

# Vagal Afferent Inhibition of Spinothalamic Cell Responses to Sympathetic Afferents and Bradykinin in the Monkey

W. Steve Ammons, Robert W. Blair, and Robert D. Foreman

From the Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

**SUMMARY.** Effects of stimulating the left thoracic vagus nerve on the responses of spinothalamic neurons to electrical stimulation of cardiopulmonary sympathetic afferent fibers and to intracardiac injections of bradykinin were determined. Experiments were performed on 39 monkeys (*Macaca fascicularis*) tranquilized with ketamine and anesthetized with  $\alpha$ -chloralose. The 30 spinothalamic cells studied had the following characteristics. They were excited by manipulation of their somatic receptive fields, were excited by electrical stimulation of cardiopulmonary sympathetic afferent fibers, and exhibited viscerosomatic convergence. Responses of 15 of 19 cells to sympathetic afferent test stimuli were inhibited by conditioning stimuli applied to left thoracic vagus nerve. A conditioning-test interval of 40–50 msec resulted in maximal inhibition of responses to both A $\delta$  and C fiber sympathetic afferents. A long time course of inhibition was present to at least a conditioning-test interval of 200 msec. Left thoracic vagus nerve stimulation inhibited 14 of 14 cells responding to intracardiac injection of bradykinin. Entrainment of activity of five cells to the cardiac cycle occurred in response to bradykinin. In each case, left thoracic vagus nerve stimulation, in addition to reducing frequency of cell discharge, disrupted the cardiac related pattern of cell activity. Bilateral cervical vagotomy abolished all inhibitory effects of left thoracic vagus nerve stimulation. These results demonstrate that vagal afferents may participate in processing of information related to cardiac pain. (*Circ Res* 53: 603–612, 1983)

CARDIAC PAIN, associated with angina pectoris or myocardial infarction is the result of neural activity carried by sympathetic afferent fibers that enter the spinal cord via the T<sub>1</sub>–T<sub>4</sub> rami communicantes (White et al., 1933; White, 1957). Within the spinal cord, sympathetic afferent activity may activate segmental circuits leading to cardiocardiac reflexes (Malliani et al., 1969). For anginal pain to occur, sympathetic afferent activity must also activate cells in the spinal cord which transmit information to regions of the brain involved in perception of pain. Recent studies from our laboratory showed that electrical stimulation of cardiopulmonary sympathetic afferent fibers excites spinothalamic tract (ST) neurons in the upper thoracic spinal cord (Blair et al., 1981). Bradykinin—a potent algescic agent that activates sympathetic afferents (Baker et al., 1980; Lombardi et al., 1981), produces pseudoaffective responses (Guzman et al., 1962), and appears in the coronary sinus of the ischemic heart (Kimura et al., 1973)—also activates ST neurons (Blair et al., 1982). All ST neurons which responded to bradykinin or electrical stimulation of sympathetic afferents were also activated by somatic stimuli (Blair et al., 1981, 1982). Convergence of cardiac sympathetic afferents with somatic afferents may explain why a large percentage of patients with myocardial ischemia have pain referred to the chest wall or arms (Scherf and Boyd, 1958). Taken together, these studies sug-

gest that painful sensations of cardiac origin are due to activation of neural circuits which include upper thoracic ST neurons and sympathetic afferent fibers.

Vagal afferents, as well as sympathetic afferents, influence the activity of ST neurons. We have shown that electrical stimulation of cardiopulmonary vagal afferents inhibits the background activity of ST neurons as well as their responses to somatic stimuli (Ammons et al., 1983). Thus, vagal afferent information, presumably by way of bulbospinal networks, converges with sympathetic afferent information onto the same ST neurons. This finding suggests that—in addition to their known roles in mediating associated hypotension, bradycardia, and decreased sympathetic nerve activity (Kezdi et al., 1974; Felder and Thames, 1979; Thames and Abboud, 1979)—vagal efferents which are activated by myocardial ischemia may also play a role in transmission of information related to cardiac pain. To provide evidence for a role of vagal afferents in modulation of cardiac pain, it is necessary to determine whether stimulation of the vagus nerve alters the ST cell response to sympathetic afferent stimuli. In our earlier study, we tested for vagal effects on somatic, but not visceral, stimuli, and it is possible that the inhibition we observed in that study was a specific effect exerted only on responses to somatic afferents. Thus, the present study was carried out to provide specific evidence for a role of the vagus

nerve in processing of information concerning noxious cardiac events. We examined the effects of electrical stimulation of the left thoracic vagus nerve on responses of ST neurons to electrical stimulation of sympathetic afferents and to intracardiac injections of bradykinin.

## Methods

Experiments were performed on 39 monkeys (*Macaca fascicularis*) weighing between 2.7 and 6.7 kg. They were tranquilized with ketamine (10–20 mg/kg) and anesthetized with  $\alpha$ -chloralose (80 mg/kg). During the recording procedure, a constant infusion of sodium pentobarbital (2–4 mg/kg per hour) was used to maintain the anesthetic level, and either gallamine triethiodide (1–2 mg/kg per hour) or pancuronium bromide (0.15  $\mu$ g/kg per hour) was used to maintain muscle paralysis.

The surgical procedure has been described in detail elsewhere (Blair et al., 1981, 1982). Briefly, artificial ventilation was provided, respiratory  $\text{CO}_2$  was maintained between 3.5% and 4.5%, and temperature was maintained at  $37 \pm 1^\circ\text{C}$ . A catheter was placed in a femoral artery to measure arterial pressure. Throughout the experimental procedure, blood pressure, heart rate, and the ECG were recorded. Catheters were also placed in a femoral vein for fluid infusion and in the left atrial appendage for injection of bradykinin.

A bipolar electrode was placed on the cardiopulmonary sympathetic fibers, as previously described (Blair et al., 1981, 1982). An additional bipolar electrode was placed on the left thoracic vagus nerve just cephalad to the aortic arch. In two experiments, an electrode was also placed on the cardiac branch of the vagus. The electrodes were held in place with low melting point wax to prevent the nerves from drying out. Ligatures were placed around both vagi in the neck so that bilateral vagotomy could be performed at a later time. In two experiments, vagal cooling coils were placed around each cervical vagus nerve in order to perform vagal cold block. At this point, the animals were mounted in a stereotaxic frame. A bipolar stainless steel electrode was positioned in the right ventral posterior lateral nucleus (VPL) of the thalamus with the aid of evoked responses of the nucleus to somatic manipulation of the left forelimb and chest. In any one experiment, three segments of the spinal cord were exposed by laminectomy, although the three segments chosen for study varied from experiment to experiment, ranging from  $\text{T}_1$  to  $\text{T}_5$ .

The protocol was as follows. Gray matter on the left side of the spinal cord was searched, with tungsten or carbon-tipped glass microelectrodes, for cells which were antidromically activated by stimulating VPL (100  $\mu$ sec duration, 2 mA, 10 Hz). Cell activity was recorded and displayed by means of standard techniques. All of the cells studied met the standard criteria for antidromic activation (Trevino et al., 1973).

Once a ST neuron was identified, the type of sympathetic afferent fiber input it received and its somatic receptive field were determined. Sympathetic afferent input was analyzed by constructing post-stimulus histograms. Stimulus intensities varied from 0.7 to 33 V and 100- to 500- $\mu$ sec duration. One or more response peaks occurred in these histograms. Minimum afferent conduction velocity (Foreman et al., 1975) was calculated and voltage thresholds determined for each peak. Each cell had either an A $\delta$  response peak or A $\delta$  and C fiber peaks. C fibers

were judged to be activated when the later peak had a minimum afferent conduction velocity of 2 m/sec or less, and a threshold greater than the first peak. All cells in this study responded to activation of somatic and sympathetic afferent fibers, and thus received viscerosomatic convergent inputs.

All cells were initially tested for responsiveness to left thoracic vagus nerve (LTV) stimulation. Cells with little or no background activity were tested for responses to LTV stimulation during activity elicited by a somatic stimulus such as pinching the skin. Vagal stimuli 2 msec in duration were delivered at a frequency of 20 Hz. The stimulus intensity ranged from 2 to 35 V and was chosen in order to obtain a near maximal response without appearance of the stimulus artifact in the window of the discriminator. Cells inhibited by LTV stimulation were tested for vagal inhibition of responses to sympathetic afferent fiber stimulation, intracardiac injection of bradykinin, or both.

The conditioning-test (CT) technique was used to demonstrate vagal inhibition of cell responses to sympathetic afferent stimulation. Initially, a post-stimulus histogram of the cell's response to the sympathetic afferent test stimulus, only, was constructed as described above. Next, another post-stimulus histogram was constructed when the conditioning stimulus was applied to LTV 20 to 200 msec before the sympathetic afferent test stimulus. The vagal conditioning stimulus consisted of a 10-msec train of stimuli (100  $\mu$ sec, 10–33 V, 333 Hz). The period between the conditioning and test stimuli is called the CT interval. Histograms were generated for several different CT intervals for each cell. Periodically during the procedure, control histograms of the test response alone were repeated. The number of spikes elicited by the test stimulus was determined from the histograms for each CT interval. These values were expressed as a percentage of the number of spikes resulting from the test stimulus alone and were plotted (Fig. 1).

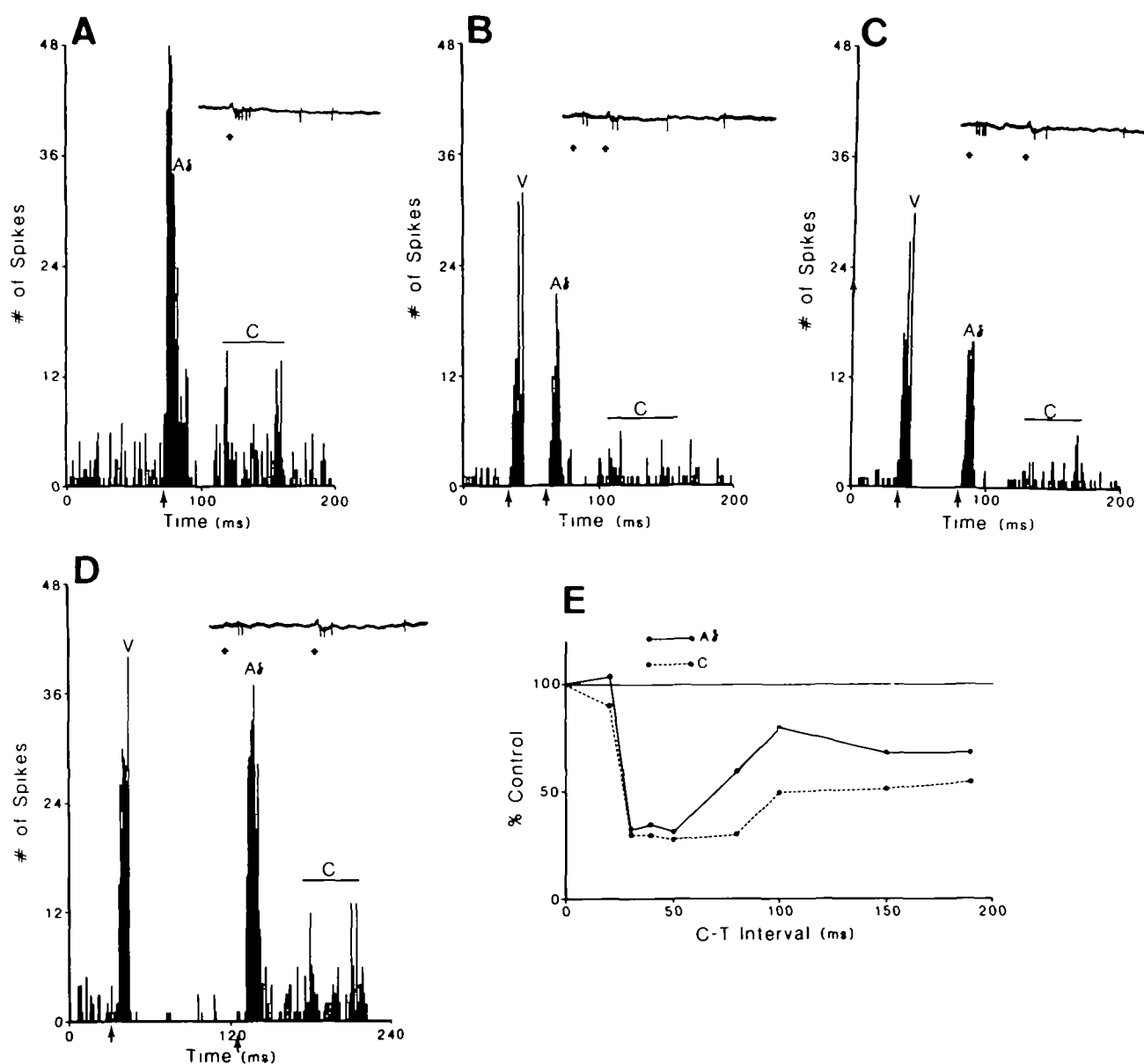
Bradykinin (in a dose of 2  $\mu$ g/kg) was injected into the left atrium. In an earlier study, we demonstrated that this route of bradykinin injection activates primarily cardiac receptors and not receptors outside the heart (Blair et al., 1982). If the cell increased its discharge rate, LTV was stimulated at a point at which cell activity appeared to have reached a peak.

## Anatomy

When study of a cell was completed, its location was marked by passing DC current (50  $\mu$ A for 20 seconds) through the recording electrode. At the end of the experiment, the portion of the spinal cord studied was removed and placed in 10% formalin. After at least 3 days, the cord was frozen and cut into 60- $\mu$ m sections. Sections containing the lesions were projected and drawn on paper. Laminar location of the cell was extrapolated from laminae of the cat spinal cord (Rexed, 1954). The location of the thalamic electrode was marked by passing DC current (20  $\mu$ A for 20 seconds) through the thalamic-stimulating electrode. In each case, the electrode was located in VPL.

## Statistics

All data are expressed as mean  $\pm$  SE. Comparisons between background activity and a single response were made by means of a *t*-test for paired data. Multiple comparisons were made with a one-way analysis of variance. When *F* ratio values were found to exceed the critical value ( $P < 0.05$ ), Dunnett's test was used to compare means. Analysis of variance was also used to determine linearity of regression (Zar, 1974).



**FIGURE 1.** Example of a conditioning-test experiment. Panel A: response of the cell to the sympathetic afferent test stimulus alone. The post-stimulus histogram was generated by stimulating the sympathetic afferents with one pulse per second for 50 seconds (100 sec, 13.2 V). Thus, the number of spikes in each 1-msec bin represents the sum of 50 cell responses. The arrow indicates the onset of sympathetic afferent stimulation. The oscilloscope tracing above the histogram shows the cell response to one stimulus presented at the point indicated by the arrow. Panel B: effect of the vagal conditioning stimulus on the test response. In the oscilloscope tracing, the first arrow indicates the point at which the vagus nerve was stimulated. The stimulus was a 10-msec train of four pulses (100  $\mu$ sec, 16.7 V, 333 Hz). The two spikes occurring shortly after the vagal stimulus were due to vagal excitation of the cell and correspond to the first peak in the histogram. The second arrow indicates the onset of the sympathetic afferent test stimulus 30 msec after the vagal stimulus. Only two early spikes occurred, indicating inhibition of the response to A $\delta$  fiber input. The second peak in the histogram shows this diminished response as a smaller A peak compared with the A $\delta$  peak in panel A. The response to C fiber input was also less. Panel C: response when the interval between the two stimuli was increased to 50ms. At a CT interval of 100 msec (panel D), the test response had recovered to some degree. Panel E: CT curve for the cell.

## Results

Seventy-one ST neurons with viscerosomatic convergent inputs were tested for responses to LTV stimulation. Forty-three (61%) were inhibited, nine (13%) were excited, three (4%) were excited and then inhibited, and 16 (22%) did not respond. A detailed analysis of these responses, as well as vagal inhibitory effects on responses to somatic stimuli,

was reported elsewhere (Ammons et al., 1983). Of the neurons inhibited by LTV stimulation, 30 were tested for inhibitory responses to LTV stimulation during electrical stimulation of sympathetic afferent fibers, during intracardiac injections of bradykinin, or both. Twenty-five of these cells had some degree of background activity which was reduced from  $13.2 \pm 3.1$  to  $2.1 \pm 1.7$  spikes/sec ( $P < 0.001$ ) by LTV stimulation. In addition, mean arterial pressure de-

creased from  $88 \pm 7$  to  $74 \pm 9$  mm Hg ( $P < 0.01$ ), and heart rate decreased from  $202 \pm 8$  to  $173 \pm 11$  beats/min ( $P < 0.001$ ). Cell activity usually began to decrease immediately after the beginning of LTV stimulation and recovered shortly after stimulation ended, whereas the cardiovascular changes occurred more slowly.

Histological reconstruction of laminar sites was successful for 27 of the 30 cells. Cells were located in the 2nd to 5th thoracic segments, but 21 of the 27 were in the 3rd or 4th segments. Sixteen (59%) were located in lamina IV of the dorsal horn, 8 (30%) were found in lamina V, and only 3 (11%) were in lamina VII. None were found in lamina I. It should be noted, however, that in another study we found a few lamina I cells which could be inhibited by LTV stimulation (Ammons et al., 1983).

### Sympathetic Afferent Stimulation

The CT technique was used to examine the time course of the interaction between vagal afferents and sympathetic afferents for 19 ST cells. This information is helpful in determining whether the interaction occurs at a presynaptic site. Conditioning stimuli applied to LTV reduced sympathetic afferent test responses of 15 of these cells. Figure 1A shows a post-stimulus histogram of a cell response to the sympathetic afferent test stimulus only. The cell responded with an early peak of activity which had an onset latency indicative of A $\delta$  fiber input. The oscilloscope tracing above the histogram shows this early cell activity as a burst of five spikes 4 msec after the stimulus artifact. Also shown in the tracing are two later spikes that occurred at a latency of 33 msec, indicative of C fiber input. C fiber activity is shown in the histogram as a second, more prolonged response peak. Figure 1B shows the cell response when the vagal conditioning stimulus was applied 30 msec before the test stimulus. The train of vagal conditioning stimuli was applied at the time indicated by the first arrow. A brief cell discharge followed LTV stimulation. The histogram of Figure 1B shows the initial vagal excitation as a peak prior to the test response. This initial brief excitation, followed by prolonged inhibition, occurred for five cells when LTV was stimulated at 1 Hz, but inhibition only was observed at higher stimulus frequencies. The reduced number of spikes occurring after the second arrow indicates that LTV stimulation did inhibit the response to the sympathetic afferent test stimulus. In this particular tracing, two late spikes were present, but over the course of 50 stimulus trials, the number of later spikes was substantially reduced. The test response was reduced to 33% of control for the A $\delta$  peak and 30% for the C fiber peak. Figure 1C shows the response when the CT interval was increased to 50 msec, the interval of maximal inhibition. The test responses for both fiber groups began to return toward control at longer CT intervals, as shown in Figure 1D. In Figure 1E, the data for cell responses to A $\delta$  and C fibers are plotted as the percent of the control test response.

The inhibitory effect of LTV stimulation could have resulted from excitation of afferents from a variety of sources, including abdominal and thoracic viscera. To determine whether cardiac afferents participated in these responses, we compared LTV stimulation and stimulation of the cardiac branch of the vagus for two cells. For both cells, stimulation of the cardiac branch elicited inhibition that was similar in magnitude to that resulting from LTV stimulation. Thus, for these two cells, most of the inhibition appeared to originate from cardiac vagal afferents.

Figure 2 shows the averaged CT curve for all 15 cells. The curve demonstrates the triphasic nature of vagal effects on responses to A $\delta$  sympathetic fiber activation. Following maximal inhibition near a CT interval of 50 msec, the response began to recover near a CT interval of 80 msec. However, there followed a secondary, prolonged inhibitory trend. The CT curve was simpler for the three cells examined for C fiber input. The point of maximal inhibition occurred slightly earlier, and there was a more prolonged period of inhibition without the intervening recovery phase.

CT curves of four cells were obtained after vagotomy. In each case, little or no inhibition occurred at each CT interval. Two of these cells evoked an initial burst of cell activity followed by inhibition similar to the cell described in Figure 1. Vagotomy abolished the inhibitory response but left intact the excitation. For these four cells and one additional cell, the number of spikes evoked by each sympathetic afferent stimulus before vagotomy was compared with that obtained after vagotomy. The results are shown in Table 1. Stimulus parameters for a given cell were the same before and after vagotomy. The results show that, in four out of five cases, vagotomy increased the number of spikes evoked by sympathetic afferent stimulation. Because of the small number of cells, this effect did not reach the level of significance.

### Bradykinin

Twenty cells whose background activity was reduced by LTV stimulation were tested for responses to injection of bradykinin (2  $\mu$ g/kg) into the left

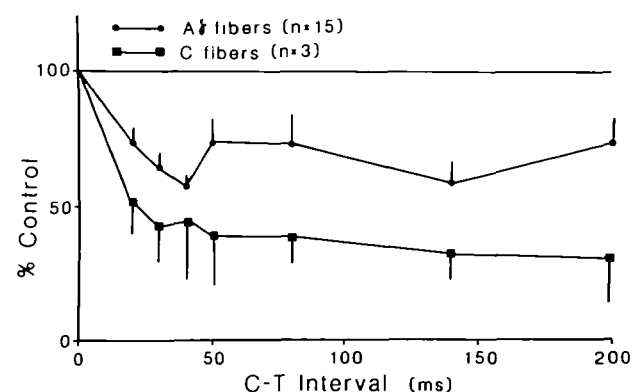


FIGURE 2. The averaged CT plot for cell response to A $\delta$  fibers and C fibers.

TABLE 1  
Effects of Vagotomy on Responses to Sympathetic Afferent Stimulation and Bradykinin

Sympathetic afferent stimulation (spikes/stimulus)			Bradykinin (spikes/s)				
Cell	Before vagotomy	After vagotomy	Cell	Before vagotomy		After vagotomy	
				Control	Bradykinin	Control	Bradykinin
063	1.8	3.4	081	7	19	6	18
081	1.3	1.9	234	30	60	27	65
141	2.4	2.4	484	3	22	5	33
234	5.6	5.7	484	3	22	5	33
574	6.6	7.2					
Mean $\pm$ SE	3.5 $\pm$ 1.2	4.1 $\pm$ 1.1		11 $\pm$ 8	30 $\pm$ 12	14 $\pm$ 6	39 $\pm$ 11

atrium. Bradykinin increased the discharge rate of 14 cells. Figure 3 shows an example of this response, as well as the effect of LTV stimulation. Six seconds after administration of bradykinin, the cell discharge rate increased rapidly from 9 spikes/sec to a peak of 43 spikes/sec, 3 seconds later. Stimulation of the thoracic vagus near the peak of the response resulted in reduction of cell activity from 40 to 9 spikes/sec. Stimulation at a point later in the response reduced

impulse rate from 22 to 5 spikes/sec. Bradykinin injection also caused a decrease in mean arterial pressure beginning within 4 seconds, from a control level of 96 mm Hg to 44 mm Hg, 27 seconds later. Heart rate was 212 beats/min before bradykinin injection, increased transiently, and then decreased to a rate of 180 beats/min 23 seconds post-injection. LTV stimulation during the response to bradykinin had little effect on either cardiovascular parameter,

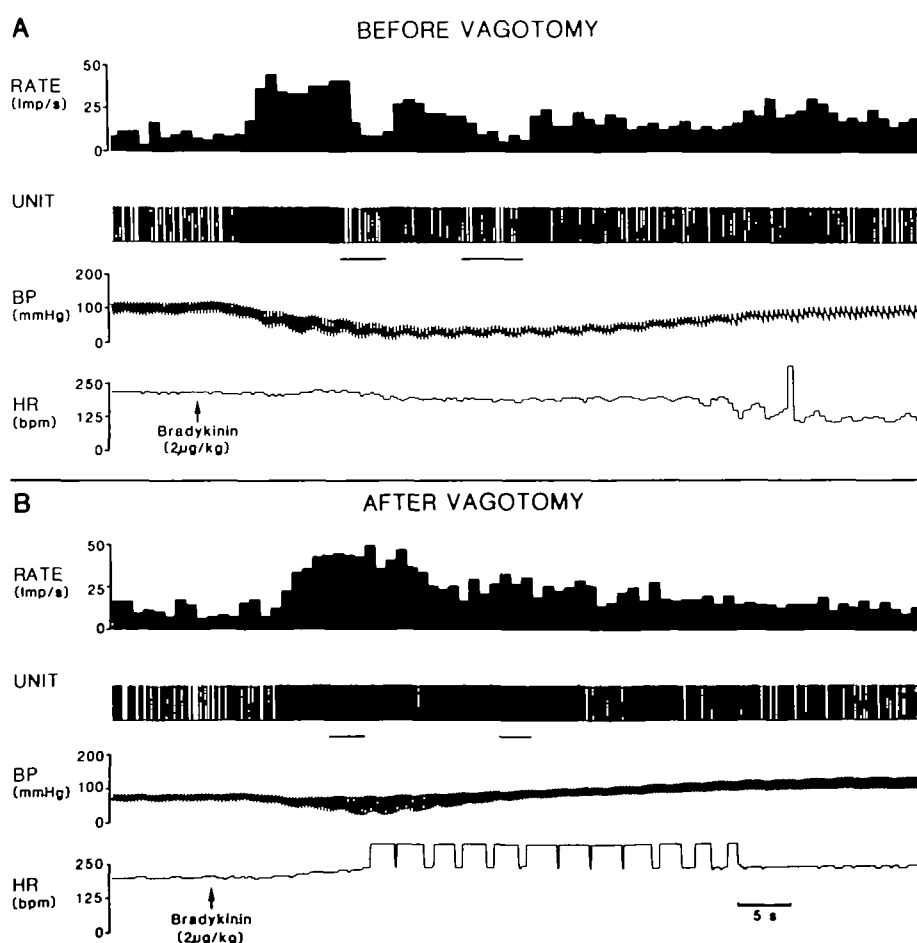


FIGURE 3. Example of a response to intracardiac injection of bradykinin before (panel A) and after (panel B) vagotomy. LTV stimulation (2 msec, 6.9 V, 20 Hz) occurred during the period shown by the bars below the second trace. The rate meter averages cell activity over a 1-second period, and its output is 1 second behind unit discharge. Rate = cell discharge rate in impulses per second; Unit = output of the window discriminator with each deflection due to one cell spike; BP = blood pressure; HR = heart rate in beats/min.

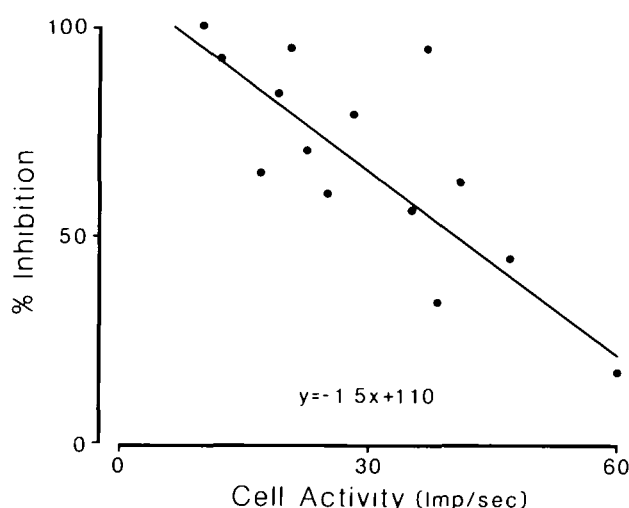


FIGURE 4. Linear regression plot for vagal inhibition. There was a significantly linear relation between the two variables ( $P < 0.05$ ).

even though it caused hypotension and bradycardia in the absence of bradykinin. Figure 3B shows that, after bilateral vagotomy, LTV stimulation had no effect on the response to bradykinin. The hypotensive response was present before and after vagotomy, but was less severe after vagotomy.

The relation between cell activity elicited by bradykinin administration and the inhibitory effect of LTV stimulation is plotted in Figure 4. Regression analysis was used to show that the relationship was linear in nature. Analysis of variance indicated that this linear relationship was statistically significant ( $P < 0.05$ ). The negative slope indicates that, with increasing cell activity elicited by bradykinin injection,

the relative potency of LTV stimulation became less.

Effects of LTV stimulation on cellular and cardiovascular responses to bradykinin are summarized in Figure 5. Bradykinin caused an increase in cell discharge rate from an average of  $9.1 \pm 2.1$  spikes/sec to a level of  $29.2 \pm 3.7$  just before LTV stimulation (Fig. 5A). Vagal stimulation reduced activity by 18 spikes/sec to an average of  $11.7 \pm 4.0$  ( $P < 0.001$ ). Cessation of vagal stimulation caused cell activity to return to the bradykinin-stimulated level. Bradykinin invariably caused hypotension and, usually, bradycardia (Fig. 5B). LTV stimulation sometimes, but not always, intensified these effects. For four of the 14 cells that responded to bradykinin, it was possible to compare responses before and after vagotomy. Before vagotomy, LTV stimulation reduced the cell activity elicited by bradykinin from  $28.4 \pm 10.1$  to  $8.5 \pm 4.1$  spikes/sec. After vagotomy, cell activity during the bradykinin response, and just before LTV stimulation, was  $34.7 \pm 12.5$ , and during LTV stimulation, was  $37.2 \pm 15.8$  spikes/sec. Table 1 shows the effect of vagotomy on the peak response to bradykinin. For three of the four cells, vagotomy increased the response. As in the case of sympathetic afferent stimulation, the number of cells tested were too small for the effect to reach the level of significance.

Five of the 14 cells not only increased their discharge rates in response to bradykinin, but also began to burst in rhythm with the cardiac cycle. An example of one of these cells is shown in Figure 6. Figure 6A shows a 6-second period of cell activity near the peak of its response to bradykinin injection. The cell burst with two to five impulses just after

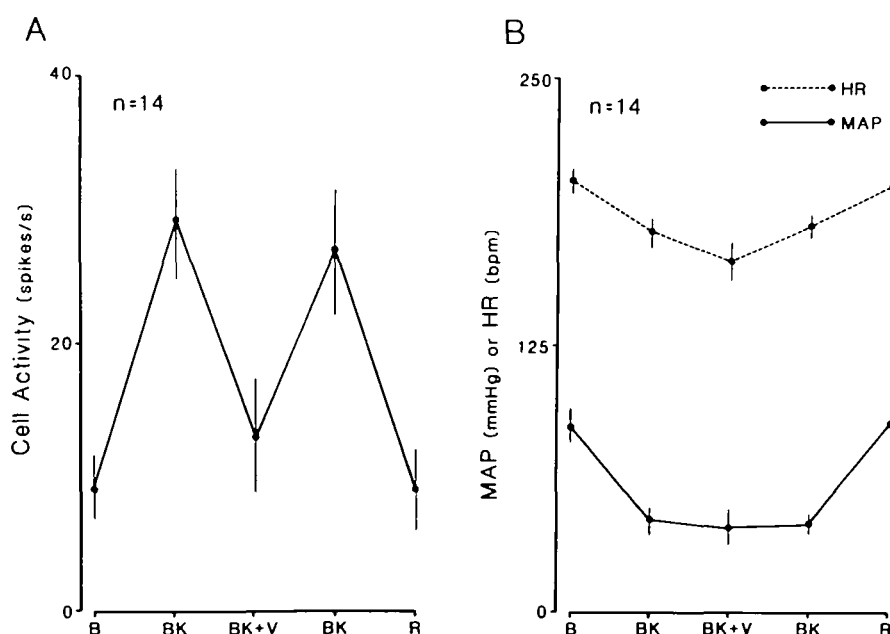
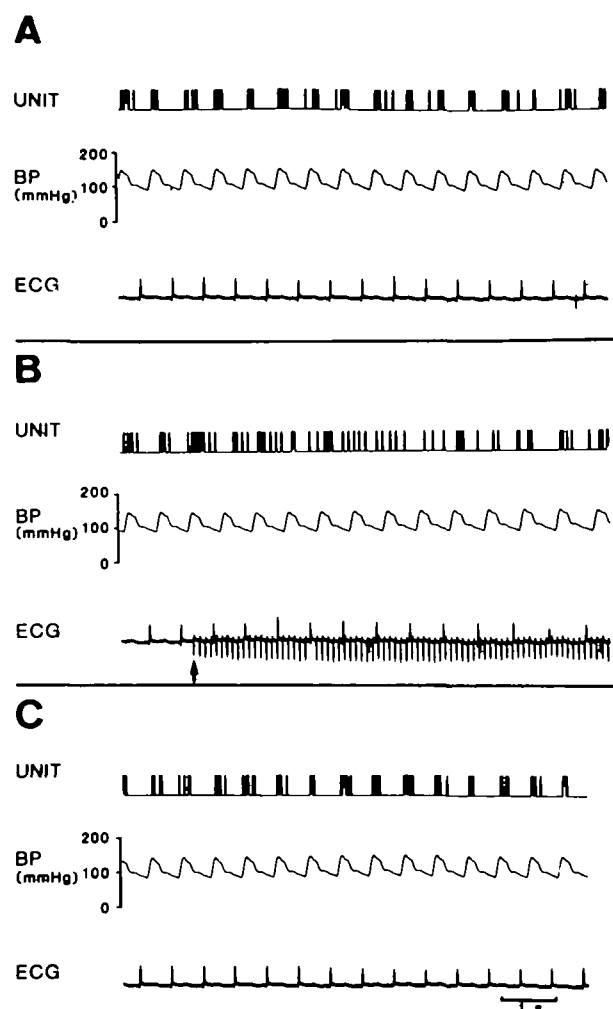


FIGURE 5. Averaged responses to intracardiac injection of bradykinin and LTV effects on those responses. Panel A: cell responses; panel B: blood pressure and heart rate responses. B = background or control levels for each parameter; BK = level during the response to bradykinin just before or just after LTV stimulation; BK + V = peak response to LTV stimulation; R = recovery value; MAP = mean arterial pressure; HR = heart rate.



**FIGURE 6.** Example of effect of LTV stimulations on entrainment of cell activity to the cardiac cycle by bradykinin. Panel A shows an 8-second period of activity near the peak of the response to bradykinin. The cell burst with 2 to 5 action potentials near the peak of each pressure wave. Panel B: LTV stimulation began at the point indicated by the arrow. Note that cell discharge rate slowed as stimulation continued and that bursting of the cell discontinued and firing became random. Panel C: recovery of entrained cell activity following the end of LTV stimulation. ECG = electrocardiogram.

the arterial pressure wave had peaked. In Figure 6B, another 6-second period of activity is shown, during which the vagus nerve was stimulated. During this period, cell discharge frequency decreased. Furthermore, as the LTV stimulation continued, the bursting pattern of cell activity became less and less apparent. Finally, cell discharge occurred randomly throughout the cardiac cycle—that is, LTV stimulation disrupted the entrainment of the cell to the cardiac cycle by bradykinin. After the end of LTV stimulation, the cell returned to its entrained pattern of activity (Fig. 6C).

### Discussion

The important finding of this study is that stimulation of vagal afferent fibers inhibits ST neuronal

activity elicited by cardiopulmonary sympathetic afferents. We chose two methods of activating sympathetic afferents. The first, electrical stimulation, was used with the CT technique to examine the time course of the vagosympathetic interaction. The CT paradigm also gives information concerning mechanisms that may be involved in the inhibition. The CT curves which were generated suggest that the inhibition is effective against activity elicited by both A $\delta$  and C fiber sympathetic afferents, and that it is long-lasting, and complex in nature. The long-lasting nature of the inhibition is consistent with, although not proof of, the presence of a presynaptic inhibitory mechanism (Schmidt, 1971; Foreman et al., 1976). Presynaptic inhibition is the result of depolarization of axon terminals resulting in decreased release of neurotransmitter. This type of inhibition lasts for 400 msec or more (Eccles et al., 1962). Post-synaptic inhibition occurs with a time course of 50 msec or less (Eccles and Sherrington, 1931). In our study, post-synaptic inhibition could be occurring and could be masked by the presynaptic mechanism. We cannot state at what point in the nervous system the inhibition is occurring. Most likely, the inhibition occurs through synaptic links with the sympathetic afferent terminals themselves, or the terminal of an interneuron which links the sympathetic afferent with the ST cell. The CT curve for A $\delta$  sympathetic afferents revealed two points of maximal inhibition (Fig. 2). Our results do not indicate the reason for the two inhibitory maxima; however, we can speculate that LTV stimulation evoked activity in two sets of vagal fibers of different sizes. Thus, the effect of slower-conducting vagal fibers would be exerted on ST cell responses some time after the effects of larger, faster-conducting vagal afferents. Alternatively, different pathways within brain stem circuits may have been activated (see below).

The second method chosen to activate sympathetic afferents was intracardiac injections of bradykinin. For every cell tested, LTV stimulation inhibited the activity of ST neurons elicited by bradykinin. Our regression analysis showed that a linear relation existed between the level of activity elicited by bradykinin and the percent inhibition caused by LTV stimulation. In addition to reductions in frequency of cell activity, LTV stimulation also caused a change in the discharge pattern. This effect is dramatically shown in Figure 6, where the entrainment of a cell's activity to the cardiac cycle in response to bradykinin was completely lost during vagal stimulation. Together, these findings suggest that vagal activity can completely alter the form of the messages being transmitted from ST neurons to the brain, whether the coding of the messages is in the form of frequency or in the form of a pattern such as the bursting pattern of our experiments.

Our experiments show clearly that vagal fibers responsible for inhibition of ST cells are afferent fibers coursing to the brain. In each case in which

bilateral vagotomy was performed, the inhibition was abolished. We did not attempt to localize precisely the source of the receptor endings of these afferents. However, there is reason to believe they are located in the cardiopulmonary region. In this study, we found two cells which could be inhibited by stimulation of the cardiac branch of the vagus to a degree similar to that produced by LTV stimulation. In a previous study (Ammons et al., 1983), stimulation of the vagus nerve below the heart did not inhibit ST cell activity. Thus, it appears that afferents originating from the cardiopulmonary region are likely to be the source of the inhibition. Vagotomy failed to eliminate the brief excitation of two cells by LTV stimulation. Consequently, sympathetic fibers in the vagus (Randall and Armour, 1977) must have mediated this effect.

The physiological significance of our results is related to mechanisms which account for cardiac pain. Bradykinin is a pain-producing substance that causes pseudoaffective responses (Guzman et al., 1962) and is released into the coronary sinus during myocardial ischemia (Kimura et al., 1973). Bradykinin activates cardiac A $\delta$  and C fiber sympathetic afferents (Baker et al., 1980; Lombardi et al., 1981), and its injection into the left atrium activates primarily cardiac receptors and not receptors outside the heart (Blair et al., 1982). Thus, bradykinin administration into the left atrium can be considered a noxious cardiac stimulus. This conclusion is important, because our results are the first to demonstrate that stimulation of the vagus nerve modulates neural circuitry involved in pain sensations resulting from noxious cardiac events. One possible criticism of this interpretation is that bradykinin also activates vagal chemosensitive C fibers, although not A or C mechanoreceptor fibers (Kaufman et al., 1980). If activation of vagal receptors inhibits ST cell activity, then it may seem surprising that vagotomy yielded only a slight potentiation of the ST cell response to bradykinin. There are two possible explanations. First, repeated injections of bradykinin into the left atrium may result in tachyphylaxis of the ST cell response (Blair et al., 1982). Thus, a greater response to bradykinin following vagotomy may have been masked by this phenomenon. Another, more likely explanation is that the vagal chemosensitive fibers activated by bradykinin make a moderate contribution to the inhibition, but the excitatory input of the sympathetic afferents is much more potent. Electrical stimulation of LTV must have either excited the chemosensitive fibers to a greater degree than bradykinin, or, more likely, other vagal afferents were activated, perhaps vagal mechanoreceptors or aortic arch baroreceptors. Regardless of which possibility is true, the potential importance of our findings is not diminished. Bradykinin injected onto the left atrium affects a large number of receptors throughout the heart. However, myocardial ischemia normally occurs in more restricted regions of the heart.

Furthermore, ischemia is associated with activation of vagal A $\delta$  and C fiber mechanoreceptors, as well as chemosensitive endings (Thorén, 1976). It is well established that the pain of angina pectoris or myocardial infarction is due to activity in sympathetic afferents (White et al., 1933; White, 1957). The vagal afferents that are activated mediate circulatory changes, including hypotension and decreased efferent sympathetic nerve activity (Kezdi et al., 1974; Felder and Thames, 1979; Thames and Abboud, 1979). The occurrence of ischemia in a restricted area of the myocardium could provide a different balance of sympathetic and vagal afferent input to ST neurons, compared with the global effects of bradykinin observed in our experiments. Indeed, it is conceivable that preferential activation of vagal afferents, rather than sympathetic afferents, could result in little or no increase in activity of the spinothalamic tract. A series of studies by Thames and co-workers (Thames et al., 1978; Walker et al., 1978; Felder and Thames, 1979) showed that preferential activation of vagal afferents can occur. They found that occlusion of the left circumflex artery was more effective in evoking vagal reflexes than was left anterior descending artery occlusion (Thames et al., 1978). Thus, the area of the myocardium over which ischemia occurs may determine the degree to which vagal afferents are activated. These findings may explain why myocardial infarction of the inferior wall results in bradycardia and hypotension but anterior myocardial infarction results in the opposite cardiovascular changes (Randall et al., 1978). Studies showing preferential activation of vagal afferents, along with our results, may also explain the large number of reports of cardiac ischemia with little or no pain (Master et al., 1969; Margolis et al., 1973). Post-mortem examinations have revealed evidence of occluded arteries in individuals who never reported pain (Master et al., 1969). Electrocardiographic evidence of myocardial infarction also occurs in patients who report little or no pain (Margolis et al., 1973). It is possible that, in these patients, vagal afferent activity is predominant during ischemia, and that activation of ascending systems involved in pain perception is prevented by this vagal activity.

The pathways over which vagal afferents exert their inhibitory effects probably include brain stem networks and descending bulbospinal pathways. Vagal afferents terminate in or near the nucleus of the solitary tract (NTS; Beckstead and Norgren, 1979). These afferents cause reflex vasodilation and inhibition of efferent sympathetic outflow by interacting with brainstem vasomotor circuits regulating blood pressure and cardiac function (Mark et al., 1973; Thorén, 1976). These effects presumably are exerted by bulbospinal pathways which control sympathetic preganglionic neurons. Descending spinal pathways also control the activity of dorsal horn neurons, including ST neurons. Electrical stim-



ulation of the hypothalamus (Carstens et al., 1982), midbrain periaqueductal gray (Carstens et al., 1979), nucleus reticularis gigantocellularis (Haber et al., 1978), and nucleus raphe magnus (Fields et al., 1977; Gerhart et al., 1981) powerfully inhibits dorsal horn neurons. Furthermore, these areas, particularly the midbrain and nucleus raphe magnus, are implicated in modulation of responses to pain in conscious animals (Mayer and Price, 1976) and humans (Richardson and Akil, 1977). An anatomical link between the NTS and the intermediolateral column was demonstrated (Loewy and Burton, 1978), although NTS projections to the dorsal horn have not been described. The NTS does project to the hypothalamus and medial reticular formation (Ricardo and Koh, 1978; Loewy and Burton, 1978). Thus, it is reasonable that vagal inhibition of ST neurons may be mediated by activation of descending systems involved in pain modulation. In support of this concept is the recent description of altered catecholamine and serotonin levels following coronary artery occlusion in areas of the rat brain related to systems involved in analgesia, including nucleus raphe magnus (Sole et al., 1983). The extent to which overlap occurs between brain circuits controlling blood pressure and those which modulate processing of sensory information is unknown. It is of interest, however, that analgesic drugs may cause changes in blood pressure (Eckenhoff and Oech, 1960), and that rats made hypertensive are less sensitive to pain (Zamir and Segal, 1979). Thus, it appears that some interaction between the two systems occurs. Clearly, interactions between vagal afferents and at least one ascending sensory channel, the ST tract, do exist. Whether such interactions occur tonically or are elicited only under unusual conditions, such as myocardial ischemia, remains to be determined.

In conclusion, this study shows that vagal afferent fibers inhibit responses of ST neurons to cardiopulmonary sympathetic afferent activation, including their activation when a noxious cardiac stimulus, bradykinin, is used. Our results are the first to suggest a role for the vagus nerve in the sensation of cardiac pain. Furthermore, these results may offer an explanation for the number of reports of myocardial ischemia with little or no pain.

*We wish to thank Diana Holston for expert technical assistance during the experiments, and Mary Martindale and Tammi Williams for preparing the figures. We thank Lula Campbell for typing the manuscript.*

*This work was supported by NIH Grants HL22732 and HL07430. W.S. Ammons is the recipient of NIH Postdoctoral Fellowship NS07114. R.W. Blair was supported by NIH Postdoctoral Fellowship HL06318. R.D. Foreman is the recipient of Research Career Development Award HL00557.*

*Address for reprints: W. Steve Ammons, Ph.D., Department of Physiology & Biophysics, OU Health Sciences Center, BMSB-653, P.O. Box 26901, Oklahoma City, Oklahoma 73190.*

*Received March 28, 1983; accepted for publication August 25, 1983.*

## References

- Ammons WS, Blair RW, Foreman RD (1983) Vagal afferent inhibition of primate thoracic spinothalamic neurons. *J Neurophysiol* 50: 926-940
- Baker CG, Coleridge HM, Coleridge JCG, Nerdrum T (1980) Search for a cardiac nociceptor: Stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat. *J Physiol (Lond)* 306: 519-536
- Beckstead RM, Norgren R (1979) An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal, and vagal nerves in the monkey. *J Comp Neurol* 184: 455-472
- Blair RW, Weber RN, Foreman RD (1981) Characteristics of primate spinothalamic neurons receiving viscerosomatic convergent inputs in T<sub>1</sub>-T<sub>5</sub> segments. *J Neurophysiol* 46: 797-811
- Blair RW, Weber RN, Foreman RD (1982) Responses of thoracic spinothalamic neurons to intracardiac injections of bradykinin in the monkey. *Circ Res* 51: 83-94
- Carstens E (1982) Inhibition of spinal dorsal horn neuronal responses to noxious skin heating by medial hypothalamic stimulation in the cat. *J Neurophysiol* 48: 808-822
- Carstens E, Yokota T, Zimmerman M (1979) Inhibition of spinal neuronal responses to noxious skin heating by stimulation of mesencephalic periaqueductal gray in the cat. *J Neurophysiol* 42: 558-568
- Eccles JC, Sherrington CS (1931) Studies on the flexor. II. The reflex response evoked by two afferent valleys. *Proc R Soc Lond [Biol]* 107: 535-556
- Eccles JC, Kostyuk PG, Schmidt RF (1962) Presynaptic inhibition of the central action of flex or reflex afferents. *J Physiol (Lond)* 161: 258-281
- Eckenhoff JE, Oech SR (1960) Effects of narcotics and antagonists upon respiration and circulation in man. *Clin Pharmacol Ther* 1: 483-524
- Felder RB, Thames MD (1979) Interaction between cardiac receptors and sinoaortic baroreceptors in the control of efferent cardiac sympathetic nerve activity during myocardial ischemia in dogs. *Circ Res* 45: 728-736
- Fields HL, Basbaum AI, Clanton CH, Anderson SD (1977) Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons. *Brain Res* 126: 441-453
- Foreman RD, Applebaum AE, Beall JE, Trevino DL, Willis WD (1975) Responses of primate spinothalamic tract neurons to electrical stimulation of hindlimb peripheral nerves. *J Neurophysiol* 38: 132-145
- Foreman RD, Beall JE, Applebaum AE, Coulter JD, Willis WD (1976) Effects of dorsal column stimulation on primate spinothalamic tract neurons. *J Neurophysiol* 39: 534-546
- Gerhart KD, Wilcox TK, Chung JM, Willis WD (1981) Inhibition of nociceptive and non-nociceptive responses of primate spinothalamic cells by stimulation in medial brain stem. *J Neurophysiol* 45: 121-136
- Guzman R, Braun C, Lim RKS (1962) Visceral pain and the pseudoaffective response to intraarterial injection of bradykinin and other algic agents. *Arch Int Pharmacodyn Ther* 136: 353-383
- Haber LH, Martin RF, Chatt AB, Willis WD (1978) Effects of stimulation in nucleus reticularis gigantocellularis on the activity of spinothalamic tract neurons in the monkey. *Brain Res* 153: 163-168
- Kaufman MP, Baker DG, Coleridge HM, Coleridge JCG (1980) Stimulation by bradykinin of afferent vagal C-fibers with chemosensitive endings in the heart and aorta of the dog. *Circ Res* 46: 476-484
- Kezdi P, Kordenat RK, Misra SN (1974) Reflex inhibitory effects of vagal afferents in experimental myocardial infarction. *Am J Cardiol* 33: 853-860
- Kimura E, Hashimoto K, Furukawa S, Hayakawa H (1973) Changes in bradykinin level in coronary sinus blood after

- experimental occlusion of a coronary artery. *Am Heart J* 85: 635-647
- Loewy AD, Burton H (1978) Nuclei of the solitary tract: efferent projections to the lower brain stem and spinal cord of the cat. *J Comp Neurol* 181: 421-450
- Lombardi F, Della Bella P, Casati R, Malliani A (1981) Effects of intracoronary administration of bradykinin on the impulse activity of afferent sympathetic unmyelinated fibers with left ventricular endings in the cat. *Circ Res* 48: 69-75
- Malliani A, Schwartz PJ, Zanchetti A (1969) A sympathetic reflex elicited by experimental coronary occlusion. *Am J Physiol* 217: 703-709
- Margolis JR, Kannel WS, Feinleib M, Dawber TR, McNamara PW (1973) Clinical features of unrecognized myocardial infarction silent and symptomatic. Eighteen year followup: The Framingham Study. *Am J Cardiol* 32: 1-7
- Mark AL, Abboud FM, Schmid PB, Heistad DD (1973) Reflex vascular responses to left ventricular outflow obstruction and activation of ventricular baroreceptors in dogs. *J Clin Invest* 2: 1147-1153
- Master AM, Geller AJ (1969) The extent of completely asymptomatic coronary artery disease. *Am J Cardiol* 23: 173-179
- Mayer DJ, Price DD (1976) Central nervous system mechanisms of analgesia. *Pain* 2: 379-404
- Randall WC, Armour JA (1977) Gross and microscopic anatomy of the cardiac innervation. In: *Neural regulation of the heart*, edited by WC Randall. New York, Oxford Press
- Randall DC, Hasson DM, Brady JV (1978) Acute cardiovascular consequences of anterior descending coronary artery occlusion in unanesthetized monkey. *Proc Soc Exp Biol Med* 58: 135-140
- Rexed B (1954) A cytoarchitectonic atlas of the spinal cord of the cat. *J Comp Neurol* 100: 297-379
- Ricardo JA, Koh ET (1978) Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res* 153: 1-26
- Richardson DE, Akil H (1977) Pain reduction by electrical brain stimulation in man. Part 1: Acute administration in periaqueductal and periventricular sites. *J Neurosurg* 47: 178-183
- Scherf D, Boyd LJ (1958) *Cardiovascular Diseases*. New York, Grune & Stratton
- Schmidt RF (1971) Presynaptic inhibition in the vertebrate central nervous system. *Ergeb Physiol* 63: 20-101
- Sole MJ, Versteeg DHG, deKloet ER, Hussain N, Lixfield W (1983) The identification of specific serotonergic nuclei inhibited by cardiac vagal afferents during acute myocardial ischemia in the rat. *Brain Res* 265: 55-61
- Thames MD, Abboud FM (1979) Inhibition of renal sympathetic nerve activity during myocardial ischemia mediated by left ventricular receptors with vagal afferents in dogs. *J Clin Invest* 63: 395-402
- Thames MD, Klopfenstein HS, Abboud FM, Mark AL, Walker JL (1978) Preferential distribution of inhibitory cardiac receptors with vagal afferents to the inferoposterior wall of the left ventricle activated during coronary occlusion in the dog. *Circ Res* 43: 512-519
- Thorén PN (1976) Activation of left ventricular receptors with nonmedullated vagal afferents during occlusion of a coronary artery in the cat. *Am J Cardiol* 37: 1046-1051
- Trevino DL, Coulter JD, Willis WD (1973) Location of cells of origin spinothalamic tract in lumbar enlargement of the monkey. *J Neurophysiol* 36: 750-761
- Walker JL, Thames MD, Abboud FM, Mark AL, Klopfenstein HS (1978) Preferential distribution of inhibitory cardiac receptors in left ventricle of the dog. *Am J Physiol* 235: H188-H192
- White JC (1957) Cardiac pain. *Circulation* 16: 644-659
- White JC, Garrey WE, Atkins JA (1933) Cardiac innervation: Experimental and clinical studies. *Arch Surg* 26: 765-786
- Zar JH (1974) *Biostatistical Analysis*. Englewood Cliffs, NJ, Prentice-Hall
- Zamir N, Segal M (1979) Hypertension-induced analgesia: Changes in pain sensitivity in experimental hypertensive rats. *Brain Res* 160: 170-173

INDEX TERMS: Antinociception • Cardiac pain • Cardiopulmonary sympathetic afferents • Inhibitory bulbospinal pathways • Myocardial ischemia