Delayed Afterdepolarizations Elicited In Vivo by Left Stellate Ganglion Stimulation

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Activation of cardiac sympathetic nerves is recognized as a triggering factor for cardiac arrhythmias. However, the mechanisms involved have only been speculated. Because evidence from studies in vitro has established a relation between catecholamines, delayed afterdepolarizations (DAD), and triggered rhythms, it seemed possible that in vivo adrenergic activation also might lead to the development of DAD. Because very little evidence was available for DAD in vivo, we have evaluated whether monophasic action potential (MAP) recording with a contact electrode could be a suitable technique for the detection of DAD from the endocardium of anesthetized cats. In six animals, atrial pacing and graded aortic constriction were performed during MAP recording to assess MAP stability during hemodynamic changes, and in no cases were modifications of the baseline observed. In 11 cats, calcium gluconate (0.5 g) and G-strophanthin (100 μg) were administered. Action potential duration at 50% (APD$_{50}$) and 90% (APD$_{90}$) repolarization were reduced (from 138 ± 16 to 122 ± 18 msec, $p<0.02$, and from 163 ± 23 to 149 ± 20 msec, $p<0.025$, respectively). In eight of 11 (73%) animals, DAD were elicited with a mean amplitude of 1.2 ± 0.4 mV. In 14 cats, the left stellate ganglion was stimulated for 45 seconds. APD$_{50}$ and APD$_{90}$ decreased (from 153 ± 15 to 145 ± 16 msec, $p<0.005$, and from 176 ± 18 to 165 ± 13 msec, $p<0.001$, respectively). DAD were induced in 10 of 14 animals (71%) with a mean amplitude of 1.2 ± 0.3 mV. These results show that DAD can be induced in vivo by administration of calcium and digitalis and by activation of the cardiac sympathetic nerves. This latter finding further strengthens the existing link between adrenergic activation and ventricular arrhythmogenesis and suggests triggered activity as a likely mechanism. (Circulation 1988;78:178–185)

Although the arrhythmogenic role of sympathetic activity has been established,1-5 the mechanisms involved remain speculative. A reduction in action potential duration, refractory period, and enhanced automaticity have been invoked.1,6,7 The relation between the sympathetic nervous system and "triggered activity" is not well defined: while evidence has been obtained in studies in vitro,8 there is limited information in vivo relating cardiac sympathetic innervation and delayed afterdepolarizations (DAD).

A large body of evidence indicates that left-side cardiac sympathetic nerves are particularly arrhythmogenic under a variety of circumstances and that their removal has significant antiarrhythmic and antifibrillatory actions.9,10 From this notion, a number of practical clinical applications have already been derived.11,12 In vitro, DAD can be elicited by catecholamines,13-15 as well as other factors,16-18 and are enhanced by increasing the driving rate of the preparation.19 Because catecholamine release and increased heart rate are consequences of augmented sympathetic nervous activity, we explored the possibility that DAD might be induced by activation of cardiac sympathetic nerves.

To date, there is no direct evidence for the presence of DAD in vivo. A technique20-22 for registering the monophasic action potential (MAP) in intact animals has allowed the demonstration in vivo of early afterdepolarizations (EAD).23 Using this technique, we attempted to demonstrate that DAD can be produced in vivo by the interventions effective in vitro, such as cardiac glycosides and calcium,14,24 and to assess the potential of direct cardiac sympathetic nerves stimulation to induce DAD. (Preliminary data have been reported.25)

Materials and Methods

Surgical Preparation

Experiments were performed on 31 adult cats (2.3-3.4 kg) sedated with ketamine (20 mg/kg body wt i.m.) and anesthetized with α-chloralose (70...
mg/kg i.v.). Ventilation with room air was maintained by means of a tracheal cannula connected to a respirator (model 607, Harvard Apparatus, South Natick, Massachusetts). Tidal volume and respiratory rate were adjusted to keep blood gases and pH within the physiological range throughout the experiment. The body temperature was constantly recorded by a thermistor probe (model 43TA, Yellow Springs Instruments, Yellow Springs, Ohio) and maintained in the normal range by a heating pad and an infrared lamp. Polyethylene catheters were inserted in the femoral artery and vein for blood pressure recording and drug administration. The second to fifth ribs were removed on the left side, and through this opening, the left stellate ganglion was carefully isolated from the surrounding tissue and prepared for electrical stimulation. The proximal connections were cut to decentralize the ganglion to avoid excessive and nonphysiological increases in heart rate through reflexes mediated by the right stellate ganglion.28 A silver suture was gently passed underneath the thoracic aorta to perform graded aortic constriction. The heart was then exposed and suspended in a pericardial cradle. To allow the endocardial recording of MAP, a 4F silver–silver chloride bipolar contact electrode (Meditec, Parma, Italy) was introduced through a stab wound in the free wall of the left ventricle. The recording pole of the electrode was 1 mm in diameter, and the reference electrode located 5 mm proximal to the recording end was 0.5 mm. The recording tip was placed against the distal end of the septum, and the catheter was secured to the epicardial surface by a silk suture. The MAP signals were amplified with DC coupled differential amplifiers (model 9853C, Beckman, Schiller Park, Illinois) at a frequency range from 0.16 to 100 Hz.

Two plunge electrodes were inserted in the right atrium for pacing at twice the diastolic threshold by a programmed stimulator with constant current output (Digitimer 4279, Eursan, Milan, Italy). Blood pressure, intracavitary electrocardiogram (ECG) recorded through a bipolar catheter, and MAP were recorded throughout the experiment (model R612, Beckman) and stored on magnetic tape (Racall Store 7 DS, Southampton, England) for subsequent analysis.

Phases of the MAP were defined according to definitions used for transmembrane action potentials.27 The amplitude of the MAP was defined as the difference between phase 2 and phase 4; action potential duration (APD) was measured from the foot of the action potential to 50% (APD50) or 90% (APD90) repolarization. DAD were defined according to what has been described in vitro.28 The coupling interval of the DAD was defined as the interval between phase 0 of the action potential and the peak of the afterdepolarization.

APD50, APD90, cycle length, and coupling of delayed afterdepolarization were measured with a digital oscilloscope (model 1425, Gould, Hainauld, Essex, England) with data storage and analysis functions using a cursor moved at intervals of 0.5 msec.

Control for Recording Stability and Movement Artifacts

The minimal acceptable amplitude of the MAP was 15 mV; if the signal had a lower amplitude, the electrode was moved to an adjacent site. Signals accepted for this protocol were always stable for more than 1 hour without requiring any additional change in depth or position of the recording tip. To avoid artifacts, control recordings for at least 20 minutes were made in each animal to identify a stable baseline: if this was not obtained, the recording site was changed and experimental protocol performed only when a constant shape of the MAP and a stable resting potential were consistently recorded. In six animals, atrial pacing at different cycle length (range, 200–330 msec) and graded aortic constriction (25–50-mm Hg increase in systolic blood pressure) were performed to assess what modification of the MAP could be induced with interventions that mimic the hemodynamic and chronotropic effect of digitalis and sympathetic stimulation.

Experimental Protocol

After control recordings were made for 20 minutes, 11 animals received Ca2+ gluconate (500 mg) and G-strophanthin (100 μg) as intravenous bolus over 5–10 minutes. In 14 animals, after the control recording was made, the left stellate ganglion was stimulated for 45 seconds by a pulse generator (model 388, Grass, Quincy, Massachusetts) connected to a stimulus-isolation unit (model PSIU6D, Grass) with a constant current output. Pulses used for the stimulation consisted of square wave of 2 msec, 15–20 Hz, and 2–4 mA. A total of three stimulation trials was performed in each animal at three different recording sites, all located in the septum or very close, allowing 15–20 minutes between them for recovery. At the end of each experiment, the position of the recording electrode was verified by careful localization of the endocardial lesion by the catheter tip.

In five experiments, after development and stabilization of DAD, the relation between cycle length and coupling interval or amplitude of the afterdepolarization was studied by performing atrial pacing at several cycle lengths.

Statistical Analysis

The effects of Ca2+ and digitalis administration and of stellate ganglion stimulation on cycle length and APD were analyzed by a paired t test. The relation between cycle length, the coupling, or amplitude of the DAD was assessed by linear regression analysis. Data are expressed as mean ± SD. Significance was accepted for p<0.05.
Results

Thirty-one animals entered the study, and they were divided into three groups: group A (n = 11), which received G-strophalin and calcium gluconate; group B (n = 14), which had the left stellate ganglion stimulated; and group C (n = 6), which had the stability of phase 4 of the MAP during pacing and aortic constriction tested.

Group A

Calcium and G-strophalin significantly modified hemodynamic and electrophysiological parameters: systolic blood pressure increased from 120 ± 25 to 160 ± 18 mm Hg, p < 0.001; mean cycle length increased slightly from 304 ± 35 to 317 ± 49 msec, NS; APD$_{50}$ diminished from 138 ± 16 to 122 ± 18 msec, p < 0.02; and APD$_{90}$ was reduced from 163 ± 23 to 149 ± 20 msec, p < 0.025.

DAD were induced in eight of 11 (73%) animals; their mean amplitude was 1.2 ± 0.4 mV, that is, approximately 6% of the total MAP amplitude (20 ± 4 mV). No significant relation was found between the changes observed in blood pressure, APD$_{50}$, APD$_{90}$, and cycle length and the induction of DAD.

Figure 1 shows the recording of MAP and blood pressure after administration of digitalis, and calcium increased blood pressure and induced DAD. The administration of these drugs induced ventricular arrhythmias in five of 11 (45%) cats. The appearance of premature beats was always preceded by DAD. In one cat, atrioventricular junctional rhythm developed and then degenerated into ventricular fibrillation; in this animal, a rising notch on the T wave of the intracavitary electrocardiogram was observed in the very early beats preceding the onset of ventricular fibrillation (Figure 2).

Group B

Left stellate ganglion stimulation (n = 14) significantly modified hemodynamic and electrophysiological parameters with no differences among the three trials performed in each animal. Blood pressure increased from 130 ± 18 to 173 ± 15 mm Hg, p < 0.0001; cycle length was shortened from 291 ± 34 to 258 ± 33 msec, p < 0.001; APD$_{50}$ was shortened from 153 ± 15 to 145 ± 13 msec, p < 0.005; and APD$_{90}$ decreased from 176 ± 18 to 165 ± 13 msec, p < 0.001.

The protocol included three trials of stimulation for each animal; therefore, a total of 42 trials was performed. DAD were induced in 20 of the 42 trials (42%); in three (21%) of the 14 animals, DAD appeared at all recording sites; in four animals (28%), DAD appeared at two sites; and in three animals (21%), DAD appeared at only one site. Overall, 10 of the 14 (71%) animals developed DAD during sympathetic stimulation; their mean amplitude was 1.2 ± 0.3 mV, that is, approximately 6% of the total MAP amplitude (21 ± 3 mV). The mean time of onset of DAD from the beginning of the stimulation was 19 ± 8 seconds.

Figure 3 shows the recording of MAP and blood pressure in control conditions (Figure 3A) and after left stellate ganglion stimulation (Figure 3B). Note in Figure 3B the increase in blood pressure and the appearance of DAD. Figure 4 shows the MAP recording at higher amplification during left stellate ganglion stimulation after the induction of DAD.

DAD lasted 53 ± 22 seconds after the end of the left stellate ganglion stimulation, by far exceeding the effect on blood pressure that returned to control values after 28 ± 18 seconds. In all cases, after the DAD disappearance, the baseline of the MAP was quite comparable with that observed in control condition. In seven animals, before each of the 21 trials of left stellate ganglion stimulation, a systolic blood pressure of 180–200 mm Hg was obtained by graded aortic constriction: this maneuver neither modified the MAP baseline nor induced DAD (Figure 5).

Among the 10 cats that developed DAD, premature beats were produced by left stellate ganglion stimulation in four cats (40%) and were always preceded by DAD. In one additional animal, a junctional rhythm was induced and was as well preceded by DAD.

Group C

In six animals, the aorta was constricted to raise systolic blood pressure from 130 ± 20 to 190 ± 15 mm Hg, p < 0.0001. DAD were never induced by augmenting blood pressure in these cats, as well as in the five animals that developed DAD during left stellate ganglion stimulation.

Cycle length was reduced by atrial pacing, starting at the first capturing frequency and progressively increasing up to 5 Hz by steps of 0.5 Hz. DAD were never induced by accelerating heart rate. This group served also to assess the time course of the stability of the MAP signal. The MAP mean amplitude in this group of animals was 25 ± 6 mV and had only minor (23 ± 5 mV, NS) differences after 1 hour.

![Figure 1. Simultaneous recording of monophasic action potential (MAP) (upper trace) and blood pressure (BP) (lower trace) after administration of calcium (0.5 g) and G-strophalin (100 μg). Note presence of delayed afterdepolarization and occurrence of one premature beat at peak of delayed afterdepolarization.](http://circ.ahajournals.org/content/78/1/180.full)
Delayed Afterdepolarization Response to Cycle Length Modifications

To evaluate if and how amplitude and coupling interval of the DAD were influenced by changes in heart rate, cycle length was modified by atrial pacing. In five animals in which DAD had been induced by sympathetic stimulation (n=3) or by strophanthrin and calcium (n=2), atrial drive of increasing frequency was performed in subsequent trials.

DAD induced by sympathetic stimulation showed a progressive increase in amplitude as cycle length was reduced; the correlation coefficient of the linear regression analysis was 0.92, p<0.0001 (Figure 6). DAD induced by calcium and strophanthrin behaved differently in that they first increased and then decreased as cycle length was reduced (Figure 6).

As the driving frequency increased, the coupling interval between DAD and MAP gradually decreased independently of the intervention used to generate the DAD; the correlation coefficient of the linear regression analysis was 0.86, p<0.0001 (Figure 7).

Discussion

This study shows that MAP recording allows the detection of delayed afterdepolarizations in vivo from the endocardial surface of anesthetized cats. The major finding is the induction of DAD by a physiologically relevant intervention known to be highly arrhythmogenic, such as left stellate ganglion stimulation.29,30 The responses, amplitude and coupling interval, of DAD recorded in vivo to changes in cycle length are consistent with observations made in vitro.8

Monophasic Action Potentials and Recording of Delayed Afterdepolarizations

A silver–silver chloride contact electrode as described by Franz21 has been used in our experiments to record MAP. Compared with the suction electrode, this method allows a longer and more stable recording period. The correspondence between MAP and transmembrane action potential during repolarization has been confirmed by simultaneous recordings.21,22 Furthermore, by using such a contact
electrode for MAP recording, Levine et al.\textsuperscript{23} were able to detect DAD induced by digitalis in vitro as well as EAD induced by cesium chloride in vivo.

A major problem related to this technique is the distinction between true electrical activity and changes in the MAP baseline secondary to variations in the inotropic state of the myocardium. Useful guidelines can be derived by a critical article by Hoffman et al.\textsuperscript{31} They reported that with a suction electrode for MAP recording it is possible to observe artifacts during the early diastolic phase. Such afterpotentials "were usually seen at the end of the action potential, their amplitude ranged from one-eighth to one-fifth of the total amplitude of the action potential, were greater after long diastolic phases and their duration was approximately equal to that of the action potential." This description clearly indicates that those afterpotentials are not related to the DAD recorded in our experiments that 1) were not potentiated by long pauses and 2) were much shorter in duration than the action potential.

Besides these theoretical considerations, the possibility of baseline changes induced by movement artifacts has been directly explored with a contact electrode, in a previous study\textsuperscript{23} as well as in the present study. Levine et al.\textsuperscript{23} showed that increases in blood pressure, heart rate, contractility, and regional wall motion (induced by phenylephrine infusion) never induced changes in MAP compatible with the interpretation of afterdepolarizations. We performed atrial pacing and aortic constriction to mimic the hemodynamic consequences of digitalis administration and of sympathetic activation: changes in the MAP that could be defined as DAD were never observed. Nonetheless, the potential for artifactual recording of MAP related to changes in inotropic state and the uncertain role of cellular injury at the recording site cannot be positively dismissed.

**Digitalis Induced Delayed Afterdepolarization**

Toxic levels of cardiac glycosides in Purkinje fibers induce DAD that can reach threshold and generate ventricular arrhythmias.\textsuperscript{32,33} These oscillations in membrane potential differ from normal automatic mechanisms that can arise in a quiescent preparation in that they are strictly dependent on an immediately preceding cardiac depolarization. Calcium movements appear to play a major role in the

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**FIGURE 3.** Simultaneous recording of monophasic action potential and blood pressure (BP) in control conditions and during stimulation of the left stellate ganglion (LSG stim). Note rise in BP and presence of delayed afterdepolarization after LSG stim.

**FIGURE 4.** Monophasic action potential recording at high amplification in control conditions and after left stellate ganglion stimulation (LSG stim) when delayed afterdepolarizations are present.

**FIGURE 5.** Simultaneous recording of monophasic action potential (MAP) (upper trace) and blood pressure (BP) (lower trace). When BP is raised by constricting the aorta (arrow), no changes in MAP are observed.

**FIGURE 6.** Plot of relation between cycle length and amplitude of delayed afterdepolarization (DAD). After digitalis and Ca\textsuperscript{2+} (left, n=2), DAD amplitude first increased and then decreased when cycle length was reduced. During left stellate ganglion (LSG) stimulation (right, n=3), a reduction in cycle length is associated with an increase in DAD amplitude.
genesis of DAD, and very high concentrations of calcium can indeed evoke oscillatory afterpotentials, even in the absence of digitalis. This was not the case in our model in which the administration of calcium without strophanthin did not induce DAD. This difference is likely to be related to the extremely high concentration of calcium used in vitro (>10 mM) that is not attained with studies in vivo. Combined administration of calcium and digitalis was able to induce DAD in vivo according to that described in vitro. The two drugs together also modified the MAP duration as it has been reported in vitro for transmembrane action potential.

Attempts have already been made to record DAD induced by digitalis in vivo. Levine reported preliminary data in which DAD had been recorded from the endocardial surface of anesthetized dogs. However, he "met with little success" later in attempting to reproduce these earlier findings. It is possible that the use of dogs, in which the heart is much larger than in cats, may be responsible for a less reproducible recording in the intact heart of focal phenomena as DAD. To the best of our knowledge, no other attempt has succeeded in detecting DAD in vivo. The present findings show the feasibility of recording DAD in vivo and further support the existence of a link between digitalis administration, development of DAD, and ventricular ectopy.

**Sympathetic Stimulation and Delayed Afterdepolarizations**

The role of augmented sympathetic activity in the genesis of arrhythmias is well recognized, and the specific arrhythmogenic potential of left-side cardiac sympathetic nerves is now firmly established. However, the underlying mechanisms have not yet been conclusively identified. The demonstration that stimulation of the left stellate ganglion improves conduction in acutely ischemic hearts has suggested that automaticity is more likely to be involved than reentry. The present data support the view that one additional mechanism by which sympathetic stimulation may generate arrhythmias, independent of myocardial ischemia, is represented by triggered activity. This is particularly important because the origin of ventricular tachycardia and fibrillation usually depends on the first "initiating" ventricular premature beat, and DAD could play such a "trigger" role.

Several lines of evidence had already provided data indicating or hinting to a relation between sympathetic activity and DAD. In vitro, superfusion with adrenergic agonists of Purkinje fibers, and of atrial fibers elicited DAD and arrhythmias. In vivo, the evidence for this relation has so far been indirect. The possibility of identifying with accuracy the mechanisms underlying specific arrhythmias by methods available in the electrophysiology laboratory has many inherent limitations. Therefore, interpretative attempts have focused on responses to drugs known to affect DAD in vitro and on repolarization changes observed on the electrocardiogram. Calcium entry blockers showed a striking protective effect against ventricular arrhythmias specifically induced by the interaction of sympathetic stimulation and myocardial ischemia. These results, independent of drug-induced hemodynamic effects, might be interpreted as suggesting a role of calcium ions in the genesis of these arrhythmias, thus pointing to triggered activity as a possible mechanism for such arrhythmias. In a patient with long QT syndrome, deflections in the T wave of intracavitary electrograms appearing during catecholamine infusion and having a close temporal relation with ventricular arrhythmias have been interpreted as reflecting DAD. The limitations of these indirect interpretations stress the need for the actual demonstration in vivo of neurally mediated DAD.

In the present study, we first demonstrated that DAD induced with an intervention classically used in isolated fibers can be recorded in vivo; then we explored the possibility that activation of cardiac sympathetic nerves could also evoke DAD. Although in this protocol the left stellate ganglion was stimulated for 45 seconds, the time necessary for the DAD appearance was 19 ± 8 seconds. When sympathetic activity is reflexly increased by a physiologically relevant stimulus such as acute myocardial ischemia, the neural excitation persists unabated for the first 1–2 minutes. The fact that DAD were not always detected at all three recording sites may indicate that in the intact heart DAD have a patchy origin, as already suggested on the basis of experimental and clinical observations. Sympathetic stimulation led also to the development of ventricular ectopy: arrhythmias were always preceded by DAD and originated from the peak of the DAD. However, it is fair to note that only in an isolated preparation can the timing of the DAD and of a premature beat be carefully assessed. In fact,
DAD may reach the threshold in an area of the myocardium distant from the recording site to which it then propagates electrotonically.

**Clinical Implications**

The present study demonstrates that DAD can be induced in normal hearts by an increased sympathetic activity mediated by the left stellate ganglion. This finding is clinically relevant.

For a long time, the frequently lethal arrhythmias of the idiopathic long QT syndrome have been related to augmented activity of the left-side cardiac sympathetic nerves. Based on the results in patients resistant to full dose β-blockade, the surgical ablation of these nerves has proven the most effective long-term treatment. Also, Priori et al. and Schechter et al. have proposed that β-adrenergic–dependent DAD might be the responsible mechanism for these arrhythmias, a concept supported by the initial observations on the efficacy of verapamil in long QT syndrome patients.

Left-side sympathetic nerves easily precipitate life-threatening arrhythmias in ischemic hearts, there is recent evidence for a significant reduction in the incidence of sudden death among high-risk patients with post–myocardial infarction treated with high thoracic left sympathectomy.

This growing clinical evidence, in conjunction with the hypothesis that neurally induced DAD may give rise to the first initiating premature beat capable of triggering sustained ventricular tachycardia or fibrillation, may contribute to the current attempt for a more focused approach to the prevention and treatment of cardiac arrhythmias.

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KEY WORDS • sympathetic nervous system • digitalis • delayed afterdepolarizations • ventricular arrhythmias • triggered activity
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