Mechanisms for Vagal Modulation of Ventricular Repolarization and of Coronary Occlusion–Induced Lethal Arrhythmias in Cats

Leonid Rosenshtraukh, Peter Danilo, Jr, Evgeny P. Anyukhovsky, Susan F. Steinberg, Vitalyi Rybin, Kimberly Brittain-Valenti, Victor Molina-Viamonte, Michael R. Rosen

Abstract Our goal was to better understand the mechanisms underlying muscarinic receptor actions on the ventricle in vivo. Therefore, we studied the effects of vagal stimulation on ventricular repolarization and of vagal tone on lethal arrhythmias induced by 30 minutes of left anterior descending coronary artery ligation in anesthetized cats. Experimental groups included normal control cats subjected only to coronary ligation and cats pretreated with atropine, pertussis toxin (PTX), or propranolol. All cats received bilateral cervical vagal stimulation (Vstim) at 1, 3, and 5 Hz for 1 minute at 10-minute intervals. Before coronary ligation, Vstim slowed sinus rate, prolonged the PR interval, and lowered blood pressure. Most important from the point of view of electrophysiological function was a vagally induced acceleration of ventricular repolarization in paced and unpaced hearts, which could be explained by the effects of acetylcholine (i.e., shortening the subepicardial muscle action potentials). The effect on repolarization was blocked by atropine or PTX but not by propranolol. The extent of sinus slowing and acceleration of repolarization was directly related to the level of functional PTX-sensitive G protein (P<.05). Coronary occlusion was performed during atrial pacing such that the heart rate in all groups was equal. The incidence of ventricular fibrillation (VF) was 10% in the control group and 50% and 54% in atropine and PTX groups, respectively (P<.05). During atrial pacing before coronary occlusion, a vagal index was calculated as percent QT, shortening during Vstim. When the vagal index was 13% to 26%, the incidence of VF during occlusion was zero. When the vagal index was 0% to 12%, VF was 52% (P<.01). Conclusions are as follows: (1) Vstim accelerates ventricular repolarization in cats via a pathway that incorporates a PTX-sensitive G protein and involves an altered gradient between epicardium and endocardium. (2) Removal of vagal tone during ischemia favors VF, as predicted by a vagal index. (Circ Res. 1994;75:722-732.)

Key Words • β-adrenergic blockade • pertussis toxin–sensitive GTP regulatory protein • vagal stimulation • ventricular fibrillation

Parasympathetic influences appear protective against the arrhythmogenic effects of myocardial ischemia in animal models and in human subjects. Specifically, the incidences of ventricular tachycardia and ventricular fibrillation are reduced, and/or their time of onset is delayed when vagal tone is high. The mechanisms underlying parasympathetic effects on the heart are the subject of ongoing investigation. Vagal modulation of normal cardiac electric activity is attributed in part to the actions of acetylcholine on cardiac ion channels. For example, in the atrium acetylcholine acts through the GTP regulatory protein G, to activate the acetylcholine-activated potassium current (I_{K,ac}), accelerate repolarization, and counteract sinus node pacemaker depolarization. Via a separate pathway, acetylcholine also suppresses the pacemaker current, I_{I}. The interaction between vagal and sympathetic effects on rhythm and arrhythmogenesis has also been studied, and the phenomenon of accentuated antagonism, whereby acetylcholine reduces β-adrenergic catecholamine effects on sinoatrial rate, atrioventricular conduction, and ventricular repolarization has been described previously. β-Adrenergic receptor occupancy leads to increased adenylate cyclase activation via the stimulatory G protein, G_{s}. This results in the formation of cAMP, cAMP-dependent phosphorylation of calcium channels, and an increase in cytosolic calcium, which promotes a variety of arrhythmogenic events. Muscarinic receptor activation counteracts β-adrenergic effects by inhibiting adenylate cyclase activity through a G-dependent pathway.

In the present study, we examined the effects of vagal stimulation on ventricular repolarization and the protective role of vagal tone in the setting of myocardial ischemia. To this end, we studied chloralose-anesthetized cats in which ventricular fibrillation was induced by coronary artery ligation. Interventions included vagal stimulation, β-adrenergic blockade, and interruption of receptor-effector coupling at the level of the muscarinic receptor with atropine, or at the level of the inhibitory G protein, G_{i}, with pertussis toxin (PTX). Because heart rate has been shown to be an independent risk factor with respect to fibrillatory arrhythmias during ischemia, experiments were conducted both on spontaneously beating hearts and on electrically paced hearts.

As shall be demonstrated, vagal effects on ventricular repolarization are readily demonstrable on ECG and

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are abolished by atropine or PTX pretreatment but not by β-adrenergic blockade. The antifibrillatory effects of vagal tone occur independent of heart rate changes and are lost after muscarinic receptor blockade with atropine or partial G-protein inactivation with PTX.

Materials and Methods

Intact Animal Studies

Cats weighing 2.0 to 2.9 kg were sedated with ketamine (12.5 mg/kg IM) and anesthetized with α-chloralose (75 mg/kg IV). Additional amounts of α-chloralose were given as needed during the experiment. A Harvard model 707 respirator connected to a tracheal cannula was used to maintain ventilation with room air. Arterial blood gases and pH were measured with a Corning model 158 pH/blood gas analyzer (Corning Medical Instruments). Cats with blood gas values greater than or less than two standard deviations from the mean were omitted from the study since these factors could contribute to arrhythmogenesis. The following values obtained were within the physiological range:25,26: pH 7.32±0.01; PCO₂, 33±1.1 mm Hg; and PO₂, 91±1.9 mm Hg (all mean±SEM).

Polyethylene catheters were inserted into a femoral artery and vein to record blood pressure and to administer drugs, respectively. Body temperature was maintained via a heating pad. A left lateral thoracotomy was performed through the fifth intercostal space, and an incision was made in the pericardial sac. Blunt dissection was used to isolate the left anterior descending coronary artery (LAD) at its origin, proximal to any branches. A 3–0 silk ligature was placed around the LAD, and the ends were threaded through a piece of tubing. The tubing was clamped down on the LAD to produce occlusion, which was maintained for 30 minutes. Throughout the experiments the ECG and the arterial blood pressure were monitored and continuously recorded on a Gould chart recorder and video display system. Arterial blood pressure was transduced with a Gould P23 strain gauge, which was calibrated with a sphygmomanometer.

The animals in group I (Fig 1) were studied first in sinus rhythm and then subjected to atrial pacing and to vagal stimulation. To pace the heart, a bipolar plaque electrode with silver contacts was sewn to the surface of the right atrial appendage. Constant current pulses twice diastolic threshold and of 2 milliseconds in duration and 5 to 10 V were used at frequencies of 1, 3, and 5 Hz.

Fig 1. Experimental protocols. See text for discussion. PTX indicates pertussis toxin.

After stimulation of each anesthetized animal in group I, the vagal stimulation protocol was performed during sinus rhythm. Effects on heart rate, PR interval, QRS complex, and the QT interval were recorded, and the rate-corrected QT interval (QTc) was calculated by using the Bazett formula.27 Group IB animals were then given atropine (0.1 mg/kg IV), and the vagal stimulation protocol was repeated. All group I animals were then paced atrially at a rate 5% to 10% faster than sinus rate, and pacing was continued throughout the remainder of the study. The vagal stimulation protocol was repeated during pacing, and after stabilization, the LAD was occluded.

Study groups IIA and IIB (control and PTX treated, respectively) provided nonpaced, non-vagally stimulated controls. Hence, the animals were permitted to remain in sinus rhythm throughout the study. Animals used for studies in groups IC and IIB received PTX (30 μg/kg IV) ~60 hours before the experiment. PTX catalyzes the ADP-ribosylation and inactivation of a family of G proteins and was used to manipulate functional G protein levels in cat ventricular myocardium.

The occurrence of arrhythmias was quantified by counting the number of ventricular premature depolarizations (VPDs), episodes of ventricular tachycardia (defined as four or more consecutive ventricular premature depolarizations), and occurrences of ventricular fibrillation.

Assay of PTX-Sensitive G Proteins

On completion of the experiments on PTX-treated animals and matched control animals, ventricular tissue was frozen for measurements of the PTX-sensitive G protein(s). Membranes were prepared, and PTX-dependent ADP-ribosylation was performed according to methods previously described.28 Specifically, membranes were prepared by resuspending myocardium in 5 mL sucrose (0.25 mol/L)–histidine (0.03 mol/L) buffer (pH 7.6) containing 2 mmol/L EDTA and 0.1 mmol/L phenylmethylsulfonyl fluoride and homogenizing at 4°C with a Polytron. The homogenate was centrifuged at 6000g for 15 minutes, and the supernatant containing myocardial membranes was saved. Myocardial membranes were pelleted by centrifugation at 43 600g for 45 minutes and resuspended in 100 mL of 0.03 mol/L histidine buffer (pH 7.4) containing 2 mmol/L MgCl₂ and 1 mmol/L EDTA.

ADP-ribosylation assays were performed as follows: Myocardial membranes were incubated in 20 μL of a 50 mmol/L Tris-chloride buffer (pH 8.0) containing 2 mmol/L MgCl₂, 1 mmol/L EDTA, 10 mmol/L dithiothreitol, 0.1% Lubrol PX, 10 mmol/L thymidine, 10 μmol [³²P]NAD (1.5 μCi per assay), and 20 μg/mL PTX for 1 hour at 37°C. The reaction was terminated by addition of sodium dodecyl sulfate–polyacrylamide gel sample buffer and boiling for 5 minutes. Electrophoresis was performed on vertical slab gels (resolving gel, 12%; stacking gel, 3.9% acrylamide). Two protein bands of similar molecular weight were specifically labeled by PTX. Radioactivity in these bands was quantified by proportional counting of the gel for 1 hour with a Betascan, and PTX-sensitive protein levels were determined by relating the number of counts in the two protein bands specifically labeled by PTX to the specific activity of the [³²P]NAD and to the protein concentration. Each sample was assayed at two different protein concentrations, and a twofold increase in [³²P] incorporation into the specifically labeled bands was routinely observed. Results reported represent the average of duplicate determinations on a single preparation from each experimental animal.

Isolated Tissue Studies

Adult cats (2 to 3 kg) were anesthetized with sodium pentobarbital (35 mg/kg IP). After thoracotomy, hearts were removed and immersed in ice-cold Tyrode’s solution equilibrated with 95% O₂/5% CO₂ and containing (mmol/L) NaCl 131, NaHCO₃ 18, KCl 4, CaCl₂ 2.7, MgCl₂ 0.5, NaH₂PO₄ 1.8, and 2 millisecond in duration and 5 to 10 V were used at frequencies of 1, 3, and 5 Hz.
TABLE 1. ECG and Hemodynamic Characteristics Before Pacing and Occlusion in Group I Animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>Sinus Rate, bpm</th>
<th>PR, ms</th>
<th>QRS, ms</th>
<th>QTc</th>
<th>Systolic</th>
<th>Diastolic</th>
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<tbody>
<tr>
<td>IA</td>
<td>Control</td>
<td>10</td>
<td>180±8</td>
<td>76±3.2</td>
<td>29±0.8</td>
<td>394±11</td>
<td>113±7</td>
<td>72±7</td>
</tr>
<tr>
<td>IB</td>
<td>Before atropine</td>
<td>10</td>
<td>193±8</td>
<td>76±2.7</td>
<td>35±2.4</td>
<td>383±19</td>
<td>120±9</td>
<td>79±10</td>
</tr>
<tr>
<td>IB</td>
<td>After atropine</td>
<td>10</td>
<td>190±8</td>
<td>77±2.6</td>
<td>35±2.4</td>
<td>385±19</td>
<td>103±10</td>
<td>68±7</td>
</tr>
<tr>
<td>IC</td>
<td>PTX</td>
<td>13</td>
<td>214±6</td>
<td>73±2.1</td>
<td>31±3.0</td>
<td>341±6</td>
<td>123±9</td>
<td>55±6</td>
</tr>
</tbody>
</table>

n indicates the number of cats; bpm, beats per minute; QTc, rate-corrected QT interval; BP, blood pressure; and PTX, pertussis toxin. Values are mean±SEM.

dextrose 5.5. Epicardial and endocardial strips (10×10×1 mm) were filleted with a surgical blade from the right and left ventricular free walls, placed in a tissue bath, and superfused with Tyrode’s solution warmed to 37°C. The pH of Tyrode’s solution was 7.35±0.05. Solutions were pumped through the tissue bath at a flow rate of 12 mL/min, changing chamber content three times per minute. The bath was connected to ground using a 3 mol/L KCl/Ag/AgCl junction.

All preparations were impaled with 3 mol/L KCl-filled glass capillary microelectrodes with tip resistances of 10 to 20 MΩ. The maximum rate of rise of phase 0 of the action potential (Vmax) was obtained by electronic differentiation with an operational amplifier. The electrodes were coupled by an Ag/AgCl junction to an amplifier with high-input impedance and input capacity neutralization (model Duo 773, WPI). The transmembrane action potentials and Vmax were displayed on an oscilloscope (model 4074, Gould) and recorded on a plotter (model 7470A, Hewlett-Packard). For stimulation of preparations, standard techniques were used to deliver square-wave pulses 1.0 millisecond in duration and 2.0 times threshold via bipolar Teflon-coated silver electrodes.

After placement in the tissue bath, all preparations were allowed to equilibrate in control Tyrode’s solution. During equilibration, significant changes in the duration of action potentials were observed. Experiments were not started until the preparations were fully recovered and had displayed stable electrophysiological characteristics for at least 30 minutes. Time between the placement of preparations in the bath and the start of drug superfusion varied from 3 to 5 hours. The effects of graded concentrations of acetylcholine were studied at two drive-cycle lengths: 900 and 300 milliseconds. Data were collected 20 minutes after each change in concentration. To investigate accentuated antagonism, the effects of isoproterenol (10−7 mol/L, 10 minutes of exposure) were determined. Then preparations were superfused with control Tyrode’s solution, and after 60 minutes of isoproterenol washout, they were superfused for 15 minutes with 10−3 mol/L acetylcholine. The effects of isoproterenol were studied again in the continued presence of acetylcholine.

Statistical Analysis

For studies comparing the means of two groups, Student’s t test was used. For studies of the effects of a sequence of interventions, such as graded vagal stimuli, we used ANOVA, and when the F value was statistically significant, Bonferroni’s test was used.28 Linear regression analysis was used to assess the relation of vagal inhibition to G protein levels. Fisher’s exact test was used to evaluate the differences in the incidence of arrhythmias across study groups.

All results are expressed as mean±SEM. A value of P<.05 was considered to indicate statistical significance.

Results

Control Data for Group I Animals

Table 1 shows the control ECG and arterial pressure data from the group I animals whose hearts were subsequently paced. Although PTX-treated cats tended to have faster sinus rates and shorter QT, than the other groups and had the widest pulse pressures, there were no significant differences among the groups (all P>.05).

Effects of vagal stimulation on the sinus rates of control, atropine-treated, and PTX-treated animals are shown in Fig 2. A marked decrease in sinus rate occurred in the control animals and attained significance at a frequency of 1 Hz. The response was partially attenuated in PTX-treated animals, where significance was attained at 3 Hz. Finally, the response to vagal stimulation was completely lost in the presence of atropine, and the results here differed significantly from the control values. A comparable result was obtained for the PR interval (data not shown). The QT interval also changed significantly as a function of vagal stimulation (Fig 3). Hence, marked shortening of the QT, occurred on vagal stimulation in the control animals. This response was largely attenuated in PTX-treated animals and totally blocked in those treated with atropine.

The observation that PTX only partially attenuated vagally induced changes in ECG parameters could imply that the PTX treatment protocol used in the present study resulted in incomplete in vivo ADP-ribosylation and inactivation of susceptible G proteins. Therefore, PTX-sensitive G protein levels were measured in membranes prepared from hearts of control and PTX-treated animals. The mean PTX-sensitive G protein levels were 12.98±0.82 and 8.50±0.60 pmol/mg protein in control animals and PTX-treated animals, respec-

Fig 2. Graph showing effects of vagal stimulation at 1, 3, and 5 Hz on sinus rate (animals from Fig 1, group I). In control animals, there is a marked reduction in sinus rate, which is partially attenuated in the pertussis toxin (PTX) group and abolished in the atropine group. The inset shows the three curves expressed as percent decrease in sinus rate. Control values for the three groups do not differ significantly.
vagal responsiveness. Similar sample, respectively. 7.2 and 14.3 were a and proteins in sinus critically protein PR interval. However, the PTX-sensitive G protein levels in PTX-treated cats varied substantially (in some instances approaching the control levels). This was surprising in view of our previous finding that this PTX-treatment protocol is associated with >90% in vivo ADP-ribosylation and inactivation of G proteins in the rat and dog. Nevertheless, this result permitted us to test whether vagally induced changes in ECG parameters are related to the level of functionally intact PTX-sensitive G protein. Fig 5 demonstrates that the effects of maximal vagal stimulation (5 Hz) on sinus rate (Fig 5, top) and QTc (Fig 5, bottom) diminish significantly with decreasing functional G protein levels, with respective r values of −.80 and −.77. These results suggest that a PTX-sensitive G protein critically transduces vagally induced effects on sinus rate and QTc and that conditions that lead to reduced PTX-sensitive G protein levels may impair vagal responsiveness. Similar results were seen for the PR interval (data not shown).

Control data for systolic and diastolic blood pressure and the effects of vagal stimulation in the three groups are shown in Fig 6. Note that at ≥1 Hz in control animals, blood pressure fell significantly. This response was attenuated in the PTX-treated animals and completely blunted in those treated with atropine.

The experimental design for the group I animals raises some concerns: First, the actions of vagal stimulation on the QT might reflect experimental error inherent in the use of the Bazett formula. Second, the shortening of the QT interval might reflect a β-adrenergic action of vagal trunk stimulation. These potential concerns were addressed in the following way: In six additional cats, we performed the identical experimental protocol as in group I, with vagal stimuli of 1, 3, and 5 Hz. We then paced the atria at a constant rate.

![Graph showing effects of vagal stimulation at 1, 3, and 5 Hz on the rate-corrected QT interval (QTc) (Fig 1, group I). Control values show a significant decrease in the QTc, which is abolished by atropine and by pertussis toxin (PTX) pretreatment.](http://circres.ahajournals.org/)

FIG 3. Graph showing effects of vagal stimulation at 1, 3, and 5 Hz on the rate-corrected QT interval (QTc) (Fig 1, group I). Control values show a significant decrease in the QTc, which is abolished by atropine and by pertussis toxin (PTX) pretreatment.

![Graph showing effect of maximal vagal stimulation (5 Hz) on percent decrease in sinus rate (upper panel) and rate-corrected QT interval (QTc, lower panel) expressed as related to pertussis toxin (PTX) substrate assayed. Note the significant correlation for both variables, implying a relation between the level of PTX substrate and the ability of vagal stimulation to influence them. All animals are from Fig 1, group IC (PTX treated).](http://circres.ahajournals.org/)

![Graph showing effect of maximal vagal stimulation (5 Hz) on percent decrease in sinus rate (upper panel) and rate-corrected QT interval (QTc, lower panel) expressed as related to pertussis toxin (PTX) substrate assayed. Note the significant correlation for both variables, implying a relation between the level of PTX substrate and the ability of vagal stimulation to influence them. All animals are from Fig 1, group IC (PTX treated).](http://circres.ahajournals.org/)

![Graph showing effects of vagal stimulation at 1, 3, and 5 Hz on arterial pressure (Fig 1, group I). There is a significant reduction in arterial pressure in control animals that is partially attenuated in pertussis toxin (PTX)-treated animals and completely abolished in animals treated with atropine.](http://circres.ahajournals.org/)

![Graph showing effects of vagal stimulation at 1, 3, and 5 Hz on arterial pressure (Fig 1, group I). There is a significant reduction in arterial pressure in control animals that is partially attenuated in pertussis toxin (PTX)-treated animals and completely abolished in animals treated with atropine.](http://circres.ahajournals.org/)
TABLE 2. ECG and Arterial Pressure of Propranolol-Treated Cats

<table>
<thead>
<tr>
<th>Vagal Stimulation</th>
<th>0</th>
<th>1 Hz</th>
<th>3 Hz</th>
<th>5 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus rhythm (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>235±19</td>
<td>215±19</td>
<td>195±17</td>
<td>170±15*</td>
</tr>
<tr>
<td>PR, t ms</td>
<td>78±3.3</td>
<td>80±3.2</td>
<td>83±4.9</td>
<td>87±4.9*</td>
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<tr>
<td>QRS, ms</td>
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<td>27±4.2</td>
<td>28±4.0</td>
<td>27±4.2</td>
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<tr>
<td>QTc, t</td>
<td>340±31</td>
<td>308±13</td>
<td>296±16</td>
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<tr>
<td>BP, mm Hg</td>
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<tr>
<td>Systolic†</td>
<td>170±15.4</td>
<td>165±15.1</td>
<td>161±15.3</td>
<td>161±15.2</td>
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<td>Diastolic†</td>
<td>109±10.3</td>
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<td>Propranolol (n=6)</td>
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<tr>
<td>HR, bpm</td>
<td>163±10</td>
<td>148±11</td>
<td>132±15*</td>
<td>119±14*</td>
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<tr>
<td>PR, t ms</td>
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<td>86±9.3</td>
<td>89±10.8</td>
<td>94±13.8*</td>
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<td>QTc, t</td>
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<td>330±9</td>
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<td>BP, mm Hg</td>
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<td>Systolic†</td>
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<td>Diastolic†</td>
<td>93±14.0</td>
<td>92±12.7</td>
<td>86±14</td>
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<tr>
<td>Paced rhythm (n=5)</td>
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<td>SR, t ms</td>
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<td>113±10.2</td>
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<tr>
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<td>Systolic†</td>
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<td>146±22.7*</td>
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<tr>
<td>Diastolic†</td>
<td>113±12.4</td>
<td>105±19.4</td>
<td>99±15.6*</td>
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<td>Propranolol (n=5)</td>
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<tr>
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<tr>
<td>SR, t ms</td>
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<td>124±10.7</td>
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<td>QRS, ms</td>
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<tr>
<td>BP, mm Hg</td>
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<tr>
<td>Systolic†</td>
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<td>124±23.7</td>
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<td>79±17.6</td>
<td>78±17.0</td>
<td>85±19.8</td>
<td>87±20.0</td>
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</tbody>
</table>

HR indicates heart rate; bpm, beats per minute; QTc, rate-corrected QT interval; and BP, blood pressure. Values are mean±SEM.

*P<.05 vs values at 0 Hz (Bonferroni).
†Significant (P<.05) response (ANOVA).

and repeated the vagal stimulation protocol. We subsequently infused five of these cats with propranolol (0.75 mg/kg IV) for 10 minutes and repeated the vagal stimulation protocol with the animals first in sinus rhythm and then during atrial pacing.

Complete data from these experiments are presented in Table 2. Note that during sinus rhythm in the presence of propranolol there is a significant slowing of sinus rhythm and reduction in arterial pressure. The vagally induced slowing of sinus rate, prolongation of the PR interval, and reduction in arterial pressure occurred as described for group I cats. Moreover, in the presence of propranolol, as in its absence, rapid stimulation caused a reduction in the QTc.

In the animals that we paced atrially (Table 2), the effect of propranolol was such that vagal stimulation no longer significantly prolonged the stimulus–R wave interval nor lessened arterial pressure. Nonetheless, as shown in Fig 7, the QT interval in paced hearts was shortened by vagal stimulation both in the absence and presence of β-blockade. Hence, the effect of vagal stimulation to shorten the QT interval is neither the result of any artifact of rate correction when using the Bazett formula nor the result of β-adrenergic stimulation.
Effects of Acetylcholine on Subepicardial and Subendocardial Action Potentials in Isolated Tissues

At a basic cycle length of 900 milliseconds, acetylcholine produced little or no change in action potential characteristics of isolated epicardial and endocardial tissues (Table 3). The ability of acetylcholine to antagonize the actions of β-adrenergic agonists was examined in experiments in which epicardial and endocardial preparations were treated with acetylcholine (10^-3 mol/L), which had no effects on action potential duration of both tissues. Acetylcholine completely abolished the effects of isoproterenol in both tissues. The data from these experiments are summarized in Table 4.

Fig 8 illustrates the effects of acetylcholine on epicardial and endocardial action potentials at a cycle length of 300 milliseconds. Acetylcholine (10^-3 mol/L) produced no changes in endocardium. In contrast, the same concentration of acetylcholine decreased epicardial action potential duration. Effects of acetylcholine at a cycle length of 300 milliseconds are summarized in Table 5.

Ischemia and Associated Arrhythmias

Throughout the control period, there were no cardiac arrhythmias, either before or during atrial pacing in group I animals. The arrhythmias that occurred after the onset of ischemia are summarized in Table 6. Only 1 of 10 control animals fibrillated, whereas 5 of 10 atropine-treated and 7 of 13 PTX-treated animals fibrillated. This difference between controls and the other two groups in the incidence of fibrillation was significant (P<.05 by Fisher's exact test). When those animals that did and did not fibrillate were compared, there were no significant differences in any group for sinus rate or paced heart rate. The time to fibrillation after the onset of ischemia averaged <3 minutes in the PTX- and atropine-treated groups, whereas it was 22 minutes in the lone control animal that fibrillated. There were fewer VPDs in the animals that fibrillated, reflecting, no doubt, the brief interval before fibrillation occurred. Moreover, the differences among groups were insignificant, given the large variances seen. No significant differences occurred among the groups in ventricular tachycardia (all runs of which were <30 seconds in duration).

Although the paced heart rates for groups IA, IB, and IC did not differ significantly, one might be concerned about a tendency for pacing rates to be faster in animals that fibrillated than those that did not (see Table 6). However, our study had been designed in such a way that heart rate could be eliminated as a variable. This was done as follows: For the animals in group I, we had paced seven sets of cats (each set consisting of one control, one atropine-treated, and one PTX-treated animal) at the same atrial rate. Although for each member of a set atrial rate was the same, each of the seven sets was driven at a different rate, such that the mean for all was 229±5.3 beats per minute. In these experiments, fibrillation occurred in one of seven control animals, four of seven PTX-treated animals, and five of seven atropine-treated animals. Fisher's exact

**Table 3. Effects of Acetylcholine on Action Potential Parameters of Feline Ventricular Muscle at a Cycle Length of 900 Milliseconds**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10^-9 mol/L</th>
<th>10^-7 mol/L</th>
<th>10^-5 mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epicardium (n=6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDP, mV</td>
<td>-86±2</td>
<td>-87±2</td>
<td>-87±1</td>
<td>-88±1</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>100±3</td>
<td>100±3</td>
<td>101±3</td>
<td>100±3</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt;, V/s</td>
<td>158±20</td>
<td>161±20</td>
<td>162±19</td>
<td>161±19</td>
</tr>
<tr>
<td>APD&lt;sub&gt;50&lt;/sub&gt;, ms</td>
<td>138±21</td>
<td>140±20</td>
<td>139±20</td>
<td>141±21</td>
</tr>
<tr>
<td>APD&lt;sub&gt;90&lt;/sub&gt;, ms</td>
<td>179±19</td>
<td>178±20</td>
<td>182±17</td>
<td>182±18</td>
</tr>
<tr>
<td><strong>Endocardium (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDP, mV</td>
<td>-86±1</td>
<td>-86±1</td>
<td>-86±1</td>
<td>-87±1</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>112±4</td>
<td>112±4</td>
<td>111±4</td>
<td>112±4</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt;, V/s</td>
<td>169±21</td>
<td>170±21</td>
<td>173±20</td>
<td>173±20</td>
</tr>
<tr>
<td>APD&lt;sub&gt;50&lt;/sub&gt;, ms</td>
<td>138±9</td>
<td>140±8</td>
<td>140±10</td>
<td>143±10</td>
</tr>
<tr>
<td>APD&lt;sub&gt;90&lt;/sub&gt;, ms</td>
<td>173±15</td>
<td>171±17</td>
<td>174±15</td>
<td>177±14</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential; amplitude, action potential amplitude; V<sub>max</sub>, maximum rate of rise of phase 0; and APD<sub>50</sub> and APD<sub>90</sub>, action potential duration at 50% and 90% of full repolarization, respectively. Values are mean±SEM.
The experiments described above suggest that in the presence of an intact vagal neuroeffector junction, there is a low incidence of fibrillation during ischemia and that after muscarinic blockade or PTX treatment to functionally inactivate G\textsubscript{i}, the incidence of fibrillation increases. Moreover, they suggest that vagal influences on heart rate do not significantly influence the phenomena described.

As an additional control condition, we determined the incidence of fibrillation in the presence of completely intact vagal innervation in 10 cats. Here, the heart was not paced, and the vagi were neither isolated nor stimulated (Fig 1, group IIA). Control heart rate was 170±10 beats per minute. This decreased to 138±7 beats per minute on coronary occlusion (P<.05). Ventricular fibrillation did not occur, and the animals manifested 103±36 VPDs and 3.0±1.2 instances of ventricular tachycardia. All these findings are consistent with those in the control group in Table 6 (P>.05). Another 6 animals (Fig 1, group IIB) were pretreated with PTX, as described above. Here, control heart rate was 167±11 beats per minute, and after coronary ligation it was 162±13 beats per minute (P>.05). Again, pacing and vagal isolation and stimulation were not performed. The 2 animals that did not fibrillate had a mean G protein level of 13.1 pmol/mg; the 4 animals that did fibrillate had a mean level of 5.6±0.9 pmol/mg.

For the animals in groups IA, IB, and IC, we attempted to identify a predictor of ventricular fibrillation during ischemia. Hence, we calculated a vagal index before LAD occlusion by expressing the QT\textsubscript{c} obtained on maximal vagal stimulation as a percentage of that before vagal stimulation. The range of changes in QT\textsubscript{c} were 0% to 26% shortening. Fisher's exact test was used for the analysis as follows: The 33 animals in group I were divided into two groups: those that had maximal shortening of the QT\textsubscript{c} on vagal stimulation before ischemia (ie, 13% to 26% shortening) and those that had the least shortening of the QT\textsubscript{c} (0% to 12%). In all, 8 animals had maximal QT\textsubscript{c} shortening, and none subsequently fibrillated during ischemia. In contrast, 25 animals had QT\textsubscript{c} shortening of 0% to 12%, and 13 animals fibrillated. The difference between the groups

![Image](http://circres.ahajournals.org/attachment/107799/fig8.png)

**Fig 8.** Tracings showing effects of acetylcholine (Ach, 10^{-5} mol/L) on epicardial and endocardial action potentials at a basic cycle length of 300 milliseconds. Note the shortening of action potential duration that occurs in epicardium only.
TABLE 5. Effects of Acetylcholine on Action Potential Parameters of Feline Ventricular Muscle at a Cycle Length of 300 Milliseconds

<table>
<thead>
<tr>
<th>Acetylcholine</th>
<th>Control</th>
<th>10⁻⁷ mol/L</th>
<th>10⁻⁸ mol/L</th>
<th>10⁻⁹ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardium (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDP, mV</td>
<td>84±2</td>
<td>84±1</td>
<td>84±1</td>
<td>84±1</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>99±2</td>
<td>99±1</td>
<td>99±1</td>
<td>98±2</td>
</tr>
<tr>
<td>Vₘₐₓ, V/s</td>
<td>167±17</td>
<td>163±17</td>
<td>162±17</td>
<td>163±18</td>
</tr>
<tr>
<td>APD₅₀, ms</td>
<td>74±5</td>
<td>76±4</td>
<td>80±6</td>
<td>59±5*</td>
</tr>
<tr>
<td>APD₉₀, ms</td>
<td>138±6</td>
<td>139±6</td>
<td>145±7</td>
<td>118±5*</td>
</tr>
<tr>
<td>Endocardium (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDP, mV</td>
<td>85±1</td>
<td>85±1</td>
<td>85±2</td>
<td>86±1</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>108±3</td>
<td>109±3</td>
<td>109±3</td>
<td>108±3</td>
</tr>
<tr>
<td>Vₘₐₓ, V/s</td>
<td>173±21</td>
<td>173±21</td>
<td>173±21</td>
<td>170±17</td>
</tr>
<tr>
<td>APD₅₀, ms</td>
<td>93±5</td>
<td>93±5</td>
<td>91±4</td>
<td>90±4</td>
</tr>
<tr>
<td>APD₉₀, ms</td>
<td>140±6</td>
<td>140±6</td>
<td>139±7</td>
<td>138±7</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential; amplitude, action potential amplitude; Vₘₐₓ, maximum rate of rise of phase 0; and APD₅₀ and APD₉₀, action potential duration at 50% and 90% of full repolarization, respectively. Values are mean±SEM.

*P<.05 vs control.

was highly significant (P<.01), indicating that a high vagal index for QTc in cats is a good predictor of protection from ventricular fibrillation during ischemia.

**Discussion**

We have reported the following significant findings: (1) Vagal stimulation induces a shortening of the QT interval consistent with a direct action on the normal feline ventricle. (2) The response of the feline heart to vagal stimulation before ischemia predicts the likelihood of a lethal event during ischemia. (3) Vagal tone protects against lethal arrhythmias during 30 minutes of ischemia.

**Vagal Effects in the Normal Ventricle**

For many years it was thought that neither the vagus nor its primary neurotransmitter, acetylcholine, had significant effects on ventricular tissues. This was in part the result of early cellular electrophysiological studies that demonstrated no effects of acetylcholine on ventricular myocardial action potentials (reviewed in Reference 34) and in part the result of studies that suggested effects attributable to vagal stimulation might also be interpreted as secondary to the sinus or idio-ventricular rate changes induced by vagotonia. Several lines of investigation stimulated the reexamination of vagal effects on the ventricle. First, histochemical studies indicated the presence of acetylcholinesterase in the canine and human ventricle, consistent with vagal innervation of the ventricle. Innervation was thought to be especially prominent in the proximal ventricular conducting system. Second, ligand binding studies demonstrated M₂ receptors in ventricular tissues. Third, studies of accentuated antagonism demonstrated that whereas muscarinic agonists might have little effect to accelerate repolarization in ventricular and Purkinje fibers in their own right, the same agonists effectively counteracted the ability of ß-adrenergic agonists to accelerate repolarization. The ß-adrenergic effect was attributed to the Gₛ-dependent stimulation of cAMP formation leading to an increase in Ca⁺²⁺ and K⁺ conductance. The muscarinic action was attributed to an M₂ receptor action via Gₛ to attenuate ß-adrenergic stimulation of cAMP formation. The net result of muscarinic stimulation was to lessen the shortening of action potential duration induced by ß-agonist. Vagally induced prolongation of the effective refractory

**Table 6. Arrhythmias in Group I Control, Atropine-Treated, and Pertussis Toxin-Treated Cats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Heart Rate, bpm</th>
<th>Paced Heart Rate, bpm</th>
<th>VPDs, Total No.</th>
<th>VT, No. of Runs</th>
<th>Incidence of VF, %</th>
<th>Time to VF, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−VF</td>
<td>+VF</td>
<td>−VF</td>
<td>+VF</td>
<td>−VF</td>
<td>+VF</td>
</tr>
<tr>
<td>IA: Control (n=10)</td>
<td>193±10</td>
<td>214</td>
<td>220±11</td>
<td>222</td>
<td>175±105</td>
<td>480</td>
</tr>
<tr>
<td>IB: Atropine (n=10)</td>
<td>183±15</td>
<td>197±7</td>
<td>203±18</td>
<td>223±3</td>
<td>124±55</td>
<td>9±6</td>
</tr>
<tr>
<td>IC: PTX (n=13)</td>
<td>202±8</td>
<td>221±9</td>
<td>204±32</td>
<td>240±7</td>
<td>136±109</td>
<td>9±8</td>
</tr>
</tbody>
</table>

bpm indicates beats per minute; −VF, cats not fibrillating; +VF, cats fibrillating (1 control, 5 atropine treated, and 7 pertussis toxin [PTX] treated); VPDs, ventricular premature depolarizations; VT, ventricular tachycardia; and VF, ventricular fibrillation. Values are mean±SEM.

*P<.05 vs atropine and PTX groups.
period in endocardial and epicardial canine cardiac tissues, especially in the face of sympathetic tone, has been demonstrated in the intact canine heart. This result, too, is consistent with accentuated antagonism.

Direct vagal actions on ventricular repolarization also have been described, although information here is contradictory. For example, in sheep Purkinje fibers, acetylcholine prolongs action potential duration, an effect attributed to a reduction in potassium current carried by both inward and outward rectifiers. In contrast, in guinea pig ventricle, acetylcholine accelerates repolarization, an action attributed to blockade of the slow inward calcium current. Acetylcholine also accelerates repolarization in the ferret ventricle, an effect attributed to activation of I_{k,AC}. Whereas early studies had suggested no effect of acetylcholine on canine ventricular myocardial repolarization, these experiments were performed on endocardium. More recent investigation of canine subepicardial and subendocardial muscle suggests that acetylcholine has no effect on the latter but markedly accelerates repolarization in the former.

With respect to the feline heart, acetylcholinesterase staining has been demonstrated, consistent with the presence of vagal innervation of the ventricles. Acetylcholine has been assayed in feline ventricle, where its concentration is 1/7 to 1/2 that of pooled atria. Finally, acetylcholine has been shown to modulate contractility in cat ventricular trabeculae and papillary muscles. In this case, accentuated antagonism of catecholamine effects by acetylcholine was evident: no direct effects of acetylcholine or carbamylcholine were demonstrated.

What is, perhaps, most clear from this brief review of the literature is that a diversity of conflicting information exists with respect to vagal effects on the ventricle and that both species and tissue differences appear important. Moreover, in light of the number of studies performed previously, it is somewhat surprising that the effects of vagal stimulation on ventricular repolarization of the intact heart have been referred to only minimally. Whether this is the result of lack of interest in vagal effects on the QT interval or failure to see them is impossible to state. Two studies of canine heart reported that vagal stimulation and acetylcholine altered T-wave amplitude. Although this result is consistent with a vagal action, no measure of QT interval was reported in either study.

In human subjects, vagal tone has been shown to prolong the effective refractory period (QT intervals were not reported). Although this result might appear at odds with our demonstration of a shortened QT interval, all the patients reported had intact sympathetic function. In the presence of sympathetic tone, which accelerates repolarization, accentuated antagonism resulting from vagal stimulation would prolong repolarization and refractoriness. Hence, there is no inconsistency between this study and our own.

What is clear from the present study is that vagally induced acceleration of repolarization not only occurs but that it is abolished by atropine and by pretreatment with PTX. This suggests a pathway involving muscarinic receptors and a PTX-sensitive G protein. Our studies also implicate a direct muscarinic receptor action rather than one uniquely dependent on accentuated antagonism. We state this for several reasons: First, in the cat, the vagus appears to have few cardiac sympathetic nerves accompanying it; hence, it can be thought of as a relatively “pure” parasympathetic nerve. Second, our experiments using propranolol to block β-adrenergic receptors indicate that even in the absence of a catecholamine effect, there is vagal modulation of repolarization. It should be noted as well that were an important sympathetic component present, the use of atropine or PTX to block the vagal effect should have enhanced any sympathetic shortening of the QT interval. Yet, this did not occur. That our results were not an artifact of the rate correction for the QT interval is seen in the fact that they occurred regardless of whether hearts were beating spontaneously or were paced atrially.

Other investigators have considered the effects of vagal stimulation on endocardial repolarization in feline heart and have reported no change in monophasic action potential recordings. However, no ECG recordings were reported here. Our own studies of feline subepicardial and subendocardial muscle indicate that at long cycle lengths, there is no direct effect of acetylcholine (consistent with Reference 49 in dogs), but accentuated antagonism is clearly demonstrated. They show, as well, that at a basic cycle length in the range of normal feline heart rate, acetylcholine induces a decrease in epicardial but not endocardial action potential duration. This result is entirely consistent with studies of canine ventricular epicardial and endocardial action potentials in vitro, in which only the epicardium was found sensitive to acetylcholine effects.

Hence, the results of prior studies, as well as our own, are consistent with a vagal effect via the muscarinic receptor and a PTX-sensitive G protein to accelerate ventricular repolarization, resulting in a shortened QT interval. Thus, although these studies do not rule out a role for muscarinic receptor–dependent accentuated antagonism of sympathetic effects, our results argue strongly for an important direct effect of muscarinic agonists on ventricular repolarization.

The Response of the Feline Heart to Vagal Stimulation Is a Predictor of Lethal Arrhythmias During Coronary Occlusion

The search for predictors of lethal events that follow coronary occlusion is not new. In a canine model of chronic anterior myocardial infarction complicated by exercise and acute ischemia in the left circumflex distribution, Schwartz and associates predicted susceptibility to ventricular fibrillation by infusing phenylephrine and monitoring baroreceptor responsiveness. Those animals with a brisk baroreceptor response before exercise and ischemia were resistant to ventricular fibrillation; those with a blunted response were susceptible. Moreover, the modulation of vagal effects on the canine heart by PTX has suggested that the muscarinic actions are mediated by G_{m}.

Studies in patients have also suggested that baroreceptor responsiveness might be a useful indicator of cardiovascular mortality after myocardial infarction.
Not only baroreceptor sensitivity but other tests of vagal effect have been used in human subjects, centering mainly on heart rate variability. The fact that we could calculate an index based on vagal effects on PR or RR intervals, modulate these effects with PTX, and find them predictive of the occurrence of ventricular fibrillation during ischemia emphasizes the linkage that exists between the extent of ADP ribosylation and inactivation of the G protein, the expression of vagal action, and the eventual outcome. Our results are consistent with the idea that upregulation of vagal tone at any of several levels of the receptor-effector pathway will improve outcome, whereas downregulation will favor a negative outcome.

Vagal Protection Against Ventricular Fibrillation

The occurrence of sympathetic-parasympathetic interactions and accentuated antagonism in the setting of myocardial infarction in the intact animal has been documented, as has the occurrence of both sympathetic and parasympathetic hyperactivity during coronary occlusion. That an intact muscarinic receptor pathway reduces mortality after LAD ligation in the cat also has been previously documented. Although the effect of vagal tone on heart rate is protective against lethal arrhythmias in the cat and dog, these earlier studies have suggested that not only the heart rate change but a vagal effect in its own right provides protection. These studies have not necessarily distinguished between direct muscarinic receptor-dependent effects or indirect actions via accentuated antagonism and a β-adrenergic receptor response. However, vagal elevation of the ventricular fibrillation threshold reportedly does not occur in dogs pretreated with propranolol, suggesting that accentuated antagonism is important to vagal modulation of this variable. Our own results indicate that even in the absence of extrinsic vagal stimulation, the maintenance of basal vagal tone during ischemia reduces the incidence of lethal arrhythmias and that reduction of the basal tone by atropine or PTX increases arrhythmogenesis. It should be emphasized that the basal tone referred to is that in the presence of a decentralized vagus and intact neurorheoceptor junction in group I animals and in the presence of an intact vagus in group IIA animals.

In conclusion, we have examined the extent to which the muscarinic receptor and its associated PTX-sensitive G protein contribute to normal repolarization and to ischemic arrhythmias. We have documented the presence of a vagal effect on ventricular repolarization that is independent of vagal effects on heart rate and of β-adrenergic receptor stimulation. We have found that the estimation of vagal tone, via an index calculated by using PR or RR intervals, predicts the likelihood of lethal arrhythmias after coronary occlusion. Finally, we have found that the occurrence of arrhythmias on occlusion is enhanced by atropine or by PTX treatment.

These studies emphasize the complex role of the vagus in its own right and in its sympathetic interactions in modulating the electrophysiology of the normal and the ischemic ventricle.

Acknowledgments

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L Rosenshtraukh, P Danilo, Jr, E P Anyukhovsky, S F Steinberg, V Rybin, K Brittain-Valenti, V Molina-Viamonte and M R Rosen

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