Pericardial Prostaglandin Biosynthesis Prevents the Increased Incidence of Reperfusion-Induced Ventricular Fibrillation Produced by Efferent Sympathetic Stimulation in Dogs

Toshihisa Miyazaki, MD, and Douglas P. Zipes, MD

This study tested the hypothesis that sympathetic neural stimulation increases the prevalence of reperfusion-induced ventricular fibrillation and explored the mechanisms by which this occurs and how it may be prevented. In anesthetized, autonomically denervated dogs, we examined the effects of bilateral ansae subclaviae stimulation (SS) and of induction of pericardial biosynthesis of prostaglandins, an intervention that reduces SS effects by acting at presynaptic sites. A 5-minute occlusion of the left anterior descending coronary artery distal to the first or second diagonal branch was performed during SS. Heart rate was maintained constant by atrial pacing. In the absence of SS, one of 23 dogs developed ventricular fibrillation during occlusion, and three of the remaining 22 dogs developed ventricular fibrillation upon reperfusion. SS did not increase the prevalence of occlusion-induced ventricular fibrillation (four of 23 dogs) but increased the prevalence of reperfusion-induced ventricular fibrillation (12 of the remaining 19 dogs, \( p = 0.01 \)). SS did not affect occlusion-induced decrease in local electrogram amplitude recorded from the ischemic myocardium or myocardial blood flow to the ischemic myocardium during occlusion or reperfusion. SS, however, prevented occlusion-induced increase in diastolic excitability threshold. Instillation into the pericardial cavity of arachidonic acid solution (3 \( \mu \)g/ml) resulted in release of prostacyclin, measured by radioimmunoassay as a stable metabolite 6-ketoprostaglandin F\(_{1\alpha} \) (63.1±11.3 ng/ml, \( n = 11 \), mean±SEM), and of prostaglandin E\(_2 \) (7.0±0.9 ng/ml, \( n = 11 \)). This pericardial solution blunted SS-induced increase in mean arterial blood pressure and reduced the prevalence of ventricular fibrillation during reperfusion (six dogs to one dog, \( p < 0.05 \)). Blood flow to the ischemic myocardium remained unaffected. Indomethacin, when added to the solution (3 \( \mu \)g/ml), reversed the effects of prostaglandin release and arrhythmia development. These data indicate that efferent sympathetic stimulation during a coronary occlusion and reperfusion sequence increases the prevalence of reperfusion-induced ventricular fibrillation that is reduced by pericardial biosynthesis of prostaglandins. Pericardial prostaglandin synthesis may serve as a unique antiarrhythmic function by regulating efferent cardiac sympathetic nerve effects. (Circulation 1990;82:1008-1019)

The effects of sympathetic stimulation during acute coronary occlusion have been well studied.\(^1\)\(^-\)\(^9\) Efferent sympathetic input to the heart increases in the first several minutes after acute myocardial ischemia or infarction,\(^1\)\(^-\)\(^5\) with maximal activation of cardiac sympathetic preganglionic fibers at 5 minutes.\(^4\) Ventricular tachyarrhythmias\(^4\) and a significant decrease in ventricular fibrillation (VF) threshold may result.\(^4\) Recently, ischemia was shown to also interrupt postganglionic neural transmission to the heart in a spatially heterogeneous manner,\(^9\) in part because of the effects of ischemic metabolites that accumulate in the ischemic area.\(^10\) The resultant regional autonomic imbalance may be important in the generation of early ventricular arrhythmias.

In contrast to this body of knowledge, sympathetic neural effects on reperfusion-induced arrhythmias have been less well studied. The mechanisms responsible for arrhythmias during reperfusion are most
probably quite different from those after coronary occlusion.11–16 α-Adrenoceptor stimulation appears to be important because the α-adrenoceptor blocking agents phentolamine and prazosin reduce ischemia-and reperfusion-induced arrhythmias in cats,13 and phentolamine also reduces reperfusion-induced arrhythmias in dogs.17 However, because of their multiple pharmacological properties,18–20 some of the effects of these α-adrenoceptor blockers may be through extra-adrenergic actions, such as a membrane-stabilizing effect similar to that of class I antiarrhythmic agents.4,21–23 Furthermore, Lombardi et al4 found in dogs that bilateral stellectomy actually augmented the decrease in VF threshold upon reperfusion. They suggested that cardiac sympathetic neural discharge exerts an antifibrillatory effect on reperfusion arrhythmias, an action opposite to that noted during coronary occlusion. However, they did not determine autonomic effects on the prevalence of spontaneous reperfusion-induced VF. In fact, we are not aware of any study determining the effects of efferent sympathetic neural stimulation on the prevalence of spontaneous reperfusion-induced arrhythmias.

This study tested the hypothesis that stimulation of bilateral ansae subclaviae (SS) increased the prevalence of spontaneous reperfusion-induced VF and explored the mechanisms by which this occurs and how it may be prevented. Specifically, we examined whether induction of pericardial biosynthesis of prostaglandin, an intervention that we have shown to suppress SS effects on cardiac electrophysiological properties by acting at presynaptic sites,24 blunted the effects of SS on reperfusion-induced VF.

Methods

Surgical Procedures

Studies were performed in 43 mongrel dogs of either sex weighing 16–30 kg. Dogs were anesthetized initially with pentobarbitol (30 mg/kg i.v.), and additional doses were given as needed to maintain anesthesia. Dogs were intubated and ventilated with room air by a constant volume-cycled respirator (model 607, Harvard Apparatus, South Natick, Mass.). A fluid-filled cannula was placed in the right femoral artery and connected to a transducer (p-23Db, Statham, Cleveland, Ohio) to monitor arterial blood pressure. A femoral venous cannula was used to infuse normal saline at a rate of 100–200 ml/hr to replace spontaneous fluid losses. The chest was opened through a median sternotomy. The ansae subclaviae were isolated as they exited from the stellate ganglia, were doubly ligated, and were cut. The cervical vagi were also isolated, doubly ligated, and transected. A small incision was made in the anterior surface of the pericardium. The edges of the incision were tied with sutures at four points so that tension applied to the sutures produced a square opening approximately 2.5×2.5 cm.24 A thermister (model 400, Yellow Springs Instruments, Yellow Springs, Ohio) was used to monitor epicardial temperature, which was maintained between 36° and 38° C by the use of a heating pad and by adjusting the proximity of an operating table lamp.

Through the pericardial opening, the left anterior descending coronary artery (LAD) was dissected free at a site distal to the first or second diagonal branch; care was taken not to damage the perivascular nerves. A silk suture was positioned around the LAD, passed through a plastic tube, and used as a noose to obtain complete occlusion and reperfusion of the LAD by tightening and releasing the suture.

Electrode Placement and Electrographic Recording

Through the pericardial opening, three pairs of bipolar plunge electrodes (tips 1 mm apart) were inserted into the midwall of the left ventricle in the center and well outside of the expected ischemic zone and into the right atrium. The signals were amplified, filtered between 30 and 1,000 Hz, and recorded simultaneously with lead II electrocardiogram and arterial blood pressure on a recorder (model 2800, Gould Brush, Cleveland, Ohio) at a paper speed of 5 or 25 mm/sec. The heart rate was maintained constant by unipolar right atrial pacing. The basic cycle length for pacing was selected in each dog to overdrive the sinus cycle length during a brief test period of SS and remained constant throughout the experiment.

Stimulation of Bilateral Ansae Subclaviae

Shielded bipolar electrodes were placed on the right and left anterior and posterior ansae subclaviae to stimulate the efferent cardiac sympathetic nerves with constant-current isolators driven by a programmable stimulator (Pulser 4, Frederick Haer, Brunswick, Me.). Stimuli were 4-msec pulses at a frequency of 2–4 Hz at 2–4 mA.

Measurement of Diastolic Excitability Threshold

To monitor the changes in diastolic excitability threshold after LAD occlusion (in protocol 3, see below), constant-current stimuli25 were used. A unipolar electrode was inserted into the expected center of the ischemic area. The test site was driven at a basic cycle length of 370 msec with a 2-msec rectangular cathodal stimulus delivered by a programmable stimulator (Krannert Medical Engineering, Indianapolis, Ind.) and a constant-current isolator (Krannert Medical Engineering). An anodal electrode was placed in the abdominal wall, and an additional bipolar electrode was placed near the stimulating electrode in the left ventricle. The ventricular responses were recorded from the bipolar electrode and from the lead II electrocardiogram and were displayed on a storage oscilloscope. The test site was driven initially at twice the diastolic threshold. Then, the stimulating current was reduced gradually until ventricular capture was lost. When failure to capture occurred, the current setting was noted, and the current was increased to a higher intensity until ventricular capture was regained. This
sequence was repeated every 15–30 seconds after the onset of LAD occlusion.

**Measurement of Regional Myocardial Blood Flow**

In protocol 4 (see below), regional myocardial blood flow was measured by the microsphere technique. Approximately two million, 15-μm carbonized plastic microspheres labeled with cobalt-57, nio-
bium-95, ruthenium-103, or tin-113 (Biotechnology System, DuPont de Nemours, Dover, Delaware) were injected through the cannula in the left atrial appendage, followed by a 10-ml saline flush. Beginning 15 seconds before the injection, reference blood samples were drawn from the left brachial and femoral arteries at a rate of 2.06 ml/min until each sample volume reached 10 ml. After the protocol was completed, the epicardial cyanotic border produced by a transient LAD occlusion was marked with methylene blue dye. The heart was electrically fibrillated and removed. The heart was cut perpendicular to the distribution of LAD into four or five slices. Two myocardial tissue samples were taken from the central cyanotic region of the anterior left ventricle of the two slices, one sample from the marginal region of the anterolateral left ventricle, that is, a region corresponding to the cyanotic border, and two samples from the normal zone of the uninvolved posterior left ventricular wall. Each sample was subdivided into subepicardial, midmyocardial, and subendocardial thirds. Thus, 15 pieces were obtained. Each piece was weighed; the average weight of the pieces was 0.515 g.

Blood and tissue sample radioactivities were measured for 1 min/sample on a gamma counter (model 5530, Packard Instruments, Downers Grove, Ill.). Data on net counts per minute for each isotope were provided after corrections of raw count data for interisotope interference, background, and decay. Myocardial blood flow (ml/min/g) was calculated according to the formula: tissue flow is equal to (tissue counts multiplied by reference flow count divided by (reference counts multiplied by tissue weight), where reference counts is the mean of the values obtained from the brachial and femoral artery samples. Myocardial blood flow values taken from the ischemic zone and from the normal zone were averaged.

**Radioimmunoassay of Prostaglandin**

Radioimmunoassay was used to determine the concentrations of prostaglandin (PG) E₂ and 6-keto-PGF₁α, the stable metabolite of prostacyclin (PGI₂), in the pericardial superfusate during epicardial superfusion (in protocol 2, see below). At the end of each superfusion, 10 ml of the fluid sample was taken from the pericardial cavity and placed in a tube that contained indomethacin (10 μg/ml), then frozen until assayed with commercially available kits (Amersham, U.K.).

The assay was performed in tubes containing [³H]bicyclic PGE₂ or [¹²⁵]I-6-keto-PGF₁α, each antibody, and either standards or samples. After vortexing, the unbound bicyclic PGE₂ was precipitated with dextran-coated charcoal, and after centrifugation, the supernatant was decanted into the vials containing scintillation fluid. The antibody-bound 6-keto-PGF₁α was reacted with the Amerlex-M second antibody that is bound to magnetizable polymer particles and then was separated from free fraction by magnetic separation. The amounts of bound [³H]bicyclic PGE₂ and [¹²⁵]I-6-keto-PGF₁α were determined by a beta scintillation counter (model 2000, Packard Instruments) and a gamma counter (model 5530, Packard Instruments), respectively. The concentration of unlabeled bicyclic PGE₂ or 6-keto-PGF₁α in the sample was then determined from each standard curve.

**Experimental Protocol**

Protocol 1. Effects of SS on spontaneous arrhythmia development during occlusion and reperfusion. Twenty-three dogs received a 5-minute LAD occlusion and reperfusion twice in the absence of SS. Then, the sequence was repeated in the presence of SS. Thirty-minute intervals were allowed for recovery between occlusions. During these sequences, heart rate was held constant by atrial pacing at a cycle length ranging from 250 to 400 msec (mean, 324±42 msec). Pacing was started 90 seconds before LAD occlusion and continued for 90 seconds after reperfusion. SS, when it was applied, was begun 1 minute before LAD occlusion and was continued for 1 minute after reperfusion (Figure 1). The occurrence of arrhythmias during occlusion and upon reperfusion was compared in the absence and in the presence of SS. The effects of SS on ischemia-induced changes in local electrogram amplitude was also determined.

If VF occurred during LAD occlusion, the occlusion was discontinued, and the heart was defibrillated with a direct-current shock of 5–20 J applied with paddles to the epicardium. If a second shock of the
same intensity failed, then a shock with a higher energy level (up to 40 J) was applied. In such cases, therefore, reperfusion-induced arrhythmia could not be categorized. If VF occurred upon reperfusion, direct-current shock was applied immediately.

**Protocol 2. Effects of pericardial biosynthesis of prostaglandin on arrhythmia development during occlusion and reperfusion in the presence of SS.** In eleven of 23 dogs in which the effects of SS on arrhythmia development had been examined (protocol 1), Tyrode's solution containing arachidonic acid (3 µg/ml, Sigma Chemical Company, St. Louis, Mo.) was instilled into the pericardial cavity. Epicardial superfusion with this solution was started 15 minutes before the onset of subsequent LAD occlusion and continued for 2 minutes after reperfusion. After a 10-ml fluid sample was taken from the pericardial cavity for radioimmunoassay of prostaglandins, the rest of the solution was removed completely by suction. The pericardial cavity was then washed out three times with Tyrode's solution that did not contain arachidonic acid. These dogs received subsequent superfusion with Tyrode's solution containing arachidonic acid (3 µg/ml) plus indomethacin (3 µg/ml, Sigma Chemical Company), a cyclo-oxygenase inhibitor. Then, the ischemia and reperfusion sequence in the presence of SS was repeated, followed by the fluid sampling. Heart rate was held fixed by atrial pacing at a constant cycle length of 250-400 msec (mean, 324±55 msec).

**Protocol 3. Effects of SS on changes in diastolic excitability threshold produced by the occlusion and reperfusion sequence.** These parameters were examined in a separate group of three dogs. The order of pacing, SS, and LAD occlusion was performed as shown in Figure 1. Heart rate was fixed by atrial pacing at a cycle length of 370 msec.

**Protocol 4. Effects of SS and epicardial superfusion with arachidonic acid solution on changes in regional myocardial blood flow of the ischemic, marginal, and normal zones during the occlusion and reperfusion sequence.** These parameters were determined in another separate group of 12 dogs. In these 12 dogs, blood flow to the ischemic myocardium was measured in the absence and in the presence of SS during atrial pacing at a constant cycle length ranging from 300 to 380 msec (mean, 335±24 msec). In five of 12 dogs, myocardial blood flow immediately after reperfusion was also determined in the absence and in the presence of SS. In another five of 12 dogs, the effect of instillation of arachidonic acid solution on regional myocardial blood flow was determined.

In protocol 4, the LAD was occluded at its more distal portion, that is, distal to the second through the fourth diagonal branches to minimize the prevalence of VF because the presence of VF would preclude the blood flow measurement.

**Data Analysis**

When the effects of interventions on arrhythmia development were assessed, we used data obtained during the second occlusion and reperfusion sequence in the absence of SS as control. These data were chosen because the arrhythmogenic effect of the initial occlusion may be different from the effect of subsequent occlusions because the former produces more severe electrical disturbances than does the latter.26-28

Data were expressed as mean±SEM. The difference among mean values was determined with an analysis of variance for repeated measurements. Paired or unpaired t test was used when two measurements were compared. The effects of interventions on the prevalence of arrhythmias were analyzed with McNemar's test.29 Statistical significance was set at a p value less than 0.05.

**Results**

**Experimental Protocols**

**Protocol 1. Effects of SS on spontaneous arrhythmia development during occlusion and reperfusion.** The occurrence of ventricular arrhythmias during the second (control) occlusion and reperfusion sequence in the absence of SS and during the subsequent occlusion and reperfusion sequence in the presence of SS is shown in Figure 2. Arrhythmias were classified into four categories of increasing severity: no arrhythmias, premature ventricular complexes (single or couplets, PVCs), self-limiting ventricular tachycardia (three or more consecutive ventricular complexes, VT), and VF.8 For each occlusion and reperfusion sequence in each dog, the highest category was scored.

SS increased the severity of arrhythmias induced by reperfusion. In the absence of SS, one of 23 dogs (4%) developed VF during occlusion, and three of the remaining 22 dogs (14%) developed VF upon reperfusion. In the presence of SS, four of 23 dogs (17%, NS versus control) developed VF during occlusion and 12 of the remaining 19 dogs (63%, p<0.01 versus control) developed VF upon reperfusion. The combined prevalence of VT and VF during occlusion in the presence of SS (15 of 23 dogs, 65%) was also significantly higher than during the second (control) occlusion in the absence of SS (five of 23 dogs, 22%, p=0.01).

The percent change in the amplitude of the local bipolar electrogram recorded from the ischemic myocardium as a function of time after LAD occlusion is depicted in Figure 3. The first and second occlusions in the absence of SS, and the subsequent occlusion with SS all resulted in a significant decrease in the electrogram amplitude compared with each control value (p<0.001 at 5 minutes). The electrogram changes noted during the first 2 minutes after the first occlusion were significantly (p<0.05) greater than those during subsequent occlusions. Otherwise, there was no difference in the electrogram changes among occlusions. Thus, SS did not reduce further the ischemia-induced changes in local electrogram amplitude.
Protocol 2. Effects of pericardial biosynthesis of prostaglandin on arrhythmia development during occlusion and reperfusion in the presence of SS. The occurrence of arrhythmias in 11 dogs that received epicardial superfusion is depicted in Figure 4. For each occlusion and reperfusion sequence in each dog, the highest arrhythmia category was scored. In these dogs, SS increased the prevalence of VF during occlusion and reperfusion compared with the second (control) occlusion (from one of 11 dogs to eight of 11 dogs, p<0.05) by augmenting the development of VF upon reperfusion (from one to two dogs during occlusion; none to six dogs during reperfusion, p<0.01). During epicardial superfusion with arachidonic acid solution in the presence of SS, two dogs developed VF during occlusion and another one dog upon reperfusion. Thus, arachidonic acid solution did not significantly reduce the prevalence of VF throughout the occlusion and reperfusion sequence as a whole (eight of 11 dogs to three of 11 dogs, p=0.06), but it did suppress the development of VF during the reperfusion phase (from six to one dog, p<0.05). During superfusion with arachidonic acid plus indomethacin, the prevalence of VF increased again (six of 11 dogs; one dog during occlusion and five dogs during reperfusion).

An example from a dog showing a precipitating effect of SS on the development of reperfusion-induced VF and prevention by epicardial superfusion with arachidonic acid solution is shown in Figure 5. In the absence of SS (panel A), VT occurred within several seconds of reperfusion. In the presence of SS (panel B), VT occurred immediately after reperfusion and accelerated into VF. Epicardial superfusion with arachidonic acid prevented the occurrence of reperfusion VF in the presence of SS (panel C). During superfusion with arachidonic acid plus indomethacin in the presence of SS (panel D), VF occurred again upon reperfusion.

Instillation into the pericardial cavity of arachidonic acid solution resulted in release of prostacyclin, measured as the stable metabolite, 6-keto-PGF1α (63.1±11.3 ng/ml, n=11) and of PGE2 (7.0±0.9 ng/ml, n=11) (Table 1). This solution blunted the SS-induced increase in mean arterial blood pressure immediately before coronary occlusion (29±3 mm Hg without superfusion to 17±4 mm Hg with superfusion, n=11, p<0.05). SS increased the mean arterial blood pressure during coronary occlusion compared with that during control occlusion (86±6 versus 113±6 mm Hg, n=11, p<0.01). However, in the presence of superfusion with arachidonic acid, this increase was insignificant (99±6 mm Hg, n=11). Superfusion with arachidonic acid plus indomethacin reversed the effects of arachidonic acid on prostaglandin release and blood pressure (Table 1).

Protocol 3. Effects of SS on changes in diastolic excitability threshold produced by occlusion and reperfusion sequence. Changes in diastolic excitability threshold produced by the occlusion and reperfusion sequence in the absence and in the presence of SS are depicted in Figure 6 as percent changes from each control value obtained immediately before coronary occlusion. The control values were 158±50 μA (n=3) in the absence of SS and were 165±40 μA...
Myocardial blood flow measured in the ischemic and marginal zones immediately after reperfusion was also similar in the absence and in the presence of SS (Figure 7B). The myocardial blood flow on reperfusion in the ischemic zone was 4.69±1.09 ml/min/g \((n=5)\) in the absence and was 4.52±1.04 ml/min/g \((n=5)\) in the presence of SS. Myocardial blood flow to the normal zone upon reperfusion, however, was higher in the presence than in the absence of SS.

Regional myocardial blood flow measured during coronary occlusion in the absence and in the presence of epicardial superfusion with arachidonic acid solution is shown in Figure 8. Arachidonic acid superfusion did not significantly affect myocardial blood flow to the normal, marginal, and ischemic zones.

**Discussion**

**New Observations**

The present results indicate that stimulation of efferent cardiac sympathetic nerves during coronary occlusion and reperfusion sequence increases the prevalence of spontaneous VF during the reperfusion phase of an occluded coronary artery. This facilitatory role of sympathetic neural stimulation was not accompanied by a measurable change in myocardial blood flow distribution to the ischemic and marginal zones during coronary occlusion and reperfusion but was accompanied by preserved excitability in the ischemic myocardium. Pericardial biosynthesis of prostaglandin, induced by instillation of arachidonic acid solution into the pericardial cavity, prevented efferent sympathetic-induced VF during reperfusion.

**Possible Mechanisms for Efferent Sympathetic Modulation of Reperfusion-Induced Ventricular Arrhythmias**

Efferent sympathetic modulation of reperfusion arrhythmias can be mediated by multiple processes that include direct or indirect electrophysiological changes and hemodynamic or blood flow processes. However, until the mechanism of reperfusion-induced ventricular arrhythmias is determined, it is impossible to know with certainty how interventions like sympathetic stimulation modulate that event. Temporal dispersion of recovery of excitability\(^{10}\) augmented by ischemia-induced heterogeneous interruption of efferent neural action\(^{9,10}\) is one possibility by which sympathetic stimulation can predispose the heart to fibrillation. A second potential mechanism is the preservation of local diastolic excitability of the ischemic myocardium\(^{31}\) by efferent sympathetic neural stimulation (Figure 6). If preserved excitability prevents electrical uncoupling of the ischemic zone from the rest of the myocardium, then sympathetic neural effects may facilitate the propagation of excitation from within the ischemic zone to adjoining myocardium.\(^{8}\) This may permit exit of “focal” arrhythmogenic mechanisms, such as abnormal forms of automaticity and triggered activity, and is consis-
tent with the findings of Pogwizd and Corr. They noted that arrhythmias induced by reperfusion were most often initiated and maintained by nonreentrant "focal" mechanisms. In their studies, acceleration of a rapid nonreentrant tachycardia led to VF. Some other possibilities include an increased local catecholamine concentration that may stimulate the "focal" mechanisms such as delayed afterdepolarizations and early afterdepolarizations during coronary occlusion and reperfusion, changes in the heterogeneous accumulation of extracellular potassium, which may be affected by stimulation of cardiac sympathetic nerves and free radical formation.

Sympathetic stimulation may affect myocardial blood flow distribution during coronary occlusion and reperfusion in the conscious animals. However, the distribution of myocardial blood flow in the central ischemic and marginal zones during coronary occlusion and upon reperfusion remained unaffected in the present model (Figure 7), perhaps in part because of abbreviation of diastole by the fast rates. Also, the decrease in local electrogram amplitude after coronary occlusion, a change related quantitatively to the degree of regional myocardial blood flow reduction, remained unaffected by neural stimulation. Therefore, we have no evidence that the arrhythmogenic action of bilateral ansae subclaviae stimulation on reperfusion-induced VF in the present model can be explained by its potential effect on coronary circulation. However, it is difficult to draw any definite conclusion concerning the changes in the degree of ischemia by efferent sympathetic stimulation because we did not measure oxygen consumption in the ischemic myocardium, in which active contraction has already ceased, to determine whether it is still modified by sympathetic neural effect. Janse et al demonstrated an increase by left stellate stimulation in the degree of TQ segment depression of the local extracellular electrograms in the ischemic zone and suggested a worsening in the degree of ischemia after sympathetic stimulation.

The facilitation of reperfusion-induced arrhythmias by cardiac sympathetic stimulation appears concordant with previous studies demonstrating that sympatholytic interventions including pretreatment with 6-hydroxydopamine and reserpine suppress reperfusion-induced arrhythmias, although the effects of β-adrenoceptor blocking agents alone on reperfusion-induced arrhythmias are inconsistent. It is also concordant with the observations that vagal stimulation has a protective effect on reperfusion-induced arrhythmias, suggesting the importance of sympathetic-parasympathetic interaction in the development of reperfusion-induced arrhythmias.

**Antiarrhythmic Function of Pericardial Biosynthesis of Prostaglandin**

We demonstrated that instillation of arachidonic acid solution into the pericardial cavity in vivo results in release of large amounts of prostacyclin and PGE₂. These findings are concordant with previous reports that showed that the pericardium in vitro produced prostaglandins in greater concentrations than the myocardium and was a major source of prostacyclin. In contrast, the amount of thromboxanes produced by the pericardium in vitro was...
negligible. Pericardial prostaglandins superfuse the epicardial sympathetic nerves, reducing their effects on sinus cycle length, AH interval, and ventricular effective refractory periods, and they antagonize the facilitatory action of angiotensin II by acting at presynaptic sites. We postulated in that study that pericardial biosynthesis of prostaglandin may help suppress arrhythmia development in various situations, especially during acute myocardial ischemia, when efferent sympathetic stimulation to the heart is maximally increased.

In the present study, we tested this hypothesis and found it to be true. Pericardial fluid prostaglandins blunted sympathetic stimulation-induced blood pressure rise as well as the increase in VF prevalence. The blood pressure changes may have affected VF prevalence. The arachidonic acid solution did not increase myocardial blood flow to the ischemic and marginal zones, although it tended to increase the flow to the normal myocardium (Figure 8).

Coker et al found that low coronary venous concentrations of prostacyclin and elevated thromboxane B2 levels released from the ischemic myocardium correlated with an increased severity of ventricular arrhythmias in dogs after coronary artery ligation. These findings suggest that stimulation of

![Figure 5](image)

**Figure 5.** Tracings from a dog showing the effect of stimulation of bilateral ansae subclaviae (SS) on reperfusion-induced ventricular fibrillation and reversal by epicardial superfusion with arachidonic acid solution. Simultaneous recordings of lead II electrocardiogram (ECG II), mean arterial blood pressure (BP), and local bipolar electrograms of the left ventricular normal (NZ) and ischemic (IZ) zones immediately after reperfusion are shown. Arrows in the ECG II indicate the stimuli from right atrial pacing. AA, epicardial superfusion with Tyrode's solution containing arachidonic acid (3 μg/ml); AA+IND, epicardial superfusion with arachidonic acid (3 μg/ml) plus indomethacin (3 μg/ml). See text for details.

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prostacyclin synthesis and inhibition of thromboxane B₂ synthesis in the heart might prove beneficial during the early stages of acute myocardial ischemia or infarction. The present results support this possibility in terms of an interaction between prostaglandins and efferent cardiac sympathetic activity. Also, several studies have shown an antiarrhythmic effect of intravenous or intracoronary exogenous prostaglandins during coronary occlusion⁴⁶–⁴⁸ and reperfusion.⁴⁹ However, systemic hypotension and increased heart rate after intravenous prostaglandin administration, that is, a reflex increase in sympathetic drive, may reverse these effects.⁵⁰ In this regard, intracoronary administration of prostaglandins⁴⁹,⁵⁰ or stimulation of cardiac biosynthesis of prostaglandin may prove beneficial.

**Consideration of the Experimental Model and Limitations of the Study**

In the present study, we examined the effects of several interventions in dogs that underwent repeated 5-minute occlusions of the LAD. Although the extent of myocardial blood flow reduction and the size of the myocardium at risk produced by coronary occlusion differs greatly from dog to dog, variations within the same dog appear negligible because brief periods of coronary occlusions do not alter the collateral flow to the ischemic myocardium.⁵¹,⁵² Therefore, we used McNemar's test²⁹ to compare correlated proportions instead of a χ² analysis to determine the effects of the intervention on arrhythmia prevalence.

Repeated coronary occlusions in the same dog, however, do not necessarily produce similar electrophysiological alterations or arrhythmias. The initial occlusion may cause more severe electrical disturbances than subsequent occlusions.⁶⁶–⁶⁸ Therefore, we used the second occlusion and reperfusion sequence as the control sequence in the present study.

Austin et al⁵³ described a positive correlation between the extent of the myocardium at risk and the incidence of VF not only during coronary occlusion, but also upon reperfusion. With a very proximal occlusion of a coronary artery, the prevalence of arrhythmia may already be high and neither denervation nor efferent sympathetic stimulation do much to modulate it. To test autonomic effects, therefore, we occluded the LAD at its midportion, that is, after the first or second diagonal branch.

We selected a 5-minute period of coronary occlusion for several reasons: 1) electrophysiological effects of a 5-minute occlusion are reversible, 2) the maximal activation of efferent cardiac preganglionic fibers after coronary occlusion does not persist beyond this time period,⁶,⁴ and 3) the duration of

**FIGURE 6.** Plot of effects of stimulation of bilateral ansae subclaviae (SS) on changes in diastolic excitability threshold produced by occlusion and reperfusion. In each dog, the percent change from the control value in diastolic excitability threshold after coronary occlusion (O) and reperfusion (R) was compared in the absence and in the presence of SS. In dog 2 and dog 3 (middle and right panels), ventricular fibrillation was induced 3 minutes after coronary occlusion and upon reperfusion in the presence of SS, respectively, and the subsequent measurements were discontinued. See text for details.
anginal episodes in patients decreased during this time period. Thus, the present model appears suitable to examine the role of neural activity in affecting spontaneous arrhythmia development during the occlusion and reperfusion sequence. However, a 5-minute period of ischemia is also a limitation of the present study because our conclusions may not apply to occlusions of longer duration. For example, the present results appear contrary to those reported by Lombardi et al,4 mentioned earlier. They showed in dogs that bilateral stellate ganglionectomy augmented the decrease in VF threshold upon reperfusion after a 10-minute period of LAD occlusion. Logical extension of that conclusion is that efferent sympathetic stimulation would suppress the occurrence of VF upon reperfusion, which is opposite to the present results. It is possible that differences in the duration of occlusion are responsible for the contradictory results. It is also possible that changes in VF threshold measured at the normal right ventricle4 do not necessarily reflect the likelihood of spontaneous occurrence of VF during reperfusion.

Last, we stimulated the ansae subclaviae during the entire period of coronary artery occlusion and reperfusion. It is obvious that changes during occlusion that may have been produced by sympathetic stimulation, for example, an increase in the degree of ischemia, would probably affect the prevalence of reperfusion-induced arrhythmias. Thus, although sympathetic stimulation only increased the prevalence of reperfusion-induced VF, an action that was blocked by pericardial synthesis of prostaglandin, the nature of the protocol does not exclude an effect of these interventions on the myocardium during occlusion as well.

**Clinical Implication**

The ventricular tachycardia that occurs upon reperfusion after a reversible episode of ischemia frequently culminates in VF,11,14 and pretreatment with a spectrum of antiarrhythmic agents fails to prevent the VF.54 The present data demonstrate that efferent cardiac sympathetic stimulation significantly increases the vulnerability to developing spontaneous VF upon reperfusion. It is probable that increased sympathetic neural activity during reperfusion in patients increases the incidence of sudden cardiac death. An intervention that modulates presynaptic sympathetic neural transmission, for example, inducing pericardial biosynthesis of prostaglandins, or vagal stimulation may reduce the
influence of such neural activity and reduce the prevalence of sudden cardiac death. Of importance, such interventions modulate not only β-adrenoceptor stimulation, but also α-adrenoceptor stimulation. The role of the latter in reperfusion-induced arrhythmias is well known.13,17

Acknowledgments

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KEY WORDS: arrhythmias, reperfusion-induced, sympathetic modulation, blood flow, myocardial, diastolic excitability threshold
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T Miyazaki and D P Zipes

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