

Nature of differential sympathetic discharges in chemoreceptor reflexes

(laterality in sympathetic action/cardiac reactions/vasomotor nerve response/baroreceptor reflex)

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ABSTRACT In a study of autonomic reflexes it was found that some produce a generalized, bilaterally uniform response whereas others have an asymmetric or laterality of action. Recordings from vertebral nerve fibers (mainly vasoconstrictors to forelimb muscles), right and left cardiac sympathetics, and renal nerves show that baroreceptors evoke a bilaterally uniform inhibition but chemoreceptors of the carotid sinus and aortic arch initiate a differential discharge. In the chemoreceptor reflex the vagi are activated and bradycardia generally occurs. Vertebral and renal sympathetic fibers increase their activity bilaterally commensurate with the increase in arterial pressure. Sympathetic discharges to the heart, however, are not uniform; they show ipsilateral inhibition and a strong contralateral increase in activity. Stabilization of blood pressure or inactivation of baroreceptors abolishes the ipsilateral inhibition. In isolation, therefore, the chemoreceptor-induced cardiac sympathetic discharge is just quantitatively stronger contralaterally. In the absence of vagi, heart rate changes differ depending on which chemoreceptors are stimulated, because the pacemaker is on the right. Asymmetrical discharges do occur and, in the eventual response to stimulation of chemoreceptors, reflex interactions actually augment the laterality of effects. Peripheral interactions, in the sense that changes effected by one may induce another reflex, are responsible in part for the balances of autonomic activity ultimately seen as the body reacts to stimuli.

A long-lasting concern of those who study functions of the autonomic nervous system has been to determine how its central control mechanisms can respond to certain stimuli by producing a differential response whereas other stimuli cause the sympathetic division to discharge as a whole. The responses to baroreceptor and chemoreceptor stimulations provide examples of the two categories of effect, and we thought an analysis of their discharge patterns, particularly those evoked by stimulation of the chemoreceptors, would be instructive. Although it is well known that stimulation of arterial chemoreceptors produces augmentation of respiration and pressor responses, the effects on heart rate are controversial. Some investigators have found that, in anesthetized animals with controlled ventilation, bradycardia is evoked by chemoreceptor stimulation (1-4). Others have reported that a moderate tachycardia is obtained when chemoreceptors are stimulated (5, 6).

In previous studies (7, 8) we found that stimulation of the carotid chemoreceptors led to a decrease in cardiac sympathetic discharge but an augmentation in the activity of vertebral nerve fibers that have a vasoconstrictor action on vessels of the forelimb. Similar differential discharges have been recorded by others from splanchnic and cardiac sympathetics during chemoreceptor reflexes (9). In further investigations of the mechanisms whereby differential responses of the sympathetic system are evoked, we have identified two factors that contribute to this phenomenon.

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METHODS

Preparation. These experiments were performed on 29 cats anesthetized with α -chloralose, 70 mg/kg, given intraperitoneally. After paralysis with intravenous injection of 4 mg/kg gallamine triethiodide (Flaxedil), the animals were artificially ventilated, positive pressure being applied at a rate of 31/min. The respiratory volume was adjusted to a maximum which, if exceeded, caused phrenic nerve discharges to become irregular or to cease due to hyperventilation. This produced a 4.0-4.5% end-expiratory CO₂ level, as measured from time to time during surgery and later phases of the experiments. In some experiments the volume discharge of the respiratory pump was increased or decreased deliberately to achieve changes in tonic sympathetic discharges.

A femoral artery and vein were cannulated, and arterial blood pressure was measured by use of a Statham transducer. Heart rate was recorded by a tachometer (Grass 7P4) triggered from the pressure pulse. Blood flow through an axillary artery was recorded with an electromagnetic flowmeter (Statham SP2202) on the right, skinned forearm (paw occluded). Rectal temperature was maintained at 37.5°-38.5° by a heating pad and a lamp.

Stimulation of Chemoreceptors. In order to stimulate carotid chemoreceptors, the lingual arteries of both sides were cannulated with fine polyethylene tubes whose tips were positioned in the carotid sinus. Lobeline chloride (10 μ g) or NaCN (30 μ g) in 0.1 ml of Ringer's solution, was injected through one or both cannulas to stimulate sinus chemoreceptors. In some experiments the drug was also injected into the aorta through the subclavian artery. Recent as well as old studies (10, 11) indicate that such drugs do produce a selective activation of chemoreceptors. In some experiments, sinus nerves, cut peripherally, were electrically stimulated through fine silver bipolar electrodes. Stimulating pulses of 1-2 V (0.2 msec at 20/Hz) were usually adequate to excite mainly fibers from chemoreceptors without activating those from the baroreceptors (12), but intensities had to be varied in some cases to produce the desired selectivity. Blood pressure changes and patterns of sympathetic discharges evoked by sinus nerve stimulation generally could be relied upon as indicators of selective chemoreceptor stimulation (7).

Recordings from Autonomic and Phrenic Nerves. The inferior cardiac nerves and vertebral nerves were exposed by a retropleural approach on both sides where they emerged from the stellate ganglia. When possible, branches of vertebral nerves that innervated muscle vessels were dissected out so that fiber action potentials could be recorded. Such fibers were tonically active and thus considered to be vasoconstrictors because muscle vasodilators are known to show no tonic activity (13). In order to spare sympathetic efferents to the heart as much as possible, we normally prepared only one branch of a cardiac sympathetic nerve on each side for use in recording, because we wished to correlate changes in heart rate with changes in sympathetic

effluent activity during reflex reactions. A phrenic nerve was also regularly prepared for recording of respiratory responses and, in a few experiments, bilateral recordings of phrenic nerve activity were taken. These nerves were placed on bipolar platinum electrodes and protected by covering them with warm mineral oil throughout the experiments.

Nerve action potentials were amplified (Tektronix 122, 0.8–1.0 KHz), displayed on oscilloscopes (Tektronix 565 and 5000 series), and photographed on moving films. For quantitative measurement of electrical activity of sympathetic nerves, area integrators (Grass 7P10) were used; base lines were determined at the end of each experiment when nerve discharges ceased. Phrenic nerve activity was also integrated as recorded (Grass 7P3). Integrated records for every 1 or 2 sec from both right and left cardiac sympathetic, vertebral, and phrenic nerves were simultaneously recorded on the Grass polygraph together with blood pressure and heart rate. Records were also stored on tape for later detailed analysis of nerve activity.

Method for Stabilizing Blood Pressure. In certain experiments the systemic blood pressure was stabilized to completely eliminate baroreceptor influences. To do this, a T cannula was inserted into the abdominal aorta, and one end of it was connected to a reservoir containing 50 ml of heparinized blood from another cat. The blood in the reservoir was kept at body temperature. The air space above the blood in the closed reservoir was connected to a plastic balloon or a 50-liter air container in which the pressure could be adjusted to a desired level by a pump. Before insertion of the T cannula, the pressure of the reservoir system was adjusted to the animal's actual blood pressure level. Changes in volume of the blood in the reservoir were recorded on the Grass polygraph, through a strain gauge, as variations in weight. After each manipulation requiring pressure stabilization, the blood volume in the reservoir was allowed to return to its original level and then a long "rest period" of at least 30 min was given before the next experimental procedure was attempted. In such experiments the procedure of stabilizing the blood pressure was repeated only two or three times; this was essential to the maintenance of normal cardiovascular responses.

RESULTS

Laterality of Right and Left Cardiac Sympathetic Nerve Responses to Chemoreceptor Stimulations. Fig. 1 is a typical example of the sympathetic discharges that occur in a chemoreceptor reflex evoked by injection of lobeline into the carotid sinus of an intact animal. The stimulus evoked a mild pressor response together with a large decrease in heart rate. Activity in vertebral nerve fibers increased, suggesting a vasoconstriction in the muscle; this was substantiated by a distinct decrease in blood flow in forearm muscles. Cardiac nerve activity decreased whereas that of a renal nerve showed only a transient decrease followed by a slight increase. Of course, phrenic nerve discharges were greatly enhanced, although the rate diminished at the beginning of stimulation. Results were similar when aortic chemoreceptors were excited by an injection of lobeline into the arch of the aorta.

When simultaneous recordings were made from vertebral and cardiac sympathetic nerves of both sides of the body, chemoreceptor stimulation produced unexpected but interesting results. This is illustrated in Fig. 2. Stimulation of the right sinus nerve, by using the specific stimulus conditions required for exciting only the chemoreceptors, produced a typical pressor response and a large decrease in heart rate. However, with stimulation of the left sinus nerve with the same conditions, the heart rate increased instead of decreased although the pressor response was the same as that evoked by stimulation of the

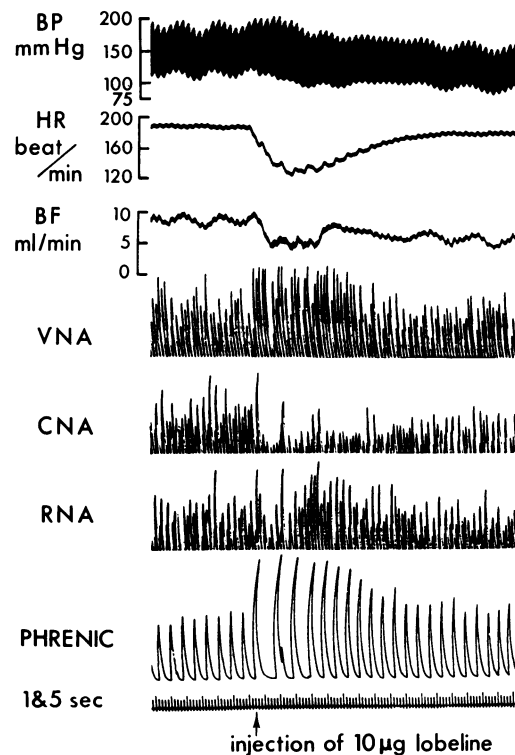


FIG. 1. Effects of chemoreceptor stimulation by lobeline injected into the carotid sinus on sympathetic nerve activities; vagi intact. BP, systemic blood pressure; HR, heart rate; BF, forearm blood flow; VNA, integrated activity of vertebral nerve; CNA, of cardiac sympathetic nerve; RNA, of renal and phrenic nerves.

opposite side. This occurred in the same animal under similar conditions (both nerves were stimulated alternatively). The difference in the cardiac response observed was mainly due to a difference in cardiac sympathetic nerve discharges because the vagi were sectioned in this preparation. When chemore-

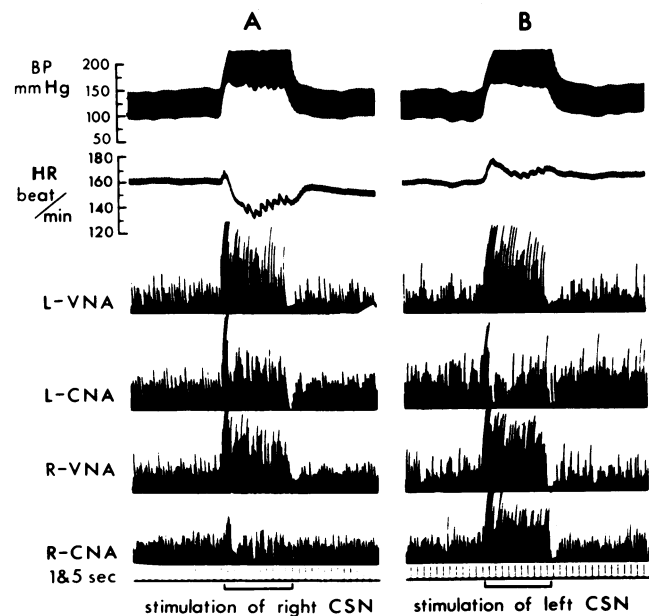


FIG. 2. Effects of chemoreceptor stimulations on sympathetic nerve activities in a vagotomized cat. The carotid sinus nerves (CSNs) of both sides were stimulated under conditions (1 V, 0.2 msec, 20 Hz) that permitted selective excitation of chemoreceptors only. Recordings were made from the right and the left vertebral (VNA) and cardiac (CNA) nerves.

ceptors of the right side were excited, although the activity of the left cardiac nerve was augmented, that of the right was somewhat inhibited and the heart rate decreased. On the other hand, stimulation of chemoreceptors on the left side produced the opposite reactions—i.e., an augmentation of the right cardiac nerve activity and a reduction of discharges on the left. Thus, stimulation of chemoreceptors resulted in an inhibition of activity of cardiac nerves ipsilateral to the stimulation site but augmented discharge of the contralateral nerves. The activity of vertebral nerves of both sides was increased, regardless of the side of stimulation; a laterality of response to chemoreceptors was observed only in cardiac sympathetic nerves.

Because the heart rate generally follows changes in the right cardiac sympathetic activity, in vagotomized animals it usually increased when the left sinus chemoreceptors were excited, whereas stimulation on the right side evoked a decrease in heart rate (Fig. 2). This emphasizes the importance of sympathetic tonic activity; even a reduction in tone can produce a marked decrease in heart rate. In animals with vagi intact, on the other hand, changes in heart rate due to chemoreceptor stimulation could not be easily predicted. In some cases the heart rate followed changes in the right cardiac sympathetic nerve activity, probably because vagal control to the heart was not sufficiently strong, but in others the heart rate decreased due to augmentation of vagal activity by chemoreceptor stimulation, regardless of changes in sympathetic nerve activity (see Fig. 3).

Fig. 3 shows an example of chemoreceptor reflexes in a cat with vagi intact. In this case, chemoreceptors were excited by local injections of lobeline into the carotid sinus of the right and left sides. Stimulation of chemoreceptors on the right produced distinct inhibition of the ipsilateral cardiac nerve discharges while augmenting activity in the contralateral cardiac nerve as well as in both vertebral nerves (Fig. 3A). When the left

chemoreceptors were excited by lobeline, the opposite responses were obtained (Fig. 3B). In both cases the blood pressure increased but the heart rate decreased, presumably due to the augmented vagal activity.

In order to understand the mechanism of such laterality in response of the two cardiac sympathetic nerves, we examined more closely their patterns of discharges during chemoreceptor stimulation. The background or tonic discharge rate in the left and the right cardiac nerves was found to be very much the same, (Fig. 4A). An injection of lobeline into the left carotid sinus evoked an augmentation of right cardiac nerve discharges but a depression of nerve activity on the left side. Synchronous with the first large phrenic burst after the injection, activity of both cardiac nerves temporarily increased, but subsequently, when phrenic discharge was absent or decreased, the right sympathetic activity increased greatly but the left cardiac nerve became quiescent for a while (Fig. 4B). The discharge pattern of the left sympathetic was very much like that of the phrenic. This inhibition resulted in a decrease in overall activity of the left sympathetics and an increased discharge on the right during chemoreceptor stimulation (Fig. 4C). A similar phrenic-locked pattern of discharges caused by chemoreceptor excitation has been reported (7).

The laterality of response of cardiac sympathetic nerves in chemoreceptor reflex does not depend on changes in tonic discharges of sympathetic nerves. In hypoventilation or hyperventilation, background discharges of all sympathetic fibers change greatly. However, it was found that responses of the left and the right cardiac nerves still were different regardless of ventilatory changes. When sympathetic discharges were very much decreased during hyperventilation (Fig. 5A) or very much increased by hypoventilation (Fig. 5B), chemoreceptor stimulation still produced different responses in the cardiac nerves

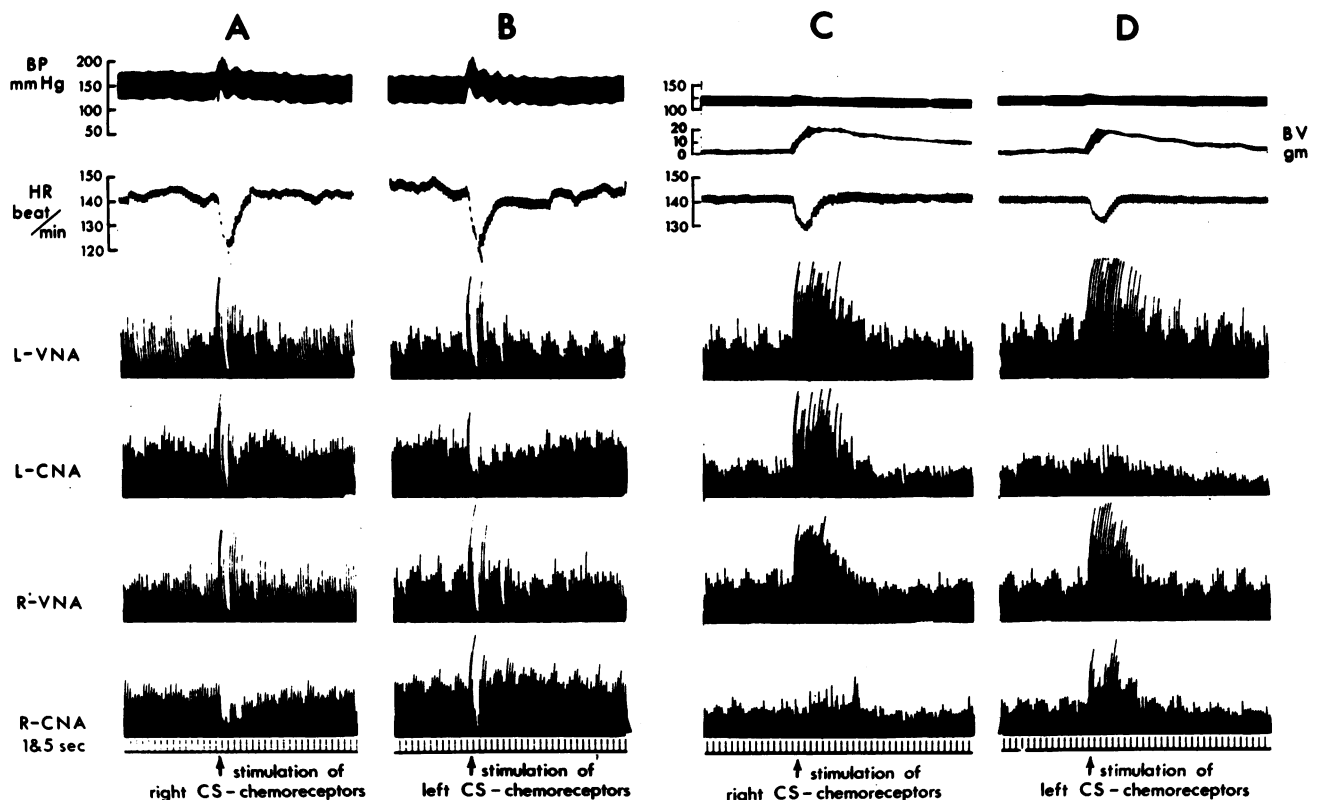


FIG. 3. Effects on sympathetic nerve activities of chemoreceptor stimulation, in cat with intact vagi, by injection of lobeline (10 μ g) into the carotid sinus (CS) of either side. (A and B) Under normal condition. (C and D) Blood pressure, kept constant by stabilization. In C and D, blood volume (BV) is shown as grams moved from the circulatory system to the extracorporeal reservoir in maintaining blood pressure constancy. See Fig. 1 for abbreviations.

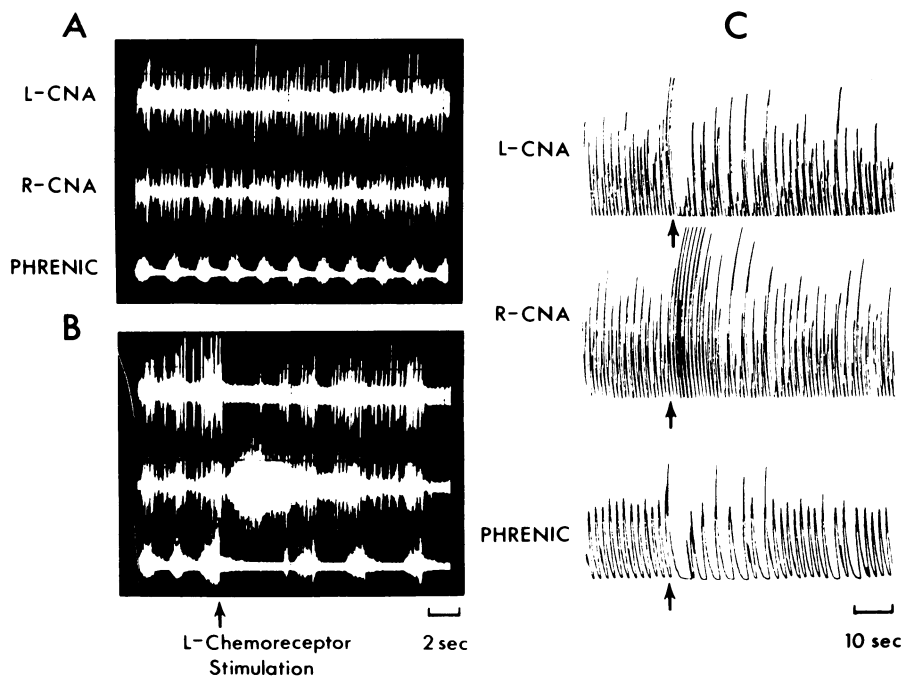


FIG. 4. (A and B) Discharge patterns (oscilloscope tracings) of the left and right cardiac (CNA) and phrenic nerves during a control period (A) and during left chemoreceptor stimulation (B); 10 μ g of lobeline was injected into the left carotid sinus at the time indicated by the arrow. (C) Integrated records of response in B.

of the two sides. The change in blood pressure was much less during hypoventilation when the pressure was already high due to the increased activity of the vasomotor nerves and the respiratory system.

Among 29 cats studied, we did not observe a single exception to the laterality of response to chemoreceptor stimulation. On the other hand, stimulation of baroreceptors or sensory afferents, such as those of the tibialis posterior of the leg, evoked no distinct difference in responses of the two nerves, nor did the right atrial stretch produce different reactions in the two nerves (14). At present it is not known why differences exist in the responses of the two cardiac nerves to chemoreceptors. Possibly, they are due to some special central connections between chemoreceptor afferents and sympathetic neuronal pathways. We found no laterality in response of the left and the right phrenic nerves to chemoreceptor excitation: simultaneous re-

cordings from both phrenic nerves showed exactly the same pattern of activity before and during stimulation of chemoreceptors (cf. figure 3 of ref. 15). The differential patterning did not reside within the respiratory system.

Effects of Blood Pressure Stabilization. Because chemoreceptor stimulation produces a pressor response, it occurred to us that the decreases in heart rate observed might be due, at least in part, to secondary effects of baroreceptor activation. To test this concept, blood pressure was maintained at a constant level by use of a pressure-stabilizing device during the responses to chemoreceptor excitation. The blood pressure was prevented from rising during chemoreceptor stimulation by permitting blood to flow from the circulatory system into the reservoir. During stimulation of the right carotid sinus chemoreceptors, blood pressure remained constant and a remarkable augmentation of discharge of the right and the left vertebral nerve occurred (Fig. 3 C and D). Left cardiac sympathetic activity also increased and the degree was much greater than that observed when the blood pressure was not controlled (Fig. 3C compared to Fig. 3A). Activity in the right cardiac nerve, ipsilateral to the stimulation side, was not inhibited as previously (Fig. 3A) but was slightly augmented. However, there was still a distinct difference in the degree of excitation produced by the right carotid chemoreceptor stimulation in left and right cardiac nerves. A similar phenomenon was observed when the left chemoreceptors were stimulated (Fig. 3D)—i.e., the left cardiac nerve activity hardly changed but activity in the right cardiac nerve increased markedly. In animals with vagi intact (Fig. 3), heart rate was decreased by stimulating chemoreceptors of either side but the magnitude of reduction was less when those of the left side were stimulated. This is explained by differences in response of the right cardiac nerve because these are more important to control of heart rate.

Fig. 3C and D also illustrates the contribution of the cardiac vagus nerve during chemoreceptor reflex. It shows a decrease in heart rate accompanied by an increase in cardiac sympathetic nerve activity. This cardiac deceleration has to be attributed to an augmented activity of cardiac vagal fibers caused by an excitation of chemoreceptors. On the other hand, when vagal innervation to the heart was eliminated (Fig. 2A and B),

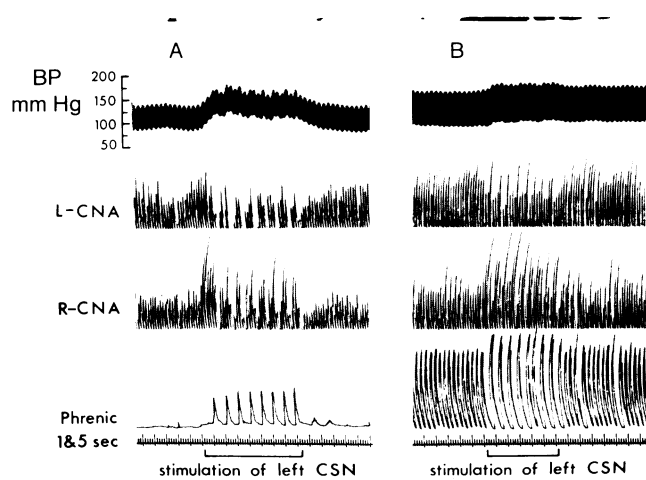


FIG. 5. Effects of excitation of chemoreceptors on cardiac sympathetic and phrenic nerve activities during hyperventilation (A) and hypoventilation (B). Carotid sinus nerve (CSN) was stimulated, under conditions ensuring specific excitation of chemoreceptor afferents only, and responses of the left and right cardiac nerve activities (CNA) were compared.

it was found that the heart accelerated in response to the left chemoreceptor stimulation because right cardiac nerve activity increased. Increase in vagal nerve discharges due to chemoreceptor stimulation has been observed by others (16).

DISCUSSION

The major point of this work is that an apparently qualitative differential action of chemoreceptors on sympathetic nerves innervating the heart and blood vessels is in fact due to action of a secondarily produced baroreceptor reflex and to the fact that chemoreceptors have little effect on the ipsilateral cardiac nerve. We have shown that the chemoreceptor reflex is essentially excitatory to vertebral forelimb muscle vasoconstrictors as well as to cardiac sympathetics, with the ipsilateral cardiac nerve alone being less affected. This quantitative difference in the bilateral effects of chemoreceptors can be turned into qualitative differential discharges of the sympathetic nerves when the response to baroreceptor stimulation is superimposed (see Fig. 3).

Although the functional significance of the laterality of cardiac nerve response observed to occur when the chemoreceptors of one carotid are excited is not clear, a stronger contralateral than ipsilateral effect on cardiac nerves is not surprising because crossed effects are typical of the central nervous system. A degree of reciprocal action—contralateral excitation and ipsilateral inhibition—such as observed to occur in cardiac reflexes might be expected because reflex interactions have now been demonstrated. Just why this asymmetry of response should occur in the cardiac nerves and not in the vasomotor nerves is not easily explained. There is a simpler, lesser duality in the innervation of the peripheral vascular tree than in the heart and this may provide a reason.

One possible explanation of why chemoreceptors and baroreceptors produce different patterns of response is that morphological pathways involved are different. The conclusion that the sinus nerve projects into the nucleus tractus solitarius in the medulla is now well documented, but neither anatomical nor electrophysiological studies have thus far separated the baroreceptor and chemoreceptor pathways. These nuclei of the tractus solitarius project bilaterally to nuclei of the reticular formation and must be destroyed bilaterally if baroreceptor and chemoreceptor reflexes are to be abolished (see ref. 17). These reactions have not been selectively destroyed by brain lesions; therefore, any differences in anatomical pathways carrying impulses from baroreceptors and chemoreceptors to contralateral and ipsilateral cardiac sympathetics must occur after the second or third synaptic connections. It has been reported that bradycardia due to chemoreceptor stimulation occurs only in animals with intact supramedullary structures (17). Our previous work also showed that the differential control of sympathetic cardiac and vasoconstrictor nerve activity is lost after decerebration. We thus believe that the laterality or differential effects of chemoreceptors are organized at supramedullary levels.

The fact that some investigators have found that the chemoreceptor reflex produces a bradycardia (1–4) while others report a mild tachycardia (5, 6) is not surprising because a number of variables can affect what occurs: (i) the chemoreceptor that is stimulated; (ii) the magnitude of the pressor response that can evoke a counter action from baroreceptors; (iii) the degree of vagus involvement (the vagi are activated in chemoreceptor responses) and (iv) the presence or absence of functionally active supramedullary structures. If the vagi and baroreceptors are inactivated, chemoreceptor stimulation generally causes a tachycardia, although sympathetic activity on the two sides of the heart is different. It is the balance between sympathetic and vagal action that determines rate change. In conclusion, it also can be said that a major implication of this work is that peripheral interactions, in the sense that an initial reflex response may initiate secondary responses that also affect the autonomic effectors, determine in part the ultimate responses of the autonomic system. Not all integrative action is in response to the initial stimulus; secondary signals are also determinant.

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* Brooks, C. McC. (1977) *Proceedings of the 18th International Congress of Neurovegetative Research Tokyo*, pp. 7–11.

1. Alanis, J., DeFillo, B. & Gordon, S. (1968) *Arch. Int. Physiol. Biochim.* **76**, 214–235.
2. Daly, M. deB. & Scott, M. J. (1962) *J. Physiol. (London)* **162**, 555–573.
3. Downing, S. E. & Siegel, J. H. (1963) *Am. J. Physiol.* **204**, 471–479.
4. Neil, E., Redwood, C. R. M. & Schweitzer, A. (1949) *J. Physiol. (London)* **109**, 259–271.
5. Landgren, S. & Neil, E. (1952) *Acta. Physiol. Scand.* **25**, 286–290.
6. Little, R. & Öberg, B. (1975) *Acta Physiol. Scand.* **93**, 34–51.
7. Kollai, M. & Koizumi, K. (1977) *Brain Res.* **138**, 159–165.
8. Koizumi, K., Kollai, M. & Brooks, C. McC. (1977) *Proc. Int. Union Physiol. Sci.* **13**, 99.
9. Iriki, M. & Kozawa, E. (1975) *Brain Res.* **87**, 281–291.
10. Jacobs, L., Sampson, S. R. & Comroe, J. H., Jr. (1971) *Am. J. Physiol.* **220**, 472–476.
11. McQueen, D. S. (1974) *Neuropharmacology* **13**, 829–835.
12. DeGroat, W. C. & Lalley, P. M. (1974) *Brain Res.* **80**, 17–40.
13. Koizumi, K. & Sato, A. (1972) *Pfluegers Arch.* **332**, 283–308.
14. Kollai, M., Koizumi, K., Yamashita, H. & Brooks, C. McC. (1978) *Brain Res.* **150**, 519–532.
15. Koizumi, K. & Kollai, M. in *Central Interaction Between Respiratory and Cardiovascular Control*, ed. Koepchen, H. P. (Springer, Berlin), in press.
16. Davidson, N. S., Goldner, S. & McCloskey, D. I. (1976) *J. Physiol. (London)* **259**, 523–530.
17. Korner, P. I. (1971) *Physiol. Rev.* **51**, 312–367.