Responses of Thoracic Spinothalamic Neurons to Intracardiac Injection of Bradykinin in the Monkey

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SUMMARY. Bradykinin stimulates afferent fibers arising in the heart and may be involved in the mediation of anginal pain and the pain associated with myocardial infarction. The sensation of pain requires that noxious information reach the brain. The purpose of the present study was to determine whether the spinothalamic tract is involved in transmitting noxious information from the heart to the brain. Bradykinin was injected (0.3–3.5 μg/kg) into the heart via a catheter in the left atrium while we recorded from single spinothalamic cells in the C_8 to T_5 spinal segments. Thirty-one of 41 cells responded to bradykinin. The responses of 12 cells were characterized by both an increase in discharge rate and entrainment of cell activity with the cardiac cycle. Eighteen cells responded with only an increased rate, and one cell exhibited only entrainment of cell activity with the cardiac cycle. The mean onset of increased cell activity occurred 15 seconds following drug injection, and the average duration of the response was 54 seconds. Thirty cells increased their mean discharge rate from 11 ± 2.5 to 29 ± 4.4 spikes/second. Thus, some spinothalamic cells probably received input from both mechanosensitive and chemosensitive afferents. Tachyphylaxis to repeated doses of bradykinin was observed in 41% of cells. Cells responding to bradykinin had a spontaneous discharge rate that was significantly greater than that of nonresponding cells. Cells did not require input from C-fiber afferents to respond to bradykinin. No statistically significant relationships were found among anatomical locations (laminae and segments) and responses to bradykinin, although cells in lamina I seemed to be less responsive than more ventrally located cells. We conclude that the spinothalamic tract may be involved in the sensation of cardiac pain. (***Circ Res 51: 83–94, 1982***)

THE spinothalamic tract is involved in the mediation of somatic pain in humans (Kuru, 1949). It may also mediate visceral pain, although definitive data supporting this hypothesis are lacking. One important component of visceral pain is the phenomenon of referral, whereby pain originating in viscera is referred to somatic structures. Ruch (1948) suggested, without direct evidence, that afferents from visceral and somatic structures converge on the same pool of neurons at some point in the pain pathways. Normally, this pool of neurons signals information from somatic structures, so that when visceral afferents are activated, the brain misinterprets the true origin of pain, and refers the pain to a somatic field. This theory is called the convergence-projection theory of pain referral. A pathway that might be expected to receive both visceral and somatic inputs is the spinothalamic tract. Recent studies have confirmed that spinothalamic neurons in the lumbosacral, lower thoracic, and upper thoracic spinal segments receive viscerosomatic convergent inputs (Hancock et al., 1975; Blair et al., 1981b; Foreman et al., 1981b; Milne et al., 1981).

Clinically, two of the most significant kinds of referred pain are anginal pain and the pain associated with myocardial infarction. This pain originating in the heart is often referred to the chest wall or arms (White and Bland, 1948; White, 1957). The pain is conducted to the spinal cord via sympathetic afferents in the T_1–T_4 rami communicantes (White et al., 1933; White and Bland, 1948; White, 1957). The stimulus that excites afferents which ultimately result in the sensation of pain has not been conclusively demonstrated. However, several studies have presented data which suggest that bradykinin may be one substance involved in the mediation of anginal pain. For example, intracardiac injection of bradykinin in cats and dogs can elicit pseudaffective responses, suggesting the production of pain (Guzman et al., 1962; Uchida and Murao, 1974). Occlusion of the left anterior descending coronary artery produces an increase in the amount of endogenous bradykinin released into coronary sinus blood (Kimura et al., 1973). Bradykinin excites both Aδ- and C-fiber afferents originating in the heart and coursing in sympathetic nerves to the spinal cord (Uchida and Murao, 1974; Lombardi et al., 1981; Baker et al., 1980). Taken together, these studies suggest that noxious cardiac events release bradykinin, which stimulates sympathetic afferent fibers coursing toward the spinal cord. Presumably, this information is interpreted by the brain as pain. The links in the pathway from sympathetic afferents to higher brain centers have not been examined. The present study was performed to examine one possible link: the spinothalamic tract.

The fact that information is conveyed from the periphery to the spinal cord does not necessarily mean that the same information will reach higher centers. The activity of neurons projecting to the brain is modulated not only by peripheral afferent input,

but also by descending inputs from higher centers as well as intersegmental circuits. The purpose of the present study was to determine how thoracic spinothalamic neurons respond to intracardiac injection of bradykinin. In so doing, we have begun to determine how visceral input is integrated in the central nervous system.

Preliminary reports of some of these data have been published previously (Blair et al., 1981a; Foreman et al., 1981a).

Methods

Nineteen monkeys (*Macaca fascicularis*) weighing 2.4–5.1 kg were tranquilized with ketamine (10–20 mg/kg) and were then anesthetized with α-chloralose (80 mg/kg). Throughout the recording procedure, the monkeys were continually infused with sodium pentobarbital (2–4 mg/kg per hr) to maintain a constant level of anesthesia and with either gallamine triethiodide (1–2 mg/kg per hr) or pancuronium (0.15 μg/kg per min) to maintain muscle paralysis. The animals were artificially ventilated to maintain expiratory CO_2 between 3.5 and 4.5%. Each monkey's temperature was monitored with a rectal probe, and core temperature was maintained at 37 ± 1°C.

A detailed description of our experimental setup has been published (Blair et al., 1981b). Briefly, catheters for recording aortic blood pressure and for administering drugs were implanted in the femoral artery and vein, respectively. A left thoracotomy in the second intercostal space provided an opening for placement of a bipolar electrode near the stellate ganglion, with one electrode hook located on the ansa subclavia and cardiac nerve and the other hook located between the T_2 and T_3 rami communicantes. The electrode was held in place with low melting point wax. For intracardiac injection of bradykinin, the heart was exposed through the 4th intercostal space, and a cannula with a small flanged end was inserted into the left atrial appendage. After the animals were mounted in a stereotaxic apparatus, a concentric bipolar stainless steel electrode was placed in the right ventral posterior lateral nucleus (VPL) of the thalamus by means of visual cues, evoked responses of the nucleus to somatic manipulation of the left forelimb and chest, and stereotaxic coordinates. A laminectomy was performed to expose the C_8 to T_5 segments of the spinal cord. All six segments were not exposed in each experiment; usually, three segments were exposed for study. VPL was stimulated with 100-μsec pulses of 2 mA, at 5 to 10 Hz. The contralateral (left) gray matter of the spinal cord was explored with either tungsten microelectrodes or glass microelectrodes filled with 4 M NaCl until an extracellular action potential produced by antidromic stimulation was encountered. All of the cells reported met the criteria for antidromic activation (Trevino et al., 1973).

Once a spinothalamic cell was identified, the type of visceral afferent input it received was determined using the technique of minimum afferent conduction velocity (MACV) (Foreman et al., 1975; Blair et al., 1981b). Poststimulus histograms of a cell's reponse to sympathetic afferent nerve stimulation were constructed; generally, either one or two peaks appeared. MACV was calculated and threshold voltage determined for each peak. The early peak had a MACV in the Aδ fiber range. If the late peak had a MACV less than or equal to 2 m/sec and a threshold greater than the early peak, it was inferred that C-fiber input was responsible for the second peak.

The protocol for injections of bradykinin was as follows. An initial dose was injected into the left atrium and the spinothalamic cell's response was noted. Doses of bradykinin varied from 0.3 to 3.5 μg/kg, but in most experiments 2 μg/kg were injected. Since responses to bradykinin can exhibit tachyphylaxis, a second dose of the same magnitude was injected into the heart at least 10 minutes after the first. If the cell still responded, the same dose was injected into the thoracic aorta after another 10-minute period. This injection was used as a control to determine whether a cell's response to intracardiac bradykinin was due to activation of cardiac receptors or was due merely to a nonspecific effect on the arterial circulation. Finally, a fourth dose of bradykinin was injected into the heart to confirm the responsiveness of the cell.

Although the emphasis of this study concerned visceral inputs of spinothalamic cells, each cell also was tested for somatic input as described previously (Blair et al., 1981b). All cells tested had somatic fields.

Activity recorded with the spinal microelectrodes was amplified and displayed conventionally. Once the study of a cell was finished, its location was marked by passing DC current (20–50 μA for 20 sec) through the metal electrode. If a glass electrode was used, the cell's depth was noted from the microdrive and the electrode was left in the track. At the end of the experiment, segmental location of each electrode was noted, and the cord was excised and placed in 10% formaldehyde. After at least 3 days, the cord was frozen and cut into 60-μm sections. Sections containing lesions and/or electrode tracks were projected and drawn on paper. Laminar location of each cell in the primate cord was extrapolated from the laminae in the feline spinal cord (Rexed, 1954). Similarly, the location of the thalamic electrode was determined by making a lesion (20 μA for 20 sec). The thalamic electrode tip was located in VPL in every experiment.

Statistical tests were made using analysis of variance or the *t*-test for paired data (Sokal and Rohlf, 1969). All numerical data are presented as mean ± SE. Differences were considered significant if $P < 0.05$.

Results

Responses to Bradykinin

All cells responded to afferent nerve stimulation, but a total of 31 of 41 cells tested responded to intracardiac injections of bradykinin. There were three types of responses to bradykinin. Eighteen cells exhibited an increase in discharge rate, and the activity was not related to the cardiac cycle. The response of one such cell is depicted in Figure 1. In both panels, from top to bottom, the traces represent cell discharge rate, the output of the discriminator, aortic blood pressure, and heart rate. In panel A, bradykinin (2 μg/kg) was injected into the left atrium, as indicated by the arrow. Approximately 4 seconds later, blood pressure began to decrease, but the onset of increased cell activity was 8 seconds. The latency to peak cell activity was 17 seconds. The discharge rate of this cell increased from 21 to 58 spikes/second. Panel B illustrates the effects of injecting the same dose of bradykinin into the descending thoracic aorta, just distal to the aortic arch. Although blood pressure decreased, no change in cell activity occurred.

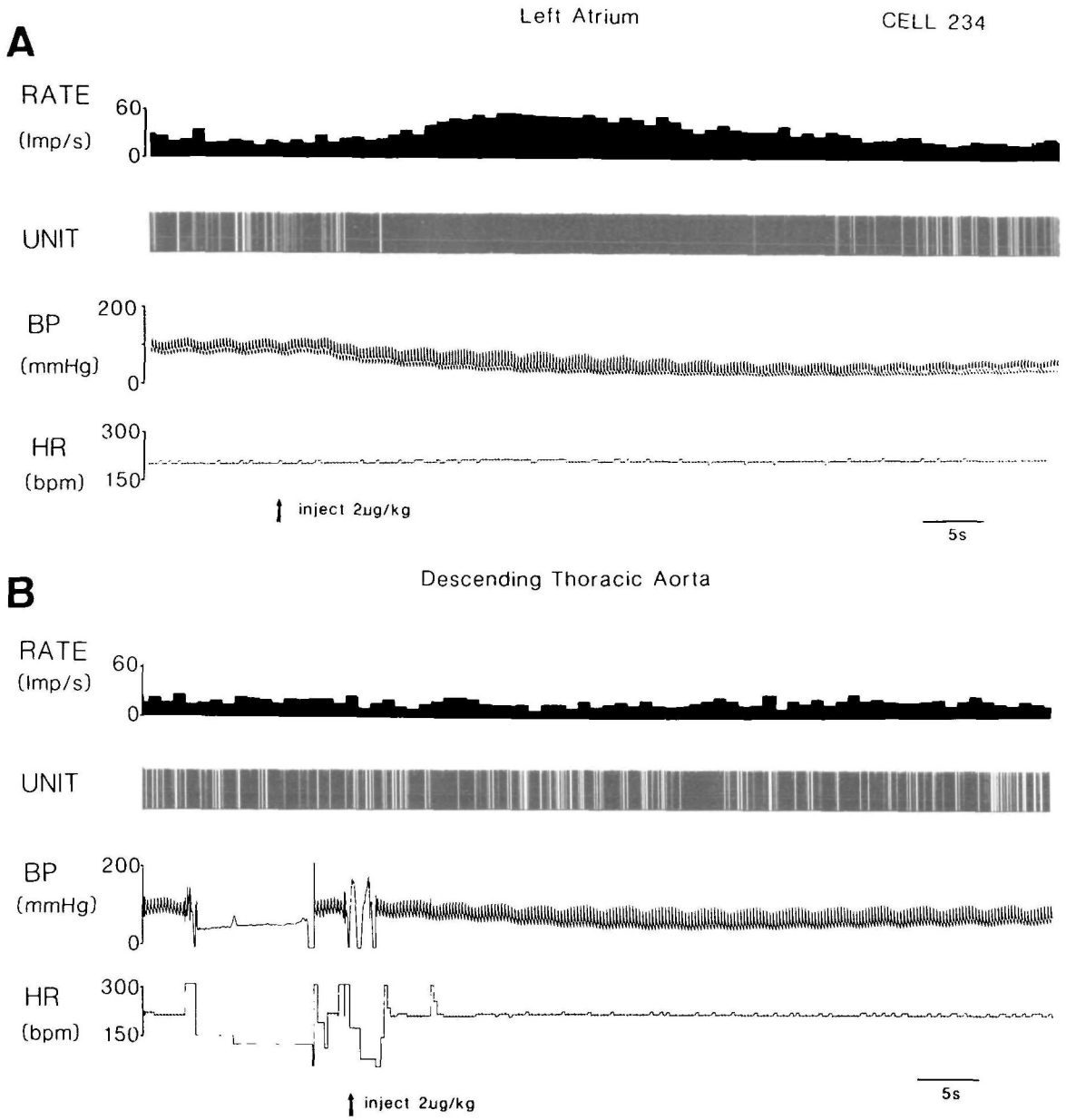

FIGURE 1. *Responses of the same spinothalamic neuron to a bolus dose of 2 µg/kg bradykinin into (A) the left atrium and (B) the aorta just distal to the aortic arch. Rate = rate of cell discharge in impulses per second (Imp/s). Unit = output of window discriminator. Each deflection (Unit) represents one cellular action potential. BP = aortic blood pressure. HR = heart rate in beats per minute (bpm). Scale bar labeled 5s indicates 5 seconds.*

The second type of response, shown by 12 cells, consisted of an increased discharge rate similar to the first group, but the increased activity became entrained with the cardiac cycle. One cell exhibited the third response, and its activity became entrained with the cardiac cycle, but the overall discharge rate did not increase. The response of this cell is shown in Figure 2. The top two traces show an analog recording of the output of the discriminator and aortic blood pressure. The bottom two traces depict a computer-generated expansion of cell discharge and blood pressure during the time indicated by the bar above the analog blood pressure trace. Bin width for the computer traces was 15 msec. Asterisks indicate cell activity obviously synchronous with arterial pressure pulses. This cell did not noticeably increase its background discharge rate to intracardiac bradykinin (in fact, cell discharge decreased with blood pressure); instead, its discharge was entrained with the cardiac cycle. No spinothalamic cell exhibited an obvious cardiac rhythmicity before the administration of bradykinin. In summary, 30 cells increased their discharge rates in response to intracardiac bradykinin; the activity of 12 of these cells became entrained with the cardiac cycle. One cell exhibited only entrainment of its activity with the cardiac cycle.

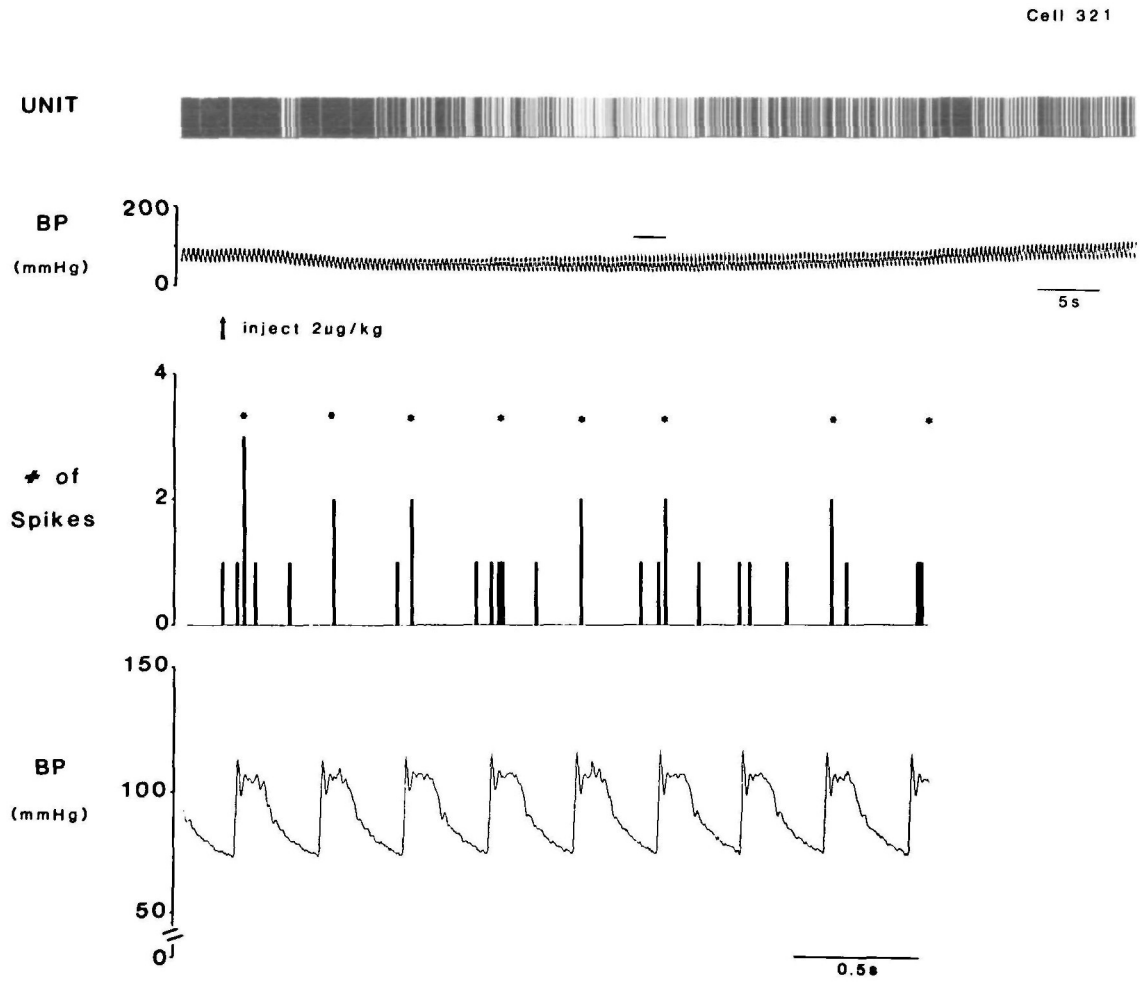

FIGURE 2. *Entrainment of discharge of one cell with the cardiac cycle in response to bradykinin. Third and fourth traces show a computer-generated expansion of period indicated by bar above analog blood pressure trace. Third trace shows cell activity; bin width = 15 msec. Activity denoted by asterisks was entrained with the cardiac cycle.*

Thirty cells increased their mean discharge rate from 11 ± 2.5 to 29 ± 4.4 spikes/second ($P < 0.001$) in response to intracardiac bradykinin. Systolic and diastolic blood pressures fell roughly equally (−36 ± 3.5 and −31 ± 2.7 mm Hg, respectively). The heart rate response was highly variable among monkeys, so that the mean change in heart rate was not significant. The average duration of increased cell activity was 54 ± 6.4 seconds; however, the duration of decreased blood pressure was significantly longer, averaging 313 ± 55 s ($P < 0.001$). The latency to onset (LTO) of increased cell activity (15.2 ± 1.6 seconds) occurred 9.2 ± 1.7 seconds later ($P < 0.001$) than LTO of the fall in blood pressure (6.0 ± 0.6 seconds). However, latency to peak responses were similar (32.0 ± 2.2 seconds for cell activity, 30.6 ± 1.9 seconds for blood pressure).

Tachyphylaxis of cell responses to intracardiac bradykinin was observed in 7/17 (41%) cells, although the response was never abolished. No additional tachyphylaxis was observed after subsequent doses of bradykinin; thus, the response of a cell to the second dose of bradykinin was often less than the response to the first dose, but responses to the third or fourth doses were similar to the response to the second dose. On the other hand, blood pressure responses to intracardiac bradykinin were not tachyphylactic.

To determine the relationships of blood pressure to the increases in cell activity in response to intracardiac bradykinin, a comparison of cell responses to bradykinin injected into the left atrium versus injection into the thoracic aorta was made (Fig. 3). Twenty cells were studied long enough to permit this comparison. The left pair of bars represent the mean change in discharge rate of all 20 cells. The activity of three cells was unaffected, seven were inhibited, and 10 were excited by bradykinin injected into the aorta. The right pair of bars represent the 10 cells excited by aortic injection of bradykinin. The average spontaneous discharge rate of the cells before injections were made is indicated by the number beneath each bar. For both comparisons, injection of bradykinin into the left atrium produced a significantly ($P < 0.001$) greater increase in cell activity than did aortic injection of the same dose. To eliminate the possibility that tachyphylaxis was responsible for the diminished

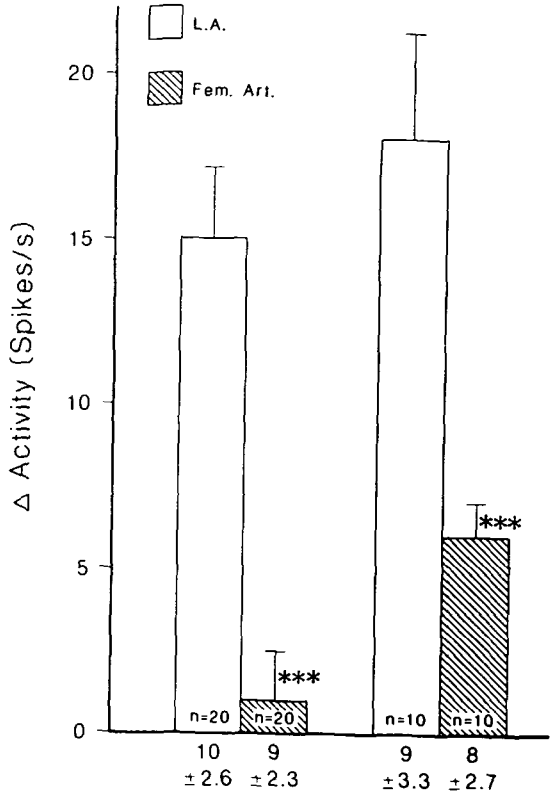

FIGURE 3. *Comparison of cell responses to bradykinin injected into the left atrium (LA) and thoracic aorta via the catheter threaded into the femoral artery (Fem. Art.). Ordinate = change in cell activity. Numbers below bars = cell discharge rate ± SE before bradykinin injection. Pair of bars on left show mean change in activity of all cells tested with both injections. Right pair of bars show mean changes in discharge rate of those cells exhibiting an increase in activity upon aortic bradykinin injection. Data used for the right pair of bars are a subset of those used for the left pair of bars. *** = statistical significance ($P < 0.001$).*

response to aortic injection, bradykinin was again injected into the left atrium after aortic injection. In each case, the cell remained responsive to this injection.

Figure 4 shows a comparison of the decreases in blood pressure produced by intracardiac versus aortic injection of bradykinin. Systolic blood pressure decreased more to left atrial than to aortic injection (−34 ± 4.8 vs. −24 ± 4.0 mm Hg, $P < 0.05$), as did diastolic blood pressure (−30 ± 3.9 vs. −23 ± 3.3 mm Hg, $P < 0.05$).

To determine whether anesthetizing cardiac receptors would diminish a cell's responsiveness to intracardiac bradykinin, the responses of two cells to bradykinin were compared before and after lidocaine was perfused around the heart. For lidocaine application, a catheter was sutured into the pericardium, and lidocaine was injected into the pericardial sac. The cells tested in this manner were not tachyphylactic to bradykinin. The responses of one cell are illustrated in Figure 5. In each panel, the traces from top to bottom show the cell's discharge rate, the output of the discriminator (note that only every fourth spike is displayed), ECG, and aortic blood pressure. Panel A shows that the cell's discharge rate increased from 27 to 38 spikes/second in response to 0.4 μg/kg bradykinin injected into the left atrium. Panel B illustrates the response of the cell to pinching skin in the cell's somatic field. Panels C and D show the cell's responses to bradykinin and skin pinch after lidocaine was perfused on the heart. Note in panel C that the response to intracardiac bradykinin (0.4 μg/kg) was abolished, but the response to skin pinch remained (panel D). Hence, the effect of lidocaine was due to an action on the heart, and not to a nonspecific depression of cell responsiveness. The other cell tested in this manner exhibited a diminished response to bradykinin, although the response was not abolished. Discharge rate increased 26 spikes/second in response to bradykinin before lidocaine, and 10 spikes/second after lidocaine. The cell's discharge became entrained with the cardiac cycle in response to bradykinin; this burst effect was abolished by lidocaine.

Recent evidence suggests that bradykinin injected peripherally may enter the brain to produce cardiovascular effects (Takahashi and Buñag, 1981). Conceivably, the effect of bradykinin on spinothalamic cells could be mediated by descending influences from the brain once bradykinin circulated to the brain. To minimize this possibility, we placed the arterial catheter in, or just distal to, the aortic arch.

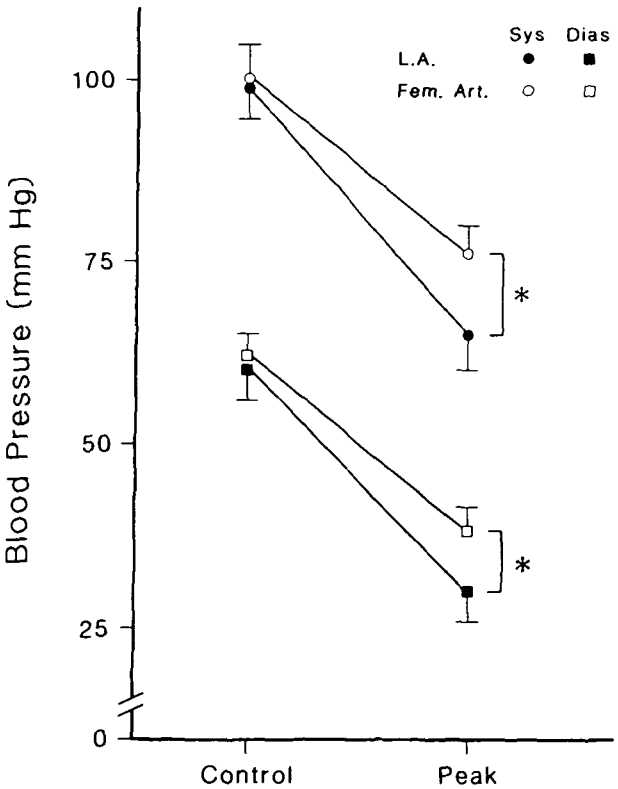

FIGURE 4. *Comparison of peak fall in blood pressure resulting from same dose of bradykinin injected into the left atrium (LA) or thoracic aorta via femoral artery (Fem. Art.) catheter. Sys = systolic blood pressure. Dias = diastolic blood pressure. *$P < 0.05$.*

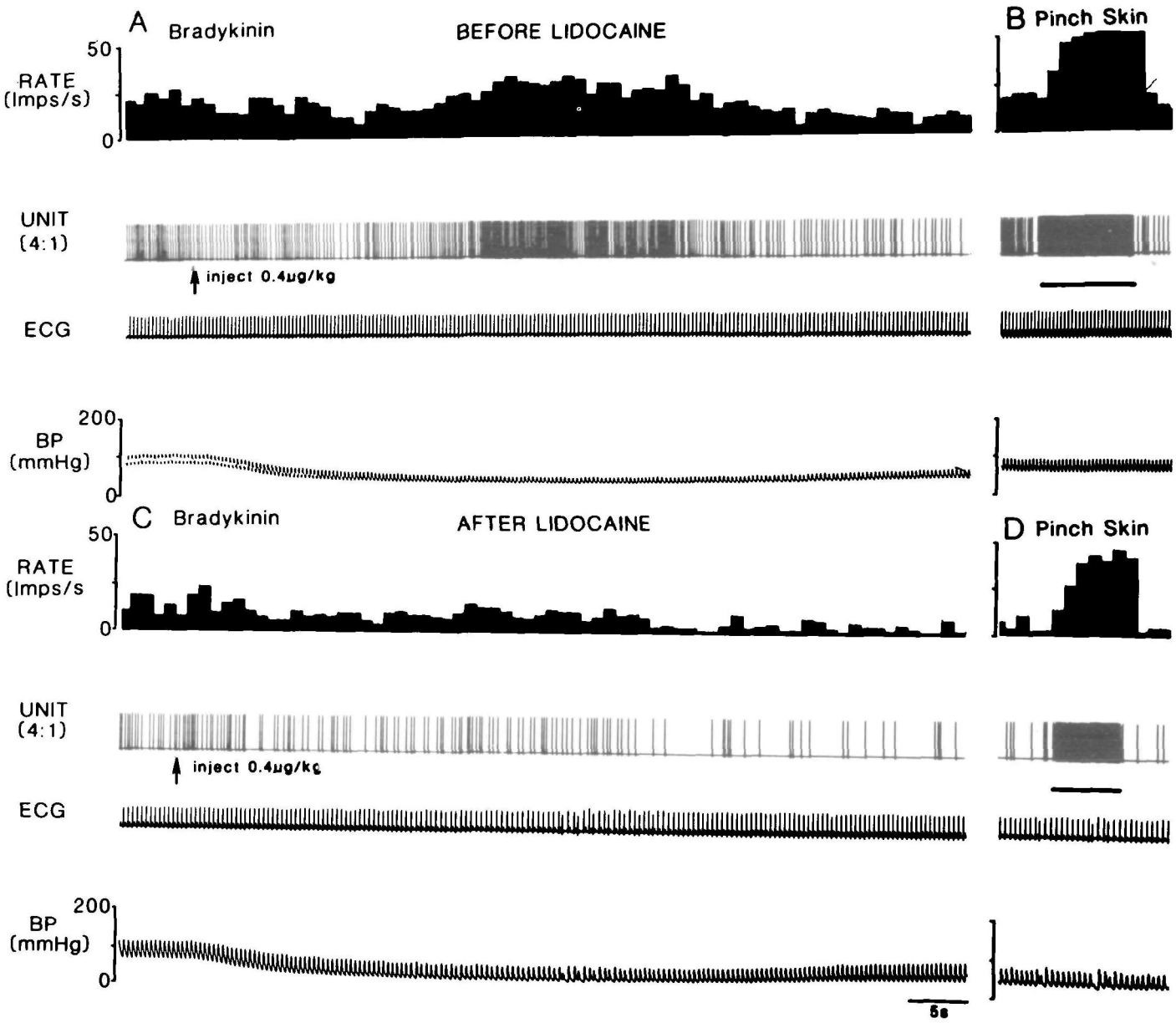

FIGURE 5. *Responses of a cell to intracardiac bradykinin (2 µg/kg) and somatic pinch before and after lidocaine perfusion over the heart. Note that for the Unit trace, one deflection represents four action potentials. Panels A and C show responses to left atrial injection of bradykinin before and after lidocaine administration. Panels B and D show cell responses to pinch of skin overlying the left triceps muscle before and after lidocaine perfusion over the heart. Duration of pinch indicated by line below Unit trace.*

Some of the bradykinin injected into this catheter probably traveled to the cranial circulation. In case that did not occur, in one experiment the arterial catheter was threaded into the right vertebral artery. The responses of two spinothalamic cells to intracardiac vs. vertebral administration of bradykinin (2 µg/kg) were compared. Both cells increased their discharge rates in response to intracardiac bradykinin, but were unaffected by the vertebral injection.

Spontaneous Activity

The mean spontaneous activity of 31 cells responding to intracardiac bradykinin was 11 ± 2.3 spikes/second; mean spontaneous activity of 10 nonresponding cells was 4 ± 2.0 spikes/second. This difference was statistically significant ($P < 0.05$).

Afferent Fiber Input

Overall, 13 of 19 (68%) cells receiving only Aδ input responded to intracardiac bradykinin, whereas 13 of 15 (87%) cells receiving Aδ and C-fiber inputs responded. One cell that received only C-fiber input did not respond.

The influence of afferent input on activity of spinothalamic cells responding to bradykinin is summarized in Figure 6. In panel A, the spontaneous activity of cells receiving only Aδ input is compared to spontaneous activity of cells receiving both Aδ- and C-

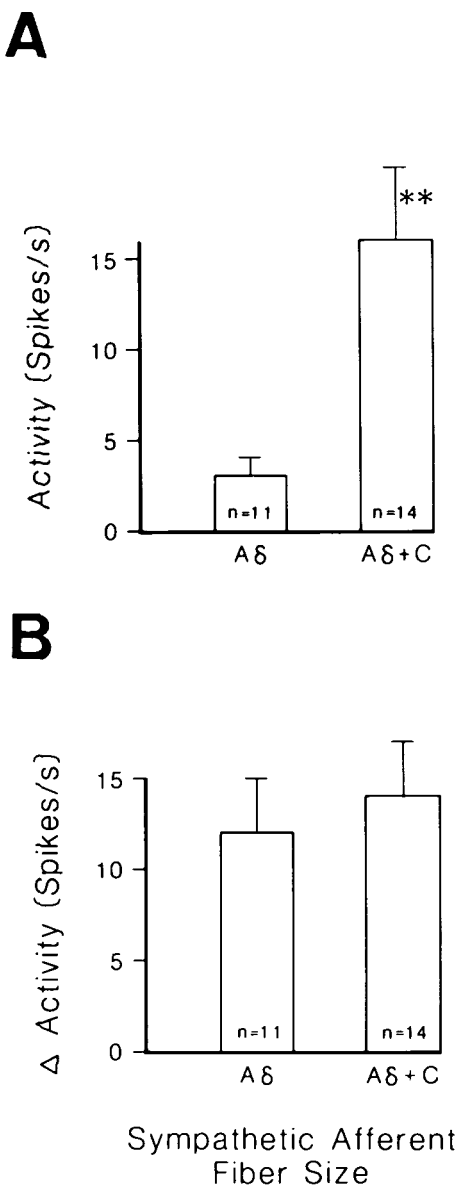

FIGURE 6. *Relationship of type of visceral afferent input on spontaneous activity (panel A) and change in activity of spinothalamic cells to intracardiac bradykinin (panel B). **P < 0.01.*

fiber inputs. Cells receiving both inputs had a significantly ($P < 0.01$) higher background discharge rate than did cells receiving only Aδ input (16 ± 3.9 vs. 3 ± 1.4, respectively). Panel B shows the effect of afferent input on change in discharge rate in response to intracardiac bradykinin. The increases in discharge rate between the two groups were not different. However, since the background discharge rate of cells receiving only Aδ input was lower, the percentage change in activity of Aδ cells (400%) was greater than for cells receiving both inputs (88%).

Anatomy

Histological reconstruction of the sites at which 35 of the 41 spinothalamic cells were located was successfully completed, and maps of representative segments are presented in Figure 7. Cells were located in the monkey's equivalent of laminae I, IV, V, and VII. Cells in more rostral segments tended to be located more dorsally in the spinal cord. Spinothalamic neurons were often found in clusters in this study. Clusters of spinoreticular and spinothalamic neurons have also been found by other investigators (Menétrey et al., 1980; G. Kevetter and W. D. Willis, personal communication). Although we made no attempt to verify this observation by anatomical techniques, the clusters extended approximately 200 μm in the rostral-caudal dimension, 100 μm mediolaterally, and 500 μm dorsoventrally.

The relationship of laminar location to spontaneous activity of cells responding to bradykinin is presented in panel A of Figure 8. Background discharge rates were generally quite variable, so that no pattern of cell spontaneity among laminae IV, V, and VII emerged. However, lamina I cells had very little spontaneous activity, although, due both to variability and

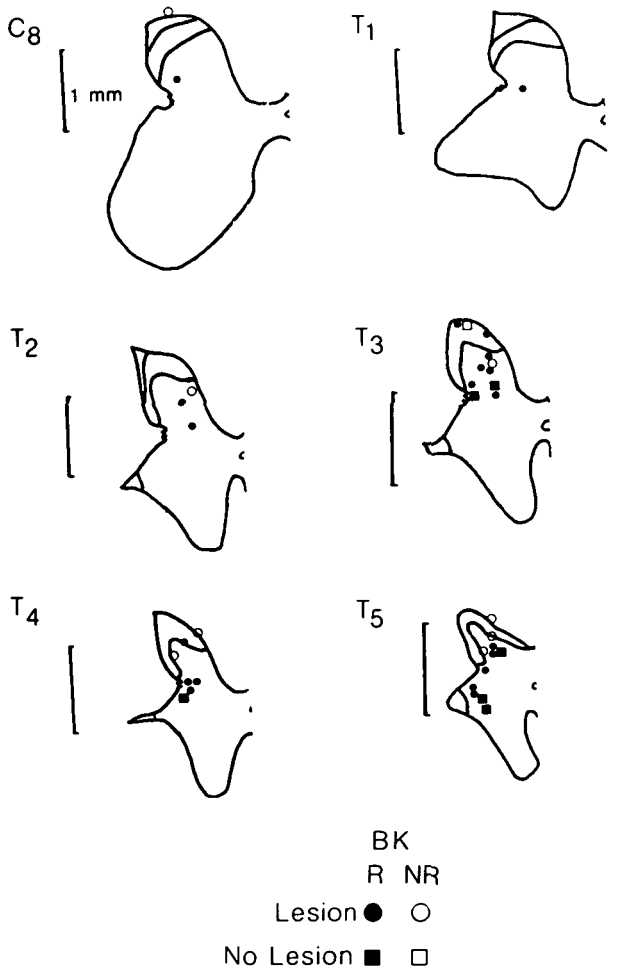

FIGURE 7. *Cord locations of spinothalamic cells. R = response, NR = no response to intracardiac bradykinin. Lesion = cell location marked by electrolytic lesion. No lesion = cell location marked by inserting glass electrode in track where cell found, and plotting cell depth, determined from microdrive reading, on the electrode track.*

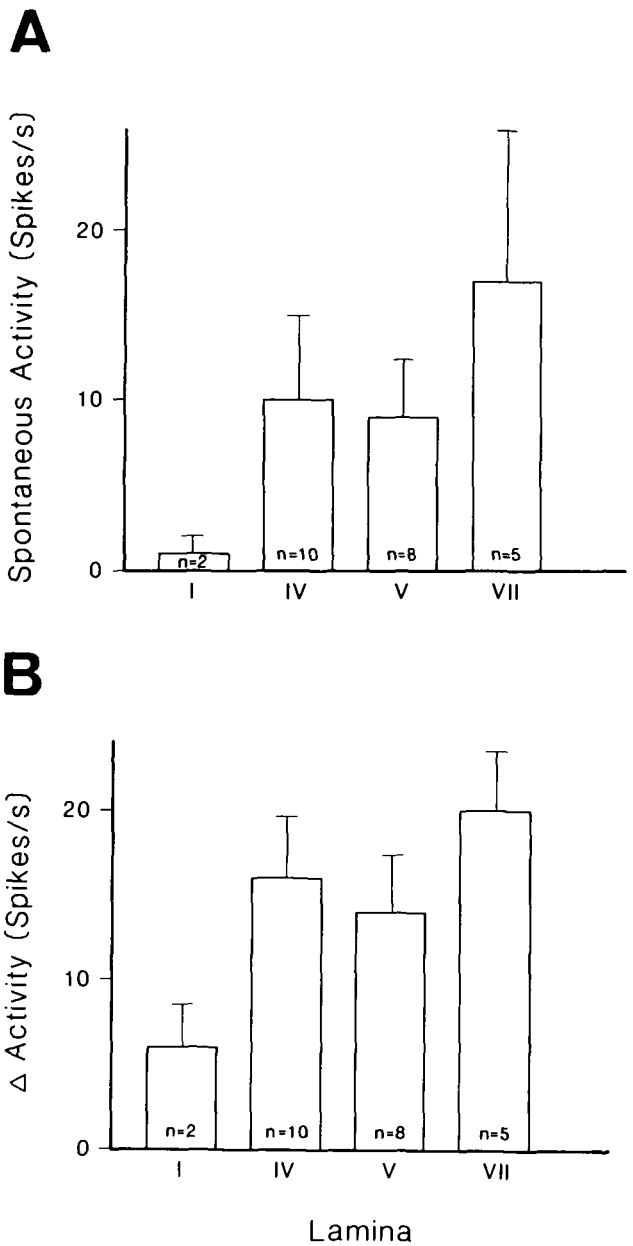

FIGURE 8. *Relationship of laminar cell location to spontaneous activity (panel A) and change in cell activity in response to intracardiac bradykinin (panel B). Only those cells marked by lesions were used for this figure.*

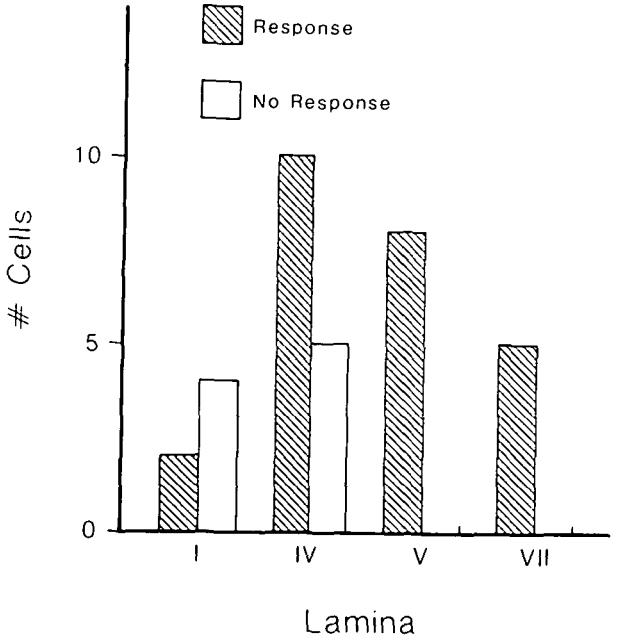

FIGURE 9. *Relationship of laminar cell location to number of cells responding and not responding to intracardiac bradykinin.*

small sample size, analysis of variance failed to detect a significant difference. Nevertheless, our population of six lamina I cells (including cells not responding to bradykinin) exhibited lower spontaneity than cells located more ventrally. Panel B of Figure 8 shows the relationship of laminar cell location to change in discharge rate in response to intracardiac bradykinin. Again, no statistical differences were found among laminae, although cells in lamina I seemed less responsive than cells in the other laminae.

Cells responding and not responding to intracardiac bradykinin are segregated according to lamina in Figure 9. Cells located in the intermediate zone and the ventral portion of the dorsal horn were more likely to respond to intracardiac bradykinin than cells in more dorsal laminae. On the other hand, there were no obvious differences in proportion of responding and nonresponding cells among segments T_3, T_4, and T_5. The number of cells studied in the other segments was not sufficient to make a conclusion.

There were no differences in the spontaneous activities or responses to bradykinin among cells in T_3, T_4, and T_5.

Discussion

Bradykinin Responses

In previous work (Blair et al., 1981b), we noted that every thoracic spinothalamic neuron studied responded to electrical stimulation of visceral afferent fibers. The present study confirms this observation. Since electrical stimulation is not a physiological means of activating spinothalamic cells, a more natural stimulus, bradykinin, was selected for "physiological" activation of afferents impinging on spinothalamic cells. We chose bradykinin because it has been implicated in the pain associated with angina pectoris (Rowe et al., 1963). Bradykinin injected into the heart stimulates Aδ- and C-fiber afferents (Uchida and Murao, 1974; Baker et al., 1980; Lombardi et al., 1981) and produces a pseudaffective response indicative of pain (Guzman et al., 1962). Furthermore, bradykinin levels in coronary sinus blood increase following coronary artery occlusion (Kimura et al., 1973). Thus, the possibility exists that bradykinin is involved in the production of pain associated with angina pectoris and myocardial infraction.

Results of the present study indicate that three-fourths of tested thoracic spinothalamic cells respond

to intracardiac injections of bradykinin. The mean latency to increased activity was 15 seconds and mean cell discharge rate increased 127%. This study is the first to demonstrate that application of a noxious stimulus to the heart can excite cells of an identified ascending tract, the spinothalamic tract, which is presumably involved in transmitting visceral noxious information to the brain. Hence, the spinothalamic tract probably is involved in the sensation of cardiac pain. This work extends the observations of Foreman and Ohata (1980), who showed that coronary artery occlusion could excite, inhibit, or have no effect on neurons not tested for ascending projections in the feline spinal cord. In the present study, 30 of 31 cells responding to bradykinin were excited; the activity of the exception became entrained with the cardiac cycle. The response of an individual cell probably depends on its function. Cells signalling a noxious event would presumably be excited, but cells involved in cardiovascular adjustments to the noxious event may have variable responses, depending upon their roles in the adjustments. The observation that not every spinothalamic cell responded to intracardiac bradykinin illustrates the complexity of spinal function, and indicates that the spinal cord is not a simple conduit to the brain. Instead, integration of peripheral and descending inputs can occur at the cord level.

The long latency to onset of cell response could indicate that receptors responsible for increased cell activity are located elsewhere than in the heart. This possibility is unlikely, since bradykinin applied epicardially, intracoronary, or into the left atrium activated Aδ- and C-fibers originating in the heart with average latencies of 12–18 seconds (Uchida and Murao, 1974; Baker et al., 1980; Lombardi et al., 1981). Hence, latency to activation of spinothalamic cells is similar to that of the visceral afferents presumably impinging on them. Thus, the long latency cannot be ascribed merely to presence of receptors other than in the heart. Instead, bradykinin may be involved in a cascade of events which must occur before afferent fibers can be activated; for example, bradykinin may interact with prostaglandins (Needleman, 1976) or be metabolized to a more active polypeptide (Marceau et al., 1981) before afferent fibers would be excited. These events would take time to occur, resulting in a long latency to response.

Evidence that most afferent fibers stimulated by bradykinin and affecting the activity of spinothalamic cells originated in the heart is provided by several observations. First, the latency to onset of the blood pressure response was 9 seconds earlier than latency to increased cell activity, and cell activity returned to control rate before blood pressure. Thus, changes in cell activity were not synchronous with alteration of blood pressure. Second, control injections were made into the descending aorta just distal to the aortic arch. Half of the spinothalamic cells exhibited either no change or a decrease in activity upon aortic bradykinin injection. The other half showed a significantly smaller increase in cell activity upon aortic bradykinin injection compared to left atrial bradykinin injection. These results indicate that, although intracardiac bradykinin will be distributed to tissues other than the heart, the most significant effect on spinothalamic cells originates from the heart. However, the circulating bradykinin could stimulate other peripheral receptors which can influence spinothalamic cell activity. Third, blood pressure responses were not tachyphylactic, but some cells were tachyphylactic to bradykinin. If cells were responding only to a blood pressure change, their discharge rates should not be tachyphylactic to repeated bradykinin injections. Fourth, lidocaine perfused over the heart diminished or abolished cells' responses to intracardiac bradykinin. Finally, bradykinin injected only into the cerebral circulation did not excite spinothalamic cells. These observations indicate that the major input on spinothalamic cells, when bradykinin was injected into the heart, originated in the heart.

Bradykinin vs. Electrical Stimulation

All thoracic spinothalamic neurons so far studied, including those in this study and a previous one (Blair et al., 1981b), could be excited by electrical stimulation of afferent fibers in the cardiac nerve and ansa subclavia or the sympathetic chain. On the other hand, only 75% of these cells responded to intracardiac bradykinin injection. If all spinothalamic cells receive inputs from visceral afferent fibers, as indicated by electrical stimulation, then an explanation of why intracardiac bradykinin did not excite all cells is appropriate. At least three possibilities exist to explain this phenomenon. (1) Some afferent fibers in the nerves stimulated do not originate in the heart, but may originate in lungs, blood vessels, or other visceral tissue. If the spinothalamic cells not responding to bradykinin received input only from noncardiac sources, then intracardiac bradykinin would have no effect on cell activity but electrical stimulation of nerves would affect it. (2) Nerve stimulation excites most, if not all, afferent fibers in nerves, but intracardiac bradykinin does not excite all afferent fibers originating in the heart. Those cells not responding to bradykinin do not receive sufficient input from the activated afferent fibers to be excited. However, electrical stimulation excites enough fibers to activate the cell. (3) Descending influences from higher centers may be different in cells not responding to intracardiac bradykinin compared to responding cells. Electrical stimulation of nerves excites a sufficient number of fibers to overcome the descending inhibition, although intracardiac bradykinin does not excite enough fibers. This possibility is likely. Cells not responding to intracardiac bradykinin had a significantly lower spontaneous activity than did responding cells. The lower spontaneity may reflect greater descending inhibition. To summarize, these possibilities provide reasonable explanations of why some cells respond to electrical stimulation of visceral fibers, but not natural stimulation by bradykinin.

Tachyphylaxis

Previous investigations indicate that the development of tachyphylaxis to bradykinin may or may not be observed. Investigators examining peripheral direct or reflex actions of bradykinin do not report the development of tachyphylaxis if at least 5 minutes elapsed between doses (Neto et al., 1974; Staszewska-Barczak et al., 1976; Tallarida et al., 1979; Reimann and Weaver, 1980). In the present study, at least 10 minutes elapsed between injections of bradykinin, and no tachyphylaxis of blood pressure or heart rate responses was observed.

On the other hand, tachyphylaxis of afferent fibers to bradykinin has sometimes been observed. Baker et al. (1980) noted tachyphylaxis in afferent fibers originating in the heart and great vessels, but other laboratories did not (Uchida and Murao, 1974; Nishi et al., 1977). Somatic afferent fibers also exhibited tachyphylaxis to bradykinin (Beck and Handwerker, 1974). In addition, the perception of pain in man was diminished upon repeated applications of bradykinin to a blister base (Elliott et al., 1960). Taken together, these studies indicate that at least some afferent fibers exhibit tachyphylaxis to bradykinin.

Tachyphylaxis of central neurons to intra-arterial injection of bradykinin has not been described. Several studies have examined the responses of central neurons to bradykinin injected into either the femoral artery or a more distal branch of the femoral artery. These studies examined how central neurons responded to chemical excitation of somatic, but not visceral, afferent fibers. Responses of feline dorsal horn cells (Besson et al., 1972; Besson et al., 1975; Randić and Yu, 1976) and primate spinothalamic cells (Levante et al., 1975; Foreman et al., 1979) were not reported as tachyphylactic to intra-arterial injection of bradykinin. Furthermore, tachyphylaxis of cells in the mesencephalic reticular formation or nucleus reticularis gigantocellularis was not noted (Guilbaud et al., 1973; Lombard et al., 1975). However, close examination of the data in some of these papers (Guilbaud et al., 1973; Besson et al., 1975; Lombard et al., 1975) reveals that tachyphylaxis of some neurons did, indeed, occur. We found 41% of thoracic spinothalamic cells were tachyphylactic to intracardiac bradykinin. Although the reasons why some central neurons exhibit tachyphylaxis and others do not are not clear, one explanation may relate to the type of afferent input elicited by bradykinin. Thus, stimulation of somatic afferent fibers by bradykinin may not be as likely to produce tachyphylactic responses of spinal neurons as stimulation of visceral afferent fibers. Furthermore, the possibility exists that the spinothalamic cells developing tachyphylaxis in the present study received input from tachyphylactic afferent fibers, while the other spinothalamic cells received input from nontachyphylactic afferents. Another possibility is that part of the tachyphylactic response may have a central origin. Although the authors did not describe this phenomenon, data from Besson et al. (1975) indicate that tachyphylaxis to intra-arterial bradykinin can be attenuated by cold blocking the spinal cord, implying that descending influences might affect the development of tachyphylaxis.

Receptors

Both chemosensitive and mechanosensitive receptors in the heart, as described by Baker et al., 1980, probably contribute to the responses of spinothalamic neurons to bradykinin. Bradykinin excites afferent fibers sensitive to mechanical events in the heart, as well as chemosensitive fibers (Baker et al., 1980; Lombardi et al., 1981). However, it has not been previously demonstrated whether these fibers provide input to spinothalamic neurons. The response characteristics of cells in the present study indirectly indicate that at least some spinothalamic cells receive both types of inputs. Eighteen cells (58% of responders) exhibited an increasing discharge rate in response to bradykinin, but exhibited no cardiac rhythmicity. This response is similar to that reported for chemosensitive afferents (Baker et al., 1980). One cell's activity became entrained with the cardiac cycle in response to bradykinin, but did not obviously increase its discharge rate. Thus, bradykinin seemed to sensitize the cell to cardiac mechanical events. This effect could be accomplished by an action on mechanosensitive afferents. The other 12 responding cells (40%) increased their discharge rates, and their activity became entrained with the cardiac cycle. These cells, then, probably received input from both mechanosensitive and chemosensitive fibers that were excited by bradykinin. The observation that some spinothalamic cells are sensitive to cardiac events under certain circumstances may provide a neural basis for the fact that people can feel abnormal ventricular beats, and the sensation is not necessarily painful (Herrmann, 1945; Scherf and Boyd, 1958). To summarize, most spinothalamic cells appear to be sensitive mainly to chemical stimulation of the heart, although a significant proportion can become sensitive to mechanical events in the heart.

Afferent Fiber Classes

As discussed earlier, intracardiac bradykinin excites both Aδ and C-fiber afferents. Baker et al. (1980) suggested that the C-fibers activated by bradykinin were cardiac nociceptors. Since spinothalamic cells are known to transmit noxious information, a logical extension of the theory is the suggestion that spinothalamic cells receiving C-fiber input would respond to bradykinin, and those cells not receiving C-fiber input would not respond to bradykinin. Guilbaud et al. (1976) presented data supporting this theory for somatic afferents. They showed that feline lumbar dorsal horn cells receiving only somatic A-fiber input were insensitive to intra-arterial bradykinin, but cells receiving only C-fiber input were responsive. Furthermore, responses of cells receiving both inputs were similar before and after cold block of the A-

fibers, again suggesting the importance of C-fiber afferents in the transmission of noxious information (Guilbaud et al., 1976). On the other hand, results of the present study do not support the hypothesis that C-fibers alone are required for visceral pain sensation. The following observations are relevant to this point. (1) Sixty-eight percent of cells receiving only Aδ input responded to bradykinin. None should have responded if C-fibers were required for response to bradykinin. (2) Three cells receiving C-fiber input did not respond to intracardiac bradykinin. This result indicates that all cells receiving C-fiber input do not respond. However, an alternative explanation, as described earlier, is that these cells were under a greater degree of descending inhibitory control; therefore, their responsiveness may have been masked. (3) The percentage increase in activity of cells receiving only Aδ input (400%) was far greater than for cells receiving both inputs (88%). The larger percentage change may signal important information regarding noxious input to the thalamus. Thus, Aδ fibers may be important in transmitting noxious cardiac information to the spinothalamic cells in the primate, and less important in transmitting somatic pain in cats. It is evident that the signaling of noxious events is a complex phenomenon, and cannot be explained simply on the basis of a change in activity of one class of afferent fibers. Instead, we agree with Guilbaud et al. (1976) that the study of pain transmission must include an examination of the output of the central neuron after afferent information has been processed.

Cells receiving both Aδ and C-fiber inputs were found to have a greater spontaneous discharge rate than cells receiving only Aδ fibers. Similar observations have previously been made (Guilbaud et al., 1976; Blair et al., 1981b). At least two explanations could account for these observations. (1) Cells with both inputs receive more synaptic input from the periphery than cells with only Aδ input, resulting in more EPSPs and an increased discharge rate. (2) Cells with Aδ input are under a greater degree of tonic descending inhibitory control than are cells with both inputs.

Location

Neither the segmental nor the laminar location of spinothalamic cells appeared to affect their responsiveness to intracardiac bradykinin, except that lamina I cells seemed to be less responsive than cells in other laminae. Foreman et al. (1979) made a similar observation for lamina I cells in the lumbosacral cord when bradykinin was injected into the sural artery. Lamina I cells may not be as strongly involved in processing input from chemosensitive afferents as cells in other laminae.

In conclusion, the present study has demonstrated that the intracardiac injection of bradykinin can excite thoracic spinothalamic cells in the primate. These results provide a potential neural substrate for the sensation of myocardial pain.

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References

Baker DG, 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

Beck PW, Handwerker HO (1974) Bradykinin and serotonin effects on various types of cutaneous nerve fibers. Pfluegers Arch **347:** 209–222

Besson JM, Conseiller C, Hamann KF, Maillard MC (1972) Modifications of dorsal horn cell activities in the spinal cord, after intra-arterial injection of bradykinin. J Physiol (Lond) **221:** 189–205

Besson JM, Guilbaud G, LeBars D (1975) Descending inhibitory influences exerted by the brain stem upon the activities of dorsal horn lamina V cells induced by intra-arterial injection of bradykinin into the limbs. J Physiol (Lond) **248:** 725–739

Blair RW, Weber RN, Holmes HR, Foreman RD (1981a) Activation of primate spinothalamic tract cells following intracardiac bradykinin injection and during coronary artery occlusion (abstr). Soc Neurosci **7:** 534

Blair RW, Weber RN, Foreman RD (1981b) Characteristics of primate spinothalamic tract neurons receiving viscerosomatic convergent inputs in T_3–T_5 segments. J Neurophysiol **46:** 797–811

Elliott DF, Horton EW, Lewis GP (1960) Actions of pure bradykinin. J Physiol (Lond) **153:** 473–480

Foreman RD, Ohata CA (1980) Effects of coronary artery occlusion on thoracic spinal neurons receiving viscerosomatic inputs. Am J Physiol **238:** H667–H674

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, Schmidt RD, Willis WD (1979) Effects of mechanical and chemical stimulation of fine muscle afferents upon primate spinothalamic tract cells. J Physiol (Lond) **286:** 215–231

Foreman RD, Blair RW, Weber RN (1981a) Effects on T_3 to T_5 primate spinothalamic tract cells of injecting bradykinin into the heart. Pain (suppl 1): S212

Foreman RD, Hancock MB, Willis WD (1981b) Responses of spinothalamic tract cells in the thoracic spinal cord of the monkey to cutaneous and visceral inputs. Pain **11:** 149–162

Guilbaud G, Besson JM, Oliveras JL, Wyon-Maillard MC (1973) Modifications of the firing rate of bulbar reticular units (nucleus gigantocellularis) after intra-arterial injection of bradykinin into the limbs. Brain Res **63:** 131–140

Guilbaud G, LeBars D, Besson JM (1976) Bradykinin as a tool in neurophysiological studies of pain mechanisms. *In* Advances in Pain Research and Therapy, Vol. I, edited by JJ Bonica, D AlbeFessard. New York, Raven Press, pp 67–73

Guzman R, Braun C, Lim RKS (1962) Visceral pain and the pseudaffective response to intra-arterial injection of bradykinin and other algesic agents. Arch Int Pharmacodyn Ther **136:** 353–383

Hancock MB, Foreman RD, Willis WD (1975) Convergence of visceral and cutaneous input onto spinothalamic tract cells in the thoracic spinal cord of the cat. Exp Neurol **47:** 240–248

Hermann G (1945) Disturbances of the heart beat. *In* Diagnosis and

Treatment of Cardiovascular Disease, vol 1, ed 3, edited by WD Stroud. Philadelphia, FA Davis Co, pp 859-942
Kimura E, Hashimoto K, Furukawa S, Hayakawa H (1973) Changes in bradykinin level in coronary sinus blood after the experimental occlusion of a coronary artery. Am Heart J **85:** 635-647
Kuru M (1949) Sensory Paths in the Spinal Cord and Brain Stem of Man. Tokyo, Sogensya
Levante A, Lamour Y, Guilbaud G, Besson JM (1975) Spinothalamic cell activity in the monkey during intense nociceptive stimulation: intra-arterial injection of bradykinin into the limbs. Brain Res **88:** 560-564
Lombard MC, Guilbaud G, Besson JM (1975) Effects of the intra-arterial injection of bradykinin into the limbs, upon the activity of mesencephalic reticular units. Eur J Pharmacol **30:** 298-308
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
Marceau F, Gendreau M, Barabé J, St.-Pierre S, Regoli D (1981) The degradation of bradykinin (BK) and of des-Arg^9-BK in plasma. Can J Physiol Pharmacol **59:** 131-138
Menétrey D, Chaouch A, Besson JM (1980) Location and properties of dorsal horn neurons at origin of spinoreticular tract in lumbar enlargement of the rat. J Neurophysiol **44:** 862-877
Milne RJ, Foreman RD, Giesler GJ Jr, Willis WD (1981) Convergence of cutaneous and pelvic visceral nociceptive inputs onto spinothalamic neurons. Pain **11:** 163-183
Needleman P (1976) The synthesis and function of prostaglandins in the heart. Fed Proc **35:** 2376-2381
Neto FR, Basil J, Antionio A (1974) Bradykinin-induced coronary chemoreflex in the dog. Naunyn Schmiedebergs Arch Pharmacol **283:** 135-142
Nishi K, Sakanashi M, Takenaka F (1977) Activation of afferent cardiac sympathetic nerve fibers of the cat by pain producing substances and by noxious heat. Pfluegers Arch **372:** 53-61
Randić M, Yu HH (1976) Effects of 5-hydroxytryptamine and bradykinin in cat dorsal horn neurons activated by noxious stimuli. Brain Res **111:** 197-203
Reimann KA, Weaver LC (1980) Contrasting reflexes evoked by chemical activation of cardiac afferent nerves. Am J Physiol **239:** H316-H325
Rexed B (1954) A cytoarchitectonic atlas of the spinal cord in the cat. J Comp Neurol **100:** 297-379
Rowe GG, Afonso S, Castillo CA, Lioy F, Lugo JE, Crumpton CW (1963) The systemic and coronary hemodynamic effects of synthetic bradykinin. Am Heart J **65:** 656-663
Ruch TC (1947) Visceral sensation and referred pain. *In* Howell's Textbook of Physiology, ed 15, edited by JF Fulton. Philadelphia, Saunders, pp 385-401
Scherf D, Boyd LF (1958) Cardiovascular Diseases. New York, Grune & Stratton, pp 635-671
Sokal RR, Rohlf FJ (1969) Biometry. San Francisco, WH Freeman and Co
Staszewska-Barczak J, Ferreira SH, Vane JR (1976) An excitatory nociceptive cardiac reflex elicited by bradykinin and potentiated by prostaglandin and myocardial ischemia. Cardiovasc Res **10:** 314-327
Takahashi H, Buñag RD (1981) Centrally induced cardiovascular and sympathetic nerve repsonses to bradykinin in rats. J Pharmacol Exp Ther **216:** 192-197
Tallarida G, Baldoni F, Peruzzi G, Brindisi F, Raimondi G, Sangiorgi M (1979) Cardiovascular and respiratory chemoreflexes from the hindlimb sensory receptors evoked by intra-arterial injection of bradykinin and other chemical agents in the rabbit. J Pharmacol Exp Ther **208:** 319-329
Trevino DL, Coulter JD, Willis WD (1973) Location of cells of origin of spinothalamic tract in lumbar enlargement of the monkey. J Neurophysiol **36:** 750-761
Uchida Y, Murao S (1974) Bradykinin induced excitation of afferent cardiac sympathetic nerve fibers. Jpn Heart J **15:** 84-91
White JC (1957) Cardiac pain. Circulation **16:** 644-659
White JC, Bland EF (1948) The surgical relief of severe angina pectoris: methods employed and end results in 83 patients. Medicine **27:** 1-42
White JC, Garrey WE, Atkins JA (1933) Cardiac innervation: Experimental and clinical studies. Arch Surg **26:** 765-786

INDEX TERMS: Cardiac pain · Sympathetic afferent fibers · Viscerosomatic convergence