Prevention of High Incidence of Neurally Mediated Ventricular Arrhythmias by Afferent Nerve Stimulation in Dogs

Xiaohong Zhou, MD; Frank L. Vance IV; Anthony L. Sims; Catherine M. Sreenan, BS; Raymond E. Ideker, MD, PhD

Background—This study tested the hypothesis that the high incidence of ventricular arrhythmias caused by hypothalamic stimulation during acute myocardial ischemia could be attenuated by afferent nerve stimulation and investigated the cardiac mechanisms for those effects.

Methods and Results—In 18 anesthetized dogs, stimulating electrodes were implanted in the hypothalamus and in the isolated left peroneal nerve. The chest was opened and approximately 100 plunge needles were inserted into the ventricles for 3-D activation mapping. Each animal underwent 4 episodes of 2.5 minutes of acute myocardial ischemia. The first and fourth episodes served as controls. During the second and third episodes, animals received either hypothalamic stimulation, peroneal nerve stimulation, or both. Hypothalamic stimulation significantly increased the incidence of ventricular arrhythmias. This high incidence was reduced 34% by simultaneous stimulation of the hypothalamus and peroneal nerve. 3-D mapping showed a focal origin for all ventricular arrhythmias. Hypothalamic stimulation increased the number of arrhythmic beats and decreased the coupling interval between each arrhythmic beat and the preceding beat. These effects were reduced by peroneal nerve stimulation.

Conclusions—Alteration in autonomic tone by hypothalamic stimulation causes a high incidence of ventricular arrhythmias during acute myocardial ischemia that can be decreased by afferent nerve stimulation. (Circulation. 2000;101:819-824.)

Key Words: arrhythmia ■ nervous system, autonomic ■ mapping

Experimental and clinical evidence supports the hypothesis that abnormal activity of the autonomic nervous system modulates the electrophysiological stability of the myocardium, especially during coronary artery disease, causing a high incidence of ventricular arrhythmias and hence sudden cardiac death. The hypothalamus is one of the major centers that control cardiovascular responses associated with emotions, stress, and defense in both humans and animals. Many experiments have demonstrated that electrical or chemical stimulation of the hypothalamus increases neural discharges in the sympathetic nervous system and induces ventricular tachyarrhythmias in animals. In addition, the cardiovascular centers in the central nervous system can be likewise modulated by afferent nerve stimulation. Stimulation of afferent nerves can produce beneficial effects for patients with coronary artery disease and reduce ventricular arrhythmias caused by hypothalamic stimulation in rabbits.

It is not known if the same effect exists in larger animals. In addition, the cardiac mechanisms for neurally mediated ventricular arrhythmias are not completely known. For example, it is not clear if these arrhythmias are caused by reentrant or focal activity. The goals of the present study were (1) to test if afferent nerve stimulation could prevent ventricular arrhythmias induced by hypothalamic stimulation during acute myocardial ischemia in dogs and (2) to investigate the electrophysiological mechanisms for these arrhythmias by cardiac mapping.

Methods
This study was approved by the Institutional Animal Care and Use Committee at The University of Alabama at Birmingham. It conformed to the American Heart Association Guidelines for the use of research animals.

Animal Preparation
Eighteen mongrel dogs (weight 12 to 15 kg) were anesthetized with thiopental (6 to 12 mg/lb) for induction and then isoflurane (1% to 3%) inhalation with 0.5% oxygen for maintenance through a respiratory (Harvard Apparatus). A catheter was inserted into the descending aortic artery through the right femoral artery to record arterial blood pressure and to obtain blood for measuring blood gas and electrolytes. Ringer’s lactate was continuously infused through a foreleg vein and supplemented with potassium chloride, sodium...
bicarbonate, and calcium chloride when needed to maintain normal metabolic status. Arterial blood pressure and the surface ECG were continuously displayed and recorded.

Preparation for Hypothalamic Stimulation
Each animal’s head was fixed with a dog stereotaxic apparatus (Stoelting Co), and a 5-mm hole was drilled through the skull. On the basis of stereotaxic coordinates (rostral, 18 mm; lateral, 1.5±1 mm; and vertical, 10.5±1 mm),11 a concentric stainless steel electrode with a 0.2-mm tip was inserted into the posterior hypothalamus, which is considered as a sympathetic center.5,12 The electrode was fixed on the skull by epoxy glue and protected by a cup.

Preparation for Peroneal Nerve Stimulation
The left peroneal nerve was isolated 1 to 2 cm along its length. A bipolar stimulating electrode was held against the nerve and the twitch threshold determined. The nerve was then cut and its central end was hooked by a bipolar hook stimulating electrode so that only the afferent fibers would be stimulated.

Isolation of the Left Anterior Descending Coronary Artery
The chest was opened through a median sternotomy and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was dissected free just above its first diagonal branch. A suture was placed around the artery and passed through a piece of polyethylene tubing for coronary occlusion.

3-D Cardiac Mapping
Approximately 100 plunge needles were used for 3-D activation mapping throughout the ventricular free walls. Electrodes consisting of 50-μm diameter silver wires were insulated with epoxy resin and placed within 21-gauge stainless steel needles. Left ventricular plunge needles contained 4 electrodes separated by 3.5 mm. Right ventricular needles contained 3 electrodes separated by 2.5 mm. Depending on heart size: 66 to 72 left ventricular plunge needles and 30 to 36 right ventricular plunge needles were used. Needles were inserted perpendicular to the epicardium to record from the ventricular free walls transmurally and from the outer portions of the septum with relatively equal spacing between needles. The electrodes were connected to a 528-channel mapping system and allowed to stabilize for at least 1 hour before the study protocol was initiated. Signals were digitally recorded simultaneously in unipolar mode with respect to a right leg electrode. The signals were recorded at 10× gain, 0.01-Hz high-pass filter, 1000-Hz low-pass filter, and 2000 samples per second per channel. Data were stored on tape and optical disk for off-line analysis.

Experimental Protocols
First, the effects of 10 seconds of hypothalamic stimulation and peroneal nerve stimulation on autonomic tone were determined. The hypothalamus was stimulated at 50 Hz with a 2-mA rectangular wave of 1-ms duration. The left peroneal nerve was stimulated at 5 Hz with a 0.3- to 0.6-mA rectangular wave of 1-ms duration, which was 2 to 4 times the muscle twitch threshold determined just before the nerve was cut. Then, each animal underwent 4 brief episodes of acute myocardial ischemia caused by occluding the LAD for 2.5 minutes with at least a 30-minute recovery interval between episodes. There were 3 groups, each having 6 animals (Figure 1). In all groups, the first and fourth ischemic episodes served as controls without neural interventions. In the hypothalamic stimulation (HS) group, HS lasting 30 seconds started 1 minute after the beginning of the second and third ischemic episodes. In the peroneal nerve stimulation (PNS) group, the left peroneal nerve was stimulated throughout the second and third ischemic episodes. In the HS+PNS group, both the hypothalamus and left peroneal nerve were stimulated during the second and third ischemic episodes with the same parameters used in the HS and PNS groups.

After the experiment, each animal was killed with a 10% potassium chloride solution given intravenously. The site of the hypothalamic electrode was marked by applying 200-μA DC to the electrode for 1 minute. The brain and the heart were then removed. The heart was perfused retrogradely through the aorta at 100 cm H2O pressure with saline for 20 minutes, with 10% formalin for another 20 minutes, and fixed in 10% formalin for 24 hours. The plunge needles were then replaced by color-coded Teflon tubes and a geometrical file was created, indicating the location of the plunge needles. All brains fixed in 10% formalin were embedded in gelatin and cut in ~1-mm sections to locate the burned spot that marked the site of HS.

Data Analysis
Activation sequences were visualized by animating the first temporal derivative (dV/dt) of the electrode recordings with a scientific workstation (Sun Microsystems, Inc). Activations were displayed on a polar representation of the ventricular myocardium on the basis of the geometrical file created for that heart (Figure 2). The dV/dt at each electrode was computed as a least-square fit to a parabola of 5 successive time values of the recorded potentials. Activation time was defined as a dV/dt ≤ −0.2 V/s. Activation wave fronts were then animated on the basis of the local activation times. Isochronal maps in this article were hand-drawn, based on individual electrode activation times and locations. The following parameters were identified by examining the animated displays. (1) The early site was defined as the site of earliest activation for each arrhythmic beat. (2) The arrhythmogenic region was considered the region where the early site(s) occurred. For this definition, each heart was divided into 15 regions (Figure 2). Six regions were at the ventricular base, 6 between the apex and base regions and 3 at the apex. (3) The arrhythmic coupling interval was the time from the earliest activation of the preceding sinus beat to the activation time of the early site for that arrhythmic beat, or the time between the earliest activation times of 2 successive arrhythmic beats. (5) The incidence of ventricular arrhythmias was the number of arrhythmic beats during an ischemic episode. (6) The ischemic region was the region supplied by the LAD and its major branches below the occlusion identified on the epicardium (Figure 2).
Heart rate and arterial blood pressure were averaged for 10 consecutive sinus beats at designated times. Changes in heart rate and blood pressure were normalized by dividing the measurements during the interventions by the measurements just before the interventions and multiplying by 100. Statistical analyses were performed with the use of ANOVA (Student-Newman-Keuls test) and the Student’s t test for paired or unpaired data. A value of $P < 0.05$ was considered significant.

**Results**

**Effects of HS and PNS on Heart Rate andBlood Pressure**

Both HS and PNS significantly affected cardiovascular autonomic tone. HS increased blood pressure and heart rate, whereas PNS decreased blood pressure and heart rate (Figures 3 and 4). These effects occurred soon after the application of the intervention and returned to baseline quickly after termination of the intervention (Figure 3), indicating that the mechanism is neural and that the autonomic tone can be quickly regulated through neural stimulation. HS rarely induced ventricular arrhythmias in normal hearts. The magnitude of the responses of heart rate and blood pressure to HS were reduced by concurrent PNS (Figures 3 and 4). Thus HS induced a surge of sympathetic excitation whereas PNS reduced the sympathetic excitation.

**Incidence of Ventricular Arrhythmias During HS and PNS**

Figure 5 shows ECG recordings for 1 animal from 1 minute to 1.5 minutes after the beginning of LAD occlusion. During the first control ischemic episode, 3 ventricular arrhythmic beats occurred, whereas HS during the third ischemic episode caused 32 arrhythmic beats. All 3 arrhythmic QRS complexes in the control episode exhibited similar morphology, whereas those during the HS episode exhibited several different morphologies, implying different origins or activation sequences for the arrhythmic beats during HS. This is confirmed by 3-D cardiac mapping, as discussed below. Figure 5 also shows that the arrhythmic coupling interval was longer for the control ischemic episode (mean value 407 ms) than the ischemic episode with HS (mean value 281 ms).

The mean number of ventricular arrhythmic beats during each episode of 2.5 minutes of acute myocardial ischemia with and without interventions is shown in Figure 6. There were no significant changes in the number of ventricular arrhythmic beats between the first and fourth control ischemic episodes in each group. This implies that any significant changes in the second and third ischemic episodes were caused by the intervention. HS significantly increased the number of ventricular arrhythmic beats compared with the first and fourth control episodes (Figure 6). HS+PNS reduced this high incidence of ventricular arrhythmias by 32% and 36% for the second and third episodes (Figure 6). PNS alone had a tendency to reduce ventricular arrhythmic beats, though there was no statistically significant difference compared with the control episodes (Figure 6).
Mechanisms of Ventricular Arrhythmias During Acute Myocardial Ischemia

All ventricular arrhythmias were shown by 3-D mapping to arise from focal activity. Maps of the 5 beats indicated by arrows in Figure 5 during HS are shown in Figure 7. Sinus activation originated from the endocardium and propagated rapidly across both ventricles (Figure 7A). During HS, an arrhythmic early site occurred focally in the subepicardial ischemic region (Figure 7B). The following arrhythmic beat originated from another location and propagated in a focal pattern (Figure 7C). Another arrhythmic beat originated in the right ventricular epicardium and slowly propagated again in a focal pattern (Figure 7D). As shown in Figure 5, this arrhythmic beat repeated once from the same location. Another arrhythmic beat originated focally from another left ventricular location (Figure 7E) and repeated for 6 beats, as shown in Figure 5B. Figure 7 demonstrates that the ventricular arrhythmias caused by HS during acute myocardial ischemia are multifocal. This multifocal activation pattern was observed in all animals with HS.

The number of ventricular arrhythmic beats during the 30 seconds of HS was 16.7±6.9, which was significantly higher than the 7.4±3.4 arrhythmic beats during HS+PNS. In both cases, the incidence of arrhythmias was higher than during the corresponding 30 seconds of PNS alone (2.9±2.3). The percentage of early sites of these arrhythmic beats from the ischemic region was 76±4% during HS, 90±11% during PNS, and 81±7% during HS+PNS and 87±12% during the corresponding 30-second interval of the control episodes. The number of regions in which arrhythmic early sites occurred during the 30 seconds of HS (4.7±1.2 per ischemic episode) was significantly higher than during the 30-second HS+PNS (2.9±0.8). Both of these values were significantly higher than during the corresponding 30 seconds of acute myocardial ischemia with PNS alone (1.5±0.9 per episode). The percentage of arrhythmic early sites that occurred in the same regions as for the control episodes was 66±22% during HS, 91±11% during PNS, and 70±8% during HS+PNS.

The incidence of 1 beat, 2 consecutive beats, 3 consecutive beats, and >3 consecutive beats during the 30-second intervention was 75, 13, 12, and 11, respectively, for HS episodes; 22, 5, 1, and 0 for PNS episodes; and 49, 14, 4, and 0 for HS+PNS episodes. The arrhythmic coupling interval for the single arrhythmic beats was 282±39 ms for HS episodes, which was significantly shorter than for HS+PNS episodes (311±54 ms) and for PNS episodes (320±59 ms). The arrhythmic coupling interval for runs lasting >2 arrhythmic beats was 250±23 ms for HS episodes, which was again significantly shorter than for HS+PNS episodes (298±32 ms) and PNS episodes (295±36 ms). Thus HS caused a higher incidence of repetitive extrasystoles and arrhythmic firing rate, whereas PNS could counteract the effects of HS.

The early focal sites of origin of the arrhythmias were more widely dispersed during HS episodes than during the control episodes. The early sites of arrhythmic beats during the control ischemic episodes in the HS group were mainly located in the endocardial (58%) and subendocardial (16%) left ventricle (Figure 8A). HS significantly decreased the percentage of early sites in endocardial tissue (39%, P<0.05 vs control), whereas the percentage of early sites in other transmural locations increased (Figure 8A). PNS did not significantly alter the percentage of early sites in endocardial tissue compared with the control (Figure 8B and 8C).

Discussion

The results of the present study in dogs are consistent with those in rabbits, for example, the high incidence of ventricular arrhythmias caused by HS during acute myocardial ischemia can be reduced by PNS. Extending these previous results, the present study also demonstrates that (1) autonomic tone can be quickly modulated by neural stimulation; (2) HS during acute myocardial ischemia causes a high incidence of ventricular arrhythmias by increasing the number of arrhythmic early sites and their firing rate; and (3) the activation pattern of neurally mediated ventricular arrhythmias during acute myocardial ischemia is focal.
It has been documented that interactions between the brain and the heart and alterations in the autonomic centers can produce significant responses in the cardiovascular system.1,2 The hypothalamus is an important autonomic center in the brain that controls cardiovascular responses.1,2 Our results demonstrated that electrical stimulation of the hypothalamus elevated heart rate and blood pressure (Figures 3 and 4), which indicates a sympathetic surge.

Many studies have shown that electrical or chemical stimulation of the hypothalamus also can produce ventricular arrhythmias.3,4,6,7 The present study also demonstrated that ventricular arrhythmias during brief acute myocardial ischemia. Our study further demonstrated that the neurally mediated ventricular arrhythmias during HS were exclusively by focal activity. Compared with acute myocardial ischemia alone, HS during acute ischemia caused a shorter arrhythmic coupling interval, more focal arrhythmic early sites as well as a more dispersed location of these focal sites, and longer runs of arrhythmic beats. These results demonstrate that the alterations in the autonomic nervous system caused by HS during acute myocardial ischemia (1) increase the arrhythmogenic sites and (2) accelerate the firing rate of these sites. Possible reasons for this may be (1) the short period of acute myocardial ischemia (2.5 minutes) and (2) the lack of old myocardial infarction serving as a substrate for reentrant activity.

It has been recognized that afferent nerve excitation can modulate autonomic activity and hence cardiovascular responses.7,8,17 Kiyono et al8 showed that low-intensity PNS caused a decrease in blood pressure and in cardiac sympathetic postganglionic nerve activity. A recent report demonstrated that median afferent nerve stimulation had an antisympathetic effect, resulting in an improvement of myocardial ischemia caused by reflexively induced sympathetic excitation.15 Cardiac autonomic tone can be regulated by afferent nerve stimulation in humans. Chauhan et al10 reported that transcutaneous electrical nerve stimulation of the chest significantly increased resting coronary blood flow and decreased arterial epinephrine concentration in patients with typical angina. However, transcutaneous electrical stimulation of the chest in heart transplantation recipients did not increase resting coronary blood flow. A human study by Nishijo et al9 showed a decrease of heart rate by transcutaneous

Figure 8. Transmural distribution of arrhythmic early sites. Data points are mean±SD of early sites for all arrhythmic beats during the 30-second intervention (episodes 2 and 3) with acute myocardial ischemia and during the corresponding 30-second interval for the control ischemic episodes without intervention (episodes 1 and 4). Ordinate gives the percentage of arrhythmic early sites for each transmural location of recording electrode given on the abscissa. A shows the results for the HS group, with a total of 200 arrhythmic beats during intervention and 39 arrhythmic beats in control episodes. B shows the results for the PNS group, with a total of 35 arrhythmic beats during intervention and 46 arrhythmic beats in control episodes. C shows the results for the HS+PNS group, with a total of 89 arrhythmic beats during intervention and 40 arrhythmic beats in control episodes. LV-1 indicates endocardial left ventricular (LV) electrodes; LV-2, subendocardial LV electrodes; LV-3, subepicardial LV electrodes; LV-4, epicardial LV electrodes; RV-1, endocardial right ventricular (RV) electrodes; RV-2, middle myocardium RV electrodes; and RV-3, epicardial RV electrodes. *Significant difference (P<0.05) compared with the control ischemic episodes.

Transmural distribution of arrhythmic early sites

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Many studies have shown that electrical or chemical stimulation of the hypothalamus also can produce ventricular arrhythmias.3,4,6,7 The present study also demonstrated that HS resulted in a high incidence of ventricular arrhythmias during brief acute myocardial ischemia. Our study further demonstrated that the neurally mediated ventricular arrhythmias during HS were exclusively by focal activity. Compared with acute myocardial ischemia alone, HS during acute ischemia caused a shorter arrhythmic coupling interval, more focal arrhythmic early sites as well as a more dispersed location of these focal sites, and longer runs of arrhythmic beats. These results demonstrate that the alterations in the autonomic nervous system caused by HS during acute myocardial ischemia (1) increase the arrhythmogenic sites and (2) accelerate the firing rate of these sites. Since increased repetitive ventricular arrhythmias are a sign of high risk of sudden cardiac death,13 the present study suggests that alteration of cardiovascular autonomic tone caused by excitation of the hypothalamus may help provoke sudden cardiac death, especially during myocardial ischemia. The precise mechanisms responsible for the induction of ventricular arrhythmias by HS are not known. Possible mechanisms include (1) direct cardiac effects of released neurotransmitters, which may cause triggered activity and increased automaticity14 and (2) HS-induced coronary vasoconstriction, which intensifies myocardial ischemia, contributing to the induction of ventricular arrhythmias.15

The present study found an increase in arrhythmic early sites in subepicardial and epicardial myocardium during HS. This may be caused by the distribution of cardiac sympathetic fibers, which mainly travel on the surface of the ventricles.16 No ventricular fibrillation or reentrant activity occurred during acute myocardial ischemia with or without HS in this study. Possible reasons for this may be (1) the short period of acute myocardial ischemia (2.5 minutes) and (2) the lack of old myocardial infarction serving as a substrate for reentrant activity.

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acupuncture stimulation of the forearm. These changes in heart rate could be affected by atropine and propranolol, which indicates that transcutaneous stimulation caused an increase in cardiac vagal activity and a decrease in cardiac sympathetic activity.2 Consistent with these reports, the results of the present study also demonstrated that the afferent nerve stimulation by means of excitation of the peroneal nerve decreased the heart rate and reduced arterial blood pressure.

The present study further demonstrated that PNS reduced the high incidence of ventricular arrhythmias caused by HS during acute myocardial ischemia. The cause for this decreased incidence may be reduction of the sympathetic surge caused by HS. One possible mechanism is that signals from afferent nerves may inhibit or reduce excitation of some of the autonomic neurons in the pathway from the hypothalamus to the spinal cord.10 A second possible mechanism may involve endogenous opioid peptides in the central nervous system, which have sympathoinhibitory action.18 Xia et al8 demonstrated that the microinjection of anti–β-endorphin, an opioid receptor antagonist, into the medulla could completely block the inhibitory effect of PNS on ventricular arrhythmias induced by HS. Yet another possible mechanism may be the improvement of coronary circulation to the ventricles caused by afferent nerve stimulation,10,17 which may increase myocardial electrical stability by decreasing ischemia.

Limitations

First, the results of the study were obtained with animals under general anesthesia. As a result, the strength of the HS and PNS was probably higher than that required for similar effects in conscious animals.

Second, the study does not provide information about the neural traffic from the hypothalamus to the heart, nor about the interaction between autonomic centers and the signals from afferent nerves, nor about the interaction between sympathetic and parasympathetic activation.

Third, repeated LAD occlusion raises the possibility of ischemic preconditioning. We used this technique because other studies have demonstrated that 2- to 5-minute periods of coronary artery occlusion can reliably induce ventricular arrhythmias.19 Ischemic preconditioning typically requires >3 periods of 3-minute coronary occlusion with 5-minute recovery intervals between episodes,20 whereas we used a 2.5-minute interval of occlusion with >30 minutes of recovery, which may not have established ischemic preconditioning.

Fourth, the definition of the ischemic region was not precise because the ischemic ventricular myocardium was not histologically examined, nor was the distribution of coronary blood flow determined.

Conclusions

Sudden cardiac death usually is caused by malignant ventricular arrhythmias, especially during ischemic heart disease. The present study shows that stimulation of the hypothalamus, an important emotional and defense center in the brain, induces a high incidence of repetitive extrasystoles, which are an index of vulnerability to ventricular fibrillation. Thus neural impulses from higher centers in the brain may be transient risk factors for sudden cardiac death. The reduction of these neurally mediated ventricular arrhythmias by PNS may be important in the prevention of arrhythmias in patients who have acute myocardial ischemia with superimposed changes in the autonomic nervous system and hence increased cardiac electrical instability. Patients may benefit from signals that counteract these changes in the autonomic nervous system and reduce the likelihood of the occurrence of malignant ventricular arrhythmias.

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