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# Nonalgebraic Interaction between Pressor and Depressor Reflexes in Dogs: Evidence for a Central Site of Action (41887)

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*Abstract.* In a previous study (Kendrick, JE and Matson, G 1979, Amer J Physiol 327:H662-H667) we demonstrated that the vascular responses in dogs to electrical stimulation of aortic nerve (AN) pressor and carotid sinus nerve (CSN) depressor afferents did not sum algebraically. We suggest this results from a reflex interaction which occurs in the central nervous system. The present study extends earlier studies by recording sympathetic vasomotor activity in chloralose-anesthetized dogs. Stimulation of the CSN reduced sympathetic activity by 51 ± 20 (SD)%. AN stimulation (2 Hz) caused a 17 ± 12% increase in sympathetic activity. Combined stimulation of the ipsilateral CSN and AN caused 0 ± 28% change rather than a 34% decrease expected by an additive interaction. The interaction recorded in this study from the sympathetic outflow is therefore consistent with the previously reported vascular responses (cited above) and implicates central nervous site(s) of action. A conditioning stimulus train to CSN inhibited sympathetic discharges to AN test stimuli. This inhibition was prevented by pairing an AN stimulus with the CSN stimulus train. The AN pressor reflexes act in part by increasing sympathetic activity and in part by suppressing the baroreflexes.

In recent publications (1-3) we reported that cardiovascular reflexes evoked by stimulating carotid sinus nerve (CSN) depressor afferents and aortic nerve (AN) pressor afferents interact in a nonalgebraic fashion. We found that the cardiac and vasomotor responses produced by carotid sinus distension or electrical stimulation of CSN baroreceptor afferents were reduced by either chemical (nicotine) or electrical stimulation of the aortic chemoreflexes. This reduction was much greater than could be accounted for by simple algebraic summation of the antagonistic reflexes. Three lines of evidence suggested that the nonalgebraic nature of the interaction was related to neural mechanisms residing within the central nervous system. First, the degree of attenuation of CSN-evoked baroreflex responses by the AN pressor reflexes was not directly related to the level of peripheral vascular tone. It therefore seemed unlikely that the nonalgebraic interaction was related to properties of vascular smooth muscle. Second, the suppression of CSN-depressor responses by AN pressor activation was more pronounced and consistent when ipsilateral CSN and AN afferents were stimulated than during contralateral CSN-AN interactions. Third, temporal pairing of the AN stimulus with the CSN stimulus resulted in greater diminution of the CSN-depressor response than occurred with uncoupled stimulation of the AN and CSN.

Other laboratories have described vascular responses produced by coactivation of pressor and depressor reflexes, and have concluded similarly that the reflex interactions take place within the central nervous system (4-7). In this report, we provide more direct electrophysiological evidence for a central nervous site of reflex interaction. We show here that baroreflex inhibition of neural discharges recorded on the sympathetic outflow from the central nervous system is attenuated by activation of AN pressor reflexes. The degree of attenuation is greater than would be expected by simply activating AN-evoked vasomotor discharges. Additional experiments further suggest that stimulation of AN pressor afferent fibers has a dual action within the central nervous system of activating pressor reflexes and suppressing CSN-evoked depressor activity.

**Methods.** Experiments were performed on adult mongrel dogs of either sex (10-12 kg). All animals were anesthetized with chloralose (100 mg/kg, iv) after pretreatment with morphine sulfate (3 mg/kg, sc). Anesthesia was maintained by continuous intravenous infusion of chloralose (approximately 25 mg/hr). After endotracheal intubation, the animals were paralyzed with Flaxedil (gallamine tri-



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ethiodide, 2 mg/kg, iv) and artificially ventilated with room air or 100% $O_2$. Ventilatory rate and volume were adjusted to maintain end tidal $pCO_2$ between 25 and 30 mm Hg. External heating devices maintained body temperature close to 38°C. Cannulas were placed in the femoral artery and vein in order to monitor blood pressure and inject drugs, respectively. Both vagus nerves were cut in the midcervical region to abolish cardiopulmonary reflexes.

Reflex responses were evoked by electrically stimulating the CSN and AN. Both nerves were prepared bilaterally in each dog. The CSN was isolated from its juncture with glossopharyngeal nerve, back to its origin in the carotid sinus. The nerve was cut close to the sinus region and gently drawn into silver ring electrodes.

Both AN were dissected from the vagosympathetic trunks and identified in a manner previously described in detail by Edis and Shepherd (8) and by us (1). These nerves were cut and their central ends drawn into similar silver ring electrodes for stimulation.

Neural activity was recorded from the left lumbar sympathetic trunk and/or from branches of the left renal nerve. The sympathetic nerves were exposed by midline laparotomy and removal of the spleen and a segment of the large intestine. A mixture of high (10%)- and low (6%)-molecular-weight dextran in saline was infused to replace the blood trapped in the extirpated intestinal segment. The sympathetic nerves were placed on bipolar silver hook electrodes for neural recording. The nerves and surrounding tissue were immersed in paraffin oil maintained at 39°C by a glass-insulated heating coil. Sympathetic discharges were amplified (preamplifier band pass filters set at 3–3000 Hz) and displayed on an oscilloscope. Continuous measurement of the frequency of compound sympathetic action potentials was obtained with a window discriminator/ratemeter whose output was recorded on a polygraph as an analog voltage that varied with the discharge frequency. Discriminator windows were set during stimulation of the CSN at frequencies and intensities which produced maximum depression of sympathetic firing. The residual sympathetic discharges were reduced sufficiently to distinguish between neural activity and background noise. The lower voltage level of the window was set above background noise and left at that level for the duration of the experiment.

In seven experiments, individual sympathetic reflex responses to ipsilateral CSN and AN stimulation were averaged by a microprocessor-based digital signal averager. This device also measured the magnitude of the reflex response by integrating the area under the averaged compound action potential.

In all experiments, the CSN and AN were first stimulated separately several times before interactions were attempted in order to be certain of consistent AN pressor and CSN depressor and sympathetic neural responses. Depressor responses and inhibition of sympathetic discharges were evoked by stimulation of the CSN with two trains of stimuli per second. Each train was 100 msec in duration and consisted of 20 rectangular pulses, each pulse 0.1 msec in duration. Stimulus intensity (1–3 V) was adjusted to produce near maximum depression of sympathetic nerve activity and blood pressure. This level of CSN stimulation had no discernible effect on breathing in unparalyzed animals. Since a 5- to 10-fold increase in stimulus intensity was necessary to cause stimulation of breathing it was assumed that the chemoreceptor afferents in the CSN were minimally involved at the stimulus intensities which caused large baroreflex responses.

The AN was stimulated at a rate of 2 Hz (2–4 V, 1.0 msec). This stimulation increased sympathetic neural discharges, caused systemic arterial pressure to rise, cardioacceleration, and increased respiratory rate and depth in unparalyzed dogs, but did not produce apneusis. The sympathetic neural discharges recorded during paralysis and artificial ventilation were 70% less than the maximum responses produced by stimulating at frequencies of 10 Hz and greater.

During combined stimulation of the nerves each AN stimulus was synchronized to the beginning of the CSN stimulus train.

Statistical comparisons were made by use of the paired $t$ test. The 95% level of confidence was selected for significance. All data are presented as the means ±SD.

**Results.** *Reflex responses during paired AN and CSN stimulation.* Figures 1–3 illustrate the changes in renal nerve and lumbar sym-

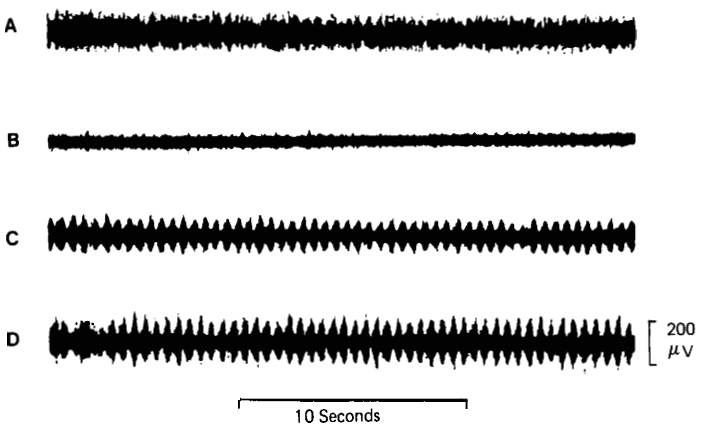


FIG. 1. Film records of neural discharges recorded from the left lumbar sympathetic trunk. (A) Spontaneous (control) discharge; (B) during stimulation of the left CSN (2-Hz trains); (C) during concurrent stimulation of the left CSN and left AN. Each AN single shock (2 Hz) is delivered simultaneously with the first shock of each CSN train. (D) stimulation of the left AN (2 Hz, single shocks) in the absence of CSN stimulation.

pathetic activity in response to stimulation of the AN and CSN, individually and in combination. The results obtained from either the renal nerves or the lumbar sympathetic trunk were qualitatively similar and therefore, not treated separately in the summary shown in Fig. 4. Film records in Fig. 1 illustrate neural discharges in the lumbar sympathetic trunk under control conditions (A), during CSN stimulation (B), during paired ipsilateral stimulation of CSN and AN (C), and during AN stimulation alone (D). Separate stimulation of the AN typically evoked a moderate increase in sympathetic firing in the lumbar sympathetic trunk (Figs. 1 and 3) and renal nerve (Fig. 2), and an increase in systemic arterial pressure. Stimulation of the CSN alone depressed sympathetic firing and blood pressure. Stimulation of the AN during CSN stimulation reinstated sympathetic firing and blood pressure. The reinstated responses were greater than expected for a strictly algebraic summation of effects. For example, in the experiment illustrated in Fig. 2, stimulation of the ipsilateral AN and CSN separately caused a 29% increase and a 64% decrease in sympathetic activity, respectively. Combined stimulation of the ipsilateral AN and CSN caused an 18% increase. If these antagonistic reflexes added algebraically combined stimulation of

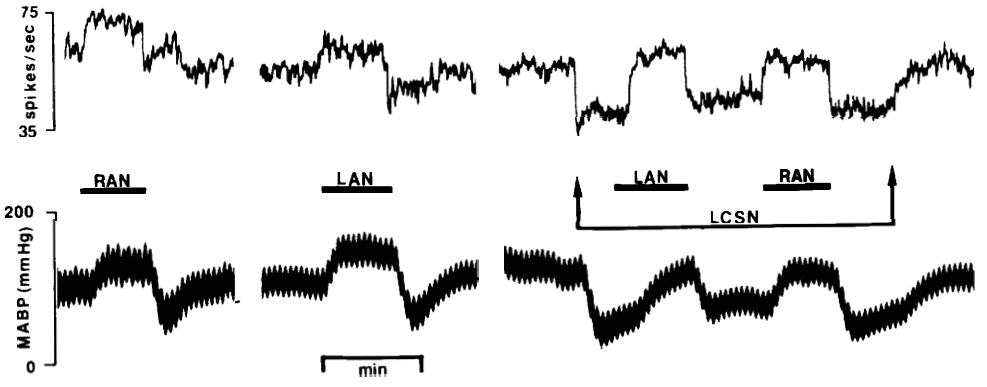


FIG. 2. Renal nerve and systemic pressure responses to left (LAN) and right (RAN) aortic nerve stimulation before and during stimulation of the left carotid sinus nerve (LCSN). Upper traces: Ratemeter records of renal nerve activity. Time constant of ratemeter = 1 sec. Lower traces: Systemic arterial blood pressure.

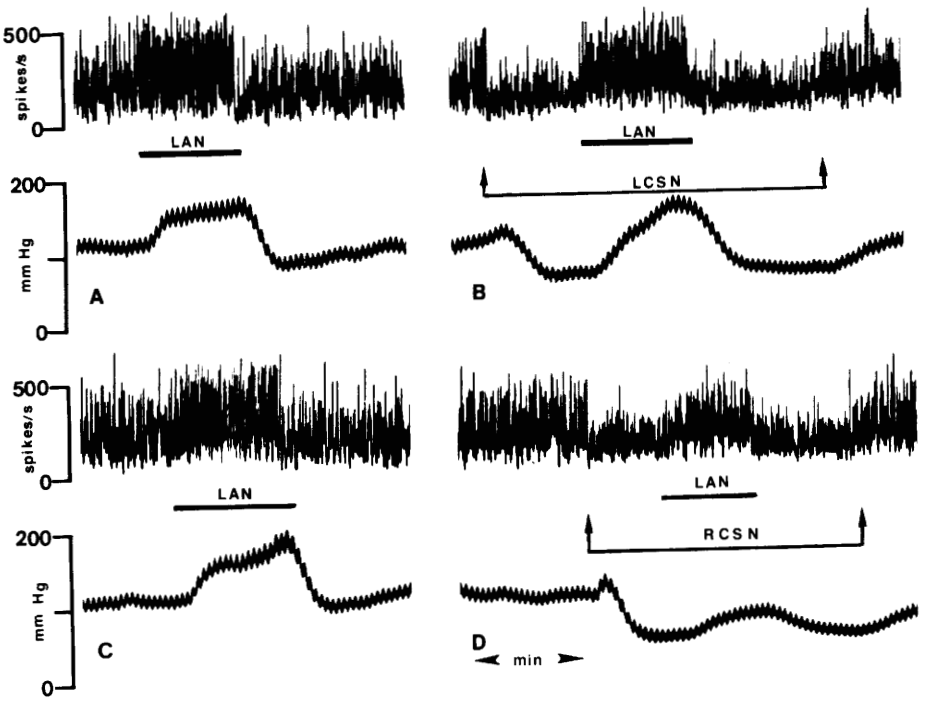


FIG. 3. Lumbar sympathetic trunk neural cativity and systemic pressure responses to stimulation of AN and CSN. Abbreviations same as Figs. 1 and 2. Upper traces: Ratemeter records (time constant = 0.1 sec). Lower traces: Systemic arterial blood pressure.

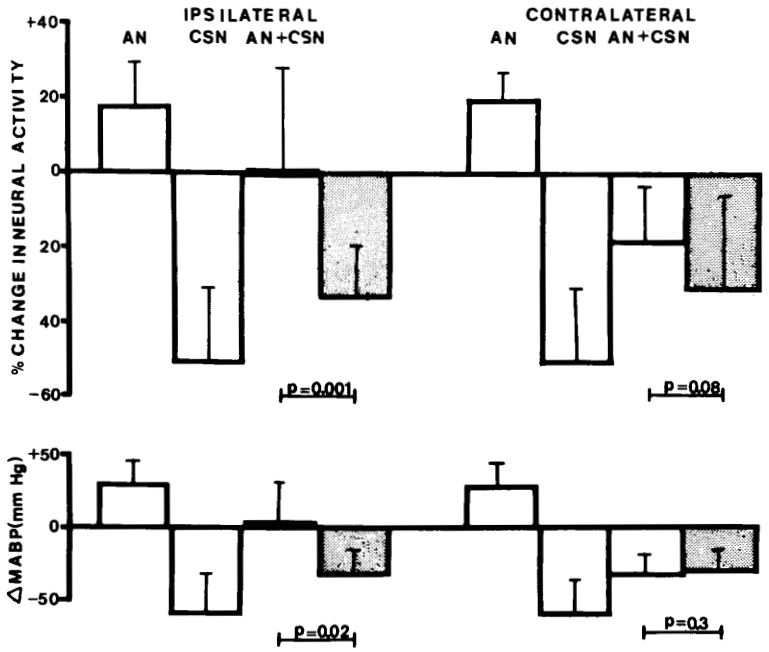


FIG. 4. Average responses (±SD) from 11 experiments of effects of separate and paired stimulation of the aortic nerve (AN) and carotid sinus nerve (CSN) on sympathetic neural activity (upper histograms) and blood pressure (lower histograms). Shaded bars represent the sum of the separate responses.

these nerves would have caused a 35% decrease in sympathetic firing. A similar nonalgebraic summation of the systemic blood pressure responses also occurred. In this case systemic pressure fell 70 mm Hg during CSN stimulation and rose 40 mm Hg during AN stimulation. No net change in pressure occurred during combined stimulation of the ipsilateral AN and CSN.

Figure 4 summarizes the results from all the experiments. In this figure, the shaded bars represent the changes in sympathetic firing which would have occurred if the responses to separate AN and CSN stimulation added algebraically. The average results obtained to stimulation of the AN and CSN on the same side (ipsilateral) are compared to those obtained when the AN on one side is stimulated in combination with CSN on the opposite side (contralateral, $N = 9$). During contralateral stimulation of the nerves, the average response of either sympathetic neural activity or systemic pressure was not different ($P = 0.08$) from the sum of the separate responses. This contrasts with the results of ipsilateral stimulation of the nerves where the response to CSN stimulation was suppressed to a greater degree in all dogs than was seen during contralateral stimulation of the nerves.

In two experiments, ganglionic transmission (lumbar trunk) was blocked by tetraethylammonium chloride (TEA, 15 mg/kg iv). In both experiments TEA caused blood pressure to fall but only a small reduction in sympathetic firing was observed. The sympathetic neural response to CSN and AN stimulation was essentially unchanged by TEA. These observations suggest that the majority of the recorded sympathetic activity was preganglionic. Therefore, we conclude that the interaction between the reflexes is not related to ganglionic processing, as might be suggested by the results of another study (9).

Collectively, the data support the hypothesis that the excitatory AN reflexes cause a suppression of the inhibitory reflexes initiated by CSN stimulation.

It was important in this investigation to determine if the restoration of sympathetic firing during paired AN and CSN stimulation was not merely a consequence of the reduced baseline sympathetic nerve activity. In other words, the same degree of activation of aortic nerve pressor afferents might result in greater sympathetic responses because CSN stimulation reduces background neural discharges thereby reducing refractoriness, leading to greater responsiveness to AN stimulation. However, this does not appear to be the case since as shown in Fig. 5, AN stimulation can prevent the CSN-evoked inhibition of firing when sympathetic background firing is high. Stimulation of the CSN alone is seen to produce a marked reduction of firing in the lumbar sympathetic trunk, whereas stimulation of the AN alone increases activity. When, however, CSN and AN are stimulated simultaneously, rather than applying antecedent CSN stimulation as in Figs. 2 and 3, the inhibition mediated by CSN stimulation is completely prevented. Fig. 5 thus demonstrates nonalgebraic summation of reflex effects in the presence of high background sympathetic activity.

*Effect of conditioning stimulation on the sympathetic reflex response to AN stimulation.* The ability of AN stimulation to restore blood pressure and sympathetic firing to control levels suggests that the sympathetic inhibition caused by CSN stimulation is suppressed by AN stimulation. In order to further test this hypothesis, in seven experiments we compared the effects of ipsilateral CSN conditioning shocks and paired ipsilateral (AN–CSN) stimulation on individual sympathetic discharges evoked by AN test shocks. The sequence of conditioning and test shocks were applied at low rates (every 3–5 sec) in order to minimize the possibility of temporal summation. Figure 6 illustrates the experimental paradigm and results obtained in one experiment. As shown in Fig. 6A, the AN test shock (denoted by arrow) evoked a discharge in the renal nerve at a latency of 180 msec. The reflex was mark-

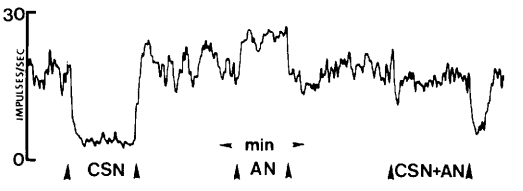


FIG. 5. Ratemeter records of neural discharges recorded from the lumbar sympathetic trunk during stimulation of CSN alone (left), AN alone (middle), and during simultaneous stimulation of the CSN and AN (right).

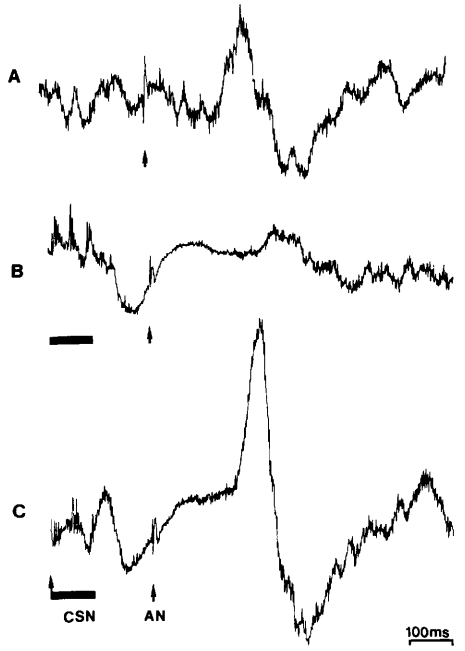


FIG. 6. Computer-averaged responses recorded from the renal nerve, evoked by single shocks applied to the AN (denoted by arrows) and by CSN trains (100-msec train, 20 shocks; denoted by horizontal bars). Each record in A–C is an average of 10 responses. (A) Sympathetic reflex evoked by AN test shock. (B) Inhibition of test response by a conditioning CSN train which precedes the test shock by 250 msec. Note also the suppression of spontaneous sympathetic firing denoted by the reduction of background fluctuations. (C) Restoration of the test response by an AN single shock, applied simultaneously with the first pulse of the CSN train. Spontaneous sympathetic firing remains depressed.

edly depressed when test shocks were preceeded (250 msec) by conditioning CSN trains (Fig. 6B). Similar results were obtained when the condition–test interval was lengthened to 500 msec. The shortest CSN–AN interval required for complete recovery (no inhibition of the test response) ranged from 700 to 1400 msec in the seven experiments. When, however, a conditioning AN single shock was applied simultaneously with the CSN train (Fig. 6C) the inhibitory effect of CSN stimulation was prevented, such that the test response to AN stimulation was reinstated. In most experiments, in fact, the test response which followed paired AN–CSN conditioning stimulation was greater than the unconditioned test response. The conditioning AN stimulus had similar restorative effects when it preceeded or followed the first shock of the CSN train by 50 msec, however, no detailed analysis of the effect of varying the temporal pattern of the AN–CSN conditioning pair was attempted. The test response in Fig. 6C occurs later than the control response (Fig. 6A) and exhibits a larger peak amplitude with less temporal dispersion. A tentative explanation for these alterations is that they are related to the earlier arrival of the CSN volley in the central nervous system. Through this inhibitory influence the CSN train could delay the AN response and also reduce temporal dispersion by suppressing spontaneous firing and refractoriness. In most experiments, the AN shock paired with the CSN train also evoked a sympathetic discharge. The first discharge had no effect on the magnitude or latency of the response to the AN test shock. The test response to the AN stimulus was not affected by a preceeding AN shock at 250- or 500-msec intervals in the absence of CSN stimulation. It is assumed, therefore, that the restoration of the reflex response to AN stimulation is not simply due to facilitation by double shocks. In summary, these results further support the hypothesis that the inhibitory effect of CSN stimulation is prevented by AN stimuli applied simultaneously.

**Discussion.** In previous studies (1–3) we investigated the vasomotor and heart rate responses to both electrical and natural stimulation of the aortic chemoreflexes and the carotid baroreflexes. The similarities between the results of natural and electrical stimulation support our belief that the excitatory response to AN stimulation is predominantly the result of stimulation of chemoreceptor afferents, while the response to CSN stimulation predominantly results from stimulation of baroreceptor afferents. The results of the present electrophysiological investigation also support earlier suggestions (2, 3) that carotid baroreflexes are suppressed within the central nervous system during aortic chemoreflex activation.

The methods of this study do not identify the central nervous sites of AN–CSN interaction. Supraspinal as well as spinal sites of action could be involved. According to our latency measurements, chemoreceptor and baroreceptor stimuli could follow quite dif-

ferent pathways before converging on a common site which influences sympathetic vasomotor activity. We previously demonstrated, as did others, that the AN pressor reflex responses are initiated by high threshold, mostly unmyelinated, slow conducting afferents, while the CSN depressor reflexes are evoked by lower threshold, faster conducting afferents (1). Thus the CSN afferent volley, or an appreciable part of the wave evoked by a train of pulses, would be expected to reach the first synapse in the medulla ahead of the AN volley. In the present study, however, single shocks to the AN, paired with the beginning of a train of stimuli applied to the CSN, resulted in abolition or suppression of the CSN inhibition of sympathetic activity. Since paired stimuli were delivered at a rate of 2 Hz in some experiments (Figs. 1–3) it might be suggested that the arrest of CSN-linked inhibition is attributable to the AN shock of the previous pair. This is unlikely, however, since the experiments exemplified in Fig. 6 show that removal of CSN inhibition still occurred when paired stimulation was applied at intervals of 3–5 sec. It seems more likely that the AN volley activates a pressor reflex pathway which has a relatively short central transit time before it merges with CSN depressor pathway. This would allow the pressor reflex to overtake and arrest the depressor activity, despite the low conduction velocity of the AN afferent volley. The site of convergence for the two reflex pathways could be spinal, supraspinal or both, as suggested from data reported by others (13, 14).

Wherever interaction might have occurred in the present study, it resulted in a generalized alteration of sympathetic vasomotor reflexes affecting both the thoracic (renal nerve) and lumbar sympathetic outflows. It is also evident that the sites of interaction are primarily ipsilateral, since the responses to stimulation of contralateral pairs of afferents (AN and CSN) were either additive algebraically ($N = 3$) or nearly so ($N = 6$).

At least two mechanisms appear to be operative in the restoration of sympathetic responses during paired AN and CSN stimulation. AN stimulation not only generates sympathetic vasomotor discharges (Figs. 1–3, 6) but also arrests the inhibition evoked by stimulation of CSN depressor afferents (Fig. 6). We cannot eliminate the possibility, although it seems unlikely, that the restored sympathetic firing during AN stimulation is produced by input from respiratory networks which are driven by AN chemoreceptor afferents, but resistant to CSN stimulation. This possibility had to be entertained, since several studies have demonstrated respiratory rhythms in spontaneous sympathetic discharges (15–17). In addition, brainstem regions which activate respiratory and vasomotor responses when stimulated have the ability to suppress baroreceptor-mediated inhibition (18, 19). In these studies, however, the hallmark of respiratory involvement is an appearance of a respiratory rhythm in the pattern of sympathetic firing. This also is the case when peripheral chemoreceptor afferent fibers are stimulated. During electrical stimulation (30–50 Hz) of the CSN chemoreceptor afferents, respiratory rhythm in phrenic nerve discharges is preserved, although the frequency and/or magnitude of inspiratory discharges increases (20, 21). Respiratory rhythm is also maintained during 2 Hz electrical stimulation of AN chemoreceptor afferents, although the depth and rate of inspiration may increase (Kendrick, unpublished observations). It is to be expected that the characteristic rhythm would be transferred to the sympathetic outflow if respiratory radiation had occurred.

In conclusion, these results demonstrate that AN pressor reflexes sum in a nonalgebraic manner with CSN depressor reflexes to determine sympathetic outflow to the peripheral vasculature. The interaction between these antagonistic reflexes occurs in the central nervous system. The interaction appears to occur much less between contralateral than between ipsilateral reflexes. The central nervous sites or the mechanisms involved in these reflex interactions have not been determined and are a goal for future studies.

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