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

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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. First published March 4, 2004; 10.1152/ajpregu.00485.2003.—Sympathetic and respiratory motor activities are entrained centrally. We hypothesize that this coupling may partially undergo 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 breathing; 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 chemoinsputs (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 mechano-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 proprio- and bulbar respiratory and sympathetic interactions that occur within brain stem 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 bulbar 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 SNA 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 reflex and cardiorepiratory response to a paw pinch after neuromuscular blockade. Anesthesia was supplemented as needed by administering one-tenth of the initial dose intravenously. Surgical procedures and experimental protocols followed National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee.

A femoral artery and vein were cannulated to monitor blood pressure and administer pharmacologic agents, respectively, and the trachea was cannulated to ventilate the animal. The cervical vagi were transected bilaterally. The animals were stabilized for neural recordings by positioning them in a stereotaxic frame. The left phrenic as well as the left splanchnic sympathetic nerves were isolated, transected, and mounted on bipolar electrodes for recording. Animals were paralyzed (Pavulon, pancuronium bromide, 0.1 mg·100 g body wt−1·h−1 iv) and ventilated with 100% O2. End-tidal PCO2 was monitored continuously and was between 33 and 38 mmHg. We recorded blood pressure, airflow, end-tidal PCO2, raw and integrated PNA, and sSNA on magnetic tape (Hewlett-Packard) and a chart recorder (Astro-Med Dash 8). Rectal temperature was maintained at 37 ± 0.5°C throughout the experiment by a servocontrolled recirculating water blanket and infrared lamps.

Experimental protocol. All animals were tested with a hypoxic gas mixture. The hypoxic gas was 8% O2 in 92% N2 and animals were ventilated with 100% O2 before and after the exposure. Brief hypoxic exposures usually lasted between 30 and 45 s (range: 20–60 s). The duration of the hypoxic exposure depended on the sensitivity of each animal to hypoxia, but the source(s) of variability in hypoxic sensitivity was (were) not identified. The exposures were poikilocepamic and end-tidal PCO2 decreased <2 mmHg.

We tested our hypothesis by comparing measurements of the coupling pattern between PNA and sSNA before (baseline) the hypoxic exposure with those made at several points during and after the hypoxic exposure. During hypoxia, we used the period of peak Fr and the end of the hypoxic exposure. After hypoxia, we used the nadir of Fr (PHFD, posthypoxic Fr decline) and 60 s after PHFD (recovery).

Data analysis. Cycle-triggered averages (CTAs) were constructed for PNA and sSNA to increase the signal-to-noise ratio of sSNA that was time locked to the respiratory cycle (Fig. 1). The reference point or time zero for the CTAs was the phase transition between inspiration (I) and expiration (E), which was identified as the steepest negative slope in the integrated PNA signal (vertical dashed line and arrows, Fig. 1; and Y, Fig. 3). Generally, we sampled the electronically integrated signal at 200 Hz (CWE, Paynter Filter, 50-ms time constant) and averaged points from 600 to 800 ms before the trigger, which was ~200 ms before the onset of I to 100–200 ms after the end of E. Onset of PNA was identified as 10% above the baseline value for PNA on the positive slope of integrated PNA signal (X, Fig. 3). The onset of the next breath was used as the end of the cycle (Z, Fig. 3).

We averaged PNA and sSNA for 10 cycles immediately before the hypoxic exposure to determine the pattern of “baseline” synchronization of sSNA and PNA. To assess changes in the pattern of entrainment during hypoxia, we averaged 3–7 cycles at peak Fr and at the end of the hypoxic exposure. The number of cycles that was chosen depended on the variability of the respiratory pattern during hypoxia. We chose at least three cycles but as many as possible that had a similar duration. To assess changes in entrainment after brief hypoxia, we averaged 10 cycles between 10 and 30 s and between 60 and 90 s after hypoxia (PHFD and recovery, respectively).

We analyzed the CTAs for the distribution pattern of respiratory-modulated sSNA. First, we superimposed the CTA for sSNA on that for PNA (solid line and thin dashed line, respectively, Figs. 1–4). We also plotted the standard deviation (long-short thick dashed line, Figs. 1 and 4) or the coefficient of variation (CoV; thick dashed line; Fig. 2) for the sSNA CTA. The DC offset on the signal was subtracted from the CTAs (arrow, Fig. 3) before calculating the average amplitude and area under the curve. We determined the DC offset from the level of signal in the absence of sSNA either after killing the animal or by immersing the nerve and electrodes in saline. The DC offset values were similar to the lowest value of sSNA recorded during the hypoxic response; consequently we used this value in our calculations. We divided sSNA CTAs into inspiratory and expiratory portions on the basis of PNA. To compare changes in sSNA activity in each phase, we divided the inspiratory and expiratory portions of the corrected CTAs in half (opposite hatched areas, Fig. 3).

We tested for significant differences between sSNA CTAs and the CoV for sSNA CTAs in the first and second halves of each phase. We applied a two-way ANOVA for repeated measures to determine significance of differences, and, if significant, we used Student-Newman-Keuls post hoc test to identify specific differences. We also correlated sSNA in the first to that in the second half of each phase.

RESULTS

As illustrated in a representative record (Fig. 1), sSNA was modulated with the respiratory cycle in anesthetized, paralyzed
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. Vertical dashed lines indicate the transition between inspiration (I) and expiration (E). B: with each respiratory cycle, sSNA bursts occurred consistently after the I-E phase transition (dashed line) but inconsistently with the E-I phase transition (note: burst of sSNA in the first 3 cycles but not in the subsequent cycles). Traces: rectified and integrated (50 ms time constant) sSNA (solid line) superimposed on integrated PNA (thin dashed line). Phrenic-triggered CTA of sSNA is formed by averaging integrated sSNA aligning the activity during each respiratory cycle by the I-E phase transition. C: CTA ± SD of sSNA (n = 10 cycles). Superimposed traces: averages of integrated PNA (thin dashed line) and integrated sSNA (thick solid line), as well as ± SD of sSNA CTA (thin long-short dashed lines). Abscissa is time in seconds and the coordinate is arbitrary units (a.u.).

Hypoxia-evoked sSNA in late E. Sympathetic activity increased significantly during hypoxia (Figs. 4–6). The evoked

Fig. 1. Analysis of splanchnic sympathetic nerve activity (sSNA); formation of cycle-triggered averages (CTAs). A: respiratory-modulated sSNA in the raw records. Traces from the top: raw sSNA, rectified and integrated (50 ms time constant) sSNA, raw phrenic nerve activity (PNA), rectified and integrated (50 ms time constant) PNA, partial pressure of carbon dioxide in the endotracheal tube (PETCO₂), air flow (pressure difference across a pneumotachograph), blood pressure (BP), and time bar (5 s). Vertical dashed lines indicate the transition between inspiration (I) and expiration (E). B: with each respiratory cycle, sSNA bursts occurred consistently after the I-E phase transition (dashed line) but inconsistently with the E-I phase transition (note: burst of sSNA in the first 3 cycles but not in the subsequent cycles). Traces: rectified and integrated (50 ms time constant) sSNA (solid line) superimposed on integrated PNA (thin dashed line). Phrenic-triggered CTA of sSNA is formed by averaging integrated sSNA aligning the activity during each respiratory cycle by the I-E phase transition. C: CTA ± SD of sSNA (n = 10 cycles). Superimposed traces: averages of integrated PNA (thin dashed line) and integrated sSNA (thick solid line), as well as ± SD of sSNA CTA (thin long-short dashed lines). Abscissa is time in seconds and the coordinate is arbitrary units (a.u.).

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).

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
Hypoxia increases sSNA during expiration.

Fig. 3. Analysis of the CTAs. A: traces: superimposed CTAs. X, onset of I; Y, end of I and onset of E; and Z, end of E (hatched areas: thin, 50% of I; thick, 50% of E; positive slope, 1st 50% of phase; negative slope, 2nd 50%). Downward arrow: DC offset as amplitude of the sSNA recording in dead animal or immersed in saline. In this example recording, three aspects of sSNA are apparent: 1) decrease in I, 2) increase after I-E phase transition, and 3) tonic activity. B: baseline respiratory modulatory pattern for all animals. Average amplitude of sSNA (arbitrary units) and its CoV (%) for each half phase. Activity in the first half of expiration was significantly greater than those in the other half phases (†) and the CoV of SNA amplitude was significantly lower in the second half inspiration than that in the second half of expiration (‡).

Fig. 4. PNA and sSNA before, during, and after brief hypoxic exposure during which respiratory frequency and blood pressure increase. During hypoxia, respiratory frequency (Fr), PNA, and sSNA increased after a brief latency (6–10 s gas “wash-in” period). Increased sSNA was associated with E, sSNA was prevalent in late E as E prolonged during and after hypoxia. This pattern of sSNA recruitment occurred in the face of increased BP. A: traces: raw PNA; raw sSNA; event marker (open, 100% O₂; solid, 8% O₂ and 92% N₂); airflow (AF); arterial blood pressure (BP); and time bar, (10 s). Circles: 1) baseline; 2) peak FR; 3) end of hypoxic exposure (45 s); 4) immediately after hypoxia (posthypoxic FR decline, PHFD). B: CTAs of integrated PNA and sSNA. 1) Before hypoxia, sSNA had a tendency to burst immediately after I; 2) at peak FR, sSNA bursts occurred in early I and throughout E; 3) at the end of the hypoxic exposure, sSNA had decreased in I and increased in the E, in particular the activity at the end of E had increased to that at the beginning of E; and 4) the late E burst persisted after hypoxia.

Discussion

Although the resting coupling pattern between respiratory and vasconstrictor 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 CoV for sSNA immediately after hypoxia was significantly less than that before hypoxia, which we interpret as reflecting the increased modulation due to 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% O2 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 novelties 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 sSNA. 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.
Using an experimental model similar to the one in this study, Koshiya and Guyenet (21) assessed the effect of the anti-hypertensive 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 biphase 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 sSNA 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 sSNA 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 sSNA was significantly greater than late I sSNA 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 sSNA was not formally tested in barodenerverated animals. However, the recruitment of sSNA 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 sSNA; however, respiratory modulation of the sSNA 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, sSNA and blood pressure increase. The increase in sSNA 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 sSNA 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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