Ischemia-reperfusion injury changes the dynamics of Ca\textsuperscript{2+}-contraction coupling due to inotropic drugs in isolated hearts

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Submitted 9 March 2005; accepted in final form 8 November 2005

Rhodes, Samhita S., Kristina M. Ropella, Amadou K. S. Camara, Qun Chen, Matthias L. Riess, Paul S. Pagel, and David F. Stowe. Ischemia-reperfusion injury changes the dynamics of Ca\textsuperscript{2+}-contraction coupling due to inotropic drugs in isolated hearts. J Appl Physiol 100: 940–950, 2006. First published November 10, 2005; doi:10.1152/japplphysiol.00285.2005.—Positive inotropic drugs may attenuate or exacerbate the deleterious effects of ischemia and reperfusion (IR) injury on excitation-contraction coupling in hearts. We 1) quantified the phase-space relationship between simultaneously measured myoplasmic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) and isovolumetric left ventricular pressure (LVP) using indexes of loop area, orientation, and position; and 2) quantified cooperativity by linearly modeling the phase-space relationship between [Ca\textsuperscript{2+}] and rate of LVP development in intact hearts during administration of positive inotropic drugs before and after global IR injury. Unpaced, isolated guinea pig hearts were perfused at a constant pressure with Krebs-Ringer solution (37°C, 1.25 mM CaCl\textsubscript{2}). [Ca\textsuperscript{2+}] was measured ratiometrically by indo 1 fluorescence by using a fiber-optic probe placed at the left ventricular free wall. LVP was measured by using a saline-filled latex balloon and transducer. Drugs were infused for 2 min, 30 min before, and for 2 min, 30 min after 30-min global ischemia. IR injury worsened Ca\textsuperscript{2+}-contraction coupling, as seen from decreased orientation and repositioning of the loop rightward and downward and reduced cooperativity of contraction and relaxation with or without drugs. Dobutamine (4 μM) worsened, whereas dopamine (8 μM) improved Ca\textsuperscript{2+}-contraction coupling before and after IR injury. Dobutamine and dopamine improved cooperativity of contraction and relaxation after IR injury, whereas only dopamine increased cooperativity of relaxation before IR injury. Digoxin (1 μM) improved Ca\textsuperscript{2+}-contraction coupling and cooperativity of contraction after but not before ischemia. Levosimendan (1 μM) did not alter Ca\textsuperscript{2+}-contraction coupling or cooperativity, despite producing concomitant increases in contractility, relaxation, and Ca\textsuperscript{2+} flux before and after ischemia. Dynamic indexes based on LVP-[Ca\textsuperscript{2+}] diagrams (area, shape, position) can be used to identify and measure alterations in Ca\textsuperscript{2+}-contraction coupling during administration of positive inotropic drugs in isolated hearts before and after IR injury.

POSITIVE INOTROPIC DRUGS are frequently administered to patients with left ventricular (LV) dysfunction to improve cardiac output (19). These agents mostly act by increasing myoplasmic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) (“upstream” mechanism) but may also enhance Ca\textsuperscript{2+} binding to troponin C on the actin (TnCA) myofilament (“central” mechanism) or alter cross-bridge kinet- ics (“downstream” mechanism). Several classes of inotropic drugs act selectively through these mechanisms to improve Ca\textsuperscript{2+}-contraction coupling. β-adrenergic and dopaminergic agonists (e.g., dobutamine and dopamine) increase myoplasmic cAMP concentrations to promote protein kinase-induced phosphorylation of TnCA (3), sarcolemmal Ca\textsuperscript{2+} channels to increase Ca\textsuperscript{2+} influx (12), sarcolemmal reticulum Ca\textsuperscript{2+} pumps to enhance Ca\textsuperscript{2+} uptake, and troponin I to reduce myofilament Ca\textsuperscript{2+} sensitivity (16). In contrast, cardiac glycosides (e.g., digoxin) do not affect cAMP levels but inhibit Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pump activity to selectively slow Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange. Levosimendan, a newer Ca\textsuperscript{2+} sensitizer, is thought to stabilize the Ca\textsuperscript{2+}-TnCA complex without increasing Ca\textsuperscript{2+} binding affinity with TnCA (11).

We recently presented novel indexes from phase-space diagrams of simultaneously measured LV pressure (LVP) vs. [Ca\textsuperscript{2+}] to understand the mechanisms of positive and negative inotropic drugs in guinea pig unpaced, isolated hearts in the absence of ischemia (27). The effects of drugs on static relationships between [Ca\textsuperscript{2+}] and LVP (peak values, rates of rise and fall, etc.) were presented in earlier articles (7, 10). For this study, we compared how positive inotropic drugs with different mechanisms of action alter the dynamic phase-space relationship between [Ca\textsuperscript{2+}] and LVP before and after ischemia and reperfusion (IR) injury. Specific indexes from LVP vs. [Ca\textsuperscript{2+}] phase-space diagrams (loops) were evaluated to determine which of these indexes best quantified dynamic Ca\textsuperscript{2+}-contraction coupling.

Central and downstream mechanisms regulating Ca\textsuperscript{2+}-contraction coupling are governed by cooperative interactions. This positive feedback mechanism is responsible for amplification in signal transduction, leading to muscle activation and force generation in response to the Ca\textsuperscript{2+} stimulus (19). To quantify cooperativity, we linearly modeled the phase-space relationship between the rate of LVP development (dLVP/dt) and simultaneous [Ca\textsuperscript{2+}] during inotropic interventions before and after IR injury. The ability to quantify changes in dynamic Ca\textsuperscript{2+}-contraction coupling and cooperativity in response to pharmacological and pathological interventions should improve our understanding of the mechanisms of cardiac force generation.

METHODS

Langendorff Heart Preparation

The investigation conformed to the Guide for the Care and Use of Laboratory Animals from the US National Institutes of Health (NIH)
no. 85–23, revised 1996). Prior approval was obtained from the Medical College of Wisconsin and Marquette University Animal Studies Committees. The preparation of guinea pig hearts at our laboratory has been described in detail (7, 9, 10, 26–28, 30–32). English short-haired guinea pigs (n = 40) were anesthetized with ketamine (30 mg intraperitoneal) and decapitated when they were unresponsive to noxious stimulation. After thoracotomy, the inferior and superior venae cavae were cut away, and the aorta was cannulated distal to the aortic valve. Each heart was immediately perfused via the aortic root with a cold oxygenated, modified Krebs-Ringer (KR) solution at a pressure head of 55 mmHg. The KR perfusate (pH 7.39 ± 0.01, PO_2_260 ± 10 Torr) was filtered (5-μm pore size) in-line and had the following calculated composition in mM (nonionized): 137 Na^+, 5 K^+, 1.2 Mg^{2+}, 1.25 Ca^{2+}, 134 Cl^−, 15.5 HCO_3^−, 1.2 H_2PO_4^−, 11.5 glucose, 2 pyruvate, 16 mannitol, 0.05 EDTA, 0.1 propranolol, and 5 insulin (U/I). Perfusate and bath temperatures were maintained at 37.2 ± 0.1°C using a thermostatically controlled water circulator. KR CaCl_2 concentration was reduced (1.25 mM) to allow for a wider range of inotropic responses at a lower control LVP. This accounts for the low control values of systolic LVP and [Ca^{2+}]_i.

Isovolumetric LVP was measured continuously with a transducer connected to a thin, saline-filled latex balloon inserted into the LV through the mitral valve from an incision in the left atrium. Balloon volume was adjusted initially to a diastolic LVP of 0 mmHg so that any subsequent increase in diastolic LVP reflected an increase in LV wall stiffness, i.e., diastolic contracture. Pairs of bipolar electrodes were placed in the right atrial appendage, right ventricular apex, and LV base to monitor spontaneous heart rate (HR) and atrial-ventricular conduction time. Coronary flow was measured by an ultrasonic flowmeter placed directly into the aortic inflow line.

**Measurement of Myoplasmic Free Ca^{2+} in Intact Hearts**

Experiments were carried out in a light-blocking Faraday cage. The heart was partially immobilized by hanging it from the aortic cannula, the pulmonary artery catheter, and the LV balloon catheter. The distal end of a trifurcated fiber-optic cable (optical surface area: 3.85 mm²) was placed gently against the LV epicardial surface through a hole in the aortic valve. A rubber O ring was placed over the fiber-optic tip to seal the hole. A rubber O ring was placed gently against the LV epicardial surface through a hole in the aortic valve. A rubber O ring was placed over the fiber-optic tip to seal the hole, and netting was applied around the heart for optimal contact with the bath. A rubber O ring was placed over the fiber-optic tip to seal the hole, and netting was applied around the heart for optimal contact with the bath. A rubber O ring was placed over the fiber-optic tip to seal the hole, and netting was applied around the heart for optimal contact with the bath. A rubber O ring was placed over the fiber-optic tip to seal the hole, and netting was applied around the heart for optimal contact with the bath.

Hearts were loaded with the Ca^{2+} indicator indo 1-AM (Sigma Chemical, St. Louis, MO) to a final concentration of 6 μM; this technique has been described in detail previously (7, 9, 10, 26, 27, 30–32). Fluorescence emissions at 385 (F_{385}) and 456 nm (F_{456}) were recorded by using a modified luminescence spectrophotometer (SLM Aminco-Bowman II, Spectronic Instruments, Urbana, IL). The LV region of the heart was excited with light from a xenon arc lamp, and the light was filtered through a 350-nm monochromator with a bandwidth of 16 nm. The arc lamp shutter was opened only for 2.5-s recordings using a simple event detection algorithm. [Ca^{2+}]_i, and the simultaneously obtained LVP signals between each consecutively detected Ca^{2+} diastolic point, were aligned and averaged on a point-by-point basis to form the averaged [Ca^{2+}]_i and LVP transient signals. Care was taken to exclude any dysrhythmic beats in the averaged Ca^{2+} and LVP transients.

**Static Indexes Derived From Averaged Ca^{2+} and LVP Transients**

Phasic LVP and [Ca^{2+}]_i, global myocardial contractility (dLVP/d_{max}) and relaxation (dLVP/d_{min}), and total myoplasmic [Ca^{2+}]_i influx (d[Ca^{2+}]_i/d_{max}) and efflux (d[Ca^{2+}]_i/d_{min}) from all cellular and extracellular sources were computed from the averaged Ca^{2+} and LVP transients. Several temporal indexes were also computed: half-maximal duration of Ca^{2+} and LVP transients; peak delay between systolic [Ca^{2+}]_i and LVP; contraction response time (delay between d[Ca^{2+}]_i/d_{max} and dLVP/d_{max}); and relaxation response time (delay between d[Ca^{2+}]_i/d_{min} and dLVP/d_{min}). These indexes have been described previously (7, 10, 27).

**Dynamic Indexes Derived From LVP vs. Ca^{2+} Phase-Space Diagrams**

Averaged LVP was plotted as a function of averaged myoplasmic [Ca^{2+}]_i over the cardiac cycle, and the following indexes were derived: LVP vs. [Ca^{2+}]_i loop area; loop orientation (ΔLVP/Δ[Ca^{2+}]_i); loop position vector, P, for spatial characterization (location, |P|, and direction, θP) computed from the origin (0 nM [Ca^{2+}]_i, 0-mmHg LVP) to the loop centroid (LC = (0.5 * phasic [Ca^{2+}]_i) + diastolic [Ca^{2+}]_i, LC = (0.5 * phasic LVP) + diastolic LVP); and loop trajectory vectorgrams, T(t), also measured from the origin, to link signal temporal information with loop shape. The loop indexes were used to quantify the efficiency of Ca^{2+}-contraction coupling and have been described previously (27).

**dLVP/dt vs. Ca^{2+} Phase-Space Diagrams**

Instantaneous dLVP/dt was plotted vs. [Ca^{2+}]_i to diagram the phase-space relationship between [Ca^{2+}]_i and the rate of LVP rise and fall before and after IR injury (Fig. 2A). The dLVP/dt vs. [Ca^{2+}]_i diagram was divided into three sections: segment 1, onset of Ca^{2+} transient to dLVP/d_{max}; segment 2, dLVP/d_{max} to dLVP/d_{min}; and segment 3, dLVP/d_{min} to end of Ca^{2+} transient. A linear regression model (Fig. 2B) was applied to all three segments during drug administration before and after global ischemia.

The slopes of each of the three segments were used to quantify the positive feedback mechanism, i.e., cooperativity, that is a character-
istic of the chemomechanical coupling in cardiac muscle (20, 35). The slope of segment 1 represents cooperativity of contraction (binding of troponin C and Ca\(^{2+}\), and actinomyosin cross-bridge formation), and the slope of segment 3 represents cooperativity of relaxation (dissociation kinetics of troponin C and Ca\(^{2+}\), and actin and myosin). Segment 2 represents the transition kinetics from contraction to relaxation.

**Statistical Analysis**

Static and dynamic indexes from LVP and \([\text{Ca}^{2+}]\) transients and linear regression models of dLVP/dt vs. \([\text{Ca}^{2+}]\) obtained during administration of inotropic drugs before and after IR were compared with control using one-way ANOVA followed by Dunnett’s comparison of means post hoc test (MINITAB Statistical Software Release 13.3, Minitab, State College, PA). Control and inotropic drug data obtained after ischemia were compared with their respective values before ischemia using Student’s paired t-test. Differences among means were considered statistically significant at \(P < 0.05\) (two-tailed). Because only one concentration of each drug was given, we did not compare drug responses among each other. All indexes were expressed as means ± SE.

**RESULTS**

*Static Indexes Derived From Averaged LVP and \([\text{Ca}^{2+}]\) Transients*

HR. HR was unchanged on reperfusion after global ischemia in control hearts (Table 1). Dobutamine and dopamine increased HR before and after ischemia. Digoxin did not alter HR. Levosimendan increased HR before, but not after, global ischemia.

Phasic LVP and \([\text{Ca}^{2+}]\). Phasic LVP decreased after ischemia in all groups (Table 1). Each inotropic drug increased phasic LVP from control before ischemia and, except for levosimendan, also after ischemia. Each drug increased systolic, but not diastolic, LVP from control before and after ischemia. Systolic LVP was unchanged after ischemia from its preischemic value in the control group, but decreased in hearts treated with the inotropic drugs. Diastolic LVP increased after ischemia in all five groups. Phasic, systolic, and diastolic \([\text{Ca}^{2+}]\) were greater after ischemia in control hearts. Each drug increased phasic \([\text{Ca}^{2+}]\) from control before ischemia. In contrast, only dobutamine increased phasic \([\text{Ca}^{2+}]\) from control after ischemia. Each drug increased systolic \([\text{Ca}^{2+}]\) com-
pared with control before ischemia. Interestingly, digoxin and dopamine reversed the increase in systolic $[\text{Ca}^{2+}]$ in the control group when administered after ischemia. Dobutamine increased diastolic $[\text{Ca}^{2+}]$ before ischemia. Digoxin decreased diastolic $[\text{Ca}^{2+}]$ after ischemia.

**Myocardial contractility and relaxation.** Each drug enhanced myocardial contractility and relaxation from control before and after ischemia (Fig. 3, A and B). This increase was lower after ischemia in all drug groups.

**$[\text{Ca}^{2+}]$ influx and efflux.** Each drug enhanced $[\text{Ca}^{2+}]$ influx and efflux (Fig. 3, C and D) before ischemia. Only dobutamine had the same effect after ischemia. Control and digoxin-treated hearts increased $[\text{Ca}^{2+}]$ influx and efflux after ischemia compared with before ischemia.

### Table 1. Effects of inotropic drugs before and after global ischemia on morphological and temporal indexes

<table>
<thead>
<tr>
<th></th>
<th>Before Ischemia</th>
<th>After Ischemia</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dobutamine</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>236±3</td>
<td>350±12*</td>
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<tr>
<td>Systolic LVP, mmHg</td>
<td>32±1</td>
<td>73±5*</td>
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<td>Diastolic LVP, mmHg</td>
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<td>2±1</td>
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<td>Phasic LVP, mmHg</td>
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<td>Systolic $[\text{Ca}^{2+}]$, nM</td>
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<td>609±48*</td>
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<tr>
<td>Diastolic $[\text{Ca}^{2+}]$, nM</td>
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<td>140±10*</td>
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<tr>
<td>Phasic $[\text{Ca}^{2+}]$, nM</td>
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<td>469±72*</td>
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<td>$\text{LVP}_{\text{0s}}$, ms</td>
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<td>78±1*</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{ISO}}$, ms</td>
<td>142±3</td>
<td>78±3*</td>
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<tr>
<td>Peak delay, ms</td>
<td>70±5</td>
<td>42±3*</td>
</tr>
<tr>
<td>Contraction response, ms</td>
<td>65±5</td>
<td>32±3*</td>
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<tr>
<td>Relaxation response, ms</td>
<td>72±5</td>
<td>33±4*</td>
</tr>
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Values are means ± SE. $\text{CaCl}_2$ concentration was 1.25 mM. LVP, left ventricular pressure; $[\text{Ca}^{2+}]$, $[\text{Ca}^{2+}]$ concentration; $\text{LVP}_{\text{0s}}$, half-maximal duration of LVP transients; $[\text{Ca}^{2+}]_{\text{ISO}}$, half-maximal duration of $\text{Ca}^{2+}$. Statistical significance was measured at $*P < 0.05$ vs. control before ischemia; †$P < 0.05$ vs. control after ischemia; ‡$P < 0.05$ after vs. before ischemia for all groups.
Half-maximal duration of Ca\(^{2+}\) and LVP transients. Each drug, except levosimendan, significantly decreased the duration of the LVP transient compared with control, before and after ischemia (Table 1). Dobutamine, dopamine, and digoxin, but not levosimendan, also shortened the Ca\(^{2+}\) transient duration before ischemia. Dobutamine and digoxin reduced the duration of the Ca\(^{2+}\) transient compared with control after ischemia. The durations of LVP and Ca\(^{2+}\) transients were unchanged after ischemia compared with before ischemia in each group.

Peak delay. Dobutamine alone reduced the delay between peak Ca\(^{2+}\) and LVP from control before and after ischemia (Table 1). Peak delay was unchanged after ischemia compared with before ischemia in each group.

Temporal sensitivity of myocardial response. Each drug shortened the contraction response time before but not after ischemia (Table 1). Contraction response time was significantly shorter after ischemia than before. Dobutamine, dopamine, and levosimendan, but not digoxin, reduced the relaxation response time from control before ischemia. Relaxation response times were shorter in control and digoxin-treated hearts after ischemia than before ischemia.

Dynamic Indexes Derived From LVP vs. Ca\(^{2+}\) Phase-Space Diagrams

Figure 4 illustrates phase-space diagrams before and after IR injury for all control hearts. After IR injury, the LVP vs. Ca\(^{2+}\) loop was flatter and wider, and the centroid was shifted rightward. These changes are quantified as unchanged loop area, decreased orientation (from 0.25 to 0.09), and increased location (P) (175–228), which signified worsened Ca\(^{2+}\)-contraction coupling.

Typical LVP-[Ca\(^{2+}\)] phase-space diagrams obtained in control hearts and during infusion of inotropic drugs before and after 30-min global ischemia are displayed in Fig. 5.

Loop area. IR injury reduced loop area in all but the control groups. Inotropic drugs increased loop area before ischemia (Fig. 6A), but only dobutamine increased loop area after ischemia.

Loop orientation. This index is a measure of the shape of the loop computed as the ratio between phasic LVP and phasic [Ca\(^{2+}\)]. A higher index value indicates that more LVP is generated for a given amount of [Ca\(^{2+}\)]. Loop orientation was reduced after ischemia in all groups. Dopamine and digoxin increased, whereas dobutamine decreased, loop orientation...
before ischemia. Dopamine and digoxin increased loop orientation after ischemia.

**Loop position vector.** The loop position vector $P$ spatially localizes the loop in the LVP-$[Ca^{2+}]$ domain. Dobutamine and dopamine altered both the location and direction of the loop centroid before ischemia (Fig. 7A). Loop centroid direction and location were reduced after ischemia in control hearts (Fig. 7B). Dopamine, digoxin, and levosimendan increased the angular location of the loop centroid but did not change its distance from the origin compared with control hearts after ischemia. In contrast, dobutamine increased the distance of the centroid from the origin but did not affect direction. Drug infusion after global ischemia returned the loop centroid positions toward the values observed before ischemia.

**Loop trajectory vectors.** Vectorgrams of loop trajectory magnitude (Fig. 8, top) and direction (bottom), with and without each drug, before and after ischemia, are illustrated in Fig. 8. $T(t)$ represents the temporal characteristics of $[Ca^{2+}]$-contraction coupling and uncoupling during the cardiac cycle. We identified four phases simultaneously (Fig. 8, insets) from $T(t)$ and $\angle T(t)$ plots: 1) onset of cardiac cycle, 2) first minimum $\angle T(t)$, 3) peak $|T(t)|$, and 4) peak $\angle T(t)$. The trajectory vectorgrams show shorter cycle lengths during drugs before and after ischemia, consistent with increased HR. The interval between onset of $[Ca^{2+}]$ rise and diastolic LVP (points 1 and 2) was prolonged after ischemia for each drug except digoxin. This interval was reduced by dobutamine before ischemia and by digoxin, dopamine, and levosimendan after ischemia. The interval between diastolic LVP and systolic $[Ca^{2+}]$ (points 2 to 3) was reduced after IR injury in the control group. This interval was shortened by each drug before ischemia, but the drugs had no effect after ischemia. The delay between systolic $[Ca^{2+}]$ and systolic LVP (points 3 to 4) was unchanged after ischemia compared with before ischemia in each group. Dobutamine alone shortened this interval before and after ischemia.

**Phase-Space Analysis of $dLVP/dt$ vs. $[Ca^{2+}]$**

The linear regression model for the phase-space plots of $dLVP/dt$ and $[Ca^{2+}]$ had averaged correlation coefficients of 0.9 or better for all segments and all interventions. Each slope (mmHg·nM$^{-1}$·s$^{-1}$) was reduced after ischemia in each group (Table 2), indicating the loss of cooperativity due to ischemic (Fig. 6B) before ischemia. Dopamine and digoxin increased loop orientation after ischemia.

![Fig. 5. Phase-space characterization of LVP vs. $[Ca^{2+}]$ loops before (A) and after (B) global ischemia in each group. Note the increased diastolic LVP and excess $[Ca^{2+}]$ loading on reperfusion in control hearts. Conversely, the drugs given after ischemia attenuated $[Ca^{2+}]$ loading.](image)

![Fig. 6. Calculated loop area (A) and loop orientation (B) before and after ischemia in each group. Dobutamine increased loop area before and after ischemia, but loop orientation was unchanged or decreased, suggesting decreased $Ca^{2+}$-contraction coupling. The other drugs increased loop area before but not after ischemia. Dopamine and digoxin improved loop orientation before and after ischemia. *Drug vs. pre- or postischemic control; †preischemia vs. postischemia, all groups: $P < 0.05$.](image)
Our analysis demonstrated that under different conditions. We found that changes in Ca$^{2+}$ DISCUSSION above ischemia in each 

B After Ischemia

Fig. 7. Loop position vector (P) before (A) and after (B) ischemia in each group. Loop direction $\theta$ was plotted vs. |P| to show that IR injury shifted the loop rightward and downward in the control group. Dobutamine moved the loop centroid rightward and downward before and after ischemia. Conversely, dopamine and digoxin moved the loop centroid upward before and after ischemia. Levosimendan moved the loop upward only after ischemia. *Drug vs. pre- or postischemic control; †preischemia vs. postischemia, all groups: P < 0.05.

injury in the binding of Ca$^{2+}$ to troponin C and/or in actinomyosin cross-bridge interactions. Dobutamine and dopamine increased the cooperativity of contraction before and after ischemia. Digoxin only increased the cooperativity of contraction after ischemia. Each drug increased the slope of segment 2 before ischemia, but only dopamine and dobutamine increased this slope after ischemia. Dopamine alone increased the cooperativity of relaxation before ischemia, whereas both dobutamine and dopamine increased this index after ischemia. Levosimendan had no effect on the cooperativity of contraction or relaxation before or after ischemia compared with control hearts.

DISCUSSION

We developed dynamic indexes to better interpret the changes in Ca$^{2+}$-contraction coupling over a cardiac cycle under different conditions. We found that 1) changes in shape, size, and position of LVP vs. [Ca$^{2+}$] phase-space diagrams individually quantify drug and ischemia-induced changes in Ca$^{2+}$-contraction coupling in different ways; and 2) each of the dynamic indexes furnishes different information about Ca$^{2+}$-contraction coupling, and each is required to understand the dynamics of the LVP-[Ca$^{2+}$] loop under different conditions. Our analysis demonstrated that 1) IR injury in the absence of positive inotropic drugs worsened Ca$^{2+}$-contraction coupling; 2) dobutamine worsened Ca$^{2+}$-contraction coupling before and after ischemia; 3) in contrast, dopamine enhanced Ca$^{2+}$-contraction coupling before and after ischemia; 4) digoxin improved Ca$^{2+}$-contraction coupling, particularly after ischemia; 5) effects of inotropic drugs on static and dynamic indexes were greater before than after ischemia, confirming that IR injury reduces contractile response to [Ca$^{2+}$]; and 6) the three identified segments of the phase-space relationship between dLVP/dt and [Ca$^{2+}$] are enhanced by inotropic drugs before and less so after ischemia, indicating changes in cooperativity affecting contractile function in response to available Ca$^{2+}$.

IR Injury and Ca$^{2+}$-Contraction Coupling

Myocardial damage due to coronary artery disease or cardiac surgical procedures may be reversible (stunning) or irreversible (infarction) during reperfusion, depending on the length and severity of the ischemic insult (8, 28, 34). IR injury results from several interrelated mechanisms that include 1) reduced maximal Ca$^{2+}$-activated force or Ca$^{2+}$ sensitivity, 2) myoplasmic Na$^+$ and Ca$^{2+}$ overload, 3) inefficient ATP synthesis and utilization associated with NADH accumulation, 4) impaired myocardial vascular perfusion, and 5) reactive oxygen species-induced damage (18, 28, 31). Our results show that IR injury decreased Ca$^{2+}$-contraction coupling. This is indicated by unchanged LVP vs. [Ca$^{2+}$] loop area, but reduced loop orientation concomitant with a shift in the loop position to the right and at a shallower angle. IR injury also reduced contractile response to [Ca$^{2+}$], as noted from increased total [Ca$^{2+}$] influx and efflux, despite unchanged contractility and reduced relaxation.

The trajectory vectorgrams indicate that IR injury prolonged the duration of LVP diastole, reduced the phase of the cardiac cycle characterized by a rapid rise in LVP immediately after the rise in [Ca$^{2+}$], and increased the duration of [Ca$^{2+}$] decline. Changes in duration of these phases were not accompanied by changes in peak delay or HR and thus reflected attenuated Ca$^{2+}$-contraction coupling with IR injury. Decrease in slopes of the three segments defining the dLVP/dt-[Ca$^{2+}$] phase-space diagram also suggests depression in the cooperative binding of Ca$^{2+}$ to troponin C and cross-bridge kinetics due to IR injury. These alterations in intracellular Ca$^{2+}$ homeostasis are likely responsible for the increase in diastolic LVP and the reduction in LV compliance after ischemia.

Dopaminergic and β-Adrenergic Agonists and Ca$^{2+}$-Contraction Coupling

Dopamine enhanced Ca$^{2+}$-contraction coupling before and after ischemia, as indicated by the increases in LVP vs. [Ca$^{2+}$] loop orientation and loop area, and by the repositioning of the loop at a steeper angle in the phase-space plot. Dopamine decreased relaxation response time before ischemia; this also suggests an increase in contractile response to [Ca$^{2+}$]. Increases in the steepness of all three segments of the dLVP/dt vs. [Ca$^{2+}$] diagrams with dopamine suggest improved cooperativity during contraction and relaxation, both before and after ischemia.

Dobutamine infusion before and after ischemia increased the LVP vs. [Ca$^{2+}$] loop area, but loop orientation was unchanged before, and reduced after, ischemia; this suggests a drug-
induced decrease in Ca\(^{2+}\)-contraction coupling before and after ischemia. A shift in the loop location rightward and at a shallower angle with dobutamine before and after ischemia also supports this hypothesis. The depression in Ca\(^{2+}\)-contraction coupling with dobutamine occurred, despite significant increases in Ca\(^{2+}\) flux and availability before and after ischemia; this suggests that dobutamine decreased the contractile response to Ca\(^{2+}\). Plots of dLVP/dt vs. [Ca\(^{2+}\)] show that, whereas dobutamine increased the cooperativity of contraction before and after IR injury, the cooperativity of relaxation was increased with drug infusion only after IR injury.

Endogenous catecholamines play a key role in regulating myocardial contractility through \(\beta_1\)-adrenergic and dopaminergic receptors in vivo. Catecholamines increase myoplasmic [Ca\(^{2+}\)], increase Ca\(^{2+}\) uptake into the sarcoplasmic reticulum, and decrease myofilament Ca\(^{2+}\) sensitivity (12, 16). The results observed in this and previous studies (10) suggest that activation of dopaminergic and \(\beta_1\)-adrenergic receptors by dopamine (21) has more potent effects than activation of \(\beta_1\)-adrenoceptors by dobutamine alone.

Cardiac Glycosides and Ca\(^{2+}\)-Contraction Coupling

Digoxin enhanced the contractile response to Ca\(^{2+}\) after ischemia, as indicated by increases in contractility and relaxation, with no changes in [Ca\(^{2+}\)] influx or efflux from the postischemic control. The duration of LVP and Ca\(^{2+}\) transients was reduced before and after ischemia, and contraction response time was shortened before ischemia. These changes occurred independently of HR. Dynamic indexes from phase-space diagrams of LVP vs. [Ca\(^{2+}\)] revealed that digoxin increased loop area, orientation, and \(\Delta P\) before ischemia, but only increased orientation and \(\Delta P\) after ischemia; this suggests enhanced Ca\(^{2+}\)-contraction coupling with digoxin after and, to a lesser extent, before ischemia. Digoxin enhanced cooperativity of contraction after, but not before, ischemia. These results indicate that digoxin had more potent effects on impaired compared with normal myocardium.

Ca\(^{2+}\) Sensitizers and Ca\(^{2+}\)-Contraction Coupling

At low concentrations, levosimendan acts as myofilament Ca\(^{2+}\) sensitizer by binding to TnCA in the presence of Ca\(^{2+}\), which stabilizes the Ca\(^{2+}\)-TnCA complex without actually increasing Ca\(^{2+}\) binding affinity with TnCA (11). At higher concentrations, levosimendan also acts as a cardiac phosphodiesterase III inhibitor, resulting in elevated cAMP and subsequently in increased [Ca\(^{2+}\)] (23, 24). Levosimendan increased phasic LVP and [Ca\(^{2+}\)] before but not after ischemia. Nevertheless, levosimendan increased contractility both before and after ischemia. These results are supported by previous studies of levosimendan and LV dysfunction in dogs (25). Levosimendan shortened contraction and relaxation response times before ischemia, possibly due to the phosphodiesterase III inhibitory effect. These changes were accompanied by an increase in HR, which indicates that levosimendan alters myocardial electrophysiological properties and is, therefore, not acting as a pure Ca\(^{2+}\) sensitizer (33). The dynamic indexes revealed that levo-

Fig. 8. Plots of average loop trajectory vector magnitude (\(T\)) (top) and phase (bottom) vs. time for each group before (A) and after (B) ischemia. Insets: averaged control LVP vs. Ca\(^{2+}\) loops before and after ischemia with four identifiable phases of the cardiac cycle marked by points a–d, derived from the vectorgram peak phases (b, d) and magnitude (a, c). These phases are shown for control \(T(t)\) and \(\Delta P(t)\) plots before and after ischemia and marked by dotted lines. The drugs changed the relative time of occurrence of each of these phases, as described in detail in the text.
suggests that Ca\textsuperscript{2+} \(0.05\) after vs. before ischemia for all groups.

Table 2. Effects of inotropic drugs before and after global ischemia on slopes of linear relationship between averaged dLVP/dt vs. [Ca\textsuperscript{2+}] phase-space diagrams

<table>
<thead>
<tr>
<th></th>
<th>Before Ischemia</th>
<th>After Ischemia</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>2.3 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>4.1 ± 0.4*</td>
<td>2.2 ± 0.4\‡</td>
</tr>
<tr>
<td>Dopamine</td>
<td>4.5 ± 0.3*</td>
<td>2.5 ± 0.3\‡</td>
</tr>
<tr>
<td>Digoxin</td>
<td>3.5 ± 0.5</td>
<td>2.2 ± 0.2\‡</td>
</tr>
<tr>
<td>Levosimendan</td>
<td>3.0 ± 0.4</td>
<td>1.4 ± 0.3</td>
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<thead>
<tr>
<th></th>
<th>Before Ischemia</th>
<th>After Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 0.4</td>
<td>3.3 ± 0.6\‡</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>12.0 ± 2.0*</td>
<td>7.0 ± 1.5\‡</td>
</tr>
<tr>
<td>Dopamine</td>
<td>16.0 ± 1.0*</td>
<td>10.0 ± 1.6\‡</td>
</tr>
<tr>
<td>Digoxin</td>
<td>10.0 ± 0.8*</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Levosimendan</td>
<td>10.0 ± 2.0*</td>
<td>4.0 ± 1.0*</td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>Before Ischemia</th>
<th>After Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−11.3 ± 1.0</td>
<td>−2.0 ± 0.3</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>−15.0 ± 2.0</td>
<td>−6.0 ± 1.3\‡</td>
</tr>
<tr>
<td>Dopamine</td>
<td>−17.0 ± 2.0</td>
<td>−6.0 ± 1.7\‡</td>
</tr>
<tr>
<td>Digoxin</td>
<td>−14.0 ± 2.0</td>
<td>−4.3 ± 0.7</td>
</tr>
<tr>
<td>Levosimendan</td>
<td>12.0 ± 1.6</td>
<td>−3.6 ± 1.0\‡</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg·mmM⁻¹·s⁻¹. Slopes of linear fit are given for the three sections of the phase-space diagram of rate of LVP development (dLVP/dt) vs. [Ca\textsuperscript{2+}], averaged over 8 experiments per group. Figure 3 shows sample traces of such segments. Segment 1 quantifies the cooperativity of contraction, segment 2 quantifies the cooperativity of relaxation, and segment 3 quantifies the transitional cooperativity dLVP/dt vs. [Ca\textsuperscript{2+}] and that the relation between positive dF/dt and [Ca\textsuperscript{2+}] during the period from peak dF/dt to peak F was linear. This linear relationship was unchanged for twitch contractions of different duration and strength in ferret papillary muscles. We extended their study by examining the phase-space diagrams of dLVP/dt vs. [Ca\textsuperscript{2+}] and found three linear relationships between dLVP/dt and [Ca\textsuperscript{2+}] over three segments of the cardiac cycle. However, we noted that the slopes of the linear relationship between dLVP/dt and [Ca\textsuperscript{2+}] were indeed sensitive to changes in contractile function due to inotropic drugs in the presence and absence of ischemia. This suggests that inotropic drugs, whatever their mode of action, affect cooperativity in the central and downstream mechanisms that regulate Ca\textsuperscript{2+}-contraction coupling.

Potential Limitations

Isolated perfused hearts are used extensively to study cardiac function. They allow for the control of pre- and postload conditions and autonomic nervous system influences that can complicate in vivo studies. However, isovolumic LVP was measured globally, whereas [Ca\textsuperscript{2+}] was measured only in a core of LV free wall under the optical probe. The fluorescence technique used to estimate myoplasmic [Ca\textsuperscript{2+}] is well established and superior to other Ca\textsuperscript{2+} measurement techniques (3, 22). This technique, however, is sensitive to changes in background autofluorescence and inner filtering. Although we routinely correct for background autofluorescence, it is difficult to estimate the loss of signal intensity due to inner filtering. Because these hearts were unpaced, changes in HR with inotropic interventions likely confound some of the conclusions drawn from the static indexes of contractility, relaxation, Ca\textsuperscript{2+} influx and efflux, and all of the temporal indexes. However, only by allowing hearts to beat at their inherent rhythm can we fully understand the physiological impact of these drugs on the intact heart. Controlling the HR would lead to changes in the peak values and kinetics of the measured Ca\textsuperscript{2+} transients and thus result in LVP values that are non-physiological.

Inotropic drugs were given before as well as after ischemia, so it is possible that the drugs induce cardiac preconditioning that could subsequently alter the static and dynamic indexes obtained after ischemia. For example, levosimendan is reported to precondition the heart and protect against subsequent IR injury (17). Conversely, digoxin is not believed to protect against IR injury and may abolish protection when coupled with ischemic preconditioning (15). The role of β-adrenoceptor agonists administered before IR injury in cardioprotection remains controversial. Some researchers have reported that β-adrenoceptor agonists produce a deleterious effect on IR injury (29), but others have demonstrated improved hemody-

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portant information by which to quantitatively assess dynamic changes in loop shape, size, and position each furnished im-
guinea pig isolated hearts during administration of positive
erescence, F385mito and F456mito. Nonmyoplasmic fluorescence was mea-
myoplasmic compartment (4). F385mito and F456mito were calculated at
was calculated similarly:

\[ R_{\text{tot}} = \frac{(F_{385})_{\text{endo-diastolic}}(F_{385})_{\text{max}}}{(F_{456})_{\text{endo-diastolic}}(F_{456})_{\text{max}}} \]  

Similar to Eqs. 1 and 2, myoplasmic [Ca\(^{2+}\)] ([(Ca\(^{2+}\)]\text{m}) was calculated as:

\[ [(Ca^{2+})\text{m}] = S_{456}K_d(R_{\text{m}} - R_{\text{min}})/(R_{\text{max}} - R_{\text{m}}) \]  

where \( R_{\text{m}} \) was derived from the ratio of the myoplasmic fluorescence, F385 and F456, calculated at each time point by effectively subtracting mitochondrial compartment Ca\(^{2+}\) ([(Ca\(^{2+}\)]\text{initio}) from [Ca\(^{2+}\)]\text{tot and multiplying the remainder by total end-diastolic fluorescence (as in Eq. 3) so that:

\[ R_{\text{m}} = [(F_{385})_{\text{max}} - (F_{385})(\text{end-diastolic})(F_{385})_{\text{min}}]/(F_{456})_{\text{max}} - \]  

Nonstimulated endothelium does not contribute significantly to [Ca\(^{2+}\)]\text{tot (6, 30).

GRANTS

The research was supported in part by American Heart Association (AHA) Grant 0425661Z to S. S. Rhodes; National Institutes of Health (NIH) Grant HL-58691, NIH Grant GM-8204–06, and AHA Grant 0355608Z to D. F. Stowe; and by the Research Service, VA Administration.


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ergic receptors hastens relaxation and mediates phosphorylation of phos-
holphamin, troponin I, and C-protein in ventricular myocardium from

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