Acute Alterations in Diastolic Left Ventricular Chamber Distensibility: Mechanistic Differences Between Hypoxemia and Ischemia in Isolated Perfused Rabbit and Rat Hearts

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Changes in diastolic chamber distensibility (DCD) during hypoxemia and ischemia were studied in isolated-buffer-perfused rabbit hearts. Two minutes of hypoxemia (low Po2 coronary flow) resulted in a shift of the diastolic pressure-volume curve to the left, i.e., distensibility was decreased (hypoxemic contracture). In contrast, 2 minutes of ischemia (zero coronary flow) resulted in an initial shift of the diastolic pressure-volume curve to the right indicating increased distensibility, which was followed by a later (30 minutes) shift to the left (ischemic contracture). Two minutes of ischemia superimposed on hypoxemia caused complete reversal of contracture. A quick stretch and release applied to the myocardium reversed late ischemic contracture but did not effect early hypoxemic contracture. The role of intracellular pH in modulating changes in DCD during hypoxia and ischemia was studied using phosphorus-31 nuclear magnetic resonance spectroscopy of isolated-buffer-perfused rat hearts that demonstrated changes in DCD similar to rabbit hearts during hypoxemia and ischemia. Intracellular pH decreased from 7.03 ± 0.02 to 6.87 ± 0.03 (p<.01) during 2 minutes of ischemia but did not change significantly during 4 minutes of hypoxemia. When 2 minutes of ischemia were superimposed on hypoxemia, pH decreased from 6.99 ± 0.01 during hypoxemia to 6.88 ± 0.02 after 2 minutes of ischemia (p<.01), concomitant with the complete reversal of hypoxemic contracture. These results suggest different mechanisms for late ischemic and early hypoxic contracture and also suggest an explanation for the opposite initial changes in DCD seen after brief periods of ischemia and hypoxemia. The early development of contracture during hypoxemia and rapid redevelopment of diastolic tension after quick stretching are consistent with the hypothesis that hypoxic contracture results from persistent Ca++-activated diastolic tension secondary to impaired calcium resequstration by the sarcoplasmic reticulum. In contrast, the late development of contracture during global ischemia and reversal by quick stretching is compatible with rigor bond formation. The initial increase in distensibility during early ischemia and the reversal of hypoxic contracture by a brief period of superimposed ischemia probably is the result of two factors present during ischemia but not during hypoxemia: 1) the collapse of the coronary vasculature and loss of the "erectile" effect and, 2) the rapid development of intracellular acidosis, which has been shown to affect myofibrillar calcium sensitivity, and this may lead to a decrease in Ca++ activated diastolic tension. (Circulation Research 1986;59:515-528)
use to refer to the slope of the pressure-volume or stress-strain curve.

Hypoxemia causes an acute decrease in diastolic chamber distensibility within 2 minutes. In contrast, global ischemia causes an initial increase in distensibility due, in part, to collapse of the pressure and volume in the coronary vasculature, termed the “erec-
tile” or “garden hose” effect. Distensibility subsequently decreases after sustained (30–60 minutes) global ischemia, an effect termed ischemic contrac-

Thus, global ischemia and hypoxemia cause opposite initial changes in diastolic distensibil-

ity; sustained ischemia and hypoxemia both decrease distensibility.

The mechanisms responsible for the decrease in dia-

stolic distensibility that occurs after 2 minutes of hy-

poxemia or after sustained global ischemia have not been defined. At least two intracellular mechanisms have been suggested, actin–myosin “rigor” bond for-

mation, and “incomplete relaxation” due to failure of the sarcoplasmic reticulum to completely reseques-
ter calcium during diastole so that Ca++-activated cross-bridge cycling: Rigor bonds are abnormal actin–myosin bonds that form when the ATP concentration decreases to very low levels; cross-bridge cycling is the normal systolic actin–myosin interaction resulting from calcium activation of the contractile proteins; thus, “incomplete relaxation” resulting from persistent cross-bridge cycling is equivalent to a state of sus-
tained partial systole. Rigor bonds are characterized by failure of tension redevelopment after a quick stretch so that post-stretch muscle tension remains markedly reduced. In contrast, a quick stretch maneuver imposed on muscle with continuous cross-bridge cy-

clling will be followed by a rapid redevelopment of tension to near the prestretch value.

We performed a series of experiments to determine whether the same mechanism was responsible for the decrease in diastolic chamber distensibility observed acutely with global hypoxemia and after sustained glo-

bal ischemia. We reasoned that if both states of de-

creased distensibility were caused by the same in-

tracellular mechanism (e.g., rigor bond formation secondary to cytosolic ATP depletion), then once the condition of decreased distensibility occurred acutely after hypoxemia, superimposed ischemia should either maintain or further decrease the degree of distensibil-

ity. In addition, if the mechanism were the same, the response to a quick-stretch should be similar for both the acute hypoxicem and sustained ischemic decreases in chamber distensibility.

To gain insight into the mechanisms responsible for the opposite initial changes in distensibility during acute hypoxemia and ischemia, we measured intracel-

lular pH by phosphorus-31 nuclear magnetic reso-
nance spectroscopy during brief periods of global hy-

poxemia and ischemia and correlated the pH changes with the changes in diastolic distensibility.

Materials and Methods

Isolated Perfused Rabbit Heart Preparation

Albino New Zealand male rabbits weighing be-

tween 1.8 and 2.0 kg were intravenously heparinized and then sacrificed by rapid intravenous injection of 3–5 ml pentobarbital. The thorax was quickly opened and the heart cooled with chilled saline solution, making it asystolic during the operative period. The aorta was dissected free, an incision was made at the level of the right innominate artery and a cannula was tied into the root of the aorta. Retrograde coronary perfusion was immediately started from a warmed perfusion res-

ervoir at a constant pressure head of 75 mm Hg. In this way, coronary perfusion was maintained while the heart was being removed from the animals and only a few seconds elapsed between the time of sacrifice and the onset of experimental coronary perfusion.

The heart was then dissected free, removed from the thorax and placed in a constant-temperature-water-jacketed chamber that kept the heart at 36–37°C (Figure 1). The pericardium was removed so that measured ventricular pressure reflected myocardial wall tension per se and could not be influenced by a pericardial effect. The means of coronary perfusion was then changed from the fixed pressure reservoir to a constant flow pump (Harvard Apparatus model 1203). Aortic pressure, which in this model is equivalent to coronary perfusion pressure, was measured via a Statham P23Db pressure transducer connected to a sidearm of the infusion cannula. The left ventricle was decom-

pressed by an apical puncture and a drain was placed in the apex of the left ventricular cavity so that it re-
mained free of fluid from Thebesian vein drainage.

The coronary venous drainage, comprising all of the flow through the right side of the heart, emerged through the pulmonary artery, which was cannulated and drained off to keep the right ventricle decom-

pressed and eliminate any influence of right ventricular pressure or volume on left ventricular distensibility.

A double-cannulated latex balloon (manufactured in our laboratory from the tip of a Trojan-Enz condom) was inserted into the left ventricle. Both cannu-

las exited through the mitral valve orifice and were tied in place at the atriovenous groove. Each cannula con-

sisted of 12–15 cm of polyethylene tubing (inner diam-

deter = 0.11 cm, intramedic polyethylene tube PE 160, Clay Adams, Inc., New York, N. Y.). One cannula was connected through a clear plastic stopcock to a saline-filled calibrated hand-held glass insulin syringe, which was used to progressively fill the left ventricle to construct pressure-volume curves as well as to rapidly expand the intraventricular balloon to deliver a “quick stretch” to the myocardium in selected experiments. Any air bubbles introduced into the system could be seen and easily withdrawn.

A right ventricular pacemaker wire was inserted through a right atrial incision and attached to a Grass S-

6 stimulator. The hearts were paced at a rate of 180/min with a 5 V unipolar stimulus of 5 msec duration. The pacing rate of 180/min consistently exceeded the animals’ endogenous pacemaker rates so that heart
rate was constant. A temperature probe was inserted into the right ventricle via the right atrium.

The perfusate consisted of a modified Krebs-Henseleit buffer adapted from the work of Neely et al. 118 mM sodium chloride, 4.7 mM potassium chloride, 2.0 mM calcium chloride, 1.2 mM potassium monophosphate, 1.2 mM magnesium sulfate, 23 mM sodium bicarbonate, 0.4 mM sodium ethylenediamine-tetraacetic acid, 5.5 mM glucose, and 1.0 mM lactate. Lactic acid was neutralized with NaOH before being added to the buffer. The potassium concentration of 5.9 mM minimizes ventricular ectopy; the EDTA was originally added to chelate trace toxic metals thought to be present in the perfusate. The perfusate was gassed with a mixture of 95% oxygen and 5% carbon dioxide, which resulted in a pO$_2$ of 542 ± 6 mm Hg and a pH of 7.40–7.45. Perfusion flow rate was adjusted to 30–38 ml/min to provide a physiologic coronary perfusion pressure of 80–110 mm Hg at an LV diastolic pressure of 5–10 mm Hg. The high pO$_2$ of the perfusate and high coronary flow rate was necessary in this model to deliver adequate oxygen since the perfusate is hemoglobin free. The stability of this preparation has been reported elsewhere. 28 The heart was submerged in warm buffer and temperature maintained between 36–37°C. A total of 26 rabbit hearts was used for these studies.

**Measurement of Mechanical Function.** Left ventricular pressure and aortic (coronary perfusion) pressure were monitored continuously throughout each experiment and recorded periodically. A photographic recorder with a high-frequency response was used (Electronics For Medicine DR8). The damping ratio of this pressure measurement system was 0.54, and calculated natural resonant frequency was 75 Hz. Thus, the system was critically damped and the amplitude and waveform of the recorded pressure should accurately reflect the true pressure at frequencies up to 90% of the natural frequency of 75 Hz. This result satisfied the range showed by Falsetti et al. to be required for accurate measurement of ventricular pressure.

The relation between the balloon and left ventricular size is critical in this perfusion technique. The balloon must be slightly larger than the left ventricular cavity; otherwise, as the balloon is filled, an increase in the pressure within the balloon will be recorded because of increasing balloon wall tension rather than ventricular wall tension. We manufactured various balloons of slightly different size so that a balloon slightly larger than the left ventricular cavity could be utilized in each experiment. The volumes used to generate the left ventricular filling curves were always on the flat part of the balloon's filling curve.

**Experimental Protocol**

**Control period.** Before beginning each experiment, the heart was perfused for 30 minutes of 30–38 ml/min coronary flow rate to achieve an LV systolic pressure of 80–110 mm Hg at an LV diastolic pressure of 5–10 mm Hg. The same coronary flow and heart rate were maintained through each experimental protocol. Hearts that could not achieve this level of stable function for at least 20 minutes were discarded (approximately 5% of preparations). After the 30-minute initial control period, the balloon volume resulting in an LV end-diastolic pressure of 5–10 mm Hg, was precisely measured. This volume became the control LV volume in each heart for the duration of the experiment.

**Measurement of LV diastolic chamber distensibility (DCD).** Ideally, LV DCD should be evaluated from a plot of the relation between diastolic pressure and volume over a wide physiologic range. In the isovolumic heart preparation used in these experiments, this could be accomplished by performing an
LV filling curve, that is, by increasing LV balloon volume by 0.2 ml increments and measuring the corresponding end-diastolic pressure, beginning with a completely collapsed (zero volume) balloon and increasing the balloon volume until end-diastolic pressure was between 30 and 35 mm Hg. To avoid any contribution of hysteresis or stress–relaxation phenomena to observed shifts in the pressure–volume relationship, LV diastolic pressure was always measured at least 7–10 seconds after each addition of volume to the LV balloon and only during successive inflation and not deflation of the balloon. Filling curves were initially obtained in duplicate to establish reproducibility. During rapid interventions when complete LV filling curves could not be measured, an abbreviated filling curve was obtained by emptying the LV balloon, then adding two measured increments of volume, sufficient to raise the LVEDP to 30–35 mm Hg. This procedure could be performed within 20 seconds. Short lengths of stiff, translucent polyethylene tubing, clear plastic stopcocks, and glass syringes were used to minimize the possibility of damping by air bubbles and to facilitate rapid, complete emptying of the LV balloon. At a recording speed of 100 mm/sec, the recorded LV diastolic pressure tracing at a heart rate of 180/min exhibited a flat diastolic plateau phase (Figure 5).

**Effect of Global Ischemia on Diastolic Chamber Distensibility.** Following determination of control LV filling curves, the LV balloon volume was returned to the control volume. Coronary flow was decreased to zero. After 2 minutes and 30 minutes of ischemia, complete filling curves were obtained.

**Effect of Hypoxemia on Diastolic Chamber Distensibility.** After control filling curves, LV balloon volume was returned to the control value. The coronary perfusion was switched to Krebs-Henseleit buffer that had the same composition as the control perfusate, but was equilibrated with a 5% CO2–95% N2 gas mixture, which resulted in a PO2 of 10–20 mm Hg. After 2 minutes of hypoxemia, a complete filling curve was obtained.

**Effect of Ischemia Superimposed on Hypoxemia on Diastolic Chamber Distensibility.** Following determination of control filling curves LV balloon volume was returned to the control value and coronary flow was turned off. Filling curves were obtained after 5 seconds and 2 minutes of global ischemia. Hearts were reperfused with oxygenated buffer for 10 minutes and filling curves were repeated. The hearts then were made hypoxic for 2 minutes and filling curves were performed. The LV balloon volume was then returned to the control volume and coronary flow was turned off, to superimpose the ischemic state on the hypoxic myocardium. An abbreviated LV filling curve was obtained after 5 seconds and a complete filling curve after 2 minutes to assess the effect of global ischemia superimposed on hypoxemia.

**Effect of a Single Stretch on Ischemia Contracture.** After 30 minutes of ischemia, all hearts manifested a decrease in DCD (increase in LVEDP). The LV balloon was rapidly inflated to impose a single rapid stretch on the myocardium. The stretch volume was between 0.8 and 1.0 ml, sufficient to transiently raise pressure in the asystolic ventricle to 130–150 mm Hg. The balloon was then emptied, quickly refilled to the original LV control volume, and LVEDP recorded.

**Effect of Single Stretch on Early Hypoxemic Contracture.** After a control period of oxygenated coronary perfusion with the LV balloon volume at the control value, hearts were made hypoxic at the same coronary flow rate for 2 or 3 minutes. All hearts manifested a decrease in DCD (increase in LVEDP). A single rapid stretch was performed as described above.

**Measurement of Intracellular pH by Phosphorous-31 NMR**

Isolated perfused rat heart. To study the relation between intracellular pH (pH_i) and changes in DCD, we used isolated rat hearts that were small enough to fit into the tube of our nuclear magnetic resonance spectrometer.

The same procedures used to prepare the isolated perfused rabbit heart (described above) were applied to Sprague-Dawley rats weighing between 400 and 500 g with the following modifications. The rats were anesthetized with 1 cc of intraperitoneal pentobarbital. The thorax and pericardium were opened, the heart resected and immediately cooled in iced Krebs buffer. The aorta was isolated and tied to an aortic cannula delivering warmed, oxygenated, phosphate-free Krebs by a constant flow pump. A left ventricular apical drain was inserted. Intra-left ventricular pressure was monitored by the balloon-in-left ventricle method described above. The pulmonary artery was transected to facilitate venous drainage. The hearts were not paced. The preparation was suspended in a 20 mm (outer diameter) NMR tube and the venous effluent was allowed to bathe the heart.

The perfusate consisted of the same modified Krebs-Henseleit buffer described above with the following differences: No potassium monophosphate was used (to insure that all phosphorous NMR signals were of intracellular origin); the concentration of calcium chloride, EDTA, and dextrose were slightly different at 1.75, 0.5, and 11 mM, respectively. The aortic and left ventricular cannulae and a suction tube, which maintained a constant fluid level above the heart, were attached to long extension tubes inside a warmed water jacket. This "umbilical cord" was necessary to permit placement of the isolated heart inside the magnet.

All hearts underwent an initial 30-minute control period of oxygenated perfusion at a coronary flow rate of 18–22 ml/min to provide an aortic (coronary perfusion) pressure of 80 mm Hg. Coronary flow rate was kept constant throughout each experiment. Left ventricular balloon volume was adjusted to produce a left ventricular end-diastolic pressure of 5–10 mm Hg. After at least 20 minutes of stable performance (left ventricular developed pressure ≥70 mm Hg at left ventricular end-diastolic pressure ≤10 mm Hg and aortic pressure of 80 mm Hg), left ventricular balloon...
Results

Isolated Rabbit Heart Studies

Reproducibility of pressure-volume curves. Pressure-volume (P-V) curves were constructed in duplicate during oxygenated control perfusion. Data representing 9 sets of duplicate curves from 5 hearts is shown in Figure 2. The first P-V curve was virtually superimposable on that obtained immediately afterwards. At an LVEDP of 15 mm Hg, the mean difference in LV volume was 0.01 ml ± 0.01 ml, p = NS by paired t test. The maximum difference was 0.06 ml.

Effect of Global Ischemia and Hypoxemia on Diastolic Chamber Distensibility. The upper panel of Figure 3 shows the effect of early (2 minutes) and late (30 minutes) ischemia on diastolic chamber distensibility (DCD). Left ventricular end-diastolic pressure (LVEDP) is plotted against left ventricular balloon volume and the position of the resultant curve represents LV distensibility. Compared with the baseline curve, 2 minutes of global ischemia resulted in a shift of the pressure-volume (P-V) curve to the right, indicating an increase in DCD. After 30 minutes of global ischemia, the curve was shifted to the left, indicating a decrease in DCD, or ischemic contracture.

The lower panel of Figure 3 compares the effects of 2 minutes of hypoxemia and 2 minutes of ischemia on DCD. In these experiments the hearts were reoxygenated length of fluid-filled tubing between the heart and pressure transducer, rapid, reproducible filling curves could not be obtained. For this reason, changes in left ventricular end-diastolic pressure at a constant left ventricular balloon volume were used to assess changes in chamber distensibility. After the control period, hearts were subjected to two minutes of ischemia followed by sequential periods of reperfusion, hypoxemia, and ischemia as described above for the isolated rabbit hearts; during these periods 31P-NMR spectra were recorded for determination of pH, A total of 6 rat hearts were used for these experiments.

31P-NMR spectroscopy. A Nicolet 1180 computer was used in the pulsed Fourier transform mode to generate 31P-NMR spectra from a Nicolet NT-360 spectrometer operating at 145.75 MHz. To homogenize the magnetic field, we used an 18-channel Oxford Instrument Shim Supply and maximized the signal intensity, thus minimizing the signal line width from sodium in the heart and its perfusate.

NMR spectra were recorded for determination of pIL. A total of 6 rat hearts were used for these experiments. 31P NMR spectra were obtained during the last 1.8-1.9 minute of each intervention. Intracellular pH (pH) was derived from the relationship between pH and the separation between inorganic phosphate and creatine phosphate resonance peaks.

Pressure–Volume Curve Analysis

Pressure–volume (P–V) curve data points between 5 and 30 mm Hg for each heart were fit to an exponential equation \( P = Ae^{bV} \), where \( P \) is diastolic pressure in mm Hg, \( V \) is the left ventricular chamber volume in ml, \( A \) and \( b \) are constants indicating position and slope of the curve, respectively. The A and b coefficients were determined by least-square linear regression analysis of the linear form of the equation \( \ln P = \ln A + bV \). Zero volume was defined as that volume that initially produced a left ventricular end-diastolic pressure of 5 mm Hg at the end of the equilibration period. The b coefficient is presented as an arithmetic mean ± the standard error of the mean (SEM). The A coefficient is presented as a geometric mean with the asymmetric range of values defined from the mean of lnA ± SEM.

Statistical Analysis

All data are expressed as the mean ± the standard error of the mean (SEM) except where otherwise noted. For comparisons over time, each heart was used as its own control and measurements after various interventions were compared by Student's paired t test. Groups of data from experiments involving multiple sequential interventions were evaluated by a one-way analysis of variance. Differences between two groups were tested and considered significant only when the F value from the analysis of variance indicated a significant difference among groups at the level of \( p < 0.05 \). When more than four comparisons were made, the \( p \) value was corrected by the Bonferroni method.
Early vs. Late Ischemia

FIGURE 3. The effect of ischemia and hypoxemia on diastolic chamber distensibility (DCD). Diastolic pressure-volume (P-V) curves obtained after 2 minutes of ischemia, 30 minutes of ischemia, and 2 minutes of hypoxemia are presented. Compared with the control curve (●), 2 minutes of ischemia shifted the P-V curve to the right (▲, upper and lower panels), indicating a decrease in DCD. After 30 minutes of ischemia, the P-V curve shifted to the left (●, upper panel) indicating a decrease in DCD. In contrast to 2 minutes of ischemia, 2 minutes of hypoxemia resulted in a shift of the P-V curve to the left (●, lower panel), comparable to the shift seen after 30 minutes of ischemia. Six hearts were assessed during 30 minutes of ischemia (upper panel). Ten different hearts were used for the 2 minute runs of ischemia or hypoxemia (lower panel); in these experiments the hearts were reoxygenated after each 2 minute ischemic or hypoxic run.

Table 1. Effects of Ischemia and Hypoxemia on Diastolic Chamber Distensibility: P-V Curve Coefficients

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia 2 min</th>
<th>Ischemia 30 min</th>
</tr>
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<tbody>
<tr>
<td><strong>A coefficient</strong></td>
<td>5.3</td>
<td>3.4**</td>
<td>42.1***</td>
</tr>
<tr>
<td></td>
<td>(5.2–5.4)</td>
<td>(2.8–3.7)</td>
<td>(34.5–51.4)</td>
</tr>
<tr>
<td><strong>b coefficient</strong></td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2*</td>
<td>4.2 ± 0.8**</td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia 2 min</th>
<th>Hypoxemia 2 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A coefficient</strong></td>
<td>5.6</td>
<td>1.3***</td>
<td>14.3***</td>
</tr>
<tr>
<td></td>
<td>(5.5–5.8)</td>
<td>(1.0–1.6)</td>
<td>(13.5–15.2)</td>
</tr>
<tr>
<td><strong>b coefficient</strong></td>
<td>3.7 ± 0.4</td>
<td>3.5 ± 0.3m</td>
<td>4.0 ± 0.4**</td>
</tr>
</tbody>
</table>

Asterisks (*) indicate significant difference vs control value by paired t test; * = p < 0.05; ** = p < 0.25; *** = p < 0.001, respectively; ns indicates the absence of statistically significant difference from the control value.

Numbers in parentheses indicate the range of values for the A coefficient.

The coefficients derived from fitting these P–V curves to an exponential equation are presented in Table 1. The A coefficient indicates the position of the curve. Because “zero volume” was defined as that volume which initially produces an LVEDP of 5 mm Hg during the control perfusion, the A coefficient is an approximation of the pressure at that volume which initially produced an LVEDP of 5 mm Hg. Thus, compared with a control value of 5.3 and 5.6 in the two groups of experiments, the lower A value of the P–V curves after 2 minutes of ischemia, 3.4 and 1.3, respectively, indicate a shift in the curve downward and to the right, i.e., increased distensibility. The higher A values of the curves after 30 minutes of ischemia (42.1), and 2 minutes of hypoxemia (14.3), indicate a shift upward and to the left, i.e., decreased distensibility.

There was an increase in the b coefficient, consistent with an increase in the slope of the P–V curve after 30 minutes of ischemia (3.3 ± 0.2 during control vs. 4.2 ± 0.8 after 30 minutes of ischemia, p < 0.05), but not after 2 minutes of hypoxemia (3.7 ± 0.3 during control vs. 4.0 ± 0.4 after 2 minutes of hypoxemia, p = NS). Results after 2 minutes of ischemia were variable: In one group of rabbits (Figure 3 and Table 1, upper panels), there was a small decrease in the b coefficient (3.3 ± 0.2 during control vs. 3.2 ± 0.2 after two minutes ischemia, p < 0.05). However, in a comparable, but larger group of rabbits represented in Figure 3 and Table 1, lower panels, the decrease in the b coefficient of the P–V curve after two minutes of ischemia did not reach statistical significance (3.7 ± 0.4 during control vs. 3.5 ± 0.3 after 2 minutes ischemia, p = NS).

Effect of Ischemia Superimposed on Hypoxemia on Diastolic Chamber Distensibility. Figure 4 and...
Effects on the diastolic P–V curve of ischemia superimposed on hypoxemia and the reversal of hypoxic contracture by acute ischemia. Each of 10 hearts was subjected to seven successive interventions in each of seven hearts. 1) Initial control (○). 2) After 5 seconds of global ischemia, the P–V curve shifted to the right (∆) indicating the increase in DCD attributable to loss of the "erectile effect." 3) After a total of 2 minutes global ischemia, there was an additional shift of the P–V curve to the right (▲). 4) Reperfusion for 10 minutes returned the P–V curve to its original control position (●). 5) After 2 minutes of hypoxemia, the P–V curve shifted to the left (■) indicating a decrease in DCD (hypoxic contracture). 6) Global ischemia was then superimposed on hypoxemia by turning off coronary flow. After 5 seconds, the P–V curve shifted to the right (◇) indicating an increase in DCD. This shift was similar to that seen after 5 seconds of ischemia in the non-hypoxic heart. 7) After a total of 2 minutes of ischemia superimposed on hypoxemia, there was a marked additional shift of the P–V curve to the right (◇) such that DCD after 2 minutes of ischemia superimposed on hypoxemia was almost the same as DCD after 2 minutes ischemia alone (▲), i.e., hypoxic contracture was reversed by 2 minutes of global ischemia. See Table 2 for P–V curve data.

Table 2 show the effects on the diastolic P–V curve of ischemia superimposed on hypoxemia and the reversal of hypoxic contracture by acute ischemia. Each of 10 hearts was subjected to seven successive interventions and P–V curves were obtained after each intervention. The position of the P–V curve is expressed as the A coefficient derived from fitting the P–V curves to an exponential equation (Table 2).

After the usual control period, hearts were made globally ischemic. After 5 seconds of ischemia, the diastolic P–V curve was shifted to the right, A = 5.6 during the control period vs. A = 2.4 after 5 seconds of ischemia, p < 0.03, indicating an increase in DCD. After an additional 115 seconds of ischemia, there was a small additional shift to the right, A = 1.3, p = NS compared with 5 seconds of ischemia. Left ventricular systolic pressure fell from 95.6 ± 9.9 mm Hg during the control period to 70.0 ± 1.0 mm Hg after 5 seconds of ischemia; after a total of 2 minutes of ischemia, left ventricular systolic pressure fell to 6.1 ± 1.3 mm Hg (see Figure 5). The hearts were then reperfused at the control flow rate for 10 minutes with return of the diastolic P–V curve to its original position, A = 5.1 after reperfusion, compared with control, A = 5.6, p = NS, verifying recovery to baseline diastolic chamber distensibility. Left ventricular systolic pressure returned to 84 ± 2.4 mm Hg. The hearts were then made hypoxic for 2 minutes, which resulted in a decrease in left ventricular systolic pressure to 44.9 ± 2.4 mm Hg. As described earlier, the diastolic P–V curve shifted to the left, A = 14.3, p < 0.006, indicating a decrease in DCD or the development of hypoxic contracture. At this time, the hearts were made globally ischemic, i.e., coronary flow was turned off to superimpose ischemia on the hypoxic state. After 5 seconds, the diastolic P–V curve shifted to the right, A = 4.1, p < 0.006 compared with A = 14.3 after 2 minutes of hypoxemia, indicating an increase in DCD. However, in contrast to the initial ischemic period in which the diastolic P–V curve shifted very little over the next 115 seconds of ischemia, the additional 115 seconds of ischemia in the hypoxic hearts resulted in a much greater shift of the diastolic P–V curve to the right, such that the position of the curve after two minutes of ischemia superimposed on hypoxemia was the same as that after 2 minutes of ischemia alone, A = 1.5 after ischemia superimposed on hypoxemia vs. 1.3 after 2 minutes ischemia alone, p = NS. Thus, these results demonstrated that acute ischemia completely reversed hypoxic contracture (Figure 4). There were no significant changes in the b coefficients (slopes) of the P–V curves in this experiment.

To determine whether repetitive 2-minute periods of ischemia would produce reproducible shifts of the P–V curve, a separate series of 5 hearts was subjected to: a) 2 minutes of ischemia, b) 10 minutes of reperfusion, and c) a second 2-minute period of ischemia (Figure 6). The coefficients of the exponential curves fit to the data are presented in Table 3. There was no difference in the A coefficient of the control versus the reperfusion P–V curves, or of the first versus the second ischemic P–V curves. Thus, changes in DCD after two repetitive ischemic periods were similar in this series of hearts.

Effect of a quick stretch on early hypoxemia and late ischemic contracture. After the usual control period of oxygenated perfusion, during which the LV balloon volume was adjusted to produce an LVEDP of 10 mm Hg, 6 hearts were made ischemic for a total of 30 minutes (Figures 7A and C). The hearts became asystolic and LV pressure increased to 36.8 ± 2.8 mm Hg, as ischemic contracture developed. The intra-LV balloon was then rapidly inflated, emptied, and reinfated to the original control volume. Following this quick stretch, LV pressure at the control LV balloon volume decreased to 5.3 ± 0.8 mm Hg (p < 0.001).

In 6 different hearts, the effect of a quick stretch on hypoxic contracture was examined (Figures 7B and C). After the usual 30-minute control period of oxygenated perfusion, during which the left ventricular balloon volume was adjusted to produce an LVEDP of 10 mm Hg, the hearts were switched to hypoxic perfusate for 2–3 minutes at the same control balloon volume. During hypoxemia, LVEDP rose to 26.0 ±
Table 2. Effects of Ischemia Superimposed on Hypoxemia: P-V Curve Coefficients

<table>
<thead>
<tr>
<th>Consecutive interventions</th>
<th>A</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>5.6</td>
<td>3.7±0.4</td>
</tr>
<tr>
<td></td>
<td>(5.5–5.7)</td>
<td></td>
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<tr>
<td>2. Ischemia 5 sec</td>
<td>2.4*</td>
<td>3.4±0.3</td>
</tr>
<tr>
<td></td>
<td>(2.1–2.7)</td>
<td></td>
</tr>
<tr>
<td>3. Ischemia 2 min</td>
<td>1.3(NS)</td>
<td>3.5±0.3</td>
</tr>
<tr>
<td></td>
<td>(1.0–1.6)</td>
<td></td>
</tr>
<tr>
<td>4. Recovery 10 min</td>
<td>5.1(NS vs control)</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td></td>
<td>(4.4–5.9)</td>
<td></td>
</tr>
<tr>
<td>5. Hypoxemia 2 min</td>
<td>14.3**</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td></td>
<td>(13.5–15.2)</td>
<td></td>
</tr>
<tr>
<td>6. Posthypoxic ischemia 5 sec</td>
<td>4.1**</td>
<td>3.9±0.3</td>
</tr>
<tr>
<td></td>
<td>(3.3–5.2)</td>
<td></td>
</tr>
<tr>
<td>7. Posthypoxic ischemia 2 min</td>
<td>1.5(NS vs ischemia 2 min)</td>
<td>3.6±0.4</td>
</tr>
</tbody>
</table>

Asterisks (*) or (NS) indicate significant or insignificant differences, respectively, from preceding or indicated value by paired t test: * p < 0.03; ** p < 0.006.

Numbers in parentheses indicate the range of values for the A coefficient.

3.3 mm Hg. A quick stretch, as described above, was then performed. Following the quick stretch maneuver, LVEDP at control volume rapidly returned to the same level seen during hypoxemia, prior to the quick stretch, 25.5 ± 4.2 mm Hg, p = NS, i.e., hypoxemic contracture was not altered by a quick stretch.

Isolated Rat Heart Studies

INTRACELLULAR pH DURING HYPOXEMIA AND ISCHEMIA IN THE ISOLATED PERFUSED RAT HEART. Table 4 shows intracellular pH (pHi) assessed by 31P-NMR in 6 isolated perfused rat hearts subjected to the same sequence of hypoxemia and ischemia as described above. Associated changes in DCD were assessed by measuring changes in LVEDP at a constant LV balloon volume that was adjusted during the control period to produce an initial LVEDP of 20.3 ± 0.6 mm Hg. Control intracellular pH was 7.01 ± 0.02. After 2 minutes of global ischemia, pHi fell to 6.87 ± 0.03 (p < 0.01) and LVEDP fell to 10.7 ± 2.4 mm Hg (p < 0.025). Both pH and LVEDP returned to baseline after a 10-minute reperfusion period; (pH = 7.01 ± 0.01 and LVEDP = 21.7 ± 1.1 mm Hg, p = NS for both, compared to control values). Hypoxemia for 4 minutes resulted in a rise in LVEDP to 47.7 ± 2.9 mm Hg (p < 0.001). A longer period of hypoxemia was used in these experiments than in the rabbit heart studies because of the time required for hypoxic perfusate to traverse the long "umbilical cord" to the perfused heart inside the magnet. There was no significant change in pHi, 6.99 ± 0.01, p = NS. When global ischemia was superimposed on hypoxemia for two minutes, pHi fell to 6.88 ± 0.02 and LVEDP fell to 10.3 ± 3.4 mm Hg, (p = NS for both compared with initial 2-minute ischemic period). Thus, the decrease in LVEDP observed after the initial 2-minute ischemic period was associated with a significant decrease in pHi. Hypoxic contracture developed in the absence of any significant change in pHi, when a brief period of ischemia was superimposed on hypoxemia, LVEDP and pHi both decreased to the same levels observed after the initial ischemic period.

Figure 5. Left ventricular pressure tracing from an isolated isovolumic rabbit heart during 2 minutes of global ischemia: Segments of the left ventricular pressure tracing of a typical experiment are shown. Left ventricular end-diastolic pressure begins to decline at the onset of ischemia and the majority of the decline (from 12 to 2 mm Hg in this example) is apparent after 5 seconds.
Role of Coronary Vascular Collapse on DCD

During global ischemia, coronary flow ceases, wall thickness diminishes and the LV becomes more distensible. In contrast, during hypoxemia, coronary flow continues, coronary vascular collapse does not occur, and the "coronary hydraulic lattice" effect to maintain diastolic LV wall stiffness is maintained. This factor may be largely responsible for the opposite initial changes in DCD after 2 minutes of ischemia compared with 2 minutes of hypoxemia. Coronary vascular collapse may also contribute to the observed reversal of hypoxemic contracture by global ischemia.

However, changes in coronary vascular turgor do not completely explain the changes in DCD that were observed when ischemia immediately followed hypoxemia. Vogel et al showed that early hypoxemia, in a constant coronary flow preparation, was associated with a decrease in coronary perfusion pressure (indicating a fall in coronary resistance, compatible with hypoxemia induced vasodilation) but an increase in wall thickness. The increase in wall thickness and associated decrease in chamber distensibility could not be attributed solely to coronary vasodilation since administration of adenosine, which induced a degree of vasodilation equal to that observed during hypoxemia, caused no change in distensibility or wall thickness when coronary flow was held constant. These observations suggested that another factor such as persistent actin–myosin cross-bridge cycling, i.e., incomplete relaxation, may contribute to the decrease in DCD during hypoxemia.

Since the decrease in DCD observed during early hypoxemia cannot be attributed solely to a coronary vascular "erectile" effect, collapse of the vascular bed on cessation of coronary flow, when ischemia immediately followed, i.e., was superimposed on hypoxemia, would be expected to result in only a partial reversal of the early hypoxemic contracture, as we observed after 5 seconds of superimposition of ischemia in the hypoxic heart. However, the further shift of the diastolic P–V curve to the right after 2 minutes of superimposition of ischemia, which completely reversed the hypoxemic contracture (Figure 4), requires additional explanation.

Role of Intracellular Acidosis

Differences in the degree of intracellular acidosis may be another factor affecting the early opposite changes in DCD in response to hypoxemia and ische-

**Table 3. Reproducibility of Changes in Diastolic Chamber Distensibility After Successive Repeated 2-Minute Periods of Ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia #1</th>
<th>Reperfusion</th>
<th>Ischemia #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.16</td>
<td>2.69</td>
<td>5.53*</td>
<td>2.20†</td>
</tr>
<tr>
<td></td>
<td>(5.05–5.26)</td>
<td>(2.39–3.03)</td>
<td>(5.47–5.58)</td>
<td>(1.72–2.83)</td>
</tr>
<tr>
<td>b</td>
<td>3.4 ± 0.32</td>
<td>3.29 ± 0.37</td>
<td>3.1 ± 0.17</td>
<td>3.1 ± 0.37</td>
</tr>
</tbody>
</table>

*Not significantly different from control.
†Not significantly different from ischemia #1.
Table 4. Changes in Intracellular pH (pH$_i$) and LVEDP During Ischemia, Hypoxemia, and Ischemia Superimposed on Hypoxemia

<table>
<thead>
<tr>
<th>Consecutive intervention</th>
<th>LVEDP</th>
<th>pH$_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.3±0.6</td>
<td>7.03±0.02</td>
</tr>
<tr>
<td>Ischemia 2 min.</td>
<td>10.7±2.4*</td>
<td>6.87±0.03**</td>
</tr>
<tr>
<td>Recovery 10 min.</td>
<td>21.7±1.1</td>
<td>7.01±0.01</td>
</tr>
<tr>
<td>Hypoxemia 4 min.</td>
<td>47.7±2.9****</td>
<td>6.99±0.01**</td>
</tr>
<tr>
<td>Ischemia, 2 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>superimposed on hypoxemia, 4 min.</td>
<td>10.3±3.4***</td>
<td>6.88±0.02**</td>
</tr>
</tbody>
</table>

Asterisks indicate significant differences compared with preceding value by paired *t* test; *p* < 0.025, **p* < 0.01; ***p* < 0.005; ****p* < 0.001.

The effect of acidosis on myocardial contractility and on intracellular Ca$^{2+}$ has been studied extensively (reviewed by Tsien, Poole-Wilson, Mandel et al). Changes in intracellular pH have been shown to alter the sensitivity of the contractile proteins to Ca$^{2+}$, i.e., a decrease in pH shifts the pCa-force relation such that tension development at any given Ca$^{2+}$-level is markedly decreased. Using the aequorin technique, Allen et al have shown that when isolated papillary muscles are made acidotic by perfusion with high Pco$_2$ buffer, intracellular calcium increases at the same time active tension is decreasing. This suggests that the inhibitory effect of H$^+$ on myofibrillar Ca$^{2+}$ sensitivity is sufficiently potent to offset the potentially positive inotropic effect of an increase in cytosolic Ca$^{2+}$.

These observations of the effect of pH on Ca$^{2+}$-activated tension development may also be relevant to diastolic function in view of recent evidence that cytosolic Ca$^{2+}$ persists throughout diastole at levels sufficient to account for some resting myofilament activation. Thus, the acidosis that occurs after 2 minutes of ischemia may contribute to the observed increase in DCD, i.e., there may be some decrease in residual...
diastolic "tone" during ischemia because of a pH-mediated decrease in myofilament sensitivity to Ca++.

**Role of Sarcoplasmic Reticular Ca++ Resequestration**

The decrease in DCD during hypoxemia is generally thought to be related to an increase in diastolic Ca++ availability resulting from impaired resequestration by the sarcoplasmic reticulum.4,21,32,51 Thus, the effect of intracellular acidosis to decrease Ca++-activated tension may contribute to the reversal of hypoxic contracture by superimposed ischemia. Further support for this hypothesis comes from studies of isolated papillary muscle preparations,52-54 in which pretreatment with acidic buffer was shown to prevent or delay the development of hypoxic contracture. Bing et al55 suggested that the mechanism was related to the impairment of contractile function that occurred when the muscles were bathed with acidic buffer, the slower rate of ATP depletion leading to a slower rate of contracture development. However, Greene and Weisfeld32 demonstrated the same protective effect of acidosis against hypoxic contracture in the absence of contractile activity, supporting the concept of a direct effect of H+ on Ca++-activated resting or diastolic tension during hypoxemia.

Contractile force was affected differently by ischemia and hypoxemia: Systolic pressure decreased more during 2 minutes of ischemia (95.6 ± 3.2 to 6.1 ± 1.3 mm Hg) than during 2 minutes of hypoxia (84.0 ± 2.4 to 44.9 ± 2.4 mm Hg). This can be attributed to two factors, the greater degree of intracellular acidosis during ischemia, which inhibits Ca++-activated tension, and a loss of sarcomere stretch as pressure and volume decrease in the coronary vasculature.55,56 Less vigorous contractions during ischemia compared with hypoxemia might result in a lesser contribution of the viscous element in muscle to chamber stiffness, thus contributing to the greater DCD during ischemia compared with hypoxemia. However, the time course of the decline in DCD after the onset of ischemia suggests this is not an important factor in this model: The major decrease in DCD, i.e., fall in LV volume (Figure 5) or rightward shift in the left ventricular pressure–volume curve (Figure 4, Table 2) had occurred by 5 seconds of ischemia at which time left ventricular systolic pressure had decreased only slightly (95.9 ± 3.2 mm Hg to 70.0 ± 2.5 mm Hg). During the subsequent 115 seconds of ischemia, DCD changed minimally despite a major decline in left ventricular systolic pressure (70.0 ± 2.5 to 6.1 ± 1.3 mm Hg). Thus, the decrease in DCD associated with cessation of coronary flow occurs before there is a substantial loss of contractile force. We have demonstrated this phenomenon in two previous studies.57

In summary: The opposite acute changes in DCD with early hypoxemia and early global ischemia can be explained by considering which of the following elements are present: 1) a pressurized coronary vasculature, which decreases DCD by the "garden hose" effect, 2) tissue hypoxia, which may result in an increase in cytosolic calcium level due to impairment of sarcoplasmic reticular resequestration of Ca+++, and 3) tissue acidosis, which decreases Ca+++-activated tension development. With hypoxemia, (1) and (2) are present, and (3) is absent or minimal; the net effect is a rapid decrease in DCD or hypoxic contracture. With global ischemia, the coronary vasculature is acutely decompressed, resulting in an immediate increase in DCD due to the loss of the "erectic effect." In addition, rapid development of acidosis may decrease resting diastolic Ca+++-activated tone, outweighing the effects of any increase in cytosolic calcium availability. The complete reversal of hypoxic contracture by superimposed ischemia can reasonably be attributed to the same two factors: Coronary vascular collapse with loss of the "erectic effect" and intracellular acidosis, which, by decreasing the sensitivity of the myofilament to Ca+++, reverses the effects of increased diastolic cytosolic Ca++ levels associated with tissue hypoxia.

In contrast to the increase in DCD after 2 minutes of ischemia, the decrease in DCD observed after 30 minutes of ischemia, i.e., late ischemic contracture, is best explained by rigor bond formation due to a progressive fall in cytosolic ATP levels with sustained ischemia.17 Evidence for rigor bond formation as the cause of late ischemic contracture comes from the quick-stretch experiment (Figure 7), wherein a quick stretch completely reversed the sustained increase in ventricular pressure that had developed after 30 minutes of ischemia. The absence of tension redevelopment after a quick stretch is characteristic of rigor bonds. In contrast, the rapid redevelopment of tension after a quick stretch in hearts with early hypoxic contracture suggests that persistent Ca+++-activated tension is present.24-26

The mechanism of the apparent reversal of rigor bond tension by a quick stretch is unclear. Previous experiments from this laboratory have shown that the reversal of ischemic contracture after a quick stretch persists for at least 5 minutes and redevelopment of contracture can be prevented by applying repetitive stretches every 5 minutes.12 Schuchlieb et al18 showed that repetitive balloon expansion applied to severely ischemic hearts after the onset of severe ischemic contracture decreased contracture pressure, but caused an increase in ultrastructural damage. However, Apstein and Ogilby12 showed that repetitive balloon expansion during a comparable period of ischemia, and applied prior to the onset of contracture, prevented contracture and resulted in better recovery of function after reperfusion, suggesting that the stretching did not cause significant damage. Whether a single quick stretch after 30 minutes of ischemia mechanically ruptures the rigor bond or exerts some other effect is not clear from these experiments. However, the fact that the effect of the quick stretch maneuver is clearly different in hearts with ischemic contracture compared to hearts with early hypoxic contracture provides strong evidence that different mechanisms cause these two types of contracture.

**Clinical correlations.** The changes in DCD that
we have observed with acute global hypoxemia and ischemia in the isolated perfused heart are probably related to alterations in DCD observed in patients with ischemic heart disease.

The decrease in diastolic distensibility, which we have observed after two minutes of hypoxemia in the isolated heart, is strikingly similar to the time course and magnitude of the shift in the diastolic pressure-volume curve seen after pacing induced angina in patients with coronary artery disease or pacing-induced ischemia in dogs with coronary arterial stenoses. In both cases there is persistent coronary flow to the hypoxic region so that coronary "turgor" is maintained and tissue acidosis is minimized. The mechanism for decreased distensibility in models of pacing-induced ischemia is thought to be the same as that causing early hypoxemic contracture, i.e., an increase in intracellular calcium availability secondary to impaired resequestration of Ca++ by the sarcoplasmic reticulum resulting in incomplete relaxation.

In contrast to global hypoxemia and the pacing-induced ischemia model, the acute increase in DCD observed after acute global ischemia in the isolated heart is comparable to the increase in diastolic segment length or diastolic chamber distensibility observed after an acute coronary ligation in intact animals. Global ischemia in the isolated heart and a complete coronary occlusion share the common features of an immediate collapse of the coronary vasculature in the ischemic region (loss of "turgor" effect) and an acute marked tissue acidosis, both of which would be expected to initially increase DCD as has been observed.

The decrease in DCD observed after sustained global ischemia in isolated hearts may be similar to changes that have been observed after prolonged ischemia during cardiac surgery. In its most florid form, this decrease in distensibility was recognized as the "stone heart syndrome." This is now rarely seen since better methods of intraoperative myocardial preservation have been used, but significant decreases in DCD are still observed in the immediate postoperative period. Although the mechanism responsible for the decrease in DCD in postoperative patients has not been defined, the time-course is similar to that of "late" ischemic contracture observed in isolated animal hearts. Because of its sensitivity to a quick-stretch, rigor bond formation is probably responsible, at least in part, for this phenomenon.

In conclusion: We have demonstrated that in isolated perfused hearts, the decrease in distensibility that occurs after 30 minutes of global ischemia, is caused by a different mechanism than the decrease in distensibility that develops early in the course of hypoxemia. We have also attempted to define the factors responsible for the initially opposite changes in distensibility seen after the brief periods of global ischemia and hypoxemia.

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Key Words • hypoxemia • ischemia • LV chamber distensibility • isolated rabbit heart • contracture • intracellular pH • diastolic properties
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