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ORIGINAL RESEARCH

Pulseless Electrical Activity as the Initial Cardiac Arrest Rhythm: Importance of Preexisting Left Ventricular Function

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BACKGROUND: Pulseless electrical activity (PEA) is a common initial rhythm in cardiac arrest. A substantial number of PEA arrests are caused by coronary ischemia in the setting of acute coronary occlusion, but the underlying mechanism is not well understood. We hypothesized that the initial rhythm in patients with acute coronary occlusion is more likely to be PEA than ventricular fibrillation in those with prearrest severe left ventricular dysfunction.

METHODS AND RESULTS: We studied the initial cardiac arrest rhythm induced by acute left anterior descending coronary occlusion in swine without and with preexisting severe left ventricular dysfunction induced by prior infarcts in non–left anterior descending coronary territories. Balloon occlusion resulted in ventricular fibrillation in 18 of 34 naïve animals, occurring 23.5±9.0 minutes following occlusion, and PEA in 1 animal. However, all 18 animals with severe prearrest left ventricular dysfunction (ejection fraction 15±5%) developed PEA 1.7±1.1 minutes after occlusion.

CONCLUSIONS: Acute coronary ischemia in the setting of severe left ventricular dysfunction produces PEA because of acute pump failure, which occurs almost immediately after coronary occlusion. After the onset of coronary ischemia, PEA occurred significantly earlier than ventricular fibrillation (<2 minutes versus 20 minutes). These findings support the notion that patients with baseline left ventricular dysfunction and suspected coronary disease who develop PEA should be evaluated for acute coronary occlusion.

Key Words: acute myocardial infarction ■ cardiac arrest ■ pulseless electrical activity ■ resuscitation

There are 560 000 victims of cardiac arrest annually in the United States, with 360 000 out-of-hospital cardiac arrests (OHCA) and 200 000 in-hospital cardiac arrests. The majority of cardiac arrests are attributable to nonshockable rhythms.[1–3] Data from the National Registry of Cardiopulmonary Resuscitation, reporting on 51 919 in-hospital arrests, found only ≈24% had an initial rhythm of ventricular tachycardia (VT) or ventricular fibrillation (VF), whereas the remaining 37% and 39% had pulseless electrical activity (PEA) or absence of electrical activity as the initial rhythm, respectively.[4] Similar trends are reported for out-of-hospital arrests.[2,5] PEA is broadly defined as the condition in which spontaneous organized cardiac electrical activity is present in the absence of blood flow sufficient to maintain organ perfusion and consciousness, and the absence of rapid spontaneous return of perfusion.[6] The survival rate for VT/VF averages 20%, and the survival rate for PEA/asystole is substantially lower, averaging 5%.[4,7] Thus, current resuscitation algorithms are inadequate for PEA arrests.

See Editorial by Gazmuri

Although the majority of cardiac arrests in the setting of ischemic heart disease are caused by

CLINICAL PERSPECTIVE

What Is New

- In the swine model, acute coronary ischemia in the setting of severe left ventricular dysfunction produces pulseless electrical activity because of acute pump failure, which occurs almost immediately after coronary occlusion.

What Are the Clinical Implications?

- Patients with baseline left ventricular dysfunction and suspected coronary disease who develop pulseless electrical activity should be evaluated for acute coronary occlusion.
- Furthermore, given the severity of left ventricular dysfunction, these patients may benefit from an aggressive resuscitation strategy incorporating rapid revascularization and mechanical circulatory support.
- Ventricular fibrillation after pulseless electrical activity may be an indicator of an acute coronary occlusion

Nonstandard Abbreviations and Acronyms

ACO	acute coronary occlusion
IPC	ischemic preconditioning
OHCA	out-of-hospital cardiac arrest
PEA	pulseless electrical activity
PKCε	protein kinase C epsilon

shockable ventricular arrhythmias such as VT and VF,[8] a substantial number of arrests caused by coronary ischemia present with an initial rhythm of PEA.[9] Several studies, including an autopsy study following witnessed OHCA caused by PEA with unsuccessful resuscitation, reported significantly more diagnoses of acute myocardial infarction than of pulmonary embolism and other noncardiac causes.[10] Furthermore, data from a large multicenter retrospective study demonstrated a high incidence of culprit lesions treated with coronary intervention in patients after cardiac arrest with initially nonshockable rhythms and without ST-segment–elevation myocardial infarction (STEMI) on ECG.[1] Similarly, in another study of patients with OHCA caused by PEA, there was a 70% prevalence of obstructive coronary artery disease, with over one third of the patients having angiographic culprit vessel lesions warranting percutaneous coronary intervention,[11] further implicating PEA as an initial acute coronary occlusion (ACO) cardiac arrest rhythm in ischemic heart disease. We hypothesized that the underlying substrate, and specifically left ventricular

(LV) function, was an important factor determining PEA versus VT/VF as the initial rhythm in cardiac arrest caused by acute coronary ischemia.

Not all nonshockable arrests remain nonshockable. Although VF cannot spontaneously develop into PEA, PEA can spontaneously progress to VF, which is associated with substantially decreased survival when compared with survival in those patients with PEA that does not degenerate to VF.[4] Features distinguishing those with PEA that do and do not progress to VF are not well characterized, but a potential explanation may be related to ischemic preconditioning (IPC). In IPC, the myocardium is subjected to brief episodes of ischemia and reperfusion, leading to rapid metabolic adaptations that render the heart less vulnerable to the consequences of a subsequent prolonged ischemic insult, including ventricular arrhythmias. Although IPC itself cannot be used in a therapeutic manner, spontaneous preconditioning, in both acute and chronic ischemic settings, likely confers protective features in the setting of a subsequent prolonged ACO.[12–16] With cardiac arrest caused by acute ischemia specifically, peri-infarct angina (ie, angina occurring within 48 hours before hospital arrival for the index STEMI), similar to acute preconditioning, is protective against out-of-hospital VF arrest and is associated with improved 5-year survival.[12,13] Although it is known that IPC decreases the likelihood of VT/VF in ACO, it is not known whether IPC decreases the likelihood or delays the onset of subsequent VT/VF in PEA.

We hypothesized that prearrest cardiac function was a significant factor determining whether an initial ischemia-induced cardiac arrest rhythm is VF or PEA, and that swine would be a good model to test this hypothesis. We further hypothesized that preconditioning could be applied in a swine model of ACO, and that in animals with severe LV dysfunction, ischemic preconditioning decreases the incidence of VF and/or prolongs the time before PEA degenerates to VF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

This was a controlled laboratory experiment performed using swine. All animals were treated in accordance with institutional Johns Hopkins Animal Care and Use Committee guidelines and in compliance with the Animal Welfare Act regulations and Public Health Service Policy.

To test our hypotheses, 2 groups of animals were studied. Group 1 comprised animals with normal left ventricular function. Group 2 comprised animals with severely reduced left ventricular systolic function. These were prepared via serial infarctions performed in the right coronary artery and left circumflex coronary artery by occluding each artery for 2 hours. The 2 infarctions

were separated by 1 week. Both groups underwent a prolonged left anterior descending coronary artery (LAD) occlusion performed via percutaneous coronary balloon occlusion (Figure 1). Before the LAD occlusion, baseline LV function was assessed via transthoracic echocardiography in 14 of 34 animals in Group 1 and 15 of 18 animals in Group 2. Ejection fraction was determined by the fractional shortening method taking the average of the parasternal long, mid, and base parasternal short views. Baseline invasive vital signs were obtained before the LAD balloon occlusion.

A subset of animals from the 2 groups, Group 1b and Group 2b, underwent IPC via short cycles of coronary occlusion and reperfusion before the final prolonged occlusion. The IPC protocols are described below. The arrest rhythms in these animals were compared with those in the animals that did not undergo IPC (Group 1a and 2a).

Study end points included the incidence of and time to VF and/or PEA. If PEA developed, the animal remained in PEA for 60 minutes or until subsequent VF developed. PEA was defined as sustained severe hypotension (mean arterial pressure <40 mm Hg) with organized cardiac electrical activity. Animals that did not have any form of arrest at 60 minutes were euthanized.

General Animal Preparation

Pigs (35±5 kg, female, American Yorkshire breed) were tranquilized with Sedazine 18 µg/kg, ketamine 0.9 mg/kg intramuscularly, and Telazol 0.23 mg/kg

intramuscularly, anesthetized with pentothal 15 mg/kg intravenously, intubated, and mechanically ventilated (Narkomed 2A, Drager) with 100% O_2 and 0.5% to 1.5% isoflurane. Percutaneous femoral venous and arterial access were established. Electrocardiographic monitoring was continuous for all protocols. An arterial sheath was placed in the femoral artery for systemic blood pressure measurements. Coronary guide catheters (6 Fr) were placed in the desired coronary arteries. Angioplasty catheters were positioned under fluoroscopy in desired coronary arteries. In addition to baseline left ventricular ejection fraction, postinfarction transthoracic echocardiography was used in a subset of Group 2 animals to assess wall motion, ejection fraction, and stroke volume during PEA.

Animal Groups/Protocols
Group 1a: Normal LV Function, No IPC: n=23

After the initial preparation, the proximal LAD was occluded for 60 minutes unless VF occurred earlier, in which case the experiment end point was achieved.

Group 1b: Normal LV Function, IPC: N=11

After initial preparation, 4 to 6 cycles of 3 to 4 minutes proximal LAD occlusions followed by 6 to 7 minutes of reperfusion were performed. After this series, the LAD was occluded at the same location for 60 minutes unless VF occurred earlier, in which case the experiment end point was achieved (Figure 2).
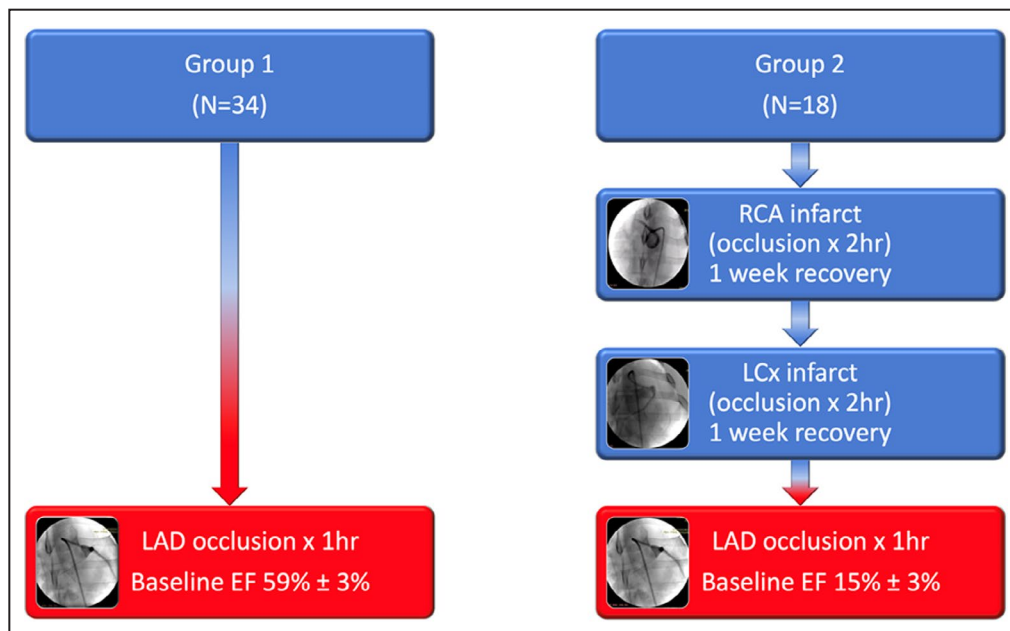
Figure 1. Animal Group 1 and Group 2 protocols.
A subset of each group underwent ischemic preconditioning described in Figure 2. EF indicates ejection fraction; LAD, left anterior descending coronary artery; LCx, left circumflex artery; and RCA, right coronary artery.
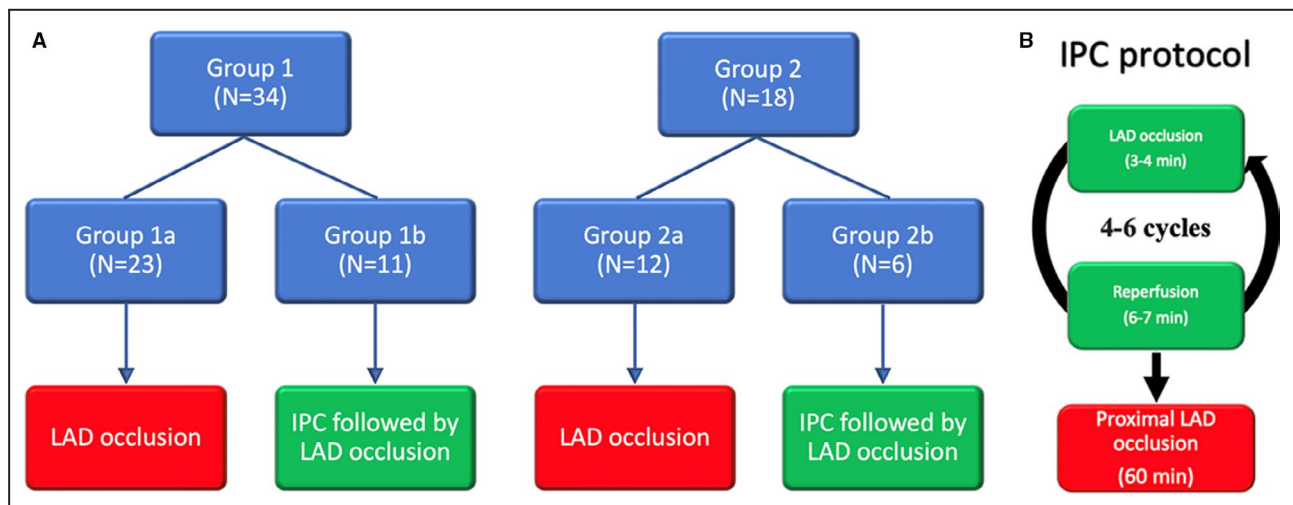
Figure 2. **Protocols for group subset allocation for ischemic preconditioning (IPC) vs no IPC.**
A, Schematic of Groups 1 and 2 and Group subsets 2a and 2b. **B**, Ischemic preconditioning protocols for Groups 1b and 2b. Group 1b underwent preconditioning using transient occlusions of the proximal left anterior descending coronary artery (LAD), whereas group 2b underwent preconditioning using transient occlusions of the distal LAD because proximal LAD occlusion led to profound hypotension.

Group 2a: Severe LV Dysfunction, No IPC: N=12

Serial infarcts were performed in 2 major coronary vascular territories (left circumflex and right coronary artery) by occluding each respective artery for 2 hours. Infarcts were separated by 1 week. If reperfusion VF occurred, it was promptly treated with defibrillation. One week after the second infarct, the proximal LAD was occluded for 60 minutes (Figure 1). The incidences of and times to the onset of PEA/VF were recorded.

Group 2b: Severe LV Dysfunction, IPC: N=6

Serial infarcts were performed in 2 major coronary vascular territories (left circumflex and right coronary artery) by occluding each respective artery for 2 hours (Video S1). Infarcts were separated by 1 week. If reperfusion VF occurred, it was promptly treated with defibrillation. One week after the second infarct, 4 cycles of 3 minutes of occlusion/7 minutes of reperfusion were performed in the distal LAD (proximal LAD preconditioning was not feasible because of profound hypotension). After this series, a final occlusion was performed in the proximal LAD for 60 minutes (Figure 2). The incidences of and times to the onset of the initial arrests were recorded. If the initial arrest was PEA, the incidence and time to subsequent VF were also recorded.

In 10 animals, we assayed for successful IPC by quantifying coronary reactive hyperemia via Doppler coronary flow wire (Volcano Therapeutics; 0.014-in diameter). In addition to hemodynamic and reactive hyperemic assessments of IPC, subcellular distribution of PKCε (protein kinase C epsilon) was assessed by immunoblotting, and its translocation from the cytosol

to the mitochondria was used as a molecular marker of successful IPC in the swine model.

Sample Preparation and Immunoblotting

Heart tissue samples were collected immediately after each experiment, rinsed in cold phosphate-buffered saline, blotted dry, and snap-frozen in liquid nitrogen. Tissue samples were homogenized in sample homogenization buffer (25 mmol/L Hepes pH=7.4, Complete Mini Protease Inhibitor Cocktail and PhosSTOP phosphatase inhibitors [Roche] in cold phosphate-buffered saline) using a chilled bead mill (Mixer Mill MM400; Retsch) for 2 minutes at 28 Hz. Tissue homogenates were centrifuged at 18 000*g* for 15 minutes at 4ºC. We henceforth refer to the pellets that are enriched in mitochondria as the particulate fraction and supernatants containing mainly cytoplasmic proteins as the cytosolic fraction. Protein concentrations were evaluated using EZQ protein quantitation kit (Thermo Fisher) in denatured samples (7 minutes at 95°C in the presence of sodium dodecyl sulfate and 1,4-dithiothreitol). Twenty micrograms of protein were separated using Tris-glycine precast gels (Criterion TGX; BioRad). After separation, proteins were transferred onto a nitrocellulose membrane and blocked in 5% nonfat dry milk (Carnation; Nestle) in Tris-buffered saline with Tween 20 for 1 hour. Membranes were incubated overnight with PKCε antibody (1:1000 dilution, rabbit, #MA5-14908; Life Technologies). Membranes were then washed 3 times for 7 minutes in Tris buffered saline with Tween 20, followed by 30 minutes incubation with the secondary antibody (1:10 000 goat anti-rabbit immunoglobulin G; Li-COR). Lastly, membranes were washed

twice for 7 minutes each in Tris-buffered saline with Tween 20, followed by a third wash with Tris-buffered saline for 7 minutes. Images were acquired using a fluorescent scanner (Odyssey; Li-COR). To measure the enrichment of mitochondria in the particulate fraction, membranes were costained with an antibody toward the β subunit of adenosine triphosphate synthase β (1:2500 mouse, #ab14730; Abcam) for 1 hour in Tris-buffered saline with Tween 20, followed by secondary antibody staining with goat anti-mouse antibody (Li-COR) in Tris-buffered saline with Tween 20 before acquisition. All membranes were poststained using the general protein staining direct blue 71 (Sigma) to control for both equal loading and transfer efficiency. Densitometry analysis was performed with ImageJ (National Institutes of Health).

Statistical Analysis

All mean values are accompanied by standard deviations. All statistical analyses were 2-sided with α=0.05. Comparisons of means between groups were performed using either unpaired t test or 1-way ANOVA test as appropriate, and $χ^2$ testing for proportions. Comparisons between groups were performed using the log rank test.

RESULTS

There were no statistically significant differences in baseline heart rate or systolic, diastolic, and mean arterial pressures across groups and subgroups. Baseline left ventricular ejection fraction was measured in 14 of 34 animals in Group 1 and was 59±3%, which is a normal left ventricular ejection fraction for swine (Video S2). The remaining animals in Group 1 were presumed to have normal LV function, because these animals did not

undergo any prior intervention. Baseline left ventricular ejection fraction was measured in 15 of 18 animals in Group 2 before the final experiment and was 15±3%, consistent with severe LV dysfunction. The marked difference in ejection fraction between groups was statistically significant ($P<0.000001$) (Tables 1 and 2).

Group 1: Normal LV Function, With and Without IPC: N=34

Balloon occlusion resulted in VF in 18 of 34 naïve animals, occurring 23.5±9.0 minutes after occlusion, and PEA occurred in 1 animal (Figures 3A and 4A). The single case of PEA in this group occurred 2.9 minutes after the onset of LAD occlusion, and subsequent VF developed 18.5 minutes from occlusion. The remaining animals did not have any cardiac arrest at 60 minutes from occlusion. Other than the single case of PEA, there was no significant hypotension before VF in any animal (Table 3).

Group 2: Severe LV Dysfunction, With and Without IPC: N=18

All 18 Group 2 animals developed PEA as the initial arrest, which occurred 1.7±1.1 minutes after vessel occlusion (Figure 3B). Sixteen of the animals developed subsequent VF, which occurred 17.6±11.0 minutes after occlusion (Table 3). The severity of prearrest cardiac dysfunction that resulted in PEA was substantial. Mean ejection fraction before the final occlusion was 15±3%, consistent with our aim of producing significant LV dysfunction before the final ischemic insult (Video S3). The mean ejection fraction at PEA onset was 5±3% (Video S4). The cardiac output observed at PEA onset was 1.6±0.4 L/min, approximately a 70%

Table 1. Baseline Group Data

Characteristic	Group 1, Naïve Animals, N=34	Group 2, Severe LV Dysfunction, N=18
Characteristic		
Baseline vitals, average±SD		
Heart rate, beats/min	91±17	95±14
Blood pressure, mm Hg, average±SD		
Systolic	99±16	91±12
Diastolic	60±13	58±8
Mean arterial pressure	73±13	69±9
Baseline LV function		
Ejection fraction (%)*	59±3	15±3
Experimental design, n (%)		
No IPC	23 (68)	12 (66)
IPC	11 (32)	6 (33)

IPC indicates ischemic preconditioning; and LV, left ventricular; and TTE, transthoracic echocardiography.

*TTE images were obtained in 14 of 34 animals in Group 1 and 15 of 18 animals in Group 2 before occlusion. The remaining studies were technically limited and inadequate for interpretation. In Group 2 animals, baseline hemodynamic data and ejection fractions listed were obtained before the final experiment.

Table 2. Baseline Subgroup Data

	Group 1a, Naïve Animals, No IPC, N=23	Group 1b, Naïve Animals, IPC, N=11
Characteristic		
Baseline vitals, average±SD		
Heart rate, beats/min	95±16	85±17
Blood pressure, mm Hg		
Systolic	96±17	104±12
Diastolic	58±14	63±11
Mean arterial pressure	71±15	77±10
	Group 2a, Severe LV Dysfunction, No IPC, N=12	**Group 2b, Severe LV Dysfunction, IPC, N=6**
Characteristic		
Baseline vitals, average±SD		
Heart rate, beats/min	91±10	103±17
Blood pressure, mm Hg		
Systolic	91±11	90±14
Diastolic	58±6	58±10
Mean arterial pressure	69±8	69±11
Baseline LV function, average±SD		
Ejection fraction, %	16±3	14±3

Baseline hemodynamics are listed before the acute coronary occlusion. IPC indicates ischemic preconditioning; and LV, left ventricular.

reduction from baseline (cardiac output of 5.0±0.8 L/min) (Table 4).

Group 1 Versus Group 2

When comparing the initial arrest rhythm that occurred after ACO, the animals in Group 1, all with normal LV function as measured by transthoracic echocardiography, were far more likely to have an initial cardiac arrest rhythm of VF than PEA, whereas all animals in Group 2, with severely reduced LV function as measured by prearrest transthoracic echocardiography, developed PEA initially (Figure 5A). Onset of PEA occurred markedly sooner than VF after coronary occlusion (1.8±1.1 versus 23.5±9.7 minutes, $P<0.0001$). When subsequent VF developed during PEA arrest, it occurred substantially later relative to the time of occlusion (1.7±1.1 versus 17.7±10.8 minutes, $P<0.0001$) (Figure 5B).

Subgroup Analysis: IPC Versus Control
Normal LV Function, Group 1a (No IPC): (N=23) Versus Group 1b: With IPC: (N=11)

The purpose of this subgroup analysis was to establish whether ischemic preconditioning is applicable in the swine model of ischemic heart disease. In Group 1a and Group 1b animals (normal LV function), IPC decreased the incidence (2/11 versus 16/23; hazard ratio [HR], 7.15; $P<0.009$) and delayed the onset of VF

(37.2±7.3 minutes versus 21.9±7.2 minutes, $P<0.012$) (Figure 5A).

Severe LV Dysfunction, Group 2a (no IPC): N=12 Versus Group 2b: With IPC: N=6

In this subgroup analysis, we tested whether IPC decreases the incidence and/or delays the onset of subsequent VF in animals with severe LV dysfunction that developed infarct-induced PEA initially. There was no impact of IPC on the incidence of PEA (12/12 in Group 2a and 6/6 in Group 2b) or in time to the onset of PEA (1.5±0.9 minutes in Group 2a and 2.3±1.2 minutes in Group 2b, $P=0.154$). However, there was a significant difference in the incidence of PEA degenerating to VF (4/6 in Group 2b versus 12/12 in Group 2a; HR, 5.5; $P<0.034$) and the time to the onset of VF (33.8±7.7 minutes in Group 2b versus 12.3±5.0 minutes in Group 2a, $P<0.00013$) (Figure 5B, Table 4).

Evidence of Successful Ischemic Preconditioning

Although VT/VF suppression is a known effect of IPC in other species, we performed additional testing to confirm successful preconditioning in the swine model. In prior animal studies (goats and rats), IPC alters the transient increase in coronary blood flow following reperfusion of a previously occluded coronary, termed the reactive hyperemia response.[17] IPC also causes a
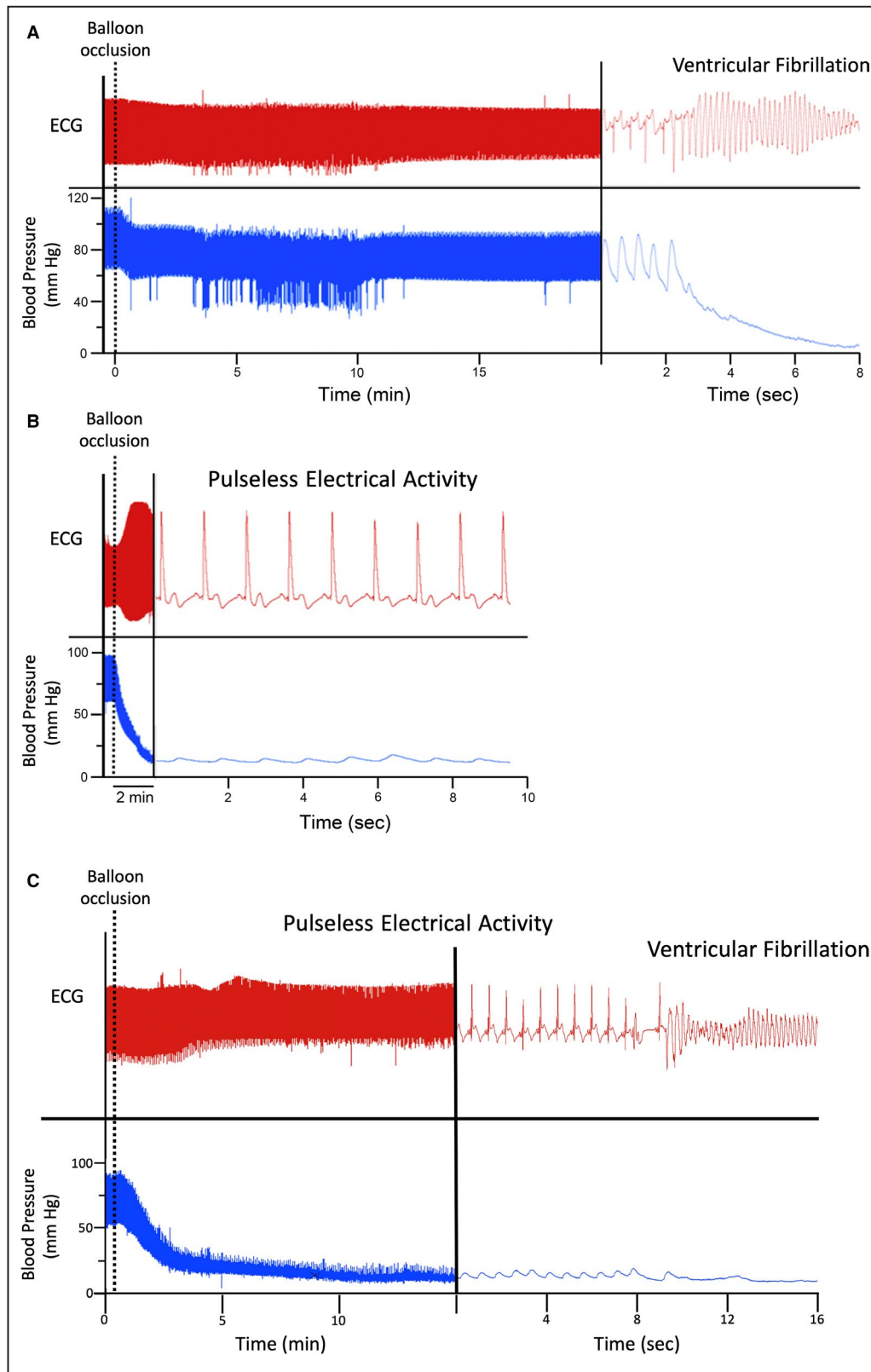
Figure 3.   Blood pressure and ECG changes over time following balloon occlusion.
A, Example of ventricular fibrillation (VF) onset in a Group 1 animal (mean onset for the group 23.5±9.0 minutes from occlusion). **B**, Example of onset of pulseless electrical activity (PEA) in a Group 2 animal (mean onset for the group was 1.7±1.1 minutes from occlusion). **C**, Example of PEA degenerating to VF in a Group 2a animal (mean onset for the VF after PE in Group 2a animals was 12.3±5 minutes from occlusion).
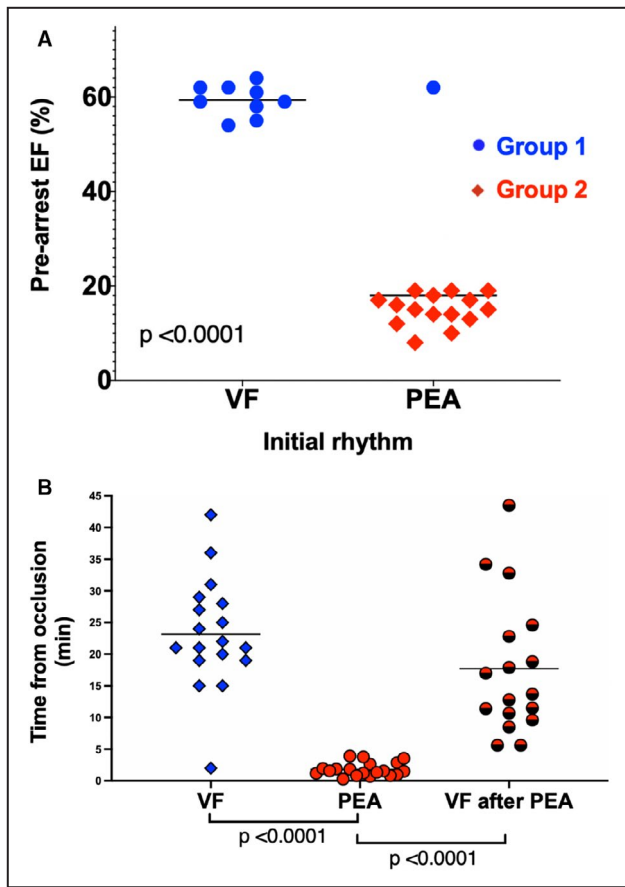
Figure 4. Initial rhythm and time to arrest following balloon occlusion.
A, Initial arrest rhythm by prearrest left ventricular (LV) function. The initial arrest rhythm was ventricular fibrillation (VF) in those with normal prearrest ejection fraction (EF) and pulseless electrical activity (PEA) in those with low prearrest EF. Prearrest LV function was determined by transthoracic echocardiography before cardiac arrest. **B**, Time to VF, PEA, and VF after PEA following balloon occlusion. Time from occlusion to PEA was significantly shorter than the time from occlusion to VF. Animals in the VF after PEA column are the same animals as the PEA column.

decrease in time to peak coronary flow on reperfusion and a decrease in total hyperemic flow. We used a coronary Doppler flow wire to quantify coronary reactive hyperemia at baseline and after IPC in 10 animals that underwent the IPC protocols. A representative coronary flow tracing from 1 of these animals is shown (Figure 6). Using area under the curve as a surrogate for total hyperemic flow, IPC caused a mean percent decrease of 50±16% in the area under the curve and a mean percent decrease in time to peak coronary flow velocity of 46±14% (Figure 6). The magnitude of these changes is comparable to the reactive hyperemia response present in other IPC studies.[17]

To provide molecular support of our reactive hyperemia data, we measured PKCε translocation to the mitochondria-enriched particulate fraction in animals

that underwent IPC and those that did not. Several studies demonstrated that PKCε translocation to the mitochondria-enriched particulate fraction is necessary to mediate IPC.[18–21] Hence, PKCε translocation from the cytosol to the mitochondria is one of the most established molecular markers of IPC. Though the exact mechanism of protection exerted by PKCε is unclear, inhibition of the mitochondrial permeability transition pore could play a major role in PKCε-mediated cardioprotection.[22] As expected, the PKCε signal ratio particulate to total was 36±8.9% SD higher in the IPC group than in controls (P=0.0045) (Figure 7A through 7C). Mitochondrial enrichment was confirmed by a 2- to 3-fold increase in adenosine triphosphate synthase β in the particulate compared with the cytosolic fractions (Figure 7A through 7C).

DISCUSSION

There are 6 major findings in this study: (1) Acute ischemia in the setting of preexisting severe LV dysfunction reliably and rapidly generates PEA. (2) Onset of PEA occurs far sooner than does VF (<2 minutes versus ≈20 minutes) in this model. (3) IPC delays the onset or prevents VF in a swine model of ACO in the setting of normal LV function. (4) Ischemic-induced PEA uniformly develops into VF arrest in the swine model in the absence of preconditioning. (5) IPC delays the onset or prevents subsequent VF in the swine model of ischemic-induced PEA (Figure 8). (6) PKCε translocation from the cytosol to the mitochondria confirms successful IPC in the swine model.

An important goal was to model clinical PEA. Of the animals in Group 2 that developed PEA, it occurred 1.7±1.1 minutes following occlusion, which is in contrast to the time for the development of VF. For example, when VF developed in Group 1a animals that had normal LV function, it occurred 23.5±9.0 minutes after coronary occlusion, and in the Group 2 animals, with severe prearrest LV dysfunction that uniformly first developed PEA, subsequent VF occurred 12.3±5.0 minutes after occlusion. Myocardial dysfunction after ACO occurs instantaneously and is evidenced by a reduction in blood pressure within seconds of proximal LAD balloon occlusion. In animals with normal baseline LV function, there is an ability to compensate and maintain adequate perfusion to the remaining uninvolved myocardium. Animals with prior infarcts involving the non-LAD territories are unable to compensate and rapidly develop sustained PEA (Figure 8). This acute pump failure is highlighted by the severely reduced stroke volume and cardiac output at the onset of PEA (Table 5). Our model focused on single-territory occlusions, but it is possible that a single coronary

Table 3. Group Results

Primary Outcome	Group 1, Naïve Animals, N=34, N (%)	Group 2, Severe LV Dysfunction, N=18, N (%)	P Value
Arrest free	15 (44)	0 (0)	0.001
Cardiac arrest	19 (56)	18 (100)	
VF	18 (95)	0 (0)	<0.0001
PEA	1 (5)	18 (100)	

Time From Occlusion, min	Average±SD	Average±SD	Difference of Means	95% CI
Initial arrest (PEA or VF)	22.6±9.7	1.7±1.1	20.90	16.27–25.53
Time to VF	23.5±9.0	*		
Time to PEA	2.9*	1.7±1.1	1.2	0.824–1.576

Incidences of cardiac arrest, VF, and PEA. Time to initial arrest and time to VF after PEA when applicable. LV indicates left ventricular; PEA, pulseless electrical activity; and VF, ventricular fibrillation.

*Not applicable.

occlusion can result in dysfunction in multiple territories if that artery is supplying, via collaterals, >1 territory. In that instance, a single occlusion in the setting of even preexisting normal LV function can result in acute pump failure and resultant PEA. Our finding that PEA is a frequent cardiac arrest rhythm with even single vessel occlusion in the setting of baseline LV dysfunction also reinforces the importance of including LV function in risk/benefit discussions of revascularization with patients. This finding is particularly relevant, because the incidence and prevalence of ischemic cardiomyopathy and LV dysfunction is on the rise because more people with chronic coronary artery disease are living longer with more severe ischemic disease because of improved therapies.[23] This may explain why the proportion of PEA cardiac arrests has also risen concomitantly over the past half century.

Our findings suggest potential benefit for early coronary revascularization in patients presenting with PEA who have known or suspected coronary artery disease or LV dysfunction with a goal of rapid reversal of ischemia and salvage of remaining viable myocardium. Because all animals with PEA developed VF in the

absence of prior preconditioning, humans that develop VF subsequent to PEA may have an even greater need for emergent revascularization because they may lack the spontaneous IPC (ie, absence of preinfarct angina) that could occur during acute myocardial infarction.

Table 4.   The Extent of Cardiac Dysfunction That Resulted in Ischemic-Induced PEA Because the Initial Cardiac Arrest Rhythm Was Substantial

	Before Final LAD Occlusion, N=12	During PEA, N=12	P Value
Ejection fraction, %	15±3	5±3	<0.000001
Cardiac output, L/min	5.0±0.8	1.6±0.4	<0.000001

Mean ejection fraction before the final occlusion was 15±3%, consistent with significant left ventricular dysfunction before the final ischemic insult. The mean ejection fraction at PEA onset was 5±3%. Cardiac output for PEA onset was 1.6±0.4 L/min, approximately a 70% reduction from baseline (cardiac output of 5.0±0.8 L/min). LAD indicates left anterior descending coronary artery; and PEA, pulseless electrical activity.
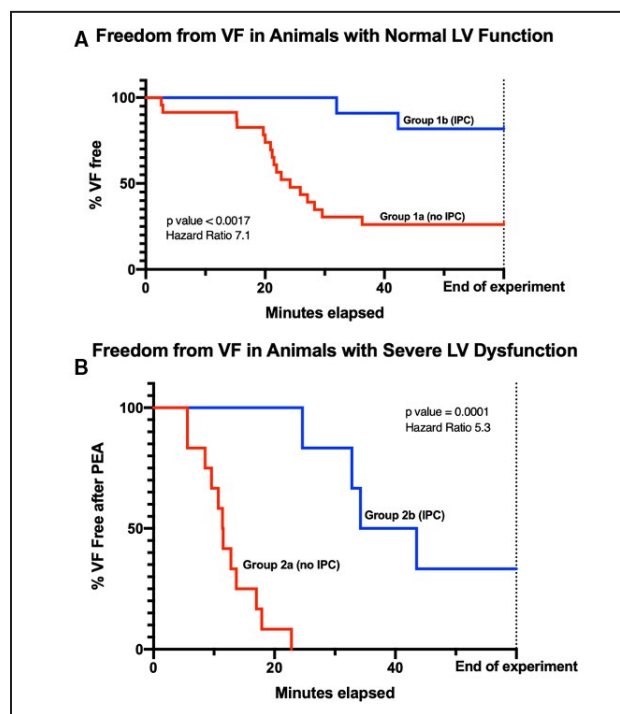

Figure 5. Effect of ischemic preconditioning on Group 1 and Group 2 animals.
A, Freedom from ventricular fibrillation (VF) in animals with normal left ventricular (LV) function following balloon occlusion. Group 1 subgroups, Group 1a and 1b, are compared demonstrating the effect of preconditioning. **B**, Freedom from VF after pulseless electrical activity (PEA) in animals with severe LV dysfunction following balloon occlusion. Group 2 subgroups, Group 2a and 2b, are compared demonstrating the effect of preconditioning. IPC indicates ischemic preconditioning.
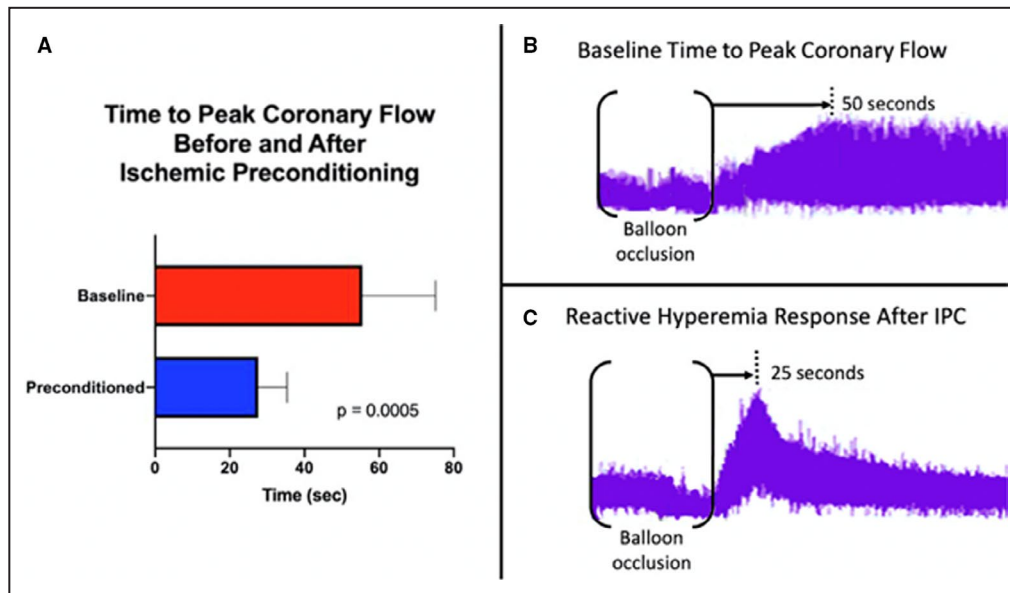
Figure 6. Coronary reactive hyperemia.
A, Time to peak coronary flow velocity during reactive hyperemia at baseline (52±15 seconds) and after ischemic preconditioning (IPC) (27±8 seconds) ($P<0.0005$, t test). **B**, Coronary flow vs time during reactive hyperemia at baseline. **C**, After IPC.

Although it is clear that immediate coronary angiography is indicated for OHCA with STEMI, the management of OHCA without STEMI remains uncertain despite a substantial incidence of coronary artery disease and ACO.[8] The European Association of Percutaneous Cardiovascular Interventions guidelines recommend coronary angiography within 2 hours for all comatose patients with OHCA after initial assessment to exclude obvious noncardiac causes of arrest,[24] whereas the American Heart Association 2015 guidelines reserve emergent angiography for patients with suspected acute myocardial infarction or predictors of ACO, and clinical instability.[25] Clinical randomized controlled trials are currently evaluating whether a universal early coronary angiography strategy improves patient-centered outcomes after OHCA in those with shockable and nonshockable rhythms and with and without STEMI on the postarrest ECG.[8]

Although in-hospital PEA arrests are traditionally thought to be predominantly attributable to respiratory and metabolic causes, PEA from acute coronary ischemia is likely underrecognized in this population. The study of >50 000 inpatient arrests by Meaney et al[4] reports that 46% of patients whose arrest has PEA as the presenting rhythm did have preexisting respiratory insufficiency. However, that same study reports that 19% of patients with a PEA arrest had an acute myocardial infarction (compared with 33% who had a VT/VF arrest), and that 31% had preexisting arrhythmias (compared with 42% who had a VT/VF arrest), strongly suggesting a substantial incidence of coronary artery disease in this patient population. That same study

reports that 34% of PEA arrests were from cardiac causes (compared with 52% who had VT/VF arrests). In addition, a quarter of PEA arrests had subsequent VF, which is consistent with the ischemic mechanisms presented in this article. Furthermore, it is likely that PEA in patients with preexisting hypotension was potentially caused by pump failure related to acute coronary ischemia, which may be underrecognized. This underrecognition of PEA is consistent with our finding that few animals had ST segment changes during the coronary occlusions that resulted in PEA. The purpose of this study was to provide a mechanistic explanation for how patients with severe coronary artery disease could have PEA as the initial arrest rhythm, especially in light of the high incidence of coronary artery disease in inpatients. The likelihood that acute ischemia may be the cause of a significant number of patients presenting with PEA arrest is important, because it could lead to more patients receiving interventions that could reverse the ischemia and salvage the patient.

Our study highlights the antiarrhythmic effect of preconditioning during the ischemic phase of ACO, which is a period of increased risk of VF in humans, because more than half of the deaths associated with acute myocardial infarction occur within 1 hour of symptom onset.[26] Thus, this is a period when potential pharmacological therapeutic interventions, which would mimic the cellular changes occurring during IPC, could have an enormous impact. Our study supports the concept of spontaneous preconditioning that occurs if there is adequate time during the transition from critical vessel obstruction and subendocardial ischemia to complete vessel occlusion
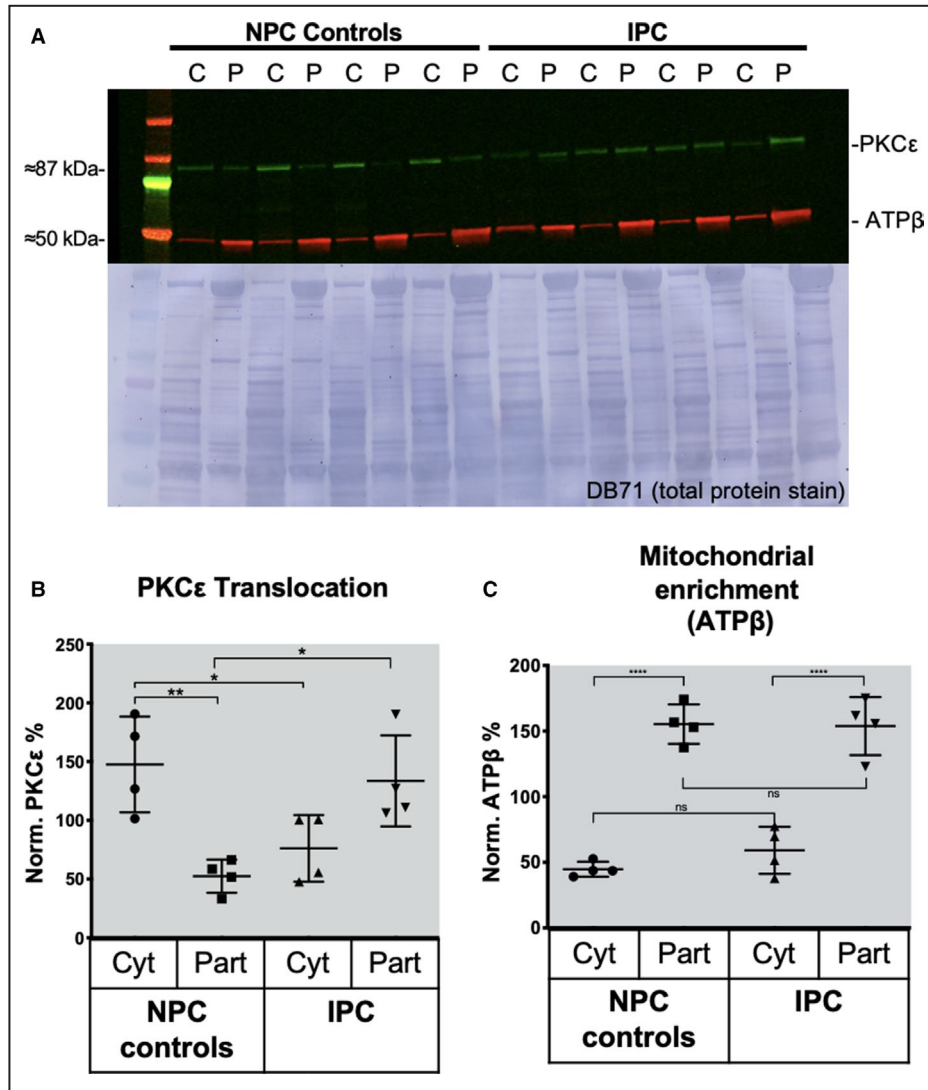
Figure 7.   Translocation of PKCε (protein kinase C epsilon) to the particulate fraction with preconditioning.

Western blot analysis was used to measure PKCε translocation to the mitochondria-enriched particulate fraction (**A**). Densitometric comparison of PKCε signal between cytosolic and particulate fractions revealed the translocation of PKCε to the mitochondria-enriched particulate fraction in the ischemic preconditioning (IPC) animals compared with no IPC controls (**B**). Mitochondria enrichment in the particulate fraction was measured using an antibody toward the β subunit of complex V adenosine triphosphate synthase β (ATPβ), showing equal enrichment in mitochondria in both no IPC and IPC samples (**C**). C indicates cytosolic fraction; DB71, direct blue 71 staining, a general protein staining to control for equal loading and transfer efficiency; and P, particulate fraction. Cyt indicates the term cytosolic fraction and Part indicates particulate fraction. An asterisk (*) indicates statistically signifant difference and more the asterisk count more statistical difference.

and transmural infarction, because this is likely the reason why there is a decreased incidence of VF cardiac arrest in patients with STEMI who experience preinfarct angina.[12] This is in contrast to STEMI that develops abruptly, which is associated with higher VF risk. Although IPC has been demonstrated to have an antiarrhythmic effect, it is not known to prevent pump failure caused by acute ischemia. Because our model is reflective of pump failure

caused by acute ischemia on a background of chronic LV dysfunction, it is reasonable to presume that IPC would not impact the time to PEA in a significant way.

Our study results demonstrate that animals with severe LV dysfunction develop ischemia-induced PEA shortly after coronary occlusion, and many then develop VF. However, like ischemia-induced VF in the normal left ventricle, IPC can prevent or reduce VF
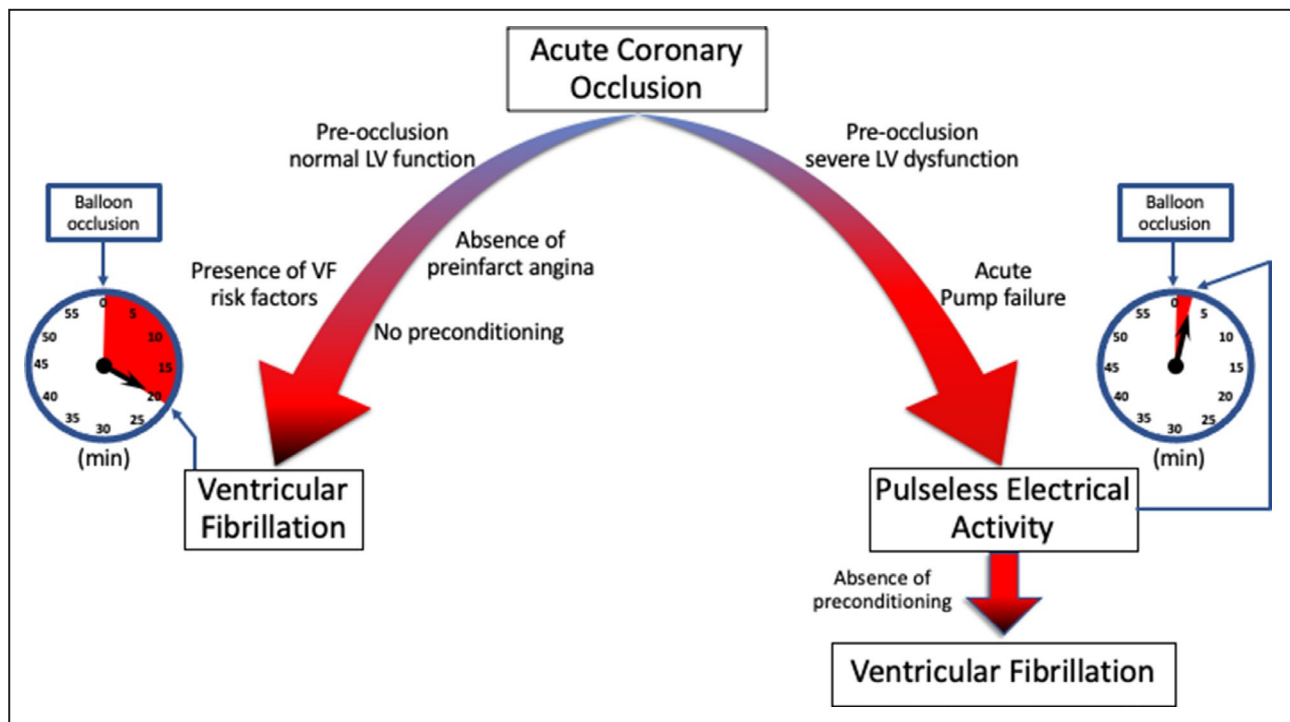
Figure 8. Ventricular fibrillation (VF) vs pulseless electrical activity (PEA) as the initial cardiac arrest rhythm.
Prearrest left ventricular (LV) function is a major factor for initial cardiac arrest rhythm during acute coronary occlusion. Onset of PEA occurs sooner than VF. VF uniformly develops after ischemic PEA in the absence of preconditioning.

in the setting of PEA. The results of our study suggest that patients who develop subsequent VF after an initial rhythm of PEA may be suitable candidates for revascularization, because VF may be a marker for acute occlusion. There is prognostic relevance as well, for as noted above, survival is better in patients with PEA who do not subsequently develop VF than those who do.[4] It is possible that spontaneous preconditioning may be the reason for this; alternatively, the better prognosis may be attributable to a noncardiac cause of PEA, which may be more readily reversible than ischemic-induced PEA.

In this study, we used PKCε translocation to the mitochondria as a molecular marker of preconditioning in addition to reactive hyperemia and prolonged time to lethal arrhythmias data as physiological markers or preconditioning. Our results demonstrate that the translocation of PKCε to the mitochondria-enriched particulate fraction, an established marker of ischemic preconditioning in rodent models,[20] is also a marker of successful ischemic preconditioning in swine.

Limitations

The main limitation of applying our study to human health is that swine are more prone to ischemic-induced ventricular arrhythmias than are humans.[27] Thus, the reproducibility of VF shown in our nonpreconditioned animals may not be directly generalizable to human coronary ischemia/VF. However, demonstrating that it is possible to delay or prevent VF in swine despite their increased susceptibility to arrhythmia is a significant finding. Furthermore, we did not test ACO in animals with mild or moderate LV dysfunction, and therefore the results of this study did not determine the extent of prearrest LV dysfunction required to develop acute pump failure and PEA.

CONCLUSIONS AND FUTURE DIRECTIONS

Our swine ischemia model recapitulates many aspects of ischemia-induced VF and ischemia-induced PEA. Acute coronary ischemia in the setting of severe LV dysfunction produces PEA caused by acute pump failure, which occurs almost immediately after coronary occlusion. After the onset of coronary ischemia, PEA occurred significantly earlier than VF (<2 minutes versus 20 minutes). In animals with normal LV function and those with severe LV dysfunction, IPC exerted an antiarrhythmic effect by preventing or delaying the onset of VF. Overall, these findings support the notion that patients with baseline LV dysfunction and suspected coronary disease who develop PEA

Table 5. Subgroup Results

Primary Outcome	Group 1a, Naïve Animals, No IPC, N=23, N (%)	Group 1b, Naïve Animals, IPC, N=11, N (%)	P Value
Arrest free	5 (22)	9 (82)	0.002
Cardiac arrest	17 (78)	2 (18)	
VF	16 (94)	2 (100)	0.009
PEA	1 (6)	0 (0)	0.7
Time From Occlusion, min	**Average±SD**	**Average±SD**	**Difference of Means** · **95% CI**
Initial arrest (PEA or VF)	20.9±8.6	37.2±7.3	16.3 · 10.17–22.4
Time to VF	21.9±7.2	37.2±7.3	15.3 · 9.9–20.7

Primary Outcome	Group 2a, Severe LV Dysfunction, No IPC, N=12, N (%)	Group 2b, Severe LV Dysfunction, IPC, N=6, N (%)	P Value
VF	0 (0)	0 (0)	NA
PEA	12 (100)	6 (100)	
Subsequent VF after PEA	12 (100)	4 (67)	0.03
Time From Occlusion, min	**Average±SD**	**Average±SD**	**Difference of Means** · **95% CI**
Time to PEA	1.5±0.9	2.3±1.2	0.8 · 0.26–1.86
Subsequent VF after PEA	12.3±5.0	33.8±7.7	21.5 · 15.16–27.83

Incidences of cardiac arrest, VF, PEA, and VF after PEA. Time to initial arrest, time to VF, time to PEA, time to VF after PEA (when applicable). IPC indicates ischemic preconditioning; LV, left ventricular; PEA, pulseless electrical activity; and VF, ventricular fibrillation.

should be evaluated for ACO. Furthermore, given the severity of LV dysfunction, these patients may benefit from an aggressive resuscitation strategy incorporating rapid revascularization and mechanical circulatory support.

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Supplementary Material

Data S1
Videos S1–S4

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Supplemental Material

Data S1. Supplemental Video Legends:

Video S1. Representative coronary angiography with balloon occlusions of the RCA, LCx and LAD. Best viewed with Windows Media Player.

Video S2. Baseline transthoracic echocardiogram, parasternal long and short views. Best viewed with Windows Media Player.

Video S3. Post RCA and post LCx transthoracic echocardiogram, parasternal long and shirt axis views. Best viewed with Windows Media Player.

Video S4. Pulseless Electrical Activity (PEA) during final occlusion – parasternal long and short axis views. Best viewed with Windows Media Player.