–1395, 1991.


