Controlled Intermittent Asystole Cardiac Therapy Induced by Pharmacologically Potentiated Vagus Nerve Stimulation in Normal and Hibernating Myocardium[1]

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Background. **Pharmacologically potentiated electrical stimulation of the right vagus nerve achieves controlled intermittent asystole cardiac therapy. The present study examined pathophysiologic consequences of repetitive intermittent asystoles on contractile function, myocardial blood flow, and vagus nerve function and morphology.**

Methods. **Open-chest anesthetized canines, with either normal left anterior descending (LAD) coronary arteries (n = 8) or severely stenotic LADs (n = 8), received pharmacologic pretreatment with pyridostigmine (0.5 mg/kg), propranolol (80 μg/kg), and verapamil (50 μg/kg) before vagus nerve stimulation. Time-matched control animals with normal (n = 4) or severely stenotic LADs (n = 6) received drugs but no vagus nerve stimulation. The vagus nerve was stimulated for 12 seconds ("on") and rested for 15 seconds ("off"). This algorithm was repeated for 15 on-off cycles, simulating using controlled intermittent asystole during the placement of 15 sutures in a distal coronary anastomosis. This 15-cycle sequence was repeated twice more, simulating a three-vessel bypass.**

Results. **Normal coronary arteries: Ninety minutes after three sets of controlled intermittent asystole, LAD blood flow was unchanged from base line (36.6 ± 4.5 versus 33.0 ± 4.2 mL/min, $p = 0.4$), and global left ventricular performance (impedance catheter, end-systolic pressure-volume relations) was similar to baseline (7.4 ± 1.2 versus7.2 ± 1.0 mm Hg/mL, $p = 0.1$). Left anterior descending coronary artery stenosis model: Ninety minutes after CIA, there were no significant differences versus control animals in regional LAD blood flow (27 ± 4 versus 29 ± 5 mL/min, $p = 0.4$) or fractional shortening of LAD myocardium (sonomicrometry; 6.2% ± 1.8% versus 5.4% ± 1.2%, $p = 0.1$). Vagus nerve conduction and morphology were unchanged from baseline.**

Conclusions. **Repetitive controlled intermittent asystole does not impair poststimulation coronary blood flow, cardiac contractile function, or vagus nerve function. Controlled intermittent asystole may be useful to facilitate off-pump or endoscopic coronary artery bypass grafting.**

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The recent rediscovery and popularization of off-pump coronary artery bypass (OPCAB) grafting challenges surgeons to construct technically perfect coronary anastomoses on the beating heart. Although some studies report excellent graft patency after OPCAB [1], there have been reports of increased anastomotic complications and graft failure rates ranging from 3% to 9% for OPCAB [2–3]. Although avoidance of cardiopulmonary bypass may be a significant advantage to the patient, it should not come at the cost of compromised anastomotic precision or completeness of revascularization.

Cardiac motion remains a significant technical challenge in OPCAB [4]. This challenge is compounded by limited working space in beating heart endoscopic procedures. Although mechanical stabilization devices are available to limit cardiac motion and stabilize the operative field, these mechanical devices may be cumbersome, may limit maneuverability and access to the problematic posterolateral target vessels, and do not completely eliminate cardiac motion at the anastomotic site. Alternatively, cardiac motion can be temporarily reduced by electrically stimulating the vagus nerve. Matheny and Shaar [5] used vagal stimulation alone in patients undergoing OPCAB. However, frequent "vagal escape" beats greatly limited the efficacy of this technique. We [6] have previously reported that brief (10 to 12 seconds) periods of arrest of the heart can be reliably achieved and repetitively applied at the discretion of the surgeon by pharmacologic potentiation of vagus nerve

[1]Controlled Intermittent Asystole[SM] cardiac therapy and surgical services and CIA[SM] cardiac therapy and surgical services are hereinafter referred to as "controlled intermittent asystole" and "CIA," respectively.

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stimulation and control of escape beats. These periods of repetitive and controlled intermittent asystole (CIA) are timed to coincide with passage of the sutures in the distal target vessel, thereby presenting a quiet field just during the suturing interval. However, cumulative episodes of brief asystole causes transient hypotension that has the potential to (1) severely reduce coronary blood flow (repetitive ischemia), (2) activate the endothelium and promote neutrophil accumulation in the target vessel myocardium and neutrophil-mediated endothelial injury [7], and (3) cause contractile dysfunction (ie, myocardial stunning). The potential for these deleterious effects during intermittent asystoles may be greater in hibernating myocardium in which blood flow is impaired by severe coronary artery stenosis [8, 9]. The present study was designed to determine whether repetitive sequences of vagal-induced CIA cause myocardial injury secondary to ischemia in either myocardium with normal coronary arteries or myocardium sensitized to ischemia–reperfusion injury by severe coronary artery stenosis. In addition, the present study determines whether external electrical stimulation causes morphologic injury or conduction abnormalities in the vagus nerve.

Material and Methods

All dogs were handled in compliance with the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (National Institutes of Health publication No. 85-23, revised 1985). The Institutional Animal Care and Use Committee approved the study protocol.

Surgical Procedure

Heart worm–free adult mongrel dogs of either sex weighing 25 to 35 kg were premedicated with morphine sulfate (4 mg/kg) and anesthetized using sodium thiopental (30 mg/kg). Anesthesia was maintained by intravenous fentanyl citrate (0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and diazepam (0.03 $mg \cdot kg^{-1} \cdot min^{-1}$). After endotracheal intubation, the dogs were ventilated with a volume-cycle ventilator, adjusted to maintain pH at 7.35 to 7.45, Po_2 greater than 100 mm Hg, and Pco_2 between 35 and 45 mm Hg. The right femoral artery was cannulated for arterial pressure and arterial blood gas monitoring. After median sternotomy, Millar MPC-500 temperature-compensating solid-state catheters (Millar Instruments, Houston, TX) were placed in the proximal aorta by means of the right internal mammary artery and through the left ventricular apex to measure instantaneous arterial and left ventricular pressures, respectively.

In the group with normal left anterior descending (LAD) coronary blood flow (normal, n = 8) and the corresponding time-matched controls (n = 6), global left ventricular function was measured by instantaneous pressure-volume loops using a 7F octapolar impedance catheter (Webster, Anaheim, CA) introduced by means of the left carotid artery and passed retrograde across the aortic valve into the left ventricle as described previously [10]. In the group in which LAD blood flow was impaired by severe LAD stenosis (stenosis, n = 8), and in respective time-matched controls (n = 4), systolic and diastolic mechanics of the LAD myocardium and left circumflex (nonischemic) myocardium were measured using pairs of segment length sonomicrometer crystals (Triton Technology, Inc, San Diego, CA) placed in the midmyocardium of the respective regions. In all experiments, phasic and mean LAD coronary artery blood flow was measured using a Doppler flow probe placed just distal to the first diagonal branch and connected to a pulsed Doppler flowmeter (model 100, Triton Technology). The right vagus nerve was carefully isolated through a cervical incision for placement of a nerve stimulation probe (Harvard Apparatus, South Natick, MA).

Experimental Protocol

All dogs received the drug combination of pyridostigmine (0.5 mg/kg) to inhibit acetylcholinesterase activity and propranolol (80 μg/kg) and verapamil (50 μg/kg) to suppress escape beats as described previously [6]. Hemodynamic and myocardial function data were acquired before and after drug administration. The dogs were then divided into the two major experimental groups: normal LAD group (n = 8) with corresponding time-matched controls (n = 6), and LAD stenosis group (n = 8) with corresponding controls (n = 4). In the LAD stenosis group, a silicone elastomer ligature was placed around the LAD coronary artery just distal to the first diagonal branch. The ligature was carefully adjusted to reduce LAD blood flow (Doppler flow probe) by approximately 90% relative to the baseline value for 1 hour before vagus nerve stimulation. In both normal and stenosis experimental groups, CIA was induced by stimulating the vagus nerve using a nerve stimulator (Grass Instrument Co, Quincy, MA) in the monopolar mode at a frequency of 40 Hz, an impulse duration of 0.4 ms, and an amplitude of 4 to 8 V. A set of vagus nerve stimulations consisted of 15 "on-off" cycles of 12 seconds of stimulation (heart arrested) and 15 seconds of no stimulation (heart beating), simulating the time used to pass a suture in a distal coronary anastomosis and to prepare for the next suture placement, respectively. There were 10 minutes between each of three sets of 15 asystoles, simulating a three-vessel bypass procedure. Time-matched controls in both groups received the drug combination but no vagus nerve stimulation. In the stenosis group, LAD stenosis was removed after completion of the second CIA set, simulating revascularization of the LAD as the second target vessel. Therefore, stenosis was imposed for a total of approximately 100 minutes. In all groups, data were acquired at 15, 30, 60, and 90 minutes after completion of the vagus nerve stimulations.

Data Acquisition and Analysis

Analog hemodynamic and cardiodynamic data were recorded on a microcomputer using an analog-to-digital converter (model DT2801A; Data Translation, Marlboro, MA) sampling at 250 Hz. Data were analyzed using an interactive videographics program as previously described [11]. In the normal LAD group in which global

left ventricular function was measured using the impedance catheter, chamber conductance was converted to volume using the Leycom Sigma 5 signal conditioner and processor (Oegstgeest, The Netherlands), as described previously [11, 12]. End-systolic and end-diastolic pressure-volume relations were measured during transient caval occlusion. Left ventricular systolic performance was described by the slope and the volume axis intercept of the linear end-systolic pressure-volume relations as described previously [12]. Left ventricular chamber stiffness (the inverse of compliance) was determined by the exponential end-diastolic pressure-volume relation (β-coefficient modulus of stiffness) as previously described [12]. Overall left ventricular performance was determined by the preload-recruitable stroke work relationship.

In the LAD stenosis group, regional function in the anterior myocardium was measured by percent segment shortening, and segmental stiffness was determined from the end-diastolic segment length data as previously described [13].

Plasma Creatine Kinase Activity

Creatine kinase (CK) activity was spectrophotometrically measured from femoral arterial blood samples as described previously [12]. Creatine kinase activity was expressed in international units per microgram of protein.

Cardiac Myeloperoxidase Activity

Neutrophils accumulate in postischemic myocardium, and contribute to coronary artery endothelial dysfunction, contractile dysfunction and infarction [7, 14]. Hence, the accumulation of neutrophils in myocardium is a sensitive marker of an inflammatory response to ischemia-reperfusion. Tissue samples from the LAD myocardium were analyzed spectrophotometrically for myeloperoxidase activity [15], expressed as the change in absorbance units per minute per 100 mg tissue.

Left Ventricular Tissue Water Content

Transmural samples from the LAD myocardium were desiccated for 72 hours, and percent water content was calculated as $100 \times [1 - (\text{dry weight/wet weight})]$.

Left Ventricular Infarct Size

The excised heart was sliced into transverse sections and soaked in a 37°C solution of 1% triphenyltetrazolium chloride (Sigma Chemical Co, St. Louis, MO) to demarcate necrotic myocardium, as described previously [13].

Vagus Nerve Conduction and Histologic Studies

To evaluate possible vagus nerve damage induced by repetitive electrical stimulation, stimulating and receiving electrodes were placed 5 cm apart on the vagus nerve on either side of the point of nerve stimulator contact. The amplitudes of conducted test stimuli (0.5 V, Grass nerve stimulator) before and after the vagal stimulation sets were compared. After sacrifice, the region of the right vagus nerve to which electrical stimulation had been applied was examined by standard hematoxylin and eosin staining.

Results

Two animals (one in each experimental group) were excluded from data analysis after vagus nerve stimulation failed to induce bradycardia before drug administration. These animals were considered as nonresponders and were used as time-matched controls.

Vagal Escape Beats

In hearts with normal LAD blood flow, there was an average of 0.86 ± 0.12 ventricular "escape" beats during the first set of 15 vagal stimulations. This was not significantly different from the 0.92 ± 0.14 and 0.90 ± 0.14 average escape beats during the second and third sets of CIA, respectively. In the hearts with LAD stenosis, there was an average of 0.59 ± 0.1 ventricular escape beats during the first set of CIA, and 0.44 ± 0.09 and 0.49 ± 0.1 average escape beats during the second and third sets of intermittent asystoles, respectively.

Hemodynamic Data

Table 1 shows the hemodynamic changes (heart rate, mean arterial blood pressure, arterial pH) before and after drug administration and during the interval after each CIA set. Whereas the pharmacologic regimen significantly reduced heart rate from base line in experimental and control groups, there were no further changes in heart rate before and after each set of asystoles. In addition, there were no differences in mean arterial pressure before and after drug administration. As expected, mean arterial pressure decreased significantly during the intervals of asystole from an average of 76 ± 5 to 28 ± 3 mm Hg in the normal LAD group, and from 72 ± 6 to 25 ± 4 mm Hg in the LAD stenosis group, with no group differences.

Left Anterior Descending Coronary Artery Blood Flow

Baseline LAD blood flow was comparable among all groups as shown in Figure 1. In the group with normal LAD (Fig 1A), blood flow decreased during each vagal-induced asystole from an average of 33 ± 4 mL/min (baseline) to 4 ± 1 mL/min, coincident with the decrease in mean arterial pressure. In the 10-minute interval between each set of 15 asystoles, LAD blood flow returned to baseline values, and there was no significant difference from respective time-matched controls at comparable times.

In the stenosis group, placement of the LAD stenosis decreased myocardial blood flow from 28 ± 3 to 4 ± 1 mL/min (Fig 1B). Left anterior descending coronary artery blood flow decreased further during each asystolic pause. However, after the stenosis was released before the third CIA set, the decrease in LAD blood flow during the third intermittent asystolic set was less pronounced than when the stenosis was in place (Fig 1B), whereas in the control group, LAD blood flow was restored to prestenotic values. Fifteen, 60, and 90 minutes after the

Table 1. Hemodynamic Variables in Group With Normal Left Anterior Descending Coronary Artery and Stenotic Left Anterior Descending Coronary Artery Groups

Variable	Group	Base Line	Postdrug	INT 1	INT 2	INT 3	60 min	90 min
HR (beats/min)								
Normal	CIA	90.5 ± 3.4	78.3 ± 2.2[a]	81.2 ± 3.1[a]	79.8 ± 4.3[a]	84.2 ± 5.0	85.6 ± 1.8	87.6 ± 3.4
	Control	92.5 ± 4.0	79.0 ± 4.8[a]	80.1 ± 2.9[a]	81.3 ± 3.4[a]	83.1 ± 3.1	83.4 ± 4.3	86.9 ± 4.1
Stenosis	CIA	86.2 ± 5.3	74.6 ± 3.1[a]	79.4 ± 4.5	79.1 ± 3.3	83.6 ± 4.8	84.6 ± 2.2	83.2 ± 2.9
	Control	88.8 ± 6.1	72.0 ± 6.7[a]	79.1 ± 4.4	80.7 ± 2.9	83.4 ± 2.1	85.9 ± 4.1	88.1 ± 5.6
MAP (mm Hg)								
Normal	CIA	77.6 ± 4.6	76.2 ± 5.6	77.8 ± 4.3	74.6 ± 5.6	76.6 ± 2.1	77.1 ± 4.3	76.9 ± 3.1
	Control	76.1 ± 3.8	74.1 ± 3.6	78.6 ± 2.1	75.9 ± 1.2	77.7 ± 4.1	74.9 ± 2.7	75.0 ± 4.9
Stenosis	CIA	79.2 ± 5.1	75.9 ± 6.6	74.3 ± 5.5	77.8 ± 4.6	75.8 ± 3.3	75.9 ± 5.1	78.9 ± 2.2
	Control	77.3 ± 4.2	77.2 ± 5.2	78.9 ± 4.8	79.0 ± 3.7	78.0 ± 5.1	76.4 ± 4.8	76.7 ± 4.5
pH (units)								
Normal	CIA	7.35 ± 0.4	7.37 ± 0.2	7.38 ± 0.4	7.35 ± 0.5	7.33 ± 0.4	7.35 ± 0.3	7.37 ± 0.5
	Control	7.37 ± 0.3	7.34 ± 0.4	7.38 ± 0.3	7.37 ± 0.4	7.36 ± 0.3	7.35 ± 0.2	7.36 ± 0.5
Stenosis	CIA	7.38 ± 0.3	7.37 ± 0.3	7.38 ± 0.2	7.36 ± 0.4	7.34 ± 0.3	7.35 ± 0.4	7.37 ± 0.5
	Control	7.39 ± 0.2	7.39 ± 0.4	7.38 ± 0.2	7.37 ± 0.3	7.38 ± 0.2	7.36 ± 0.3	7.35 ± 0.4

[a] $p < 0.05$ compared with base line.

HR = heart rate; CIA = controlled intermittent asystole; INT 1 = interval between first and second intermittent asystole; INT 2 = interval between second and third intermittent asystole sets; MAP = mean arterial pressure; 60 min, 90 min = number of minutes of recovery after last intermittent asystole set.

last asystole set, LAD blood flow returned to baseline values in both groups. Therefore, despite a total of 45 transient decreases in blood flow (three sets of 15 pauses) in the groups with normal and stenotic coronary arteries, LAD blood flow was unchanged from baseline values.

Systolic and Diastolic Function

In hearts with normal LAD, baseline global function measured by the slope of the end-systolic pressure-volume relations was comparable between the CIA and time-matched control groups (Table 2). The drug regimen did not alter systolic function relative to the predrug value (Table 2). Recovery of systolic function after each set of repetitive CIA was not significantly different compared with baseline values and values in control animals at comparable time periods. There were no time-related differences in end-systolic pressure-volume relations after each set of CIA, and there were no differences from

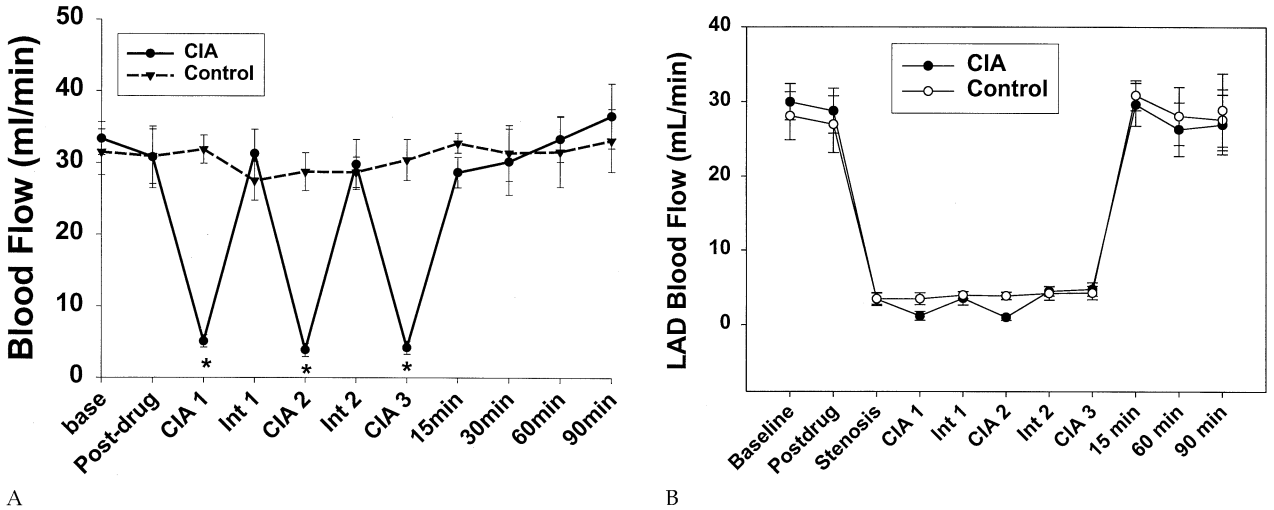

*Fig 1. (A) Left anterior descending (*LAD*) coronary artery blood flow (mL/min) in the group with normal coronary arteries. Left anterior descending coronary artery blood flow is significantly reduced during intermittent asystole pauses in the controlled intermittent asystole (*CIA*) group, but was not significantly different from time-matched controls (*Control*) during the poststimulation recovery period. (B) Left anterior descending coronary artery blood flow in the left anterior descending coronary artery stenosis group. Left anterior descending coronary artery stenosis significantly reduced blood flow from baseline; blood flow during the first and second intermittent asystoles was reduced further, but was not reduced further during the third intermittent asystole when the stenosis was resolved. *p < 0.05 versus control group. (*Base = *baseline;* Post-drug = *after administration of drug regimen;* Int = *interval between sets of vagal-induced asystoles;* 15 min, 30 min, 60 min, and 90 min = *number of minutes after the last intermittent asystole.)*

Table 2. Cardiodynamic Variables in Group With Normal Left Anterior Descending Coronary Artery and Respective Time-Matched Controls[a]

Variable	Base Line	Postdrug	Int 1	Int 2	30 min	60 min	90 min
ESPVR (mm Hg/mL)							
CIA	7.4 ± 1.2	7.3 ± 0.9	7.1 ± 1.3	7.1 ± 1.3	7.4 ± 2.0	7.0 ± 0.6	7.2 ± 1.0
Control	6.8 ± 0.9	7.0 ± 1.1	7.1 ± 1.3	7.1 ± 1.3	7.1 ± 1.3	7.2 ± 0.8	7.4 ± 1.4
PRSW [(mm Hg≅mL)/mL]							
CIA	67.8 ± 4.3	72.1 ± 4.3	73.1 ± 6.7	71.1 ± 4.8	69.5 ± 5.4	67.1 ± 3.1	69.9 ± 5.5
Control	64.2 ± 6.8	68.6 ± 5.1	66.6 ± 7.8	70.0 ± 5.4	76.4 ± 6.8	66.9 ± 5.4	70.6 ± 3.6
Modulus of stiffness							
CIA	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.04	0.08 ± 0.02	0.12 ± 0.04	0.13 ± 0.04	0.11 ± 0.01
Control	0.10 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.11 ± 0.03	0.09 ± 0.01	0.11 ± 0.03	0.10 ± 0.02

[a] Values are mean ± standard error of the mean.

ESPVR = end-systolic pressure–volume relations; CIA = controlled intermittent asystole; INT 1 = interval between first and second intermittent asystole sets; INT 2 = interval between second and third intermittent asystole sets; PRSW = preload recruitable stroke work; 30 min, 60 min, 90 min = number of minutes of recovery after last intermittent asystole set.

the time-matched control group during the 90-minute recovery period. Similar observations were made with preload-recruitable stroke work (Table 2). Hence, neither the repetitive decreases in blood pressure and LAD blood flow nor the drug regimen altered left ventricular function significantly. Baseline left ventricular chamber stiffness was comparable between the group with normal coronary arteries and its respective control group (Table 2). There was no significant change in chamber stiffness at any time compared with baseline, and there were no differences between the CIA group and the matched control group.

In the LAD stenosis group, regional systolic shortening in the LAD myocardium was not significantly affected by the administration of the drug regimen compared with the prestenosis baseline (Fig 2A). Systolic shortening was significantly decreased after 1 hour of severe stenosis compared with base line values (Table 3, Fig 2A). The three sets of CIA did not diminish the recovery of systolic shortening in the LAD myocardium relative to time-matched controls without repetitive systolic pauses. After the stenosis was relieved (interval 3 in Fig 2A), simulating revascularization of a target vessel, systolic shortening recovered to approximately 30% of baseline value in both groups. Hence, repetitive episodes of intermittent asystole did not impair contractile function in either the group with normal LAD or stenotic LAD. Stiffness in the LAD myocardium did not change with administration of

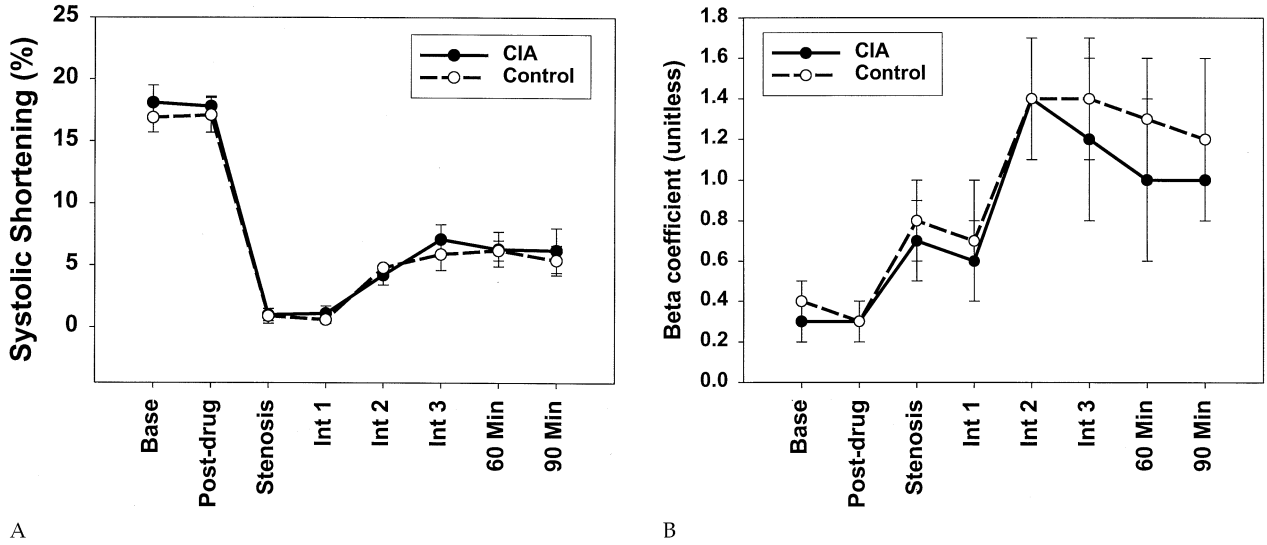

Fig 2. Systolic and diastolic mechanics in the left anterior descending coronary artery stenosis group. (A) Systolic shortening percent in the left anterior descending coronary artery myocardium was significantly reduced by stenosis, but recovery of contractile function after the three sets of intermittent asystole and resolution of the stenosis was not different from that of time-matched controls (Control). *(B) Modulus of stiffness in the left anterior descending coronary artery myocardium. Stiffness was not altered by the drug regimen, but increased as a result of the stenosis; there was no effect from the three sets of intermittent asystole compared with the time-matched controls* (Control). **p < 0.05 versus control group.* (Base = *baseline;* Post-drug = *after administration of drug regimen;* CIA = *controlled intermittent asystole;* Int = *interval between sets of vagal-induced asystoles;* 60 min and 90 min = *number of minutes after the last intermittent asystole.)*

Table 3. Regional Cardiodynamic Variables in the Left Anterior Descending Coronary Artery Stenosis Group

Variable	Base Line	Postdrug	Stenosis	Int 1	Int 2	30 min	60 min	90 min
EDL (mm)								
CIA	13.1 ± 1.6	13.5 ± 1.2	15.8 ± 0.9[a]	14.9 ± 1.6	15.3 ± 1.1	13.6 ± 1.3	13.8 ± 1.2	13.4 ± 1.5
Control	12.9 ± 1.1	13.7 ± 1.9	16.1 ± 2.1[a]	15.1 ± 1.2	14.6 ± 1.8	12.9 ± 0.9	13.1 ± 1.8	13.6 ± 1.8
ESL (mm)								
CIA	11.7 ± 1.9	11.9 ± 1.6	15.3 ± 1.6[a]	14.5 ± 2.1[a]	14.7 ± 1.7[a]	12.9 ± 1.5	13.0 ± 1.9	12.3 ± 1.1
Control	11. ± 1.7	11.8 ± 2.0	15.9 ± 1.8[a]	14.9 ± 1.5[a]	14.0 ± 2.2[a]	12.1 ± 1.4	12.5 ± 1.2	12.6 ± 1.7
% SS								
CIA	18.1 ± 1.4	17.8 ± 0.8	1.0 ± 0.5[a]	1.1 ± 0.6[a]	4.2 ± 0.8[a]	7.1 ± 1.2[a]	6.3 ± 1.4[a]	6.2 ± 1.8[a]
Control	16.9 ± 1.2	17.1 ± 1.4	0.9 ± 0.6[a]	0.6 ± 0.3[a]	4.8 ± 0.3[a]	5.9 ± 1.3[a]	6.2 ± 0.8[a]	5.4 ± 1.2[a]
Stiffness								
CIA	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.2[a]	0.6 ± 0.2[a]	1.4 ± 0.3[a]	1.2 ± 0.4[a]	1.0 ± 0.4[a]	1.0 ± 0.2[a]
Control	0.4 ± 0.1	0.3 ± 0.1	0.8 ± 0.2[a]	0.7 ± 0.3[a]	1.4 ± 0.3[a]	1.4 ± 0.3[a]	1.3 ± 0.3[a]	1.2 ± 0.4[a]

[a] $p < 0.05$ versus baseline and postdrug value.

EDL = end-diastolic length; ESL = end-systolic length; CIA = controlled intermittent asystole; INT 1 = interval between first and second intermittent asystole sets; INT 2 = interval between second and third intermittent asystole sets; %SS = percent systolic shortening; 30 min, 60 min, 90 min = number of minutes of recovery after last intermittent asystole set.

the drug regimen in the stenosis group (Fig 2B). However, LAD myocardium segmental stiffness significantly increased after the stenosis (0.7 ± 0.2, $p < 0.05$ versus baseline and drug values). Regional stiffness remained elevated 90 minutes after the last set of CIA (1.0 ± 0.2 U), but there was no difference between CIA and control groups. Hence, the increased stiffness in the LAD segment was likely related to the stenosis rather than to the three sets of CIA.

Tissue Edema

In the group with normal LAD, postexperimental myocardial water content was unchanged compared with time-matched controls. Transmural LAD myocardial tissue water content averaged 78.2% ± 3.6% in the CIA group, and 76.4% ± 4.3% in time-matched controls. In the LAD stenosis series, postexperimental tissue water content in the CIA group (78.7% ± 4.2%) was not significantly different from that in the time-matched control group (77.9% ± 5.1%). These data are consistent with the lack of group differences in chamber stiffness (β-coefficient) with repetitive episodes of CIA.

Plasma Creatine Kinase Activity

In the group with normal LAD, baseline plasma CK activity levels were comparable among groups (Table 4). The drug regimen did not alter the plasma CK levels in any group. In the LAD stenosis group, there was a significant increase in plasma CK activity after application of the stenosis relative to the baseline value. However, there was no further change in CK activity with repetitive episodes of CIA. Hence, in neither the normal nor LAD stenosis groups did repetitive episodes of CIA cause an increase in plasma CK levels.

Myeloperoxidase Activity

Repetitive episodes of CIA were associated with significant reductions in myeloperoxidase activity in the LAD myocardium relative to the respective controls in both the normal LAD group (4.2 ± 0.8 versus 7.4 ± 1.1 absorbance units/min) and the LAD stenosis group (6.7 ± 0.9 versus 9.7 ± 0.8 absorbance units/min, $p < 0.05$). These data suggest that repetitive episodes of CIA decreased neutrophil accumulation in the LAD myocardium compared with the control group which had no episodes of CIA.

Myocardial Infarction

There was no myocardial infarction identified by triphenyltetrazolium chloride in any of the hearts in any group.

Table 4. Creatine Kinase Activity

Group	Base Line	Postdrug	Int 1	Int 2	30 min	60 min	90 min
Normal							
CIA	2.34 ± 0.55	2.09 ± 1.82	5.06 ± 1.39	4.44 ± 2.01	5.47 ± 1.80	4.81 ± 0.96	4.22 ± 1.61
Control	3.09 ± 0.61	4.35 ± 0.99	5.33 ± 2.03	5.90 ± 1.11	5.38 ± 1.88	3.09 ± 1.61	4.87 ± 0.94
Stenosis							
CIA	3.45 ± 0.90	2.45 ± 0.99	8.06 ± 1.67[a]	8.06 ± 1.11[a]	6.45 ± 1.67[a]	5.81 ± 1.48[a]	5.65 ± 0.54[a]
Control	2.90 ± 0.81	3.12 ± 0.43	9.33 ± 1.78[a]	7.46 ± 1.02[a]	6.34 ± 1.34[a]	6.89 ± 1.99[a]	6.22 ± 0.71[a]

[a] $p < 0.05$ compared with baseline.

INT 1 = interval after the first intermittent asystole set; INT 2 = interval after the second intermittent asystole set; 30 min, 60 min, 90 min = number of minutes of recovery after the last intermittent asystole set; CIA = controlled intermittent asystole.

Vagus Nerve Function and Morphology

Conduction of 0.5 V through the vagus nerve resulted in a maximal amplitude of 310 ± 35 mV at baseline. Ninety minutes after the last set of CIA, maximal amplitude of this stimulus was similar to the value at baseline and in controls (302 ± 13 mV, $p = 0.8$). There were no overt pathologic changes seen at the site of stimulator placement. Myelinated and unmyelinated nerve fibers were of normal caliber, and myelin profiles were intact.

Comment

Controlled intermittent asystole was developed to create an "on-off" switch that could be applied at the surgeon's discretion to achieve brief (10–15 seconds) intervals of asystole during which sutures can be placed in a target coronary artery anastomotic site [6]. However, the physiologic consequences of repetitive episodes of asystole have not been studied in an off-pump setting in which profound hypotension during the periods of arrest cannot be counteracted by extracorporeal means. In the present study, pharmacologically potentiated vagus nerve stimulation caused immediate global arrest followed by rapid recovery of normal systolic contractions when the stimulator was turned off, thereby providing the surgeon with an "on–off" switch that can be synchronized with placement of each suture in the target vessel. There was a low incidence of escape beats in the groups with either normal coronary arteries or severe coronary artery stenosis, owing to suppressive actions of the drug regimen [6]. In addition, repetitive periods of asystole were accompanied by transient hypotension and significant decreases in coronary blood flow, with the greatest decrease in coronary blood flow during asystole being observed in the stenotic coronary artery. However, there was no persistent change in either global or segmental systolic or diastolic function after the three sets of intermittent asystole compared with prearrest conditions or time-matched controls. In addition, there was no increase in either plasma CK activity or tissue water content attributable to the repetitive episodes of CIA. Surprisingly, there was a significant reduction in neutrophil accumulation in the myocardium of both experimental models after recovery from the three sets of CIA. Therefore, repetitive periods of transient asystole showed no physiologic evidence of myocardial injury in either normal or vulnerable myocardium. Finally, the vagus nerve demonstrated no histologically apparent injury or impairment of conduction velocity near the site of stimulation.

The absence of persistent defects in cardiac function or coronary blood flow after multiple episodes of hypoperfusion is likely the result of a number of factors. First, the periods of hypoperfusion may have been insufficient to directly cause defects in function or blood flow. Second, the pharmacologic regimen may itself be cardioprotective. For example, the inclusion of a calcium-channel blocker in the drug regimen may have decreased calcium influx involved in postischemic injury. Third, the periods of arrest may have reduced myocardial oxygen demand at the same time that coronary blood flow was reduced. Hence, the periods of reduced blood flow would have coincided with low oxygen demands during the intervals of vagal-induced asystole, thereby ameliorating the oxygen supply–demand imbalance.

Interestingly, the reduction in neutrophil accumulation in LAD myocardium relative to controls was the only significant difference between the CIA groups and the time-matched control groups, and may suggest a cardioprotective effect of CIA against neutrophil-mediated injury. The neutrophil has been implicated as a primary mediator of myocardial injury during ischemia–reperfusion, and its adherence to coronary artery endothelium and subsequent accumulation in ischemic–reperfused myocardium are hallmarks of postischemic injury [7, 16–18]. Even brief periods of ischemia followed by reperfusion are associated with an accumulation of neutrophils in the involved myocardium [19]. Therefore, repetitive episodes of CIA may exert a "preconditioning-like" effect. Ischemic preconditioning is stimulated by imposing multiple brief periods of ischemia before a longer "index" ischemic episode that otherwise causes contractile dysfunction and infarction [20]. Ischemic preconditioning can attenuate contractile dysfunction under some conditions [21–23], but it is most effective in reducing myocardial infarct size [24]. A number of studies have shown a reduction in neutrophil accumulation with ischemic preconditioning [25–27]. Using a canine model simulating off-pump reperfusion, Bufkin and associates [19] reported that a brief ischemic period preceding 30 minutes of total LAD occlusion decreased neutrophil accumulation in the ischemic–reperfused zone. However, in that study [19] and in the present study, the reduction in neutrophil accumulation was not associated with a reduction in contractile dysfunction in the stenosis group.

The present study failed to show any conduction deficits in the vagus nerve across the region of electrical stimulation. The amplitude of conducted test stimuli was unchanged at the conclusion of the experiment, suggesting no functional nerve damage caused by direct electrical stimulation. Similarly, the lack of histologically apparent axonal and myelin abnormalities at the site of stimulation suggests that the procedure did not have any acute effect on the nerve fibers. Previous studies of chronic vagus stimulation for treatment of seizure disorders similarly have failed to document significant functional nerve damage [28].

In summary, this study shows that reliable and controllable cardiac asystole can be repetitively achieved by vagal stimulation with pharmacologic potentiation in an experimental model simulating a three-vessel OPCAB operation in both normal myocardium and stenotic myocardium. There was an absence of further cumulative myocardial dysfunction or injury after the three CIA episodes and associated hypoperfusion. There was, however, a reduction in neutrophil accumulation in the myocardium, which may suggest an inherent cardioprotective mechanism that may have prevented cumulative

injury. Finally, external stimulation of the vagus nerve was not associated with morphologic or functional injury. The present study is limited in that it was performed using relatively healthy hearts without chronic infarction, diffuse arteriosclerosis, hypertension, or congestive failure, which may be encountered clinically. The results of this protocol may not be similar in hearts with these clinical conditions.

Although originally conceived to facilitate OPCAB surgery, the technique of controlled intermittent asystole may be useful during on-pump coronary artery bypass grafting procedures to avoid global ischemia or application of a cross-clamp to the severely diseased aorta [29]. In addition, this technique may be useful in enabling endoscopic or robotic coronary bypass, for thoracoscopic placement of pacing leads, for endovascular deployment of aortic stents, and for a variety of electrophysiologic and percutaneous interventional procedures.

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