Entrainment pattern between sympathetic and phrenic nerve activities in the Sprague-Dawley rat: hypoxia-evoked sympathetic activity during expiration

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Dick, Thomas E., Y.-H. Hsieh, Shaun Morrison, Sharon K. Coles, and Nanduri Prabhakar. Entrainment pattern between sympathetic and phrenic nerve activities in the Sprague-Dawley rat: hypoxia-evoked sympathetic activity during expiration. Am J Physiol Regul Integr Comp Physiol 286: R1121–R1128, 2004.—Sympathetic and respiratory motor activities are entrained centrally. We hypothesize that this coupling may partially underlie changes in sympathetic activity evoked by hypoxia due to activity-dependent changes in the respiratory pattern. Specifically, we tested the hypothesis that sympathetic nerve activity (SNA) expresses a short-term potentiation in activity after hypoxia similar to that expressed in phrenic nerve activity (PNA). Adult male, Sprague-Dawley (Zivic Miller) rats (n = 19) were anesthetized (Equithesin), vagotomized, paralyzed, ventilated, and pneumothoracotomized. We recorded PNA and splanchnic SNA (sSNA) and generated cycle-triggered averages (CTAs) of rectified and integrated sSNA before, during, and after exposures to hypoxia (8% O2 and 92% N2 for 45 s). Inspiration (I) and expiration (E) were divided in half, and the average and area of integrated sSNA were calculated and compared at the following time points: before hypoxia, at the peak breathing frequency during hypoxia, immediately before the end of hypoxia, immediately after hypoxia, and 60 s after hypoxia. In our animal model, sSNA bursts consistently followed the I-E phase transition. With hypoxia, sSNA increased in both halves of E, but preferentially in the second rather than the first half of E, and decreased in I. After hypoxia, sSNA decreased abruptly, but the coefficient of variation in respiratory modulation of sSNA was significantly less than that at baseline. The hypoxie-evoked changes in sympathetic activity and respiratory pattern resulted in sSNA in the first half of E being correlated negatively to that in the second half of E (r = −0.65, P < 0.05) and positively to Te (r = 0.40, P < 0.05). Short-term potentiation in sSNA appeared not as an increase in the magnitude of activity but as an increased consistency of its respiratory modulation. By 60 s after hypoxia, the variability in the entrainment pattern had returned to baseline. The preferential recruitment of late expiratory sSNA during hypoxia results from either activation by expiratory-modulated neurons or by non-modulated neurons whose excitatory drive is not gated during late E.

hypoxic ventilatory response; chemoreflex; neural control of sympathetic nerve activity

SYMPATHETIC NERVE ACTIVITY (SNA) can be entrained with respiration, but coupling patterns vary (1, 2, 12, 26, 45). The pattern of entrainment depends on sympathetic peripheral nerve, species, and strain of animal and may be altered with respiratory mechano- and chemoinputs (5, 9). The probability of entrainment increases with increased respiratory drive (26, 45). In humans, mild hypoxia for 20 min evoked SNA both during and after the exposure (31, 47). These data indicate that the hypoxic sympathetic response may have time-dependent properties, including a form of short-term potentiation. Time-dependent properties have been described for the hypoxic ventilatory response (39). Although the excitatory hypoxic sympathetic response has been described, its time-dependent properties and their relationship to those of the hypoxic ventilatory response have not been analyzed. We examined the dynamic properties of the entrainment pattern of splanchnic SNA (sSNA) in the Sprague-Dawley adult rat before, during, and after brief hypoxia.

Both peripheral and central mechanisms determine the entrainment between SNA and phrenic nerve activity (PNA). Sensory inputs related to lung volume modulate the entrainment pattern. In this study, we were interested in determining central mechanisms underlying entrainment. Consequently, the animal preparation was vagotomized to minimize pulmonary stretch-receptor inputs, paralyzed to minimize proprioceptive inputs, and thoracotomized to eliminate ventilatory intrathoracic pressure changes and mecano-coupling between lung and chest wall. Animals were ventilated with 100% O2 to minimize peripheral chemoreceptor input before exposure to hypoxic gas. These surgical procedures reduced relevant sensory feedback to the following afferent inputs: the sympathetic afferents, the carotid baro- and chemoreceptor inputs, and inputs from cranial receptors, such as central chemoreceptors and cerebral pressure receptors (Cushing’s reflex).

Central mechanisms underlying respiratory entrainment of sympathetic outflow may include modulation of baroreceptor sensory input in the nucleus of the solitary tract (NTS) (16, 29), as well as coupled or common oscillatory output (1, 40, 48, 49). Our experimental model was designed to isolate propriothalamic and bulbospinal respiratory and sympathetic interactions that occur within brainstem networks of pattern generators and sympathetic premotor neurons. Anatomic studies have identified a potential interaction between sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) and respiratory neurons in the ventral respiratory column (35, 36). Similarly electrophysiological studies have recorded expiratory-modulated activity in bulbospinal RVLM neurons and in A5 neurons in the ventrolateral pons (14, 15, 33). These

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activities occurred in animals in which rhythmic lung afferent input had been reduced, suggesting that respiratory modulation of SNA does not depend on respiratory-modulated sensory inputs.

Brief hypoxic exposures elicit a stereotyped respiratory response that varies over the time course of the exposure (39): respiratory motor activity increases asymptotically to a maximum, whereas respiratory frequency (Fr) increases transiently, rapidly reaching a peak Fr, then progressively decreasing. After hypoxia, the pattern reverses, respiratory motor activity decreases progressively to baseline, and Fr decreases below baseline and then gradually increases to baseline. The hypoxic chemoreflex response in SNA appears to follow the monophasic pattern of inspiratory motor activity, e.g., SNA increases progressively during, and decreases immediately after, hypoxia (22, 23, 27). However, changes in entrainment pattern between SNA and PNA have yet to be analyzed quantitatively. Because sSNA entrainment to respiration increases during hypoxia and because SNA’s response pattern parallels inspiratory motor activity, we hypothesize that SNA may express a short-term, activity-dependent plasticity in its pattern after hypoxia.

At least two distinct mechanisms underlie the excitatory chemoreflex response of SNA: one is independent of respiration and the other reflects the increased synchronization with respiration (13, 20, 41, 43). Regarding the potential respiratory-independent mechanisms, sympathetic premotor neurons in the RVLM receive direct input from NTS neurons excited by chemoreceptor afferents and may themselves be intrinsically sensitive to hypoxia, resulting in a tonic increase in SNA in response to hypoxemia (17, 20). Regarding the potential respiratory-dependent mechanism, common cardiorespiratory control nuclei are activated by hypoxia (17). Neurons in the dorsolateral pons, specifically Kölliker-Fuse (KF) nucleus, are the only brain stem neurons other than those in the NTS that are activated by hypoxia and project to the RVLM (17). The dorsolateral pons also modulates the respiratory response during hypoxia and the short-term plasticity after hypoxia (37, 38). Thus a direct pontomedullary interaction between the KF nucleus and RVLM may contribute to the respiratory modulation of sympathetic activity and could mediate an enhanced sympathetic activity during and after hypoxia.

METHODS

General procedures. The methods used for this study were similar to those published previously (6). Male, adult rats (Sprague-Dawley/Zivic Miller, n = 19, 320–420 g) were anesthetized with Equithesin (30 and 133 mg/kg pentobarbital sodium and chloral hydrate, respectively). We tested the anesthetic level by evaluating the withdrawal response and 133 mg/kg pentobarbital sodium and chloral hydrate, respectively). Thus a direct pontomedullary interaction between the KF nucleus, are the only brain stem neurons other than those in the NTS that are activated by hypoxia and project to the RVLM (17). The dorsolateral pons also modulates the respiratory response during hypoxia and the short-term plasticity after hypoxia (37, 38). Thus a direct pontomedullary interaction between the KF nucleus and RVLM may contribute to the respiratory modulation of sympathetic activity and could mediate an enhanced sympathetic activity during and after hypoxia.

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and vagotomized Sprague-Dawley rats. Bursts of sSNA occurred consistently after the I-E phase transition marked by the dashed line but inconsistently with the E-I phase transition (Fig. 1B). For example (Fig. 1B), bursts of sSNA occurred at the E-I phase transition in the top three respiratory cycles but not in the subsequent cycles. Figure 1C shows the superimposition of 1) the CTA of integrated sSNA (solid line), 2) the standard deviation of the sSNA data in the cycles used to compute the CTA (long-short dashed lines), and 3) the CTA of PNA (dotted line). The reference event (dashed vertical line) for the respiratory cycle was the steep negative slope of the integrated PNA, i.e., the I-E phase transition (Fig. 1).

Although an increase in sSNA after the I-E phase transition was common among preparations, changes in activity were also associated with other phases of the cycle in subgroups of animals (Fig. 2). Respiratory-modulated sSNA could increase near the E-I transition (Fig. 2, C, E, and G) or decrease during I (Fig. 2, B, D, and F). These differences in respiratory modulation were associated with a low CoV and, thus, were consistent within these animals (Fig. 2). In contrast, apparent changes in activity could be inconsistent and associated with high CoV [Fig. 2, A (same animal as Fig. 1) and H]. Among animals, sSNA and CoV of sSNA varied across the respiratory cycle.

For the group of animals, when comparing sSNA and its CoV in each half phase (Fig. 3A), the average amplitude of sSNA in early E was significantly greater than in the other phases and the average CoV for sSNA was significantly greater in late E than in late I (Fig. 3B). In summary, under baseline conditions, the most prominent and a consistent feature in CTAs of sSNA was an increase in activity associated with the I-E phase transition (Figs. 1–3).

Hypoxia-evoked sSNA in late E. Sympathetic activity increased significantly during hypoxia (Figs. 4–6). The evoked
increase in sSNA was associated specifically with E such that average sSNA amplitudes in both early and late E were significantly greater during hypoxia than during baseline, PHFD, and recovery (Fig. 6A). In contrast, I-modulated activity did not change significantly during hypoxia (Fig. 6A), although it appeared to decrease during hypoxia (Figs. 4 and 5).

The evoked sSNA was differentially distributed within the respiratory cycle (Fig. 6). Before hypoxia, sSNA was greater in the early vs. late E. This was reversed during hypoxia: sSNA was less in early vs. late E and this difference was significant by the end of the exposure (Fig. 6A). Thus sSNA was recruited preferentially in late rather than early E (Fig. 6A). With the prolongation of TE at the end of the brief hypoxic exposure, sympathetic activity could appear as a separate burst in late E (Fig. 5, filled arrow). Before hypoxia sSNA was not significantly different in early and late I; by the end of hypoxia sympathetic activity levels in late I were significantly less than those in early I (Figs. 5 and 6A) and the CoV for late I sSNA was significantly less than those of the other half phases (Fig. 6B).

Less variable sSNA after hypoxia. Both Fr and sSNA changed abruptly on reoxygenation to end the hypoxic exposure. Immediately after hypoxia and TE lengthened, both early and late E sSNA decreased (Figs. 4 and 5) and were not significantly different from baseline E sSNA (Fig. 6A). However, late I sSNA remained less than that in early I (Fig. 6A). Furthermore, the CoV for sSNA was significantly less during PHFD than that during baseline. As recovery progressed, TE shortened and early E sSNA became greater than late E activity. The distribution of respiratory-modulated sSNA, including its CoV, returned to baseline during this period.

Correlations between sSNA within I and E. We speculated that the relationship between sSNA in early and late E would be different from that between early and late I, due to hypoxia preferentially recruiting sSNA during late E. The percent of sSNA in early E was negatively correlated to that in late E ($r = -0.65, P < 0.05$; Fig. 7A), whereas the percents of sSNA in early and late I were positively correlated ($r = 0.33, P < 0.05$; Fig. 7B). Similarly, the percent of sSNA in early E was positively correlated to TE ($r = 0.40, P < 0.05$; not shown) but that in late E was not correlated to TE ($r = -0.15, P < 0.2$).

**DISCUSSION**

Although the resting coupling pattern between respiratory and vasoconstrictor sympathetic motor activities varied, we found that that sSNA consistently increased during the early E
or post-I phase of respiration. This result is consistent with previous studies of the respiratory modulation of rat splanchnic SNA (20, 21, 30, 32). During acute hypoxic exposures, sSNA not only increased during E but also altered its pattern of entrainment to respiration. During hypoxia, respiratory modulation was enhanced primarily because sSNA decreased during late I and increased during late E. We interpret these changes as evidence for multiple respiratory-modulated influences affecting SNA. Before, during, and after hypoxia, sSNA increased during early E, indicating an excitatory input from postinspiratory neurons. During hypoxia, sSNA decreased to its lowest levels during the second half of I such that even tonic activity was blocked indicating a late-I modulated inhibitory input to spinal preganglionic or brain stem “premotor” neurons. In contrast, sSNA increased preferentially during late E, which could indicate an excitatory input during stage II E. This interpretation is based solely on temporally associated changes in sSNA, and another interpretation is that tonic activation of SNA during hypoxia is being gated during I and expressed during late E.

Short-term potentiation in sSNA after a single exposure to hypoxia is expressed as an increase in the consistency of respiratory modulation rather than an increase in average sSNA. The $\text{CoV}$ for sSNA immediately after hypoxia was significantly less than that before hypoxia, which we interpret as reflecting the increased modulation due to the activity-dependent plasticity of the respiratory system. Over the last decade, we and others have identified the lateral pons as playing a critical role in mediating this property of the respiratory pattern generator (7, 37, 38). Lateral pontine neurons that are excited by hypoxia also project to the RVLM (17) and may provide a neurologic substrate for this expression of short-term potentiation in SNA.

Critique of experimental model. Different respiratory-modulated patterns occur in SNA varying with peripheral nerve, sensory feedback, species, and strain (1, 2, 5, 8–10, 14, 15, 18, 26, 42). In this study, sSNA was recorded in anesthetized, vagotomized, paralyzed, thoracotomized, and ventilated Sprague-Dawley rats. We chose to study the sSNA because it regulates visceral vasoconstriction; reflects sympathetic premotor drive from the RVLM; has a strong chemosensory, excitatory response; and exhibits a prominent respiratory modulation (27, 32). Many of the animal’s sensory inputs were transected or attenuated to reduce peripheral feedback influences and maximize the opportunity to observe central interactions. Rhythmic information related to lung inflation was reduced after vagotomy, muscle paralysis, and chest wall expansion. Additionally, peripheral chemoreceptor input was minimized by ventilating the animals with 100% $\text{O}_2$ before and after the brief hypoxic challenge. The Sprague-Dawley rat has a robust response to hypoxia (42), and its hypoxic ventilatory response has defined time domains that include posthypoxic short-term pattern changes (39). In summary, this experimental model displays robust ventilatory and sympathetic hypoxic responses as well as activity-dependent plasticity in the respiratory pattern.

We applied a quantitative analysis of the CTA to identify hypoxia-induced changes in the distribution of sSNA throughout the respiratory cycle. Numerous investigators have used CTA to increase the signal-to-noise ratio of the respiratory modulation of sSNA (21, 30, 32). The novelities of our analysis were an analysis of the breath-by-breath variability of sSNA and a comparison of half phases of the respiratory cycle to assess shifts in the occurrence of respiratory-modulated bursts in SNA. The latter allowed us to distinguish activity changes associated with post-I vs. stage II E. The former permitted an assessment of the consistency of the modulation pattern across the sampled cycles. Because the CTA represents the average activity pattern, it is frequently assumed that this pattern occurs from cycle to cycle. Consistency of the pattern represented by the CTA is not usually measured. In a previous analysis based on CTAs, the assumption of consistency led to the development of a faulty statistic (the respiratory modulation index; see Ref. 34).

We recorded activity from sympathetic nerve bundles in this study. Consequently, changes in the entrainment pattern could reflect changes in the coupling patterns of activated neurons or...
the activation of previously quiescent populations of neurons that receive independent inputs—possibilities that could not be distinguished in our experimental model. Similarly, we could not determine whether the hypoxia-induced shift in the coupling pattern between sSNA and PNA arises from a shift in existing sympathetic premotor drive or from the recruitment of a new input.

Finally, the carotid sinus baroreceptors remained intact in these preparations and could modulate the sSNA patterns of activity. However, the baroreceptor feedback loop does not affect the recruitment pattern of sSNA during hypoxia (20) and this afferent input is gated by lateral pontine activity (16, 29). Furthermore, we observed similar changes in the entrainment patterns during and after hypoxia against a background of various blood pressures (compare Figs. 4 and 5). However, we expect that the baroreceptor feedback loop contributes to the variability in the respiratory modulation of sSNA especially during baseline and recovery periods when afferent activity is not being gated.

Multiple components of respiratory modulation in SNA. The results of our study suggest that different types of respiratory-modulated neurons affect sSNA during hypoxia. Czyzyk-Krzyska and Trzebski (9) described changes in the entrainment pattern between sSNA and PNA evoked by exposing spontaneously hypertensive Wistar rats to hypoxia. In the normotensive Wistar-Kyoto rats, SNA decreased in I and increased in early E. Exposing these animals to hypoxia increased SNA but did not alter the entrainment pattern. On the other hand, in spontaneously hypertensive rats, SNA increased in I and decreased in early E during normoxia, but during hypoxia, SNA decreased in I and increased in E. The maximal sympathetic discharge shifted from I to early E. Consequently, during hypoxia both normotensive and hypertensive animals had similar coupling patterns between respiration and SNA.

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Using an experimental model similar to the one in this study, Koshiya and Guyenet (21) assessed the effect of the antihypertensive drug clonidine on sSNA. Before injecting clonidine, sSNA was weakly modulated by respiration and activity appeared in I, postinspiration, and stage II E. Clonidine injection (200–250 µg/kg iv) caused a sustained hypertension (>10 min, 173 ± 3 mmHg) and silenced sSNA via baroreceptor activation. Subsequent injection of nitroprusside decreased blood pressure and allowed sSNA to return. In six of seven animals, this disinhibited sSNA had a single inspiratory peak. The authors noted that the stage II expiratory activity was absent and postinspiratory activity was attenuated (21).

These studies support our working hypothesis that the networks controlling SNA can be modulated by neurons involved in generating different phases of the respiratory cycle and that respiratory-modulated inputs can be differentially activated by hypoxia. Our correlative data support differential inputs affecting sSNA E activity, although these experiments did not allow us to distinguish between respiratory and nonrespiratory influences in the recruitment of late E activity. Furthermore, the studies of Koshiya and Guyenet (21) indicate that a late E component exists and may be sensitive to clonidine. In this regard, neuronal activity in the A5 region of the ventrolateral pons is inhibited by clonidine (18) and is recruited during and after hypoxia (11). We speculate that in Sprague-Dawley animals, the selective increase in stage II expiratory sSNA during hypoxia reflects the recruitment of expiratory-modulated activity in the ventrolateral pons.

Sources of respiratory modulation of SNA. Preganglionic sympathetic neurons have their cell bodies in the intermediolateral cell column and receive bulbospinal, sympathoexcitatory, respiratory-modulated inputs from RVLM, A5, and raphe (3, 14, 15). Thus respiratory modulation of the activity on postganglionic sympathetic vasoconstrictor nerves could result from the respiratory-related discharge of inputs antecedent to sympathetic premotor neurons.

During hypoxia, RVLM and A5 neurons become activated and increase their respiratory modulation, primarily seen as an E-decrementing pattern after the IE phase-transition (14). Subsequent studies showed that lesions in the RVLM blocked the hypoxic sympathetic response, whereas lesions in the ventrolateral pons attenuated the sympathetic but not the respiratory response to hypoxia (22, 23). Recordings in this laboratory have found primarily expiratory augmenting rather than decrementing activity in the ventrolateral pons (11). When activity in the ventrolateral pons was blocked, the time domains after, rather than during, the hypoxic ventilatory response were blocked, a result similar to that seen by Koshiya and Guyenet (23). Stimulating the ventrolateral pons prolongs E and activates SNA (19). Neuronal discharge in the ventrolateral pons exhibits a biphasic response to hypoxia: an initial decrease followed by an increase that persists after the hypoxic exposure (11). Thus neurons in the ventrolateral pons appear to influence the time domains of the hypoxic ventilatory response and may control those of the sympathetic response as well.

The regression analysis is consistent with differential control of expiratory sSNA during hypoxia. The percent of sSNA in early E decreased as the percent in late E increased, whereas early and late I activity was weakly but positively correlated. A partial explanation for the negative correlation of E activity is that as activity increased in late E, less of the total respiratory-modulated activity occurred in early E. However, hypoxia preferentially recruited activity in late E because by the end of the hypoxic exposure late E SNA was significantly greater than that in early E. This is consistent with early E activity being positively rather than negatively correlated to Te, although early E activity increased when Te shortened during hypoxia. The percent of early E activity was greatest in the absence of late E activity. Late E activity was the lowest during baseline and recovery when Te was greater than that during hypoxia. The positive correlation between early and late I SNA is consistent with common mechanisms determining the magnitude of this activity.

Time domains of the hypoxic sympathetic response. At least two, one excitatory and the other inhibitory, respiratory-modulated processes appeared to influence the sympathetic response to hypoxia in the Sprague-Dawley rat: sSNA was excited during E and inhibited during I. These influences progressively increased during hypoxia, along with inspiratory motor activity. This time-dependent pattern was consistent. After hypoxia, short-term potentiation of sSNA was associated with a transient decrease in the variability of the respiratory-modulated activity as indicated by the significant difference between baseline and PHFD CV for sSNA. Short-term potentiation may have occurred in early I sSNA in that average amplitude of early I SNA was significantly greater than late I SNA during PHFD but not at baseline. However, early I sSNA during PHFD was not significantly different from that at baseline so the statistical difference in I activity at PHFD resulted from a combination of insignificant changes in early and late I activity.

The role of baroreceptor feedback in determining the pattern of respiratory-modulated SNA was not formally tested in barodenervated animals. However, the recruitment of SNA in late E was observed consistently and despite various blood pressure responses. After hypoxia, the observed increase in the consistency of respiratory modulation did not supercede baroreceptor reflex inhibition of SNA; however, respiratory modulation of the SNA pattern was enhanced during PHFD at various blood pressure levels relative to baseline. Both blood pressure and the entrainment pattern of SNA returned to baseline by 60 s; this is longer than the time constant of short-term potentiation of prolongation of Te (~45 s, Ref. 7).

In summary, in response to brief exposures to hypoxia, SNA and blood pressure increase. The increase in SNA is modulated with the respiratory pattern and associated with the second half of E. After hypoxia, short-term potentiation that is expressed in respiration was evident in sympathetic activity in that SNA was more coupled to respiration during PHFD than during baseline. The enhanced respiratory modulation of SNA may have resulted from mechanisms underlying activity-dependent plasticity.

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GRANTS

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