Effects of Vagal Stimulation on Cesium-Induced Early Afterdepolarizations and Ventricular Arrhythmias in Rabbits

Naohiko Takahashi, MD; Morio Ito, MD; Shuji Ishida, MD; Takao Fujino, MD; Tetsunori Saikawa, MD; and Makoto Arita, MD

Background. Previous evidence has shown that increased sympathetic tone enhances the cesium chloride (Cs)-induced early afterdepolarizations (EADs) and ventricular tachycardias (VTs).

Methods and Results. We assessed the effects of vagal stimulation on Cs-induced EADs and ventricular arrhythmias in the rabbit heart. Monophasic action potentials (MAPs) of the left ventricular endocardium were recorded simultaneously with surface ECG. Two protocols were used: 1) While in their intrinsic sinus rhythm, 11 rabbits were given three intravenous Cs injections (1 mM/kg) 20 minutes apart, and the effects of vagal stimulation on the ventricular arrhythmias thus induced were examined. 2) Under constant atrial pacing (cycle length, 250 msec), EAD amplitude was measured after Cs injection (1 mM/kg) without (five rabbits, control group) or with (four rabbits, vagal stimulation group) vagal stimulation. We observed the following. 1) Cs produced EADs and VTs of polymorphic (PVT) and monomorphic (MVT) types. During PVT, the take-off potential of repetitive premature action potentials in MAP recordings was about the same as the peak level of EADs, and during MVT, the take-off potential was the level of full repolarization. Vagal stimulation suppressed PVT but not MVT. Vagal stimulation after spontaneous termination of MVT restarted MVT of the same morphology at a rate much slower than the preceding sinus rate. 2) EAD amplitude was significantly smaller in the vagal stimulation group than in the control group.

Conclusions. The results suggest that PVT originated from triggering by EADs, whereas MVT was of a different origin, and that vagal stimulation suppressed PVT by decreasing the amplitude of EADs. (Circulation 1992;86:1987–1992)

KEY WORDS • tachycardia, ventricular • arrhythmias • cesium

Torsade de pointes in patients with the long QT syndrome is characterized by paroxysms of polymorphic ventricular tachycardia (PVT) in which the QRS complexes twist around the isoelectric baseline.1,2 Triggered activity arising from early afterdepolarizations (EADs) has been proposed as a mechanism responsible for torsade de pointes.2–6 In vivo animal experiments, intravenous cesium chloride (Cs) injection produced EADs in the monophasic action potential (MAP) of ventricles and also caused prolongation of QT intervals and PVT resembling torsade de pointes in the surface ECG.3–6 The ECG changes and ventricular arrhythmias induced by Cs have been considered to provide an experimental animal model for the long QT syndrome,3–6 although the adequacy of this experimental design as a model of the long QT syndrome has recently been questioned by Nayebpour et al.7

In patients with idiopathic long QT syndrome, torsade de pointes is often associated with increased sympathetic tone (stress, fright, etc.) and can be suppressed by β-adrenergic blocking agents or by ablation of the left stellate ganglion.2,8,9 It has been reported that left stellate stimulation increased the amplitude of the EADs induced by Cs and facilitated the occurrence of ventricular tachycardia (VT) in dogs.5 Because vagal stimulation counteracts sympathetic activity in the heart,10 it is of interest to study the effects of vagal stimulation on Cs-induced EADs and ventricular arrhythmias. Because such information has been limited,11 we investigated it by using simultaneous recordings of left ventricular endocardial MAP and surface ECG in vivo in the rabbit.

Methods

All procedures met the guidelines of the Physiological Society of Japan on the care and use of laboratory animals.

Adult Japanese White rabbits (weight, 2.5–3.3 kg) of either sex were anesthetized with secobarbital (20 mg/kg i.v.) and given additional doses as needed to maintain a constant level of anesthesia throughout the course of the experiments. Ventilation with room air was by ventilator (SN-480-5, Shinano) through a tracheal cannula. The tidal volume and respiratory rate were adjusted to keep the blood gases and pH within the physiological range. Rectal temperature was main-
Figure 1. Simultaneous recordings of lead II of the electrocardiogram (ECG) and monophasic action potentials of the left ventricular endocardium (MAP), taken before (panel A) and at 15 seconds (panel B) and 60 seconds (panel C) after a first bolus injection of cesium. In panel B, early afterdepolarizations (solid arrows) are seen in the MAP tracings, and in panel C, premature ventricular beats occurred in association with the premature action potentials (open arrows) arising from levels close to the peaks of early afterdepolarization.

Maintained at 38–39°C by a heating pad. Saline-filled polyethylene catheters (size 4F) were inserted into the femoral artery to monitor blood pressure and into the femoral vein for drug administration. After median sternotomy, the heart was exposed and suspended in a pericardial cradle. MAPs of the left ventricular endocardium were recorded by the contact electrode technique simultaneously with a standard limb-lead ECG (lead II) and the femoral arterial blood pressure. To record MAPs from the left ventricular endocardium, a bipolar silver–silver chloride catheter electrode (size 3F) with a distal electrode 5 mm from the proximal reference electrode was introduced through a tiny stab wound made in the free wall of the left ventricle. The electrode tip was placed on the left ventricular anterior endocardium close to the apical end of the septum. MAP signals were amplified by an electrophysiological amplifier (AB-601G, Nihon Kohden) with a frequency range of 0.1–10 kHz. MAPs were accepted if the amplitude was stable and >10 mV with uniform morphology. The stability of MAP recordings thus obtained has been described previously. The action potential amplitude was defined as the difference between the potential levels at the peak of phase 2 and at the peak of phase 4. Delays or humps during phase 3 repolarization were defined as EADs. The amplitude of each EAD was measured as the potential difference between the level of phase 4 (full repolarization) and the point of initial departure from the smooth decay of phase 3 (see Figure 1, arrows). EAD amplitude was expressed as a percentage of the total amplitude of the MAPs.

The right vagus nerve was carefully dissected out of the neck and was stimulated with a bipolar electrode using 2-msec square pulses at 50 Hz (SEN-7203, Nihon Kohden). The intensity of vagal stimulation was set at the level that prolonged the sinus cycle length about 3 times control and then maintained unaltered throughout the experimental period. For constant atrial pacing, bipolar electrodes were placed on the right atrial appendage. Pacing was performed with square pulses of 1-msec duration at twice the diastolic threshold delivered by a cardiac stimulator (SEC-3102, Nihon Kohden).

Experimental Protocol

Effects of vagal stimulation on Cs-induced ventricular arrhythmias. Eleven rabbits were given three bolus injections of Cs (1 mM/kg i.v. dissolved in normal saline) 20 minutes apart. Recordings were continued until 20 minutes after the third Cs injection. All experiments were performed while the animals were in their intrinsic sinus rhythm. When ventricular arrhythmias occurred after a Cs injection, vagal stimulation was given for about 10 seconds. Vagal stimulation was also given when the ventricular arrhythmias terminated spontaneously.

Effects of vagal stimulation on Cs-induced EAD amplitude. Nine rabbits were given two injections of Cs (1 mM/kg i.v. dissolved in normal saline) 20 minutes apart. All rabbits were allowed to remain in their intrinsic sinus rhythm for the first 18 minutes after the first Cs injection. At 18 minutes, right atrial pacing was begun without (five rabbits, control group) or with (four rabbits, vagal stimulation group) vagal stimulation. Vagal stimulation was continued until 2 minutes after the second Cs injection. This experiment examined the rate-independent effects of vagal stimulation on EADs. The right atrium was paced at a cycle length of 250 msec, which was shorter than the intrinsic sinus cycle length before initiation of atrial pacing. In each group, EAD amplitude was measured at 15, 30, 60, 90, and 120 seconds after the second Cs injection.

Definitions

The following definitions were used for the ventricular arrhythmias recorded by the ECG: 1) VT, three or more consecutive premature ventricular beats (PVBs); 2) PVT, irregular VT with varying QRS morphologies; and 3) monomorphic ventricular tachycardia (MVT), regular VT of a single morphology.

Statistical Analysis

The data are expressed as mean±SD unless otherwise specified. Statistical analyses were performed by Student’s t test. The data are considered significant when p<0.05.

Results

Characteristics of EADs and Ventricular Arrhythmias Induced by Repeated Cs Injections

Before the first Cs injection, no rabbit showed ventricular arrhythmia on ECGs, and all MAPs showed a smooth repolarization with no sign of EAD (Figure 1A). The three Cs injections consistently induced EADs (Figure 1B, arrows), which appeared within a few seconds after each Cs injection and reached a maximum amplitude in about 15 seconds. Thereafter, the EADs waned with time and eventually disappeared after 15–20 minutes. The three Cs injections produced ventricular arrhythmias of somewhat varying characteristics. The first Cs injection caused isolated or coupled PVBs in nine of 11 rabbits tested but never produced VT (Figure 1C). The second Cs injection produced only PVBs in three rabbits and PVT in six. PVT was short-lasting in each case but occurred repeatedly (Figure 2). Three rabbits showed the PVT in which the QRS complex twisted around the isoelectric baseline (Figure 3A), resembling the torsade de pointes seen in patients with the long QT syndrome. In two cases, PVT eventually degenerated into ventricular fibrillation. Therefore, the third Cs injection was not given to these
and produced neither EADs nor ventricular arrhythmias. When stable ventricular arrhythmias occurred after a Cs injection, the effects of vagal stimulation were then examined. The ventricular arrhythmias thus studied included 1) bigeminal or trigeminal PVBs induced after a first Cs injection (four rabbits), 2) PVT after a second Cs injection (three rabbits), and 3) MVT after a third Cs injection (four rabbits). We also studied the effects of vagal stimulation after the spontaneous cessation of these arrhythmias.

Vagal stimulation suppressed PVBs (Figure 5) and PVT (Figure 3) but had no effect on MVT (Figures 6A–6C). More precisely, vagal stimulation abolished the PVBs associated with a significant prolongation of the PP interval from 288±23 to 757±84 msec (n=4, p<0.001) (Figures 5A and 5B). Vagal stimulation also converted PVT to intermittent PVBs (Figures 3A and 3B). Cessation of vagal stimulation resulted in a prompt recurrence of the arrhythmias observed before vagal stimulation (Figures 5C and 3C). On the other hand, in the case of MVT, vagal stimulation changed neither QRS morphology nor cycle length (276±28 msec before and 280±27 msec during vagal stimulation; n=4, p=NS) (Figures 6A–6C).

We then examined the effects of vagal stimulation on the ECG and MAPs after the spontaneous termination of arrhythmias. This test was performed only after the recovered sinus rhythm continued for at least 1 minute with no sign of ventricular arrhythmia. In the case of PVBs (four rabbits) or PVT (three rabbits), vagal stimulation given after the spontaneous termination of arrhythmias prolonged the PP interval from 266±15 to 712±57 msec for PVBs, p<0.001; and from 299±35 to 708±71 msec for PVT, p<0.05) but induced no ventricular arrhythmias (data not shown). In contrast, when given after spontaneous cessation of MVT, vagal stimulation restored the MVT and caused neither PVBs nor PVT in all four rabbits tested (Figure 6D). The QRS morphology of the MVT restored by vagal stimulation (Figure 6D) was the same as that seen before spontaneous termination (Figure 6C). However, the cycle length of the vagally restored MVT (349±38 msec) was significantly longer than both the sinus cycle length just before vagal stimulation (297±39 msec, p<0.05) and the cycle length of spontaneous MVT (276±28 msec, p<0.05). Immediately after the cessation of vagal stimulation, sinus rhythm returned at the same rate as that seen before the stimulation (Figure 6D, extreme right).

Effects of Vagal Stimulation on EADs

Figure 7 compares the time course of the changes in the amplitude of the EADs induced after the second Cs injection in the control (closed circles) and the vagal stimulation groups (open circles) measured during the right atrial pacing at a cycle length of 250 msec. In both

**Figure 2. Tracings of polymorphic ventricular tachycardia (PVT) recorded 40 seconds after a second bolus injection of cesium (ECG lead II). PVT occurred in association with the repetitive ventricular activities arising from the level of early afterdepolarization (arrows). MAP, monophasic action potentials of the left ventricular endocardium.**

ravens. After the first and second Cs injections, ventricular arrhythmias occurred only after the development of EADs. In other words, PVBs and PVT occurred when the EADs attained a certain level of depolarization, that is, 40±7% for PVBs and 44±7% for PVT. The take-off potentials of the premature action potential (in the case of PVBs, Figure 1C) or of the multiple depolarizations (in the case of PVT, Figure 2) were equivalent to the level of the EADs. As the amplitude of the EADs decreased with time, the PVBs and PVT ceased spontaneously. EAD amplitude at the disappearance of these arrhythmias was 33±5% for PVBs and 37±7% for PVT. In sharp contrast to the arrhythmias seen after the first and second Cs injections, the third injection induced a novel ventricular arrhythmia, i.e., MVT, in seven of nine rabbits (Figure 4A), although one of these seven rabbits demonstrated PVT as well. The repetitive action potentials of the MVT arose invariably from the level of phase 4 and not from the level of the EADs (Figure 4A). Spontaneous termination of MVT was always preceded by a gradual increase in the cycle length (Figure 4B), and the MVT eventually ceased when the impulses from the sinus node in a much shorter cycle length began to drive the ventricles (Figure 4B).

Arterial blood pressure was increased by each Cs injection, reaching a maximum at about 5 minutes after injection. Systolic femoral arterial blood pressures before and 5 minutes after the first, second, and third Cs injections were 96±15 and 140±15 mm Hg (n=11, p<0.001); 126±12 and 160±12 mm Hg (n=9, p<0.001); and 129±21 and 151±11 mm Hg (n=9, p<0.01), respectively.

**Effects of Vagal Stimulation on Cs-Induced Arrhythmias**

Under the control conditions (before the first Cs injection), vagal stimulation decreased the sinus rate and produced neither EADs nor ventricular arrhythmias. When stable ventricular arrhythmias occurred after a Cs injection, the effects of vagal stimulation were then examined. The ventricular arrhythmias thus studied included 1) bigeminal or trigeminal PVBs induced after a first Cs injection (four rabbits), 2) PVT after a second Cs injection (three rabbits), and 3) MVT after a third Cs injection (four rabbits). We also studied the effects of vagal stimulation after the spontaneous cessation of these arrhythmias.

Vagal stimulation suppressed PVBs (Figure 5) and PVT (Figure 3) but had no effect on MVT (Figures 6A–6C). More precisely, vagal stimulation abolished the PVBs associated with a significant prolongation of the PP interval from 288±23 to 757±84 msec (n=4, p<0.001) (Figures 5A and 5B). Vagal stimulation also converted PVT to intermittent PVBs (Figures 3A and 3B). Cessation of vagal stimulation resulted in a prompt recurrence of the arrhythmias observed before vagal stimulation (Figures 5C and 3C). On the other hand, in the case of MVT, vagal stimulation changed neither QRS morphology nor cycle length (276±28 msec before and 280±27 msec during vagal stimulation; n=4, p=NS) (Figures 6A–6C).

We then examined the effects of vagal stimulation on the ECG and MAPs after the spontaneous termination of arrhythmias. This test was performed only after the recovered sinus rhythm continued for at least 1 minute with no sign of ventricular arrhythmia. In the case of PVBs (four rabbits) or PVT (three rabbits), vagal stimulation given after the spontaneous termination of arrhythmias prolonged the PP interval from 266±15 to 712±57 msec for PVBs, p<0.001; and from 299±35 to 708±71 msec for PVT, p<0.05) but induced no ventricular arrhythmias (data not shown). In contrast, when given after spontaneous cessation of MVT, vagal stimulation restored the MVT and caused neither PVBs nor PVT in all four rabbits tested (Figure 6D). The QRS morphology of the MVT restored by vagal stimulation (Figure 6D) was the same as that seen before spontaneous termination (Figure 6C). However, the cycle length of the vagally restored MVT (349±38 msec) was significantly longer than both the sinus cycle length just before vagal stimulation (297±39 msec, p<0.05) and the cycle length of spontaneous MVT (276±28 msec, p<0.05). Immediately after the cessation of vagal stimulation, sinus rhythm returned at the same rate as that seen before the stimulation (Figure 6D, extreme right).

**Figure 3. Tracings showing effects of vagal stimulation on polymorphic ventricular tachycardia (PVT) induced after a second bolus injection of cesium. Panels A, B, and C represent recordings before, during, and immediately after vagal stimulation, respectively. Note that PVT was terminated, leaving only intermittent premature beats during vagal stimulation (panel B).**
groups, the amplitude of the EADs decreased with time. However, the amplitude of the EADs was smaller in the vagal stimulation group than in the control group throughout the period tested, with significant differences at 60, 90, and 120 seconds after the second Cs injection.

In both groups, the first Cs injection given during intrinsic sinus rhythm caused exactly the same effects, i.e., the EADs and PVBs described above (Figure 1). Moreover, vagal stimulation before the first Cs injection produced the same decrease in heart rate in both groups, suggesting that they had identical sensitivity to vagal stimulation. These observations support the notion that vagal stimulation suppressed PVBs and PVT by decreasing the amplitude of the EADs that most likely underlie the generation of these arrhythmias.

Discussion

EADs and Ventricular Arrhythmias Induced by Cs

In this study, rabbits in vivo received three injections of Cs (1 mM/kg i.v.) 20 minutes apart. Each Cs injection consistently induced EADs. The EADs attained maximum amplitude about 15 seconds after Cs injection and then decreased with time, eventually disappearing after 15–20 minutes. However, the sequential Cs injections produced ventricular arrhythmias of somewhat different characteristics. The first injection produced only PVBs with no sign of VT, whereas the second and third injections induced PVT and MVT, respectively. PVBs and PVT were always preceded by the development of EADs and occurred when the EADs attained a certain amplitude. Moreover, the take-off potential of the premature action potentials approximated the peak level of the EADs. In some cases, the induced PVT showed a “twisting of points” that resembled the torsade de pointes of patients with the long QT syndrome. In contrast, MVT was characterized by repetitive action potentials arising from phase 4 (full repolarization). These results are consistent with our previous findings and lend support to the hypothesis that PVBs and PVT are a result of triggering by EADs, whereas MVT is caused by different mechanisms. Recently, Nayebpour et al. reported that Cs caused VTs in dogs in vivo that rarely had the morphological features of torsade de pointes. They also found that the VTs occurred despite a substantial increase in serum potassium concentration and showed overdrive acceleration. These features of Cs-induced arrhythmias are inconsistent with the EAD mechanism and are quite unlike those of the clinical long QT syndrome. The observations in Nayebpour et al. as well as those in the present study suggest that not all ventricular arrhythmias induced by Cs are caused by triggered activity arising from EADs.

Effects of Vagal Stimulation

We also examined the effects of vagal stimulation on the ventricular arrhythmias induced by Cs during intrinsic sinus rhythm. Vagal stimulation suppressed PVBs and PVT with a reduction of the heart rate but had no effect on MVT. In addition, vagal stimulation significantly decreased the amplitude of EADs when compared during constant pacing. These findings strongly suggest that vagal stimulation during sinus rhythm suppressed PVBs and PVT by decreasing the amplitude of EADs, especially because the decreased heart rate secondary to vagal stimulation per se should enhance EAD amplitude. Nayebpour and Nattel also found that vagal stimulation suppressed Cs-induced VT in the dog heart. However, they did not remark any difference in vagal effects on PVT and on MVT.

Hanich et al. demonstrated in dog heart that stimulation of the left stellate ganglion increased the amplitude of Cs-induced EADs and decreased the dose of Cs required to produce VT. Increased sympathetic activity increases intracellular levels of cyclic adenosine monophosphate, thereby causing an increased Ca current through the L-type Ca channel, which plays an important role in the genesis of EADs. The increased Ca influx thus caused by sympathetic stimulation may be
Later, acetylcholine released by vagal stimulation may decrease the amplitude of EADs by antagonizing the enhanced adenylate cyclase activity caused by β-adrenergic stimulation. Moreover, acetylcholine suppresses the release of norepinephrine from sympathetic nerve ending. These effects of vagal stimulation may have combined to suppress EADs.

**Mechanisms of MVT**

It was unexpected to find that vagal stimulation after spontaneous termination of MVT led to restoration of MVT of the same morphology, whereas similar vagal stimulation never provoked PVBS or PVT after spontaneous cessation of those arrhythmias. The recurrence of MVT suggests several possibilities for the cause of MVT. Before the spontaneous termination of MVT, its rate decreased gradually, and after its termination, sinus rhythm emerged at a much faster rate than that of the MVT just before its termination. These findings suggest that the MVT was being masked by the faster sinus rhythm. Induction of MVT by vagal stimulation may thus be attributable to “unmasking” of MVT by vagal slowing of the sinus rate. Such competition between VT and sinus beats is compatible with the current understanding of the accelerated idioventricular rhythm as caused by the enhanced automaticity of a ventricular ectopic focus. In a study of dogs subjected to in vivo coronary ligation, Kerzner et al observed a similar phenomenon; vagal stimulation provoked a VT much slower than the sinus rate, which they attributed to enhanced automaticity.

Cs decreased the inward rectifying potassium current and depolarized the membrane potential. Damiano and Rosen reported that exposure of canine Purkinje fibers to 5-7.5 mM Cs for a long period or exposure to higher concentrations (10-20 mM) depolarized membrane potential and induced sustained automatic rhythms that shared the characteristic of abnormal automaticity in their response to pacing. In the present study, of the three repeated Cs injections 20 minutes apart, only the last resulted in MVT. Thus, it seems that the MVT was caused by automatic depolarization occurring at low membrane potentials secondary to a long period (≥40 minutes) of exposure to Cs in a large cumulative dose (3 mM/kg).

Another possible mechanism may be the triggered activity arising from delayed afterdepolarizations. This mechanism has been proposed as a cause of accelerated idioventricular rhythm in some patients.

In the present study, the spontaneous termination of MVT was preceded by gradual decrease in its rate, and the vagally induced MVT was slower than the spontaneous MVT. Such decrease in VT rate may be explained by a gradual decrease in serum Cs concentration, because Nayebpour et al have shown that the incidence of ventricular arrhythmias induced by bolus Cs injection decreased with a decrease in serum Cs concentration.

**Limitation**

We used only a one-lead ECG (lead II) to characterize tachycardia as polymorphic or monomorphic. This is a limitation of the present study, because simultaneous recording of multiple leads is needed to ascertain that a VT is really monomorphic.

**Implications**

Ventricular arrhythmias observed in patients with the long QT syndrome have been thought to be caused by
EADs. Intravenous administration of Cs produced QT prolongation and ventricular arrhythmias in animal hearts in vivo, and Cs injection has been used as an experimental model of the long QT syndrome. However, we found that Cs provoked two quite distinct kinds of VTs in the in vivo rabbit heart: PVT resembling torsade de pointes and regular MVT. Our results suggest that PVT was caused by EADs, whereas MVT was produced by other mechanisms, probably enhanced automaticity or delayed afterdepolarizations. In the clinical setting, both torsade de pointes and regular MVT have appeared in patients with long QT syndrome. Our observations have elucidated that these two types of VT arise by different mechanisms.

Increased sympathetic tone accelerates the development of torsade de pointes in patients with the idiopathic long QT syndrome. We therefore propose that interventions to enhance vagal tone should be effective in treating torsade de pointes associated with idiopathic long QT syndrome, because vagal stimulation suppressed the EADs that appeared likely to underlie the generation of this arrhythmia.

Acknowledgments

We thank Professors Ryosaburo Takaki and Toshihi Sakata for their kind support.

References

Effects of vagal stimulation on cesium-induced early afterdepolarizations and ventricular arrhythmias in rabbits.
N Takahashi, M Ito, S Ishida, T Fujino, T Saikawa and M Arita

doi: 10.1161/01.CIR.86.6.1987

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/86/6/1987