Effects of Ouabain and Isoproterenol on Left Ventricular Diastolic Function During Low-Flow Ischemia in Isolated, Blood-Perfused Rabbit Hearts

Beverly H. Lorell, Shogen Isoyama, William N. Grice, Ellen O. Weinberg, and Carl S. Apstein

Myocardial ischemia causes both systolic and diastolic dysfunction. A variety of positive inotropic agents with different subcellular mechanisms may be used clinically in an attempt to reverse ischemic contractile failure. We tested the hypothesis that two inotropic agents, isoproterenol (a β-adrenergic agonist) and ouabain (a sodium pump inhibitor), might have different effects on left ventricular (LV) diastolic function during ischemic failure despite an equivalent inotropic effect. Isolated isovolumic (balloon-in-LV) blood perfused rabbit hearts were paced at constant physiological heart rate (4 Hz), given either no drug (controls, n = 7), isoproterenol (n = 7), or ouabain (n = 7), and then subjected to 6 minutes of low flow ischemia (75% reduction of baseline coronary flow). The doses of isoproterenol and ouabain were selected to produce equivalent modest inotropic effects (15% increase in LV +dP/dt) in each heart during baseline perfusion conditions. During the ischemic period, there was a marked decrease in contractility, and neither isoproterenol nor ouabain demonstrated a positive inotropic effect relative to the control group. However, these agents had markedly different effects on diastolic chamber distensibility (assessed by end-diastolic pressure at constant LV volume) during ischemia. In the control and isoproterenol groups, diastolic chamber distensibility did not change during the ischemic period. In contrast, ouabain treatment resulted in a marked decrease in diastolic chamber distensibility during ischemia; this change was not completely reversible during the 10-minute reperfusion period. The mechanism by which ouabain decreased diastolic chamber distensibility relative to isoproterenol was assessed indirectly. The ouabain and isoproterenol groups were subjected to equivalent degrees of ischemia as assessed by oxygen supply/demand imbalance; during ischemia, each drug group did not differ with regard to myocardial perfusion rates, determinants of myocardial oxygen demand (heart rate, LV developed pressure, LV +dP/dt), myocardial oxygen consumption, lactate production, and ATP and creatine phosphate content. We therefore inferred that the greater decrease in diastolic distensibility in the ouabain group was not due to a greater metabolic severity of ischemia. These observations are consistent with a mechanism of cytosolic calcium overload induced by ouabain, resulting in persistent active myofilament tension development throughout diastole, to cause the observed decrease in diastolic chamber distensibility during ischemia in the ouabain group. Regardless of the subcellular mechanism, the results suggest that digitalis glycosides may have deleterious effects on diastolic function during low flow ischemia.
A variety of inotropic agents are currently used to treat heart failure resulting from myocardial ischemia. Recent studies have shown that diastolic dysfunction as well as systolic dysfunction contributes significantly to the pathophysiology of acute ischemic heart failure.1-4 Because currently used inotropic agents act by different subcellular mechanisms, we hypothesized that they may affect differently the acute diastolic dysfunction that occurs during myocardial ischemia.

To test this hypothesis, we used an experimental model that we have recently developed and that closely simulates the myocardial perfusion conditions (in terms of coronary blood flow and oxygen delivery) present in patients with severe coronary artery disease.5 This model consists of an isolated, isovolumic rabbit heart perfused with fresh, whole rabbit blood. During baseline conditions, coronary perfusion pressure is set at 100 mm Hg, and myocardial blood flow is in the physiological range. To simulate measured intracoronary pressures distal to severe stenoses,6 perfusion pressure is reduced to 20 mm Hg, and coronary flow decreases by approximately 75%. We have recently demonstrated that pacing tachycardia in this experimental preparation reproduces the reversible decrease in diastolic distensibility that occurs during pacing-induced angina in patients with severe multivessel coronary stenoses.7,8

In the current study, in the absence of pacing tachycardia, we compared two inotropic agents, ouabain and isoproterenol, which have different mechanisms of action. Ouabain exerts its positive inotropic effect by increasing cytosolic Ca\(^{2+}\) levels; this increase in [Ca\(^{2+}\)]\(_e\) after inhibition of the sarcoplasmic reticulum sodium pump by ouabain appears to result from a decrease in Ca\(^{2+}\) efflux9 and/or an increase in Ca\(^{2+}\) influx\(^{10}\) via sodium/calcium exchange. In contrast, isoproterenol is a \(\beta\)-adrenergic agonist that causes multiple intracellular events including an increase in cyclic AMP levels, an increase in the rate of sarcoplasmic reticular Ca\(^{2+}\) release, an increase in the rate of sarcoplasmic reticular Ca\(^{2+}\) resequestration during diastole, and an increase in the phosphorylation of troponin, which decreases its sensitivity to Ca\(^{2+}11,12\). Thus, it is reasonable to postulate that ouabain and isoproterenol might have different effects on myocardial relaxation during ischemia.

Our results indicate that a modestly inotropic dose of ouabain caused marked diastolic dysfunction during a period of acute ischemia, whereas an equivalent dose of isoproterenol did not. This observation suggests a potential deleterious effect of digitals glycosides in patients with pulmonary congestion secondary to acute myocardial ischemia.

**Materials and Methods**

**Perfusion Technique**

An isolated, blood-perfused rabbit heart preparation that was developed in our laboratory\(^{5}\) was used. A pair of male albino New Zealand rabbits weighing 2.0–3.5 kg were used for each experiment. One rabbit served as a donor of fresh, whole blood after intravenous anesthesia with pentobarbital sodium (50 mg/kg) and heparinization (1,000 units). The heart donor rabbit was anesthetized and heparinized as described above. After thoracotomy, the pericardium was opened and the heart was isolated and placed in a water-jacketed constant temperature chamber. A perfusion cannula was inserted into the ascending aortic stump, and the coronary arteries were perfused via the aortic root with oxygenated blood. The interval between isolation of the heart and initiation of coronary perfusion was less than 10 seconds in all experiments. The apparatus for blood perfusion consisted of a venous reservoir, variable-flow pump, oxygenator, filter of 40-\(\mu\)m pore size, and an arterial reservoir as shown in Figure 1. Coronary venous blood was recirculat-
ed by the pump through the oxygenator, filter, and arterial reservoir before entering the coronary arteries. The oxygenator was manufactured by coiling approximately 7.5 m of silastic tubing (0.58 mm i.d., 0.77 mm o.d.; Dow-Corning Medical Products, catalog #602-235), which was placed inside a large covered beaker gassed with 20% O<sub>2</sub>, 3% CO<sub>2</sub>, and 77% N<sub>2</sub>. The flow rate of the gassing mixture was varied slightly from experiment to experiment to produce a PO<sub>2</sub> of 100–110 mm Hg and pH of 7.35–7.45. The hematocrit of the blood ranged from 29% to 33%. The glucose level of the perfusion blood was maintained between 80–100 mg/dl throughout each experiment.

After initiation of coronary perfusion, a cannula was inserted via the pulmonary artery stump into the right ventricle to drain coronary venous blood to the venous reservoir and to completely empty the right ventricle. The vena cavae were ligated. A drainage cannula was placed in the apex of the left ventricle to remove any Thebesian drainage. A thermistor (Yellow Springs Instruments, Yellow Springs, Ohio) and a pacing electrode were inserted into the right ventricle through the right atrium. A collapsed latex balloon was placed into the left ventricle (LV) via the left atrium. The balloon was large enough so that no pressure was generated by the balloon itself over the range of left ventricular volumes used in the experiment. Myocardial temperature was maintained at 37° C, and heart rate was maintained at a physiological rate of 4 Hz by electrical pacing.

**Measurements**

Coronary perfusion pressure (CPP) was measured from a side arm of the perfusion cannula connected to a pressure transducer (Statham P23Db, Hato Rey, Puerto Rico). Left ventricular pressure (LVP) was measured with a high-fidelity micromanometer catheter (Millar Instruments, Houston, Texas) or short, stiff fluid-filled catheter attached to the Statham P23Db transducer. The frequency response and damping characteristics of this system have been described from this laboratory and satisfy the requirements shown by Falsetti et al for accurate measurement of ventricular pressure and its first derivative. To assess diastolic chamber distensibility, LV balloon volume was kept constant so that an increase in LV end-diastolic pressure (LVEDP) signified a decrease in distensibility. A similar approach has been used previously in isolated heart models. LVEDP was always measured after transiently turning the pacemaker off for 5 seconds to permit measurement of LVEDP after it reached its nadir during a long diastole. The coronary blood flow rate (CBF) was measured by timed samples of coronary venous effluent collected from the pulmonary artery cannula and expressed as milliliters per minute per gram LV weight. Oxygen contents of arterial and coronary venous blood were directly measured (Lex-O<sub>2</sub>-Con, Lexington, Massachusetts). Myocardial oxygen consumption was expressed as milliliters per minute per gram LV weight. Arterial and coronary venous lactate concentrations were measured by the specific enzymatic method of Apstein et al. All perfusate samples were immediately mixed with trichloracetic acid (TCA) solution (final concentration of 5% TCA) and kept under refrigeration until chemical analysis. At the conclusion of each experiment, LV wet and dry weights were determined. The stability of this isolated blood perfused rabbit heart preparation has been described in detail, and the preparation is stable for the length of time required for the protocol described below.

**Experimental Protocol**

Three groups of hearts were studied: control group (seven hearts) which received no drug; ouabain group (seven hearts), which received ouabain; and isoproterenol group (seven hearts), which received isoproterenol as described below.

In all groups, LV balloon volume was initially adjusted to achieve an LVEDP of 15 mm Hg under control conditions of CPP of 100 mm Hg and a physiological paced heart rate of 4 Hz. The balloon volume was then kept constant for the remainder of the experiment. After 30 minutes maintenance of a hemodynamic steady state, we performed baseline measurements of LV pressure and CBF, and arterial and coronary venous blood sampling for the analysis of myocardial oxygen consumption and lactate metabolism. In the groups designated to receive ouabain and isoproterenol, drugs were diluted with saline such that a volume of approximately 0.5 ml was administered into the coronary venous reservoir. A dose of each drug was chosen by titration so that the maximum LV +dP/dt increased by approximately 15% of baseline values in each heart. The concentration of ouabain and isoproterenol in the blood perfusate ranged from 0.5×10<sup>-9</sup> to 6×10<sup>-7</sup> M and from 2.5×10<sup>-9</sup> to 6.0×10<sup>-9</sup> M, respectively. In the ouabain and isoproterenol groups, drug administration was continued throughout the remainder of the experiment. We continuously observed hemodynamics for at least 10 minutes after administration of each drug, and again performed measurements and blood sampling after hemodynamics reached a steady state for 10 minutes. CPP was then reduced to 20 mm Hg for low-flow ischemia of 6 minutes. At 1 and 6 minutes of ischemia, hemodynamic measurements, and blood sampling were performed as described above. Thereafter, CPP was returned to 100 mm Hg and hemodynamic measurements and blood sampling were performed at 10 minutes of recovery.

Since ouabain administration was found to cause a deleterious effect on LV function in the setting of low-flow ischemia, we examined the effect of ouabain on LV function under the control perfusion conditions in the absence of ischemia in five additional hearts. In this study, CPP was maintained at
100 mm Hg throughout each experiment and the dose of ouabain for each heart was chosen as described above. In this study, ouabain of a moderate dose range (1 × 10⁻⁷ to 2 × 10⁻⁷M) caused a 12 ± 3% increase in LV +dP/dt and did not cause any change in LVEDP for 60 minutes of drug administration, and verified that a nontoxic low concentration of ouabain was used in those experiments.

Biochemical Assay of Adenosine Triphosphate and Creatine Phosphate Contents

Myocardial ATP and creatine phosphate (CP) contents were determined in three additional groups of hearts at the end of a 6-minute period of ischemia following the protocol described above: control group, which received no drug (seven hearts); isoproterenol group (eight hearts); and ouabain group (seven hearts). At the end of the 6-minute period of low-flow ischemia, the heart was trimmed of atria and right ventricular free wall, and the LV was rapidly frozen with Wollenberger aluminum clamps cooled with liquid nitrogen. Each frozen sample was rapidly weighed and pulverized in a mortar in liquid nitrogen. An aliquot of the frozen powder was weighed and then heated (37°C) for 48 hours to determine the frozen/dry wt ratio. The remainder of the sample was mixed with 0.6N perchloric acid, homogenized, centrifuged, and neutralized with 5 mol/l potassium carbonate. The aliquots of neutralized homogenate were placed in preweighed reagent vials and analyzed for ATP by the methods of Adams. CP was measured by the methods of Altschuld by adding an excess of creatine kinase to the ATP reaction mixture after the ATP assay had reached completion. Measurements are expressed as micromoles per gram dry weight.

Statistical Analysis

All data presented as the mean ± SEM. Statistical comparisons between the mean values in the three groups were performed using an analysis of variance (ANOVA) and the Scheffe multiple comparison test. Statistical comparisons between the baseline predrug value versus subsequent intervention values within each group were performed using Student’s paired t test with the Bonferroni correction for multiple comparisons.

Results

Figure 2 shows a representative experiment obtained from the control group, the ouabain group and isoproterenol group. At baseline before administration of any drug, LVEDP was set at 15 mm Hg in all three hearts. After administration of the drugs sufficient to cause a 13% increase in LV +dP/dt before imposition of ischemia, there was no change in either LV systolic or diastolic pressure in the hearts of the ouabain and isoproterenol groups relative to the control group. During 1 minute of low-flow ischemia, LV systolic pressure decreased markedly in all three groups. LVEDP also decreased slightly in all three groups during the initial minute of ischemia consistent with a decrease in coronary turgor. At 6 minutes of low-flow ischemia, there was no change in LVEDP in the control and the isoproterenol groups. In contrast, in the ouabain-treated heart, LVEDP at constant LV volume gradually increased during low-flow ischemia consistent with a decrease in LV diastolic distensibility.

Figure 3 shows the changes in maximum LV +dP/dt in the three groups. After administration of ouabain and isoproterenol, maximum LV +dP/dt increased by 15 ± 5% above baseline (from 1,723 ± 145 to 2,052 ± 202 mm Hg/sec, p < 0.05) in the ouabain group and by 13 ± 2% (from 1,652 ± 100 to 1,864 ± 85 mm Hg/sec, p < 0.05) in the isoproterenol group. The values in the ouabain and the isoproterenol groups were comparable at 1 and 6 minutes of low-flow ischemia.

The changes in LV developed pressure in the three groups are shown in Figure 4. After administration of ouabain and isoproterenol before the imposition of ischemia, LV developed pressure increased only slightly from 87 ± 6 to 89 ± 8 mm Hg and from 89 ± 4 to 93 ± 4 mm Hg, respectively. At 1 and 6 minutes of low-flow ischemia, LV developed pressures were decreased and comparable in all three groups. At 10 minutes of recovery, LV developed pressure returned to approximately 85% of baseline in each group.

Figure 5 and Table 1 show the changes in LVEDP in response to ischemia. LVEDP was set at a level of 15 mm Hg in all three groups at baseline. LVEDP did not change after drug administration before the imposition of ischemia, and slightly decreased at 1 minute of ischemia in all three groups consistent with a slight decrease in coronary turgor (p < 0.05 vs. baseline for each group). In the control and the isoproterenol groups, LVEDP did not change significantly during 6 minutes of low-flow ischemia. In contrast, in the ouabain group, LVEDP shifted upward at 6 minutes of low-flow ischemia to a level of 25.9 ± 3.2 mm Hg (p < 0.01 vs. control and isoproterenol groups). The recovery of the LVEDP in the ouabain group was not complete at 10 minutes of recovery.

Figure 6 and Table 2 show the changes in coronary blood flow and lactate concentration differences in the three groups. There were no significant differences in coronary blood flow at baseline, before ischemia after drug administration, during ischemia, or during recovery among the three groups. All three groups showed comparable myocardial lactate extraction at baseline. During ischemia, the myocardial lactate production values in the ouabain and isoproterenol groups tended to be slightly higher (p < 0.10) than that in the control group, but there was no difference between the ouabain and the isoproterenol groups.

Myocardial oxygen consumption values in the three groups are summarized in Table 2. Myocardial oxygen consumption increased slightly after administration of drugs in the ouabain and the
isoproterenol groups, and decreased during low-flow ischemia to a similar extent in the three groups. Left ventricular wet weights and wet/dry wt ratios were comparable in all three groups.

Left ventricular myocardial ATP and CP contents were determined at the end of a 6-minute period of low-flow ischemia in three additional groups of hearts (control [no drug], isoproterenol treatment, ouabain treatment) subjected to the identical protocol. Comparable effects on systolic and diastolic function were observed in each group of hearts studied for analysis of high-energy phosphate content in comparison with the results discussed above. The biochemical results are summarized in Table 2. These results represent about a 60% reduction in ATP content and an 80% reduction in CP content compared with normal values for rabbit myocardium as previously reported from our laboratory (ATP, 24.1 ± 0.7 μM/g dry wt; CP, 49.8 ± 0.5 μM/g dry wt). By analysis of variance (ANOVA), the CP content was significantly lower at the end of ischemia in the ouabain-treated hearts relative to the control hearts, which received no drug, but did not differ significantly with respect to the isoproterenol-treated hearts. Although there was a trend for the values of ATP content to be lower in the ouabain and isoproterenol groups with respect to the control group, there was no statistical difference in ATP content between the groups.

Discussion

A reversible decrease in left ventricular diastolic distensibility as well as systolic dysfunction has been repeatedly shown to occur during episodes of heart failure associated with acute ischemia in patients with coronary stenoses. We hypothesized that two agents that may be used clinically to reverse acute ischemic heart failure, ouabain (a sodium pump inhibitor) and isoproterenol (a β-adrenergic agonist), may have different effects on...
diastolic function in the setting of myocardial ischemia despite an equivalent inotropic effect. To test this hypothesis, we used an isolated, isovolumic rabbit heart model perfused with fresh, whole rabbit blood, and we compared hearts that were given no drugs (controls) or were given isoproterenol or ouabain and then subjected to 6 minutes of low-flow global ischemia. The doses of isoproterenol and ouabain were given to produce equivalent modest inotropic effects (15% increase in LV +dP/dt) during baseline perfusion conditions. In comparison with isoproterenol and the control state, ouabain caused a marked decrease in LV diastolic chamber distensibility (an upward shift in LV diastolic pressure at constant LV volume) during ischemia that did not completely reverse during the 10-minute reperfusion period. In each experiment, the elevated LVEDP reached its nadir and then remained constant during long diastoles with transient cessation of pacing consistent with an incompleteness of the extent of LV relaxation. In contrast, LV diastolic chamber distensibility did not decrease during ischemia in the isoproterenol and control groups.

Comparison with Demand Ischemia

The effects of ouabain on diastolic function during ischemia at a constant physiological heart rate are strikingly similar to the influence of demand ischemia. We have recently shown that the superimposition of pacing tachycardia during global low-flow ischemia (i.e., demand ischemia) in blood-perfused isovolumic rabbit hearts results in an immediate and reversible increase in LV diastolic pressure relative to LV volume with an impairment of LV relaxation, whereas brief low-flow global ischemia alone at a constant physiological heart rate (supply ischemia) causes no decrease in diastolic distensibility. A similar acute increase in LV diastolic pressure relative to volume has been observed in patients with coronary disease and in dogs with coronary stenoses during demand ischemia imposed by pacing tachycardia. Similarly, studies of ischemia in isolated hearts have shown that important differences of diastolic distensibility during myocardial ischemia are related to “demand” as opposed to low-flow “supply” ischemia; such differences are not simply due to the loss of coronary vascular turgor, repeated systolic stretch of an ischemic segment, or coronary “steal” from an ischemic to adjacent nonischemic segment.

Our present study, which showed differing effects of ouabain and isoproterenol on diastolic function during global low-flow ischemia, lends insight into these prior observations. First, our results are unlikely to be related to the magnitude of “demand” per se, or severity of the ischemic insult that was imposed. In our experimental design, coronary perfusion pressure was adjusted to the same level, and coronary flow was similar in all three groups. Glycolytic flux (as estimated by myocardial lactate production) and the determinants of myocardial energy demand ($LV +dP/dt$, developed pressure, heart rate) were comparable in the ouabain and isoproterenol groups. Furthermore, the magnitude of increased “demand” caused by ouabain and
isoproterenol was small since doses were chosen that caused a small increase in LV +dP/dt at baseline in individual hearts with no statistically detectable increase in either LV +dP/dt or developed pressure during ischemia relative to the control group. Myocardial ATP and CP contents did not differ significantly in the ouabain- and isoproterenol-treated groups in response to ischemia. Our findings are consistent with the report by Momomura et al 24 that differences in diastolic function caused by demand versus supply ischemia are not attributable to differences in myocardial high energy phosphate content.

Secondly, the disparate influence of equivalent modestly inotropic doses of ouabain and isoproterenol on diastolic function suggests that the cellular mechanism of inducing demand ischemia may be a critical factor in producing an acute decrease in diastolic distensibility. Both pacing tachycardia and ouabain share a common potential of causing an increase in cytosolic Na⁺ and Ca²⁺. Rapid repetitive stimulation may promote an increased net influx of Na⁺ and secondary increase in intracellular Ca²⁺ via the Na⁺/Ca²⁺ exchanger as well as a direct effect on intracellular Ca²⁺ due to the increased frequency of depolarizations and obligatory slow channel Ca²⁺ fluxes per minute.26 Ouabain appears to promote an increase in cytosolic Ca²⁺ via Na⁺ pump inhibition and decreased Ca²⁺ efflux (and possibly enhanced influx).9,10 The combination of either of those interventions and low-flow ischemia may simultaneously increase net cytosolic Ca²⁺ but reduce ATP availability to energy-dependent pumps in the sarcoplasmic reticulum and sarcolemma, which normally restore cytosolic Ca²⁺ and Na⁺ levels during diastole. When CPP was decreased from 100 to 20 mm Hg, we observed a slight immediate decrease in LV diastolic pressure consistent with an abrupt loss of coronary turgor in the control and isoproterenol groups with no subsequent decrease in diastolic distensibility consistent with changes observed in isolated hearts with global supply ischemia13,14 and in dogs with coronary artery occlusions.24,27-28 In this setting of low-flow or no-flow ischemia when the heart rate is physiological or slow, the myocardium is not exposed to an obligatory increase of cytosolic Na⁺ or Ca²⁺ from an increased frequency of repetitive depolarizations, and there initially may be sufficient "reserve" of energy-dependent ion pumps to adequately restore

### TABLE 1. Changes in Left Ventricular End-diastolic Pressure at Baseline, in Response to Ischemia, and at Recovery

<table>
<thead>
<tr>
<th>Group</th>
<th>Predrug</th>
<th>With drug</th>
<th>Ischemia (1 min)</th>
<th>Ischemia (6 min)</th>
<th>Recovery (10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.6±0.8</td>
<td>14.6±0.8</td>
<td>13.2±1.0</td>
<td>14.8±1.3</td>
<td>15.1±0.9</td>
</tr>
<tr>
<td>Ouabain</td>
<td>15.3±0.5</td>
<td>14.4±0.4</td>
<td>13.9±0.5</td>
<td>25.9±3.2*</td>
<td>17.8±2.0</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>14.5±0.2</td>
<td>14.5±0.4</td>
<td>12.7±0.3</td>
<td>14.0±0.8</td>
<td>12.6±0.9</td>
</tr>
</tbody>
</table>

*p-Level of significance for p<0.05 for comparisons of values during interventions vs. predrug baseline within each group using Student's paired t test with the Bonferroni correction for multiple comparisons. Statistical comparisons between the three groups were performed using analysis of variance (ANOVA) and the Scheffe multiple comparison test.
FIGURE 6. Coronary blood flow and lactate concentration difference between coronary venous and arterial blood at baseline before drug administration, during drug administration before ischemia, at 1 and 6 minutes of low-flow ischemia, and at 10 minutes of recovery in the three groups. Coronary blood flow per gram LV was comparable in the three groups before ischemia, during low-flow ischemia, and during recovery. During ischemia, the myocardial lactate concentration difference tended to be slightly higher in drug-treated groups relative to the control group (p<0.10), but there was no difference between the ouabain and isoproterenol groups. Since the determinants of myocardial energy demand (heart rate, LV developed pressure, LV +dP/dt) were similar in the ouabain and isoproterenol groups, the detrimental effect of ouabain on diastolic distensibility during ischemia does not appear to be explained by differences in the degree of ischemic insult or glycolytic flux.

cytosolic Na⁺ and Ca²⁺ levels during diastole and prevent an impairment of myocardial relaxation. This hypothesis is consistent with aequorin studies in isolated ferret muscles, which have shown that imposition of hypoxia under conditions that mitigate against cytosolic ion overload (i.e., hypothermia and a slow rate of stimulation) is associated with protection against both an acute upward shift in diastolic tension and an acute prolongation in the cytosolic Ca²⁺ transient.29

The subcellular mechanisms of action of β-adrenergic agonists are multiple, and the primary effects of these agents on myocardial relaxation during ischemia cannot be predicted a priori. β-Adrenergic agonists increase cyclic AMP, which results in phosphorylation of sarcolemmal sites that promote an increase in slow inward calcium flux and increase the rate of sarcoplasmic Ca²⁺ release.11 These actions would tend to promote an increase in cytosolic Ca²⁺ available to the myofilaments and prolong the time course of contraction and relaxation. Conversely, the increase in cyclic AMP also promotes the phosphorylation of troponin I, which results in a decrease in Ca²⁺ sensitivity of the contractile proteins, and the phosphorylation of the regulatory protein phospholamban, which increases the rate of Ca²⁺ reuptake by the sarcoplasmic reticulum, actions that would tend to enhance myocardial relaxation.11,12 In the absence of hypoxia or ischemia, the net effect of β-adrenergic agonists and an increase in cyclic AMP in cardiac muscle preparations is a lusitropic effect of an abbreviation of the time course of relaxation.30 Our observations in an isovolumic heart with constant loading conditions, global reduction of coronary flow, and constant heart rate suggest that the net effect of isoproterenol during low-flow ischemia was a lusitropic effect on diastolic function in contrast to ouabain.

Limitations of the Model

Multiple factors have been shown to modify diastolic properties during ischemia. The isovolumic blood-perfused model of global low-flow ischemia used in this preparation eliminates the contributions of 1) right ventricular-pericardial constraint; 2) changes in loading conditions; 3) segmental differences in coronary turgor; and 4) dyssynchrony of segmental contraction and diastolic recoil. As noted above, myocardial ATP content did not differ in the ouabain and isoproterenol treated hearts. Bricknell et al31 and Apstein et al21,32 have suggested that ATP produced by glycolysis rather than by oxidative phosphorylation has a critical influence on the preservation of diastolic function during cardiac ischemia or hypoxia. The present study does not address the possibility that equivalent inotropic doses of isoproterenol and ouabain may have exerted different effects on a critical pool of glycolytically produced cytosolic ATP that would not be detected by currently available standard biochemical or NMR techniques of quantitating total tissue high-energy phosphate levels. In addition, myocardial acidosis has been associated with protection against ischemia and hypoxia-induced decreases in diastolic distensibility.33,34 Changes in proton concentration are unlikely to be involved in the results we noted. Partial dissipation of the sodium gradient by ouabain would tend to impair proton extrusion via sodium/hydrogen exchange, and thus would be expected to increase [H⁺]. This would decrease [Ca²⁺]i-dependent force in diastole, an effect of ouabain opposite to what was observed.
TABLE 2. Coronary Blood Flow and Measurements of Metabolic Function at Baseline and in Response to Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Coronary blood flow (ml/min/g LV)</th>
<th>Myocardial oxygen consumption (ml/min/100 g LV)</th>
<th>Lactate concentration (Coronary venous—arterial) difference (mmol/l)</th>
<th>Myocardial ATP content (μmol/g dry wt)</th>
<th>Myocardial CP content (μmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predrug</td>
<td>With drug</td>
<td>ischemia (1 min)</td>
<td>ischemia (6 min)</td>
<td>ischemia (6 min)</td>
</tr>
<tr>
<td>Control</td>
<td>2.01 ± 0.20</td>
<td>2.01 ± 0.20</td>
<td>0.45 ± 0.06</td>
<td>0.47 ± 0.06</td>
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</tr>
<tr>
<td>Ouabain</td>
<td>2.12 ± 0.62</td>
<td>2.06 ± 0.56</td>
<td>0.40 ± 0.12</td>
<td>0.39 ± 0.75</td>
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<tr>
<td>Isoproterenol</td>
<td>1.72 ± 0.15</td>
<td>1.89 ± 0.15</td>
<td>0.43 ± 0.09</td>
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</tr>
<tr>
<td>Control</td>
<td>9.5 ± 1.3</td>
<td>9.5 ± 1.3</td>
<td>3.1 ± 0.5</td>
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<tr>
<td>Ouabain</td>
<td>8.3 ± 1.0</td>
<td>8.6 ± 1.1</td>
<td>2.5 ± 0.4</td>
<td>2.3 ± 0.6</td>
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</tr>
<tr>
<td>Isoproterenol</td>
<td>10.6 ± 2.0</td>
<td>11.6 ± 1.1</td>
<td>3.6 ± 0.8</td>
<td>3.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-0.041 ± 0.331</td>
<td>-0.041 ± 0.331</td>
<td>0.327 ± 0.116</td>
<td>1.080 ± 0.168</td>
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</tr>
<tr>
<td>Ouabain</td>
<td>-0.056 ± 0.156</td>
<td>0.232 ± 0.217</td>
<td>1.030 ± 0.245</td>
<td>1.897 ± 0.335</td>
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</tr>
<tr>
<td>Isoproterenol</td>
<td>-0.056 ± 0.234</td>
<td>0.362 ± 0.309</td>
<td>1.325 ± 0.472</td>
<td>1.861 ± 0.236</td>
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<tr>
<td>Control</td>
<td>24.1 ± 0.7*</td>
<td></td>
<td></td>
<td>11.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Ouabain</td>
<td></td>
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<td></td>
<td>8.6 ± 1.0</td>
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<tr>
<td>Isoproterenol</td>
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<td></td>
<td></td>
<td>10.6 ± 1.0</td>
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<tr>
<td>Control</td>
<td>49.8 ± 0.5*</td>
<td></td>
<td></td>
<td>12.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Ouabain</td>
<td></td>
<td></td>
<td></td>
<td>6.2 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td></td>
<td></td>
<td></td>
<td>10.8 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>

*p* Control values for rabbit myocardium previously reported from this laboratory. See text. LV, left ventricle; CP, creatine phosphate.

**Comparison With Prior Studies**

The results from our studies contrast with previous studies that have examined the influence of inotropic agents on myocardial function during ischemia and hypoxia. In dog models with coronary occlusions, prolonged ischemia, and subsequent segmental infarction, isoproterenol has been shown to cause a deterioration in systolic function, increase ST segment elevation, and enhance infarct size.35,36 In isolated papillary muscles studied during prolonged severe hypoxia, a higher dose of isoproterenol (10^-5 M) than was used in this study caused deterioration of active tension development, increased contracture, and compromised recovery.37 The effects of cardiac glycosides on mechanical function during ischemia or hypoxia have been variable and have been reported to show both a beneficial38-39 and deleterious35,40 effect on systolic mechanical function, whereas little attention has been paid to the study of diastolic properties. Vatner and Baire14 studied the influence of equipotent inotropic doses of ouabain and isoproterenol in conscious dogs with acute regional ischemia and reported that isoproterenol caused a deleterious effect on segmental length shortening and end-diastolic segment length in the ischemic zone, whereas ouabain had an opposite and beneficial influence on those parameters. These results appeared to be related to the differing influences of these agents on myocardial energy supply in that ouabain slowed heart rate (and thus prolonged the duration of diastole available for coronary flow) and increased blood flow in the ischemic zone, whereas isoproterenol increased heart rate and reduced flow to the ischemic zone. This is compatible with observations in animal models and in patients with coronary artery stenoses that isoproterenol may dilate the vasculature in nonischemic zones and promote coronary "steal" from the ischemic segment.41-43

The present study differs from these previous studies in several important aspects, including the doses and inotropic agents that were used and the animal species that were studied. In comparison with previous studies, heart rate, left ventricular load (balloon volume), and aortic pressure were held constant in our preparation and there was no influence of reflex changes in sympathetic tone. A critical difference is the nature of the ischemic insult. We studied the influence of brief, low-flow ischemia at a coronary perfusion pressure of 20 mm Hg chosen to simulate the coronary perfusion pressure distal to critical stenoses in humans.6 The present study does not address the influence of inotropic agents during prolonged no-flow ischemia and infarction with delayed reflow. Another critical difference is that our model employed global low-flow ischemia under isometric conditions. Although differences in the endocardial/epicardial perfusion ratio between the isoproterenol and ouabain groups cannot be excluded, our model did eliminate the contributions of both segmental dyssynchrony of contraction and drug-induced alterations in segmental flow. These influences may have masked any primary and differing effects of ouabain and isoproterenol on systolic and diastolic function of ischemic myocardium in the studies cited above.
Our findings in the isolated rabbit heart should not be directly extrapolated to humans. There is suggestive and controversial evidence that digitalis glycosides may have a deleterious influence in patients without chronic systolic dysfunction who experience severe ischemia or infarction. This possible detrimental influence in patients with coronary artery disease may be in part related to its arrhythmogenic effects, whereas the hemodynamic effects of digitalis glycosides on systolic and diastolic dysfunction in the setting of acute and reversible ischemia have not been well studied. Until such studies are available, our findings lend caution to the use of digitalis glycosides to manage acute ischemic heart failure with predominant diastolic dysfunction. The use of isoproterenol is not advocated by us to treat acute ischemic diastolic dysfunction in humans. In patients with coronary stenoses, any primary effect on myocardial relaxation may be outweighed by the effect of isoproterenol on regional flow and by tachycardia which may promote both an increase in energy demand as well as an obligatory net influx of Na\(^+\) and Ca\(^{2+}\) secondary to rapid depolarizations. In the future, selective lusitropic agents may become available that share isoproterenol’s effects on the rate of reuptake of calcium and abbreviation of the time course of relaxation without its potential deleterious effects on heart rate and the coronary vasculature.

In summary, our results showed that equivalent inotropic doses of isoproterenol and ouabain exerted different effects on diastolic function during 6 minutes of low-flow ischemia in isovolumic blood-perfused rabbit hearts paced at a constant physiological heart rate. Ouabain resulted in a marked increase in LV diastolic pressure relative to volume, whereas no change in diastolic distensibility occurred in either the isoproterenol or control groups. These results are not explained by differences in glycolytic flux, the determinants of myocardial energy demand, or high-energy phosphate depletion, and may be related to the propensity of digitalis glycosides to promote cytosolic Ca\(^{2+}\) overload during acute ischemia.

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KEY WORDS: diastole • relaxation • ouabain • isoproterenol
Effects of ouabain and isoproterenol on left ventricular diastolic function during low-flow ischemia in isolated, blood-perfused rabbit hearts.

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