Enhancement of Electrical Stability of Acutely Ischemic Myocardium by Edrophonium

By Lura A. Harrison, Ph.D., Lynn H. Harrison, Jr., M.D., Kenneth M. Kent, M.D., and Stephen E. Epstein, M.D.

SUMMARY

Recently we demonstrated that vagal stimulation increases ventricular fibrillation threshold (VFT) and decreases the incidence of spontaneous ventricular fibrillation during acute coronary occlusion. Assuming this protective effect of the vagus to be mediated through acetylcholine release, we hypothesized that inhibition of acetylcholinesterase, a potential clinical intervention, would also enhance electrical stability of acutely ischemic myocardium. A balloon cuff was placed around the left anterior descending coronary artery (LAD) and left ventricular and atrial electrodes were implanted in 10 dogs. Five to seven days later VFT was determined (VFT was defined as the minimum current required to produce ventricular fibrillation). During nonischemic conditions VFT was 42 ± 8 ma; when edrophonium was infused (1-2 mg/kg/min), VFT increased to 77 ± 11 ma (P < 0.005). During ischemia induced by LAD occlusion VFT fell to 19 ± 4 ma. Edrophonium increased VFT during ischemia to 52 ± 13 ma (P < 0.005), a level not significantly different from preischemic values. These results suggest that enhanced electrical stability of ventricular myocardium produced by vagal stimulation is mediated by acetylcholine release, and that inhibitors of acetylcholinesterase may provide a new means of therapy for arrhythmias occurring during acute myocardial ischemia.

Additional Indexing Words:

Ventricular fibrillation threshold Acetylcholine Vagus nerve
Cholinergic innervation Acetylcholinesterase inhibition

Previous studies have demonstrated that increased vagal tone protects against the genesis of arrhythmias during experimental acute myocardial infarction. Thus, during acute coronary occlusion, vagal stimulation is associated with a decrease in the incidence of ventricular arrhythmias, an increase in ventricular fibrillation threshold (VFT), and a decrease in the incidence of spontaneously occurring ventricular fibrillation.1-3 We also have shown that the ventricular septum of dogs is abundantly supplied by cholinergic nerve fibers, which are in close approximation to the Purkinje conduction system.4 It would therefore seem reasonable to postulate that the salutary effects produced by vagal stimulation on ventricular electrical stability are mediated through the release of acetylcholine by cholinergic nerve endings, which in turn stabilizes the ventricular conduction system.

Inhibition of the activity of acetylcholinesterase, the enzyme responsible for degradation of acetylcholine, provides an easily controlled pharmacologic equivalent of vagal stimulation. Therefore, to test the validity of the above hypothesis, and to explore the feasibility of a new pharmacologic approach to the treatment of arrhythmias occurring during acute myocardial infarction, we examined the effects of the acetylcholinesterase inhibitor edrophonium chloride (Tensilon)* on VFT before and after acute coronary occlusion produced in closed-chest anesthetized or sedated dogs.

Methods

Acetylcholinesterase inhibition would be expected to exert parasympathetic effects only if there were a reasonable amount of vagal tone present. Barbiturate anesthesia is known to decrease markedly basal vagal tone and was not used in these experiments. In addition, to avoid the vagolytic effects of acute operative procedures, a closed-chest animal model was developed in which VFT could be determined during nonischemic and ischemic conditions.

Adult mongrel dogs were prepared under halothane anesthesia and the heart was exposed through a left thoracotomy. A balloon occlusion cuff was positioned around the left anterior descending (LAD) coronary artery approximately 2 cm from its origin. A stimulation wire was

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sutured to the left atrial appendage and a ventricular myocardial electrode was placed in the area of distribution of the LAD coronary artery (i.e., the area of potential ischemia). A bipolar stimulation electrode (coated platinum wire fixed in a plastic base) was sutured to the left ventricle outside the area of potential ischemia (within the area perfused by the circumflex coronary artery). The chest was closed and the electrode wires and balloon tubing were placed subcutaneously.

Each animal was studied five to seven days after surgical preparation. Five dogs were sedated with morphine (0.5 mg/kg) and diazepam (5-10 mg/kg). Another six dogs were anesthetized with morphine (0.5 mg/kg) and α-chloralose (90 mg/kg). Heart rate, systemic arterial blood pressure, and electrocardiograms were monitored continuously. Acute coronary occlusion was induced by inflating the balloon cuff. Ischemia was documented in each animal by the elevated ST segment recorded from the electrode placed in the area of distribution of the LAD coronary artery. Each animal was ventilated with a respirator via an endotracheal tube. Arterial pH and oxygen saturation were determined. In each dog pH remained between 7.40 and 7.50 and arterial O2 saturation was greater than 92%. Ventricular fibrillation threshold was determined as follows. A train of electrical pulses (each pulse 2 msec in duration and 8 msec apart) was delivered through the bipolar platinum electrode previously sutured to the left ventricle. The train of pulses was initiated approximately 80 msec after the onset of ventricular activation and lasted for 200 msec. Thus, premature stimuli were administered throughout the ST-T segment and T wave. The current of the train of pulses was approximately 3 ma initially; the current was increased in approximately 5 ma increments (measured with a Hewlett-Packard current probe and displayed on a storage oscilloscope) until ventricular fibrillation was effected. VFT was defined as the current (ma) required to produce fibrillation. This procedure was similar to those previously described.5 Ventricular defibrillation was accomplished within 30 sec after fibrillation by a DC current (capacitor discharge) applied to the animal through 8 cm external paddles. VFT was determined during the following situations: nonischemia, ischemia (5 min after the start of occlusion of the LAD coronary artery), nonischemia with edrophonium, and ischemia with edrophonium. Occasionally, occlusion of the LAD produced ventricular ectopy. Thus, the electrocardiogram was monitored carefully during VFT determination to ensure that the train of impulses was not delivered after a premature ventricular contraction. In order to compare VFT at comparable heart rates, attempts were made to maintain heart rates constant by atrial pacing during nonischemic and ischemic conditions. This was achieved during nonischemia, ischemia, or both in seven of the 10 dogs. VFT in the presence and absence of ischemia was tested in an alternate manner before and after edrophonium. Previous studies with this model have shown that VFT is reproducible over a period of four hours with repeated interventions. This finding was reconfirmed in three control animals using the anesthetic agents employed in this study.

The dose of edrophonium was chosen by ascertaining the amount required to decrease basal heart rate in two unanesthetized dogs and in two others sedated with morphine and diazepam. Decreases of 20 beats/min in basal heart rate were usually noted after a bolus injection of 40-100 mg followed by a continuous infusion of 0.5 to 1.0 mg/kg/min; 1-2 mg/kg/min was employed in the definitive studies in order to produce a more pronounced vagal effect. To determine if this dose of edrophonium had any effects on fibrillation threshold independent of acetylcholinesterase inhibition, VFT was determined in two atropinized (0.5 mg/kg) dogs before and after administration of edrophonium.

**Results**

The values of VFT in ischemic and nonischemic ventricles are presented in table 1 and figure 1. Under nonischemic conditions, VFT in the 10 animals averaged 42 ± 8 ma. During ischemia induced by balloon occlusion of the LAD, VFT fell to 19 ± 4 ma.

### Table 1

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Control nonischemia</th>
<th>Edrophonium nonischemia</th>
<th>Ischemia</th>
<th>Edrophonium + ischemia</th>
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<tbody>
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<td>HR</td>
<td>VFT</td>
<td>SAP</td>
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<td>175</td>
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<tr>
<td>10</td>
<td>88</td>
<td>90</td>
<td>28</td>
<td>116</td>
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</tbody>
</table>

Mean values: 133 ± 7 42 ± 8 147 ± 8 77 ± 11 130 ± 7 19 ± 4 157 ± 10 52 ± 13

| P     | NS | <0.005 | <0.05 | <0.005 |

Abbreviations: HR = heart rate; SAP = mean systemic arterial pressure; VFT = ventricular fibrillation threshold.

*Paced heart rate.

P values refer to comparisons between control and edrophonium treated animals under nonischemic conditions, and to comparisons between control and edrophonium treated animals under ischemic conditions.

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VENTRICULAR FIBRILLATION THRESHOLD

Figure 1

Ventricular fibrillation threshold (VFT) values (mean ± standard error) in 10 dogs during nonischemia, edrophonium with nonischemia, ischemia, and edrophonium with ischemia. Edrophonium was administered as an initial bolus (40-100 mg) followed by an intravenous infusion (1-2 mg/kg/min). Edrophonium was given 2-3 min prior to determination of VFT. During the edrophonium plus ischemia study, edrophonium administration was begun after occlusion of the LAD coronary artery.

or approximately half the nonischemic level. A bolus of edrophonium (40-100 mg) followed by continuous edrophonium infusion (1-2 mg/kg/min) raised VFT under nonischemic conditions to 77±11 ma (P < 0.005), and during ischemia to 52±13 ma (P < 0.005). Of note, VFT in ischemic myocardium during edrophonium treatment was not significantly different from nonischemic control levels. There were no significant differences in the response of the dogs anesthetized with morphine-diazepam compared to those anesthetized with morphine-chloralose. Moreover, the protective effects of edrophonium were observed in both those dogs whose heart rates were maintained constant by atrial pacing, and those in which heart rate was permitted to change. During ischemia, edrophonium resulted in a significant increase in systemic arterial pressure (SAP). However, in the four animals (1, 3, 4, 5) in which SAP was unchanged by edrophonium, VFT still increased during the edrophonium infusion.

In order to determine whether or not edrophonium has a direct effect on VFT independent of acetylcholinesterase inhibition, four control animals received atropine (0.5 mg/kg), and afterwards were administered edrophonium in the same amount as that used in the VFT determinations above. Under these conditions, edrophonium did not alter VFT during nonischemic conditions. During ischemia, in the presence of atropine, edrophonium appeared to have a deleterious effect. Thus, the beneficial effects were not due to any direct action of the agent. These results are shown in table 2. The effect of repeated measurements of VFT was studied in three additional animals: VFT remained constant over a two hour period when it was measured every 30 min in two dogs that received morphine-diazepam, and in one dog anesthetized with morphine and α-chloralose.

Discussion

The results of this investigation demonstrate that administration of edrophonium to closed-chest anesthetized dogs leads to enhanced electrical stability of the heart, as indicated by the marked elevation in the intensity of the electric current that is required to produce ventricular fibrillation. This increase in VFT occurred both in the nonischemic heart and when myocardial ischemia was produced by acute

Table 2

Effects of Edrophonium in Dogs Previously Treated with Atropine

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>BP</th>
<th>HR</th>
<th>VFT</th>
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<th>VFT</th>
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<td>45</td>
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<td>180</td>
<td>23</td>
<td>195</td>
<td>200</td>
<td>Spontaneous VF</td>
</tr>
</tbody>
</table>

Abbreviations: Blood pressure (BP), heart rate (HR), and ventricular fibrillation threshold (VFT) results obtained in four dogs administered atropine (0.5 mg/kg).

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occlusion of the left anterior descending coronary artery. The edrophonium-induced increase in VFT appears to be contingent upon the existence of basal vagal tone, since elimination of vagal tone with atropine abolished the electrophysiologic effect of edrophonium. These data, therefore, suggest that the salutary effects of edrophonium on VFT are mediated by inhibition of acetylcholinesterase, an action that presumably leads to increased concentrations of acetylcholine at cholinergic nerve endings.

Previous studies from our laboratory demonstrated that stimulation of the cervical vagni during experimental acute coronary occlusion increases VFT, decreases the incidence of ventricular arrhythmias, and markedly diminishes mortality from ventricular fibrillation.1,3 The present demonstration that an acetylcholinesterase inhibitor also enhances ventricular electrical stability suggests that the beneficial effects produced by vagal stimulation on ventricular electrical stability are mediated by increased concentrations of acetylcholine in the vicinity of cholinergic nerve endings. Recent histochemical and electrophysiologic studies are also compatible with such a hypothesis.4 Thus, the ventricular septum of normal dogs is supplied by a rich network of cholinergic nerves intimately related to the Purkinje conduction system, and stimulation of the cervical vagni in such dogs enhances VFT. However, when these cholinergic fibers are destroyed (by infiltration of the neurotoxic agent vinblastine into the para-aortic area, the region where parasympathetic ganglia supplying the ventricle are located), the beneficial effects of vagal stimulation on VFT are abolished.

It has been shown previously that when the myocardium is ischemic, a reduction in heart rate leads to an increase in VFT.1 In the majority of dogs studied during ischemia in the present study, heart rate in the control state and during edrophonium administration was held constant by atrial pacing. In the absence of pacing, the parasympathomimetic effects of edrophonium would cause heart rate to decrease. It therefore is likely that the beneficial effects of edrophonium administration observed during ischemia would be further enhanced if heart rate had been allowed to fall.

The value of 42 ma that we obtained for VFT under nonischemic conditions is somewhat greater than values previously reported by our laboratory5 and that of others.6 We believe this is probably explained by the fact that the present studies were performed in animals that had relatively normal levels of basal vagal tone; that is, animals were not subjected to acute operative interventions or barbiturate anesthesia, both of which decrease vagal tone. Since enhanced vagal tone increases VFT, it is reasonable to conclude that the threshold of animals studied under the present circumstances would be higher than that of open-chest animals anesthetized with barbiturates.

In conclusion, the results of this investigation indicate that the protective effects of vagal stimulation on ventricular electrical stability 1) are mediated by acetylcholine release, and 2) that these salutary actions may be mimicked by administration of the acetylcholinesterase inhibitor edrophonium. Thus, if the protective effects of increased vagal tone on the development of ischemia-induced lethal arrhythmias are applicable to man, the present investigation suggests that administration of an acetylcholinesterase inhibitor, the pharmacologic equivalent of vagal stimulation, may provide a new means of therapy for ventricular arrhythmias occurring during acute myocardial infarction.

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