Basis for synchronization of sympathetic and phrenic nerve discharges

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BARMAN, SUSAN M., AND GERARD L. GEBBER. Basis for synchronization of sympathetic and phrenic nerve discharges. Am. J. Physiol. 231(5): 1601-1607. 1976.—The basis for the relationship between the discharges in the external carotid postganglionic sympathetic and phrenic nerves was studied in anesthetized cats that were vagotomized, paralyzed, and artificially ventilated. It is generally assumed that the slow rhythmic component of sympathetic nerve discharge (with the period of the cycle of phrenic nerve activity) is extrinsically imposed on central sympathetic networks by elements of the brainstem respiratory oscillator. However, a number of observations made in the present study contradict this view. First, changes in respiratory rate were accompanied by dramatic shifts in the phase relations between sympathetic and phrenic nerve discharge. Second, slow oscillations of sympathetic and phrenic nerve discharge were not always locked in a 1:1 relation. Third, the slow sympathetic rhythm persisted during hyperventilation. These results suggest that the slow periodic components of sympathetic and phrenic nerve activity are generated by independent oscillators that normally are entrained to each other.

The naturally occurring discharges of pre- and postganglionic sympathetic nerve bundles exhibit a slow rhythmic component with a period of the respiratory cycle (1, 2, 7, 12, 15, 17, 19). In addition to the sympathoinhibitory effects associated with activation of vagal lung inflation afferents (2, 8, 17), it is generally agreed that some form of direct coupling between the brainstem respiratory oscillator and the central sympathetic networks is responsible for the slow sympathetic rhythm (7, 12, 15, 18). However, the nature of the central interaction remains in question. The present investigation dealt exclusively with this problem.

Contradictory reports exist concerning the pattern of temporal relations between sympathetic and phrenic nerve discharges in vagotomized cats. Cohen and Gootman (7) and Gootman and Cohen (12) described the pattern of respiratory modulation of sympathetic and phrenic nerve discharges. Preiss et al. (18) reported that the pattern of respiratory modulation of the spontaneous discharges of single-pre and postganglionic sympathetic neurons usually was phase locked rather than phase spanning. Unitary discharge most often was augmented during inspiration and reduced during expiration, although three units also were found that fired primarily during expiration. Koizumi et al. (15) also described the pattern of respiratory modulation of SND as phase locked rather than phase spanning.

Variations in the pattern of temporal relations between sympathetic and phrenic nerve discharges make it difficult to accept the notion that "respiratory" periodicity is extrinsically imposed on central sympathetic networks by any one particular component of the respiratory oscillator. The present report entertained the alternative possibility that the slow rhythms within sympathetic and phrenic nerve discharges were generated by independent oscillators which normally are entrained to each other. Consideration of this hypothesis was warranted in view of certain experimental results reported, although not necessarily stressed, by others. First, Figure 11 of Cohen and Gootman's (7) paper is particularly intriguing. As shown by computer summation, hypocapnia eliminated the component of SND time locked to the cycle of phrenic nerve activity. Nevertheless, oscillographic traces of SND during hypocapnia showed slow oscillations that appeared similar in form to those which were locked in a 1:1 relation to the phrenic nerve discharge cycle in the normocapnic state. Thus, a reduction in arterial Pco2 appeared to unlock the phase relations between sympathetic and phrenic nerve discharge rather than to eliminate the slow rhythm within SND. Second, Koechpen and Thurau (14) observed that blood pressure and respiratory rhythms were not always synchronized. Oscillations of blood pressure in the dog most often occurred at the same frequency as the respiration, but sometimes occurred at half or twice the respiratory rate. The results presented in the current study support the view that the slow rhythms within sympathetic and phrenic nerve discharges arise from independent oscillators.

METHODS

Twenty-eight cats weighing between 2.0 and 3.5 kg were anesthetized by the intraperitoneal injection of a
mixture of sodium diallylbarbiturate (60 mg/kg), urethane (240 mg/kg), and monoethylurea (240 mg/kg). Rectal temperature usually was maintained between 36 and 37°C with a heat lamp. Blood pressure was monitored from the lumbar aorta (via a femoral catheter) and displayed on a Grass polygraph. The cervical vagus nerves were cut bilaterally in all experiments to eliminate sympathetic and respiratory reflexes associated with lung inflation.

Each cat was immobilized with gallamine triethiodide (4 mg/kg, iv) and artificially ventilated. Additional doses of gallamine (2 mg/kg) were administered as required to maintain paralysis. Pneumothoracotomy was performed to minimize the blood pressure variations that normally accompany changes in intrathoracic pressure. The respirator pump was adjusted so that the rate and amplitude of phrenic nerve discharge were comparable to that observed in the spontaneously breathing and vagotomized preparation. Arterial Pco2 levels ranged from 33-43 mmHg (Instrumentation Laboratory pH/gas analyzer, model 113) under these conditions.

Nerve recording and data analysis. The procedures used to record the discharges of the external carotid postganglionic sympathetic branch of the superior cervical ganglion and of the phrenic nerve have been described in earlier reports from this laboratory (11, 21). The cat was placed in a David Kopf Instruments stereotaxic apparatus. The right external carotid and phrenic nerves were exposed via a ventral approach in the neck after reflection of the trachea and esophagus. Nerve potentials were recorded monophasically under oil with bipolar platinum electrodes after capacity-coupled preamplification. The preamplifier band pass for the postganglionic nerve was 1-1,000 Hz, and that for the phrenic nerve was 104,000 Hz. Nerve potentials were displayed simultaneously on a Grass polygraph and a dual-beam oscilloscope. The temporal relations between sympathetic and phrenic nerve discharges were analyzed by computer summation. After RC integration (time constant, 0.05 s), nerve potentials were fed to a Nicolet 1070 computer. The sweep of the computer was triggered by a timing pulse derived near the start of inspiration (RC-integrated phrenic nerve discharge). The memory content of the computer was displayed on a second oscilloscope and photographed on 35-mm film.

Brainstem stimulation. The ventral aspect of the pons was exposed after removal of portions of the occipital and basisphenoid bones. The dura mater was opened without damage to the vertebral and basilar arteries. Pulses from a Grass S8 stimulator were applied to selected sites in the pons through bipolar concentric stainless steel electrodes (David Kopf Instruments, model SNE-100). The stimulating electrodes were positioned using the ventral median fissure and ventral surface of the brainstem as landmarks for lateral and dorsoventral orientation. The A-P level was set stereotaxically.

RESULTS

Phase relations between sympathetic and phrenic nerve discharges. Figure 1 illustrates the "respiratory" periodicity in the spontaneous discharges of the external carotid postganglionic sympathetic nerve of the vagotomized cat. As described in earlier reports from this laboratory (9, 10, 20), sympathetic nervous discharge usually was synchronized into bursts that were locked in a 1:1 relation to the cardiac cycle. The cardiac-related bursts of SND occurred almost exclusively during the inspiratory phase of the central respiratory cycle (monitored by RC-integrated phrenic nerve activity) in the example shown in Fig 1.

Computer summation was employed to analyze quantitatively the temporal relations between sympathetic and phrenic nerve discharges. As illustrated in Fig. 2, three distinct patterns were observed. The relationship between sympathetic and phrenic nerve discharges was expiratory-inspiratory (EI) in six cats (Fig. 2, A). The SND began to increase from a minimum in early expiration and reached a maximum near peak inspiration. The EI pattern was the one most commonly observed by Cohen and Gootman (7) and Gootman and Cohen (12). Eight cats exhibited an inspiratory (I) phase-relation pattern (Fig. 2, B). The SND began to increase at the start of inspiration, became maximal near peak inspiration, and then decayed in time with phrenic nerve discharge. The I pattern most closely resembled that reported by Koizumi et al. (15) and Freiss et al. (18). Finally, an
SLOW SYMPATHETIC OSCILLATOR

FIG. 2. Patterns of phase relations between spontaneously occurring phrenic and sympathetic nerve discharges in 3 vagotomized cats. Each panel shows computer-summed records (32 sweeps) of RC-integrated phrenic (top traces) and sympathetic (bottom traces) nerve activity. Address bin was 4 ms. Increased nerve discharge is shown as an upward deflection. Sweep of computer was triggered by a timing pulse derived near beginning of inspiratory phase of phrenic nerve discharge cycle. A: EI pattern. B: I pattern. C: IE pattern. Horizontal calibration is 1 s.

inspiratory-expiratory (IE) pattern was observed in five cats (Fig. 2, C). The SND began to increase after the start of inspiration and reached a maximum in early expiration. The central respiratory rate ($30.9 \pm 3.2$ cycles/min) in vagotomized cats exhibiting the IE pattern was higher than in those animals in which the phase-relation pattern was EI ($22.2 \pm 2.6$ cycles/min) or I ($23.4 \pm 2.3$ cycles/min). These values, however, were not significantly different on a group basis ($P < 0.05$).

The phase relations between the discharges of the external carotid sympathetic and phrenic nerves changed spontaneously during the course of five experiments. One such instance is illustrated in Fig. 3. Respiratory rate decreased from 31 to 24 cycles/min as rectal temperature fell from 38 to 35°C. The change in respiratory rate was accompanied by a dramatic shift in the phase relations between sympathetic and phrenic nerve discharges. The pattern changed from I (Fig. 3, A) to EI (Fig. 3, B). The shift in phase relations was further characterized by an increase in the duration of expiration and prolongation of the excitatory phase of the cycle of SND. A similar shift from an I to an EI pattern was observed in two additional experiments. In two other cats, respiratory rate increased from 18 to 25 cycles/min and from 12 to 27 cycles/min when rectal temperature was raised 2-3°C with a heat lamp. These changes were accompanied by a shift in the phase relations between sympathetic and phrenic nerve discharges from EI to I.

Dissociation of slow rhythmic components of sympathetic and phrenic nerve discharges. The observation that changes in respiratory rate were accompanied by dramatic shifts in the phase relations between external carotid sympathetic and phrenic nerve discharges raised the possibility that the slow rhythms in these nerves were generated by independent oscillators. This hypothesis is supported by the observation that slow oscillations of sympathetic and phrenic nerve discharges could be dissociated.

SPONTANEOUS DISSOCIATION. Figure 4 illustrates two of three instances in which the slow rhythmic compo-
Hyperventilation to the point of phrenic nerve quiescence was associated with disappearance of the slow rhythmic component of SND in the remaining seven experiments (Fig. 5, B). The amplitude of SND during hyperventilation in these cats was comparable to that observed during the peak of the excitatory phase of the slow sympathetic oscillation in the normocapnic state.

Persistence of the slow periodic component of SND during hyperventilation would be a strong argument in favor of the existence of an independent sympathetic oscillator, providing that phrenic nerve quiescence was due to a change in the functional state of the brainstem respiratory oscillator. That such was the case is indirectly suggested by the results of experiments in which the phrenic nerve responses elicited by single shocks (10 V; 0.5 ms) applied to inspiratory-facilitatory sites located in the dorsolateral pontine reticular formation (P3, L3.5, H-3 to H-6) were compared in the normocapnic and hyperventilated states. One of the two experiments performed is illustrated in Fig. 6. As previously reported by Cohen (5), single-shock stimulation of the dorsolateral rostral pons during inspiration elicited short latency (3.3 ms) phrenic nerve responses (Fig. 6, A1). The phrenic nerve discharge elicited during early expiration (Fig. 6, A2) was somewhat smaller in amplitude and had a longer onset latency (5.6 ms). Significantly, the phrenic nerve response evoked by pontine stimulation during hyperventilation (Fig. 6, B3) was only slightly smaller than the discharge elicited previously during expiration in the normocapnic state. These observations indicate that phrenic nerve quiescence during hyperventilation could not be attributed to inexcitability of descending inspiratory pathways or spinal inspiratory motoneurons. Rather, these results indirectly support the experiments of Cohen (5) which demonstrated that hypocapnia in the cat led to disappearance of the rhythmic components of the discharges of brainstem respiratory neurons.

**DISCUSSION**

The slow rhythm in SND of vagotomized cats is generally assumed to be extrinsically imposed upon central sympathetic networks by phase-spanning and/or phase-locked elements of the brainstem respiratory oscillator (7, 12, 15, 18). However, a number of observations made in the present study contradict this view. Rather, they support the hypothesis that the slow periodic components of sympathetic and phrenic nerve activity are generated by independent oscillators that are normally but not always entrained to each other.

First, it was observed that changes in respiratory rate were accompanied by dramatic shifts in the phase relations between the discharges of the phrenic and external carotid sympathetic nerves. The pattern of temporal relations was shifted from EI to EI when respiratory rate slowed and from EI to I when the respiratory rate was increased. These results make it difficult to accept the notion that respiratory periodicity is extrinsically imposed on central sympathetic networks by any one particular component of the respiratory oscillator. If such were the case, then the phase relations between sympa-
FIG. 5. Effect of hyperventilation on slow rhythmic components of sympathetic and phrenic nerve discharges in 2 vagotomized cats (A and B). Sequence of traces in each panel is as described in Fig. 1. Vertical calibrations are 40 μV. Time base is 1 s/division.

Third, and perhaps most definitive, slow sympathetic oscillations that were locked in a 1:1 relation to the phrenic nerve discharge cycle in the normocapnic state often persisted when respiratory rhythmicity disappeared during hyperventilation. This observation is pertinent when viewed in the light of experiments performed by Cohen (3). He reported that hypocapnia in the cat led to disappearance of the rhythmic components of the discharges of brainstem respiratory neurons. As a

FIG. 6. Effect of hyperventilation on phrenic nerve response evoked by single-shock stimulation (10 V, 0.5 ms) of an inspiratory facilitatory site in dorsolateral pontine reticular formation. Top traces show phrenic neurogram before (A) and during (B) hyperventilation. Bottom traces (records 1-3) are computer-summed phrenic nerve responses (32 sweeps) evoked by pontine stimulation. Address bin was 0.2 ms. Record 1: phrenic response elicited by shock applied during inspiration. Record 2: response evoked by shock applied during early expiration. Record 3: response evoked by shock applied once every 4 s after disappearance of spontaneously occurring phrenic nerve activity during hyperventilation. Vertical calibrations are 20 μV for phrenic neurogram and 66 μV for computer-summed evoked potentials. Horizontal calibrations are 200 ms for phrenic neurogram and 10 ms for evoked responses.
general rule, the discharges of expiratory (E) and expiratory-inspiratory neurons became continuous, whereas the discharges of inspiratory and inspiratory-expiratory neurons became sporadic and eventually ceased as the level of arterial Pco₂ was lowered. Thus, phrenic nerve quiescence produced by hyperventilation in our experiments presumably was associated with disappearance of the rhythmic discharges in those mutually inhibitory brainstem neurons (I and E, IE and EI) that comprise the central respiratory oscillator (4, 6, 13). Under these conditions, it would be impossible to attribute the generation of the slow rhythmic component of SND to a direct connection between the brainstem respiratory oscillator and the central sympathetic networks. Rather, the possibility must be considered that the slow rhythmic component of SND was generated by an independent sympathetic oscillator. The neuronal types that constitute the slow sympathetic oscillator apparently are less apt to lose their rhythmic discharge pattern during hyperventilation than are those neurons that comprise the respiratory oscillator.

Arguments may be raised concerning whether each of the observations discussed above is sufficient to support the conclusion that the slow rhythmic component of SND was generated by an oscillator independent of the one responsible for respiratory rhythmicity. Preiss et al. (18) have suggested that population recording does not allow for precise hypotheses concerning the mechanism of respiratory periodicity, since any particular pattern of phase relations between sympathetic and phrenic nerve activity might arise from the superposition of more than one firing pattern of the contributing units. It could be argued that dissociation of the slow rhythmic components of sympathetic and phrenic nerve discharges as seen in Fig. 4 might have occurred at a locus within sympathetic or respiratory networks distal to the point of direct connection between the two systems. Finally, the question whether all brainstem respiratory neurons lost their rhythmic discharge pattern at a time when the phrenic nerve became quiescent cannot be answered. Nevertheless, it is our opinion that the presently available evidence as a whole is most consistent with the independent oscillator hypothesis.

Synchronization of the two independent oscillators undoubtedly is important in coordinating sympathetic and respiratory responses to a variety of environmental conditions. The mechanism responsible for synchronization of the respiratory and slow sympathetic oscillators, however, is not clear at the present time. At least two possibilities should be entertained in future investigations. First, the two independent oscillators might, in some way, directly interact with each other. Elements of the respiratory oscillator might entrain the slow sympathetic oscillator to the cycle of phrenic nerve activity. Alternatively, the respiratory and slow sympathetic oscillators might be reciprocally connected. Second, it is conceivable that both oscillators receive common inputs from a distinct brainstem synchronizing mechanism. In this case, the respiratory and slow sympathetic oscillators would not be directly connected.

The slow sympathetic rhythm is manifested by the waxing and waning of the amplitude of more rapid periodic components within the SND. This was clearly evident in experiments in which the SND took the form of bursts locked in a 1:1 relation to the cardiac cycle. The "cardiac" periodicity (3-5 Hz) has been previously shown to be representative of a sympathetic rhythm of brainstem origin that normally is entrained to the cardiac cycle by the baroreceptor reflexes (9, 10, 20). Thus, the brainstem network responsible for generation of the 3-5 Hz periodicity apparently receives inputs from the slow sympathetic oscillator. Gootman and Cohen (12) reported that the predominant periodicity in the SND sometimes was changed from 3 to 10 Hz during inspiration in vagotomized cats. Evidence for such a change is shown in Fig. 5, B of the present study. Consequently, the slow sympathetic oscillator apparently also serves as a modulator of transmission in spinal networks that have been shown by McCall and Gebber (16) to be responsible for the 10-Hz periodic component of the SND.

In summary, the present study has extended our knowledge of the hierarchical nature of the organization of central sympathetic networks. Brainstem and spinal networks are inherently capable of synchronizing the discharge of populations of sympathetic neurons into 3- to 5-Hz and 10-Hz periodically occurring wave forms. These networks appear to be subordinate to a slow sympathetic oscillator presumably located in the brainstem. This oscillator functions as an amplitude and sometimes as a frequency modulator with regard to the mass discharges of peripheral sympathetic nerve bundles. The slow sympathetic oscillator is linked to at least one other brainstem system, i.e., the respiratory oscillator.

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REFERENCES


