Protective Effect of Vagal Stimulation on Reperfusion Arrhythmias in Cats

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The role of the autonomic nervous system in modulating reperfusion arrhythmias is still unclear. Experiments with sympathetic denervation or α- and β-adrenergic blocking agents have provided mixed results, while the effect of parasympathetic activation has not been investigated extensively. The effect of bilateral vagotomy and of vagal stimulation was studied, with and without attendant bradycardia, on the incidence of reperfusion arrhythmias in α-chloralose anesthetized cats. The left anterior descending coronary artery was occluded for 20 minutes, followed by reperfusion in 105 animals. The incidence and severity of reperfusion arrhythmias was compared in 1) neurally intact animals (heart rate 208 ± 24 beats/min), 2) animals with acute bilateral vagotomy (heart rate 233 ± 25 beats/min), 3) animals with vagal stimulation adjusted to maintain heart rate at 90–100 beats/min, and 4) animals with vagal stimulation + ventricular pacing to maintain heart rate at prestimulation values. All the neurally intact and vagotomized animals developed complex reperfusion arrhythmias, but these arrhythmias occurred in only 60 and 72%, respectively, of the animals with vagal stimulation and vagal stimulation + pacing (p < 0.005 vs. neurally intact and p < 0.02 vs. vagotomy). The incidence of ventricular fibrillation was similar in neurally intact (62%) and vagotomized (58%) animals; it was strikingly lower (7%, p < 0.01) in animals with vagal stimulation when heart rate was allowed to decrease, and it was 48% when heart rate was kept constant during vagal stimulation. A selective protection from sustained (>30 seconds duration) ventricular tachycardia was observed in animals with vagal stimulation independent of heart rate changes. These data indicate that 1) suppression of tonic vagal activity, produced by bilateral vagotomy, does not modify the occurrence of complex reperfusion arrhythmias; 2) vagal stimulation initiated shortly before reperfusion significantly reduces the occurrence of complex reperfusion arrhythmias; 3) the protective effect against ventricular fibrillation is mediated primarily by the attendant decrease in heart rate; 4) sustained ventricular tachycardia is prevented by vagal stimulation independently of heart rate changes. (Circulation Research 1987;61:429–435)

The autonomic nervous system plays an important role in the genesis of cardiac arrhythmias. However, though this concept is generally accepted in the setting of acute myocardial ischemia, it is still quite controversial in the case of those arrhythmias initiated by restoration of blood flow to ischemic myocardium, the so-called "reperfusion arrhythmias." Studies of the autonomic nervous system and reperfusion arrhythmias have almost always explored the potential role of sympathetic activity. There is a consensus that β-adrenergic blockade does not affect reperfusion arrhythmias. However, in contrast, according to most, but not all, studies, these arrhythmias appear to be prevented by chemical sympathectomy and by α-adrenergic blockade.

There is evidence suggesting that elevated vagal activity may protect against ventricular fibrillation during acute myocardial ischemia in both anesthetized and conscious animals. Whether this relation also exists during reperfusion is still unclear, even if lack of efficacy of vagal stimulation has been reported.

The purpose of this study was to evaluate specifically the role of vagal activity in modulating reperfusion arrhythmias. To avoid concomitant sympathetic activation during vagal stimulation, cats, in which a pure vagal stimulation can be obtained, were used. Also, to avoid reduction in the degree of ischemic damage secondary to a lower heart rate, vagal stimulation was initiated only a few seconds before reperfusion.

Materials and Methods

Experiments were performed on 105 adult cats of either sex, ranging in weight from 2.0 to 3.5 kg, sedated with ketamine (30 mg/kg i.m.) and anesthetized with α-chloralose (80 mg/kg i.v.). The animals were ventilated with room air by means of a tracheal cannula connected to a Harvard respirator (model 607, Millis, Mass.) with tidal volume and breathing rate adjusted to keep blood gases in physiologic range throughout the experiment; body temperature was constantly recorded by a rectal probe and maintained between 37° and 38° C by a heating pad and an infrared lamp. Polyethylene catheters were inserted in a femoral artery and vein. Vagosympathetic trunks were visualized bilaterally at a cervical level and, except for the neurally intact animals, dissected into the vagal and sympathetic components. The vagal trunks were tightly ligated and cut centrally to avoid an inhibition

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of cardiac sympathetic efferent activity at the time of stimulation. The distal ends were then prepared for electrical stimulation (model S88, SIU5 Isolation Unit, Grass Instruments, Quincy, Mass.). Rib 5 was removed on the left side. The pericardium was opened, and plunge electrodes were placed in the right ventricle for ventricular pacing. The left anterior descending coronary artery was carefully isolated at its origin. A suture was gently passed around the vessel, and its extremities were inserted into a rigid polyethylene tube.

**Experimental Protocol**

After stabilization of blood pressure and heart rate in all animals, a reversible occlusion of the left anterior descending coronary artery was obtained by pulling the two ends of the suture and by securing the ligature with a bulldog clamp. The occlusion was maintained for 20 minutes, and no attempt was made to resuscitate the animals that fibrillated during the occlusion. In animals surviving the occlusion, reperfusion was obtained by releasing the clamp and removing the suture. Reperfusion was easily confirmed by the return within seconds to a reddish color of the previously ischemic left ventricular free wall. Data were recorded for an additional 10 minutes after reperfusion.

The animals were divided into 4 groups (Figure 1): in Group 1, \( n = 34 \), the occlusion–reperfusion protocol was performed without any additional intervention; in Group 2, \( n = 16 \), the occlusion–reperfusion protocol was performed 30 minutes after bilateral vagotomy; in Group 3, \( n = 21 \), the occlusion–reperfusion protocol was performed 30 minutes after bilateral vagotomy, and a right \( n = 14 \) or bilateral \( n = 7 \) vagal stimulation was started 30 seconds before the release of the occlusion and continued for 2 minutes after reperfusion. The stimulation parameters (4 msec, 10–15 Hz, 4–10 V) were adjusted to maintain heart rate at 90–100 beats/min. In Group 4, \( n = 25 \), the occlusion–reperfusion protocol was performed 30 minutes after bilateral vagotomy. A right \( n = 13 \) or bilateral \( n = 12 \) vagal stimulation was performed as described for Group 3; right ventricular pacing was started 15 seconds before reperfusion, and heart rate was stabilized at prestimulation values. This procedure was maintained throughout reperfusion. In a separate series of experiments, the effect of ventricular pacing per se on the incidence of reperfusion arrhythmias was investigated. In these vagotomized animals \( n = 9 \), 2 died during occlusion and 7 underwent reperfusion, ventricular pacing at a rate 5–10 beats/min higher than the spontaneous heart rate was started before release of the occlusion and was continued throughout reperfusion.

**Data Recording**

In all animals, arterial blood pressure (model 4327C transducer, Beckman, Schiller Park, Ill.), instantaneous heart rate, surface electrocardiogram, and an intracavitary electrogram were continuously displayed on a Hewlett-Packard monitor (model 78304, Walling, Mass.). In some of the animals \( n = 25 \), the intraventricular pressure was recorded by a tip pressure transducer (Millar Instr., Houston, Tex.) directly introduced through the apex in the left ventricle. All these signals, including the first derivative of the intraventricular pressure \( (dP/dt) \), were recorded on an 8-channel Beckman R612 polygraph and stored on a 7-channel Racall Store 7DS magnetic tape recorder (Hythe Hampshire, UK) for subsequent analysis.

**Definition of Arrhythmias and Statistical Analysis**

Arrhythmias occurring within 2 minutes after reperfusion were considered reperfusion arrhythmias and classified as follows: VF, ventricular fibrillation; VT, ventricular tachycardia defined as 4 or more consecutive premature ventricular contractions and categorized as sustained (VTS) if lasting more than 30 seconds nonsustained (VTNS) if lasting less than 30 seconds; PVCs, premature ventricular contractions; No arr, no arrhythmias, if no more than 2 PVCs/min occurred.

Data are expressed as mean ± SD. Statistical analysis (STAT PAC, IBM Pc Computer) was performed by one-way analysis of variance (ANOVA) for multiple comparison of hemodynamic values and by paired and unpaired Student’s \( t \) tests, with Bonferroni correction as needed, for single comparisons; and by using \( \chi^2 \) test, with Yates correction when needed, for analysis of the incidence of the events. A value of \( p < 0.05 \) was considered the limit for significance of the differences observed.

**Results**

**Hemodynamic Values**

Heart rate and blood pressure for the different groups are shown in Table 1.
The vagotomized animals (Groups 2–4) had higher heart rates throughout occlusion compared with neurally intact animals (p < 0.001).

The animals with vagal stimulation had average heart rate values (92 ± 24 beats/min) approximating the desired range (90–100 beats/min). These values increased slowly, but progressively, throughout reperfusion, and at the end of the period of vagal stimulation, heart rate was 110 ± 33 (p < 0.01 vs. prerelease).

Blood pressure was similar in all groups throughout occlusion (F = 0.6). At the moment of reperfusion, blood pressure was lower in animals with vagal stimulation (91 ± 24 mm Hg) than in animals without vagal stimulation (122 ± 33 mm Hg, p < 0.001).

A significant decrease in left ventricular dP/dt max during vagal stimulation + pacing was observed (from 3,430 ± 811 prestimulation to 2,062 ± 521 mm Hg/sec poststimulation).

Ischemic Arrhythmias

The vagotomized animals (Groups 2–4) had significantly higher incidences of ischemic arrhythmias compared with control animals (48 of 62, 77% vs. 16 of 34, 47%, p < 0.05). VF was observed in 22% of the vagotomized animals and in 15% of the neurally intact animals. As mentioned in “Materials and Methods,” no attempt was made to resuscitate animals that fibrillated during occlusion (n = 21).

Therefore, 77 animals underwent reperfusion: 29 in Group 1, 12 in Group 2, 15 in Group 3, and 21 in Group 4.

Reperfusion Arrhythmias

Onset of reperfusion arrhythmias was 14 ± 12 seconds (range 1–58 seconds) in neurally intact and vagotomized animals (Groups 1 and 2), and it was 30 ± 24 (range 1–88 seconds) in animals with vagal stimulation (Groups 3 and 4). This difference is statistically significant (p < 0.001).

Complex ventricular arrhythmias (VF, VTS, and VTNS) occurred in all animals of both the neurally intact and vagotomized groups, but arrhythmias occurred in only 60% of the animals with vagal stimulation (Group 3) and in 72% of the vagal stimulation + pacing group (Group 4, Figure 2).

The incidence of reperfusion VF was similar in neurally intact (18 of 29, 62%) and vagotomy (7 of 12, 58%) groups. This incidence was decreased strikingly in the vagal stimulation group (1 of 15, 7%, p < 0.005 vs. neurally intact and p < 0.02 vs. vagotomy) but was not significantly reduced in the vagal stimulation + pacing group (10 of 21, 48%, p > 0.05) (Figure 3). In most cases, VF was observed as a degeneration of rapid runs of VT (19 ± 21 seconds in Groups 1 and 2 and 16 ± 15 seconds in Groups 3 and 4).

Reperfusion VT was significantly shorter in animals with vagal stimulation (11 ± 10 seconds) and vagal stimulation + pacing (17 ± 10 seconds) compared with neurally intact (55 ± 39 seconds) and vagotomy (53 ± 33 seconds) groups.

Interestingly, sustained VT as defined in our preparation occurred in 31 and 33% of neurally intact and vagotomy, respectively, and was prevented totally by vagal stimulation in Groups 3 and 4.

When the two life-threatening arrhythmias (i.e., VF and VTS) are analyzed together, both vagal stimulation and vagal stimulation + pacing afford significant protection (Figure 3).

The incidence of reperfusion arrhythmias in the series of animals with ventricular pacing alone was similar to control groups (VF 43, VTS 43, and VTNS 14%).

A subgroup analysis was performed to determine whether the severity of reperfusion arrhythmias may be predicted on the basis of either the occurrence of ischemic arrhythmias or the onset time of reperfusion arrhythmias. In the analysis, all groups that had a similar incidence of reperfusion VF were included; therefore, Group 3 was excluded from this analysis.

As already observed, the occurrence and severity of ischemic arrhythmias did not predict the outcome after reperfusion. In fact, the incidence of VF was similar in animals with (20 of 39, 51%) or without (14 of 23, 61%) ischemic arrhythmias. Also, no significant relation existed between time of onset and severity of reperfusion arrhythmias.

The occurrence of reperfusion VF in the different groups was not predictable on the basis of blood pressure or heart rate at the moment of reperfusion. Moreover, the incidence of reperfusion VF was not significantly different in animals with diastolic blood pressure higher or lower than the mean values: 46 vs. 71% (Group 1), 63 vs. 50% (Group 2), 12% vs. 0 (Group 3), and 60 vs. 36% (Group 4). When systolic blood pressure or heart rate was analyzed, no difference again was found.
Discussion

We have found that vagal activation can modify the occurrence of reperfusion arrhythmias and that prevention of ventricular fibrillation, but not of sustained VT, depends on the reduction in heart rate. These results and their analysis may contribute to a better understanding of the complex relation between cardiac arrhythmias and the autonomic nervous system.

Mechanisms of Reperfusion Arrhythmias

In our experimental preparation, reperfusion arrhythmias usually appeared as repetitive salvos of ventricular tachycardia that could spontaneously terminate after a few seconds (nonsustained VT), last for several seconds before returning to sinus rhythm (sustained VT), or rapidly degenerate into VF. In a few cases, the onset of reperfusion VF was sudden.

Several studies have shown that these patterns of arrhythmias may depend on a variety of mechanisms. In a feline preparation, Penkoske et al showed an increase in automaticity of ventricular muscle cells after release of a 35-minute occlusion. This increase might be due to an accentuation of normal automaticity or to the occurrence of abnormal automaticity or triggered rhythms. Indeed, the occurrence of delayed afterdepolarizations and the appearance of triggered activity has been demonstrated in fibers perfused with "normal" medium after exposure to "ischemic" medium in an attempt to mimic reperfusion.

On the other hand, reentry might be operating either at the onset of or during the self-perpetuation of arrhythmias. The possibility of reentry is enhanced by the transient, but significant, shortening of effective refractory period during the early reperfusion phase, which further accentuates the inhomogeneity of local refractory periods between normal and ischemic cells. Reentry and automaticity may be operating at the same time.

The sympathetic nervous system may enhance elec-
trical instability at the moment of reperfusion. In fact, it has been shown that catecholamines increase automaticity after reperfusion by an α-mediated effect. On the other hand, catecholamine depletion or α-blocking agents are able to reduce both the shortening of the effective refractory period after reperfusion and the incidence of reperfusion arrhythmias. 

Role of Resting Vagal Tone

The interruption of vagal activity by acute bilateral vagotomy did not affect the incidence of reperfusion arrhythmias in the α-chloralose anesthetized preparation. This is at variance with the protective role played by tonic vagal activity during ischemia and the deleterious effect of vagotomy or atropine, a finding confirmed in the present study. Indeed, the incidence of ischemic arrhythmias was greater in the vagotomized animals compared with neurally intact animals. This could be expected because acute myocardial ischemia may excite vagal afferent fibers, leading, therefore, to a reflex vagal excitation and sympathetic inhibition and resulting in a transient bradycardia during occlusion of the left anterior descending coronary artery in cats.

Corr and Gillis reported a significantly higher incidence of ventricular fibrillation during acute myocardial ischemia in vagotomized cats (60 vs. 22% in our study). The animal species, the anesthetic employed, and the artery occluded were the same in the two studies; baseline values of heart rate and blood pressure were similar. Moreover, the number of animals was adequate in this (n = 62) and in the cited study (n = 20). Thus, an explanation for this disparity is not immediately evident.

Transient vagal activation during reperfusion has been occasionally observed in animals and in man. Markers of massive parasympathetic activation, such as transient bradycardia, were not observed during reperfusion in our control group.

Effect of Vagal Stimulation

A major protective effect against reperfusion arrhythmias was observed when vagal activity was increased by electrical stimulation. This result was independent of any potential effect of parasympathetic activation on the development of ischemic damage because stimulation started only a few seconds before reperfusion. The protective effect of vagal stimulation is striking if one considers that reperfusion arrhythmias have been found very resistant to most preventive measures in a variety of different animal models.

Vagal Stimulation and Ventricular Tachycardia

A selective protection from sustained ventricular tachycardia was observed in the animals with vagal stimulation + pacing. This was due either to a prevention of the initiation of the arrhythmia (28% of the animals) or to an interruption of the self-sustaining automatic mechanism responsible for the perpetuation of the ventricular tachycardia. The experiments with ventricular pacing without vagal stimulation, in which reperfusion arrhythmias were not prevented, ruled out any role of pacing per se in mediating this protective effect. Indeed, vagal stimulation may interfere with several of the mechanisms responsible for the genesis and perpetuation of reperfusion arrhythmias.

Vagal stimulation blunts the effect of catecholamines by acting at either a presynaptic or a postsynaptic level, and therefore, it has the potential to counteract the effect of catecholamines on automaticity and refractory period. Acetylcholine has a direct effect on ventricular automaticity and prevents the occurrence of delayed afterdepolarizations induced by digitalis. Therefore, it can suppress a type of triggered activity that is believed to be very similar to that occurring during reperfusion. Finally, vagal stimulation in vivo and acetylcholine in vitro increase the effective refractory period of both ischemic and normal cells, an action that would reduce the electrical inhomogeneity at the moment of reperfusion.

In our preparation, vagal stimulation + pacing did not prevent the degeneration of VT into VF, and the incidence of VF was only slightly and not significantly lower than that of control groups. The possibility cannot be excluded that a detrimental role has been played by a catecholamine release secondary either to electrical stimulation of sympathetic fibers passing through the right ventricle or to the low blood pressure present in this group of animals. These data are in agreement with an earlier finding by Verrier et al. They found that, if heart rate was kept constant by ventricular pacing, vagal stimulation did not prevent the reperfusion-induced decrease in ventricular fibrillation threshold.

Role of Heart Rate

Vagal stimulation prevented VF only when heart rate was not controlled by ventricular pacing and when it was allowed to decrease from 230 to 100 beats/min. The protective effect of lowering heart rate is well established during acute myocardial ischemia, but it is more controversial during reperfusion. For example, Lederman et al showed that dogs paced at 125 beats/min had a lower incidence of reperfusion arrhythmias compared with dogs paced at 170 beats/min. On the other hand, in the study by Sheridan et al, propranolol was found ineffective despite a major decrease in heart rate (from about 210 to 140 beats/min). In both these studies, heart rate was lower throughout the occlusion period, and it is possible that the different results might have been caused by a different degree of ischemic damage secondary to the heart rate level. Our data indicate that a lower heart rate when obtained with vagal stimulation prevents reperfusion arrhythmias in most cases. These data are at variance with those reported by Kabell et al in which vagal stimulation during reperfusion did not prevent arrhythmias in dogs. This might be due to the concomitant activation of sympathetic fibers with stimulation of the vagosympathetic trunk in dogs.
This suggests that a lower heart rate interferes mainly with the initiation of reperfusion arrhythmias. In fact, 40% of the animals with vagal stimulation did not develop arrhythmias, compared with 0 in the control groups.

A low heart rate at the moment of reperfusion, particularly if associated with a relatively low perfusion pressure, may slow the washout of toxic metabolites, and it may buffer the rapid modification of the extracellular environment that is considered the biochemical basis for the electrophysiologic derangements occurring during reperfusion. A lower heart rate also directly decreases the possibility for triggered activity.

It would have been interesting to observe the effect on reperfusion arrhythmias of reduction in heart rate obtained without modifications in vagal activity. Unfortunately, the methods available seem to have significant limitations. Crushing of the sinus node, besides its high interindividual variability and its often transient effect, is likely to damage a large contingent of the efferent vagal and sympathetic fibers destined to the ventricles. Injections of formaline into the AV node can similarly destroy sympathetic and vagal fibers coursing in the region and can damage ventricular pacemaker cells thought to be involved in reperfusion arrhythmias. Finally, bradycardizing agents such as alinidine may have additional electrophysiologic effects that may complicate the interpretation of any result. All considered, intervention was limited in this study to vagal stimulation and pacing.

The overall data do indicate that, in cats, a parasympathetic activation that is induced by vagal stimulation initiated shortly before release of coronary occlusion is able to largely prevent reperfusion arrhythmias.

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