Comparative Effects of Hypoxia and Ischemia in the Isolated, Blood-Perfused Dog Heart: Evaluation of Left Ventricular Diastolic Chamber Distensibility and Wall Thickness


To compare the effects of hypoxia and ischemia on left ventricular (LV) diastolic function, we studied 17 isolated, isovolumic dog hearts by measuring LV diastolic chamber distensibility (LV end diastolic pressure at constant volume), wall thickness, and myocardial pH in response to hypoxia at constant coronary flow or pressure versus global ischemia (zero coronary blood flow). Hypoxic perfusates consisted of methemoglobin-containing red blood cells suspended in lactated Ringer’s solution. Brief cross-clamping of the coronary perfusion line was used to assess the contribution of coronary turgor to chamber distensibility and wall thickness. With hypoxia, left ventricles showed a significant early (5 minutes) decrease in diastolic distensibility and an increase in wall thickness, at either constant coronary perfusion pressure or flow. The increase in wall thickness was independent of hypoxia-induced changes in coronary turgor. In contrast, global ischemia produced an early increase in LV diastolic chamber distensibility and a decrease in wall thickness. When global ischemia was continued beyond 60 minutes, a decrease in LV chamber distensibility developed. This diastolic contracture was not associated with an increase in LV wall thickness. Myocardial pH decreased slightly during 15 minutes of hypoxia and markedly with 15 minutes of global ischemia. Thus, LV diastolic chamber distensibility decreased during 15 minutes of hypoxia, while an increase in distensibility was seen during global ischemia of similar duration. During hypoxia, these changes were associated with increased LV wall thickness, at either constant coronary perfusion pressure or constant coronary flow. Prolonged ischemia led to diastolic contracture without an increase in wall thickness. The differences in wall thickness with hypoxia versus ischemic contracture suggest differing mechanisms for these two types of diastolic dysfunction. (Circulation Research 1989;64:121-128)

Left ventricular (LV) diastolic dysfunction is now a well-documented explanation for the clinical and hemodynamic observations of increased filling pressures during angina.1-9 In experimental preparations, alterations in both myocardial relaxation and left ventricular diastolic distensibility are seen during demand ischemia10-12 and hypoxia.13-15 However, the pathophysiological mechanisms underlying these acute changes are controversial and appear to involve a complex interplay between mechanical (e.g., geometric, pericardial, or coronary turgor) and intracellular (e.g., prolonged and persistent contractile element interaction) factors. In fact, ischemia and hypoxia do not have uniformly concordant effects on these potential mechanisms in an experimental setting, particularly in the isolated heart model.16 While hypoxic crystalloid perfusion of an isolated heart is associated with slowed relaxation, decreased LV chamber distensibility, and increased coronary turgor due to vasodilation,13,14 ischemia produced by a reduction in coronary blood flow may have quite different effects. Acute low-flow ischemia in the absence of increased myocardial oxygen demand decreases coronary turgor and increases diastolic distensibility, with an associated increase in local accumulation of metabolites and hydrogen ion.14,17,18
More prolonged (30–60 minutes) global ischemia causes an essentially irreversible "rigor" state of the myocardium, the cellular mechanism of which is uncertain. It has been suggested that the pathophysiological basis of these reversible and irreversible types of diastolic dysfunction is related to decreased sequestration of calcium by sarcoplasmic reticulum (SR), changes in coronary turgor, changes in myocardial pH, and actin-myosin rigor bond formation. The purpose of the present study is to examine in further detail the relation between acute hypoxia and global ischemia, particularly with regards to changes in left ventricular wall thickness, an important determinant of diastolic function that has received little attention in the aforementioned studies. We performed a series of experiments in a canine isovolumic, isolated heart preparation with simultaneous assessment of LV diastolic distensibility and wall thickness changes in response to acute hypoxia, global ischemia, and changes in coronary turgor. Our results support the concept that there is an inherent mechanistic difference between the diastolic dysfunction of acute hypoxia and prolonged ischemic contracture.

Materials and Methods

Isolated Heart Preparation

Isolated perfused hearts from 17 mongrel dogs were prepared as previously described and shown in Figure 1. Briefly, in each experiment a support dog was anesthetized with chloralose and urethane and placed on mechanical ventilation. Catheters were placed in the femoral artery and vein, and the animal was anticoagulated with heparin (500 units/kg i.v.). The heart from a second dog was prepared by insertion of cannulas into the right ventricle and the carotid and subclavian arteries; the heart was then isolated by ligation of the superior and inferior vena cavae and descending thoracic aorta and was removed from the thorax. Coronary perfusion of the isolated heart was maintained by retrograde perfusion of the coronary arteries, via the carotid cannula, with blood obtained from the femoral artery of the support dog and circulated by a roller pump through a bubble trap, flowmeter, and heat exchanger to maintain blood temperature at 37°C. Coronary venous blood was drained from the right ventricle (in order to minimize ventricular interactive effects) through another flowmeter into the femoral vein of the support dog. A left ventricular drain was inserted through a stab wound in the LV apex to collect Thebesian vein flow, and an intramyocardial thermistor was placed adjacent to it in the apex.

Ventricular fibrillation was then induced in the isolated perfused heart, and the left atrium was opened. Ultrasound crystals were placed across the LV free wall, avoiding papillary muscles, to allow direct measurement of wall thickness. An inflatable latex balloon was placed in the left ventricle through the mitral anulus and secured with sutures in the anulus. A balloon size was chosen in each case such that balloon volume would be greater than LV diastolic volume, and thus pressure increments would reflect LV rather than balloon tension changes. In eight dogs, a hydrogen-ion selective polymer membrane pH electrode (Life-Span 100 TM Ph monitor, Biochem International, Waukesha, Wisconsin) was implanted in the anterior wall subendocardium to permit continuous measurement of intramyocardial pH. The heart was then submerged in a blood bath, and any remaining air was removed from the LV cavity. Following ventricular defibrillation and reinstitution of sinus rhythm, the isolated heart was allowed to equilibrate for 30 minutes at a coronary perfusion pressure of 100 mm Hg and a heart rate of approximately 120 beats/min (maintained by atrial pacing if necessary) at 37°C.

Control Measurements

To evaluate LV systolic and diastolic function, the LV balloon was inflated with saline until a systolic pressure of approximately 100 mm Hg was generated with each contraction. To allow comparisons between hearts of different sizes, this balloon volume (measured in milliliters) was arbitrarily assigned a value of 100%, and subsequent measurements are reported as percentages of this volume. Since left ventricular contractions are isovolumic in...
FIGURE 2. The coronary turgor or erectile effect on left ventricular (LV) diastolic pressure. LV pressure, perfusion pressure, and coronary flow are shown at baseline and during transient crossclamping of the aorta (mark). Coronary perfusion pressure falls rapidly, and coronary flow ceases. LV diastolic pressure decreases 6 mm Hg (the "erectile effect"), to 18 mm Hg (the "residual component").

In this model, changes in LV end-diastolic pressure (EDP) directly reflect changes in diastolic distensibility. We prefer the term diastolic distensibility to the more traditional diastolic compliance. The latter refers strictly to changes in the slope of the pressure-volume relation, while changes in distensibility indicate only that a higher diastolic pressure is needed to fill the ventricle to the same volume.

After the 30-minute equilibration period, we obtained two sets of baseline measurements of systolic and diastolic pressure, as well as LV wall thickness (at 100% volume), coronary blood flow, and myocardial pH, at 25%, 50%, 75%, and 100% of the baseline volume. The coronary perfusion line was then clamped for 30 seconds (at 75% balloon volume) to determine the coronary turgor or erectile contribution to diastolic distensibility. An example of a determination of the turgor, or erectile, contribution to LVEDP is shown in Figure 2, which demonstrates the fall in LVEDP associated with a reduction of the coronary perfusion pressure. At the mark, the perfusion line was clamped, and coronary perfusion pressure fell until coronary flow ceased. This decrease in pressure and flow was associated with a drop in the LV diastolic pressure, in this example, from 24 to 18 mm Hg. We designated 18 mm Hg the residual component of diastolic pressure, as opposed to the erectile (or coronary turgor) component, in this case the 6 mm Hg drop in pressure seen during flow cessation.

Interventions

Repeat control measurements were obtained at least 5 minutes after restoration of coronary blood flow. The perfusion system was then switched to a reservoir containing the perfusate described below, which was perfused either at constant coronary perfusion pressure or at constant coronary flow. Coronary flow and perfusion pressure, as well as LV diastolic pressure-volume curves and wall thickness, were recorded at 5, 10, and 15 minutes of perfusion. After the 10-minute curve, the coronary perfusion line was clamped once again for 30 seconds to determine the erectile contribution to diastolic pressure and wall thickness at 75% volume. After the 15-minute curve, the perfusion system was switched back to oxygenated blood from the support dog. The isolated heart was allowed 30 minutes to recover, and the experimental protocol was then repeated at constant coronary perfusion pressure or flow, whichever had not been used previously. The sequence of perfusate administration was varied randomly between experiments. After a second recovery period, global ischemia was produced by clamping of the coronary perfusion line, and measurements of left ventricular pressure, wall thickness, and myocardial pH were made at 1, 15, 30, 45, 60, and 75 minutes.

Two different myocardial perfusates were used in these experiments. During control and recovery periods, the coronary perfusate was arterial blood from the support dog. The hypoxic perfusate consisted of methemoglobin-containing red blood cells suspended in lactated Ringer's solution to obtain a hematocrit of 20%. The preparation of these methemoglobin-containing red blood cells has been
described previously.23 Briefly, washed red blood cells were placed in 0.02 M sodium nitrite (NaNO₂) to oxidize all the hemoglobin to methemoglobin. These cells were then washed four times with normal saline to remove the NaNO₂ and were suspended in lactated Ringer’s.

Relative coronary blood flow was measured with an in-line electromagnetic flow probe (Biotronex Laboratory, Kensington, Maryland).

Statistical Analysis

All data are expressed as the mean±SEM. Comparisons of paired data were made using Student’s t test. Analysis of variance was used when comparing three or more conditions in the same heart.

Results

Hypoxic Perfusion

As seen in Figure 3, hearts perfused with methemoglobin-containing hypoxic red blood cells suspended in lactated Ringer’s solution at constant coronary perfusion pressure showed a significant decrease in LV diastolic chamber distensibility (LVEDP at 100% volume increased from 14±2 to 46±5 mm Hg at 15 minutes of hypoxia, p<0.01) associated with a significant increase in LV wall thickness (16.7±0.9 to 18.6±1.1 mm, p<0.01). Both of these changes were apparent as early as 5 minutes into hypoxia. Changes in end diastolic pressure relative to volume were much more prominent at higher diastolic volumes: 25% and 50% volumes showed no significant increase in LVEDP during hypoxia.

Cessation of coronary flow resulted in an immediate increase in diastolic distensibility was greater during hypoxia than during the control period (LVEDP, 2±0.4 to 11±2 mm Hg, p<0.01). The “residual” component of LV-diastolic distensibility (LVEDP−ΔLVEDP) was also significantly altered compared with control (LVEDP at 75% volume 6±1 to 10±2 mm Hg, p<0.05). The erectile effect on wall thickness (i.e., the decrease in wall thickness induced by coronary flow cessation) did not change significantly during hypoxia (h, 0.4±0.1 to 0.5±0.3 mm, p=NS). The erectile effect on wall thickness (i.e., the decrease in wall thickness induced by coronary flow cessation) did not change significantly during hypoxia (h, 0.4±0.1 to 0.5±0.3 mm, p=NS). Thus the increase in wall thickness during hypoxia at constant pressure was not related to changes in coronary turgor but rather was due solely to a “residual” effect (h−Δh). During reoxygenation, LVEDP, the erectile effect on LVEDP, and wall thickness all returned to baseline.

Perfusion with the same solution at constant coronary flow necessitated reduction in coronary perfusion pressure to 41±10 mm Hg. Even with decreased perfusion pressure, however, this hypoxic perfusate caused a significant decrease in diastolic chamber distensibility (LVEDP at 100% volume, 13±1 to 29±6 mm Hg, p<0.01) and increase in wall thickness (16.7±0.8 to 17.3±0.9 mm, p<0.01), as shown in Figure 3. Again, these changes occur early and predominantly at larger ventricular volumes. The erectile effect, although significant during both oxygenated and hypoxic perfusion periods, did not significantly influence the decrease in distensibility (ΔLVEDP, 2±0.4 to 5±2 mm Hg, p=NS) or increase in wall thickness (Δh, 0.4±0.2 to 0.4±0.1 mm) seen with hypoxia (Figure 4). In fact, the “residual” component of diastolic distensibility and wall thickness at constant flow is not significantly different from that at constant pressure.

Global Ischemia

Abrupt cessation of coronary flow resulted in an immediate increase in diastolic distensibility (LVEDP
The effect of coronary turgor on left ventricular end diastolic pressure (LVEDP) and end diastolic wall thickness during control and 10 minutes of hypoxia. Shaded areas represent the change in LVEDP or wall thickness upon coronary vascular collapse ("erectile effect"). At constant perfusion pressure (left panel), there is a marked increase in the erectile contribution to LVEDP during hypoxia although the "residual" LVEDP is still elevated compared with control. Wall thickness decreases significantly during cross clamping, but there is no increase in the erectile contribution to wall thickness during hypoxia. During reoxygenation, all parameters (distensibility, erectile effect on distensibility, wall thickness) return to baseline. At constant coronary flow (right panel), the erectile contribution is not significantly affected by hypoxia for either LVEDP or wall thickness. (*\(p<0.05\) vs. control).

Relation Between Intramyocardial pH and LV Diastolic Distensibility

Figure 6 demonstrates the changes in intramyocardial pH during hypoxia and global ischemia. Hypoxic coronary perfusion (with methemoglobin-containing red blood cells) at either constant coronary perfusion pressure or flow was associated with a decrease in intramyocardial pH of approximately 0.2 units, while global ischemia was associated with a pH decrease approximately twice as great. At 15 minutes of hypoxic perfusion at constant coronary perfusion pressure, LVEDP at 100% volume had risen considerably while intramyocardial pH had fallen from 7.01±0.13 to 6.82±0.15. In contrast, at 15 minutes of global ischemia, a significantly greater fall in intramyocardial pH (6.91±0.11 to 6.55±0.8) was associated with a decrease in LVEDP at 100% volume (18±3 to 11±1 mm Hg).

Discussion

The pathophysiological mechanisms underlying increased left ventricular diastolic pressure relative to volume during angina pectoris have proven difficult to define. The complex interaction of such factors as normal versus ischemic wall segment, pericardial constraint, and right ventricular pressure changes have led to the use of experimental models to study the theoretically "pure" myocardial effects of ischemia. The effects of global ischemic injury on the isolated heart have been found to differ substantially from hemodynamic changes seen during angina pectoris in man. Global ischemia causes either no change or an increase in LV diastolic distensibility acutely, with a subsequent stiffening of the left ventricle only after 30 to 60 minutes of continued severe ischemia. This late diastolic stiffening is thought to be due to markedly reduced ATP levels and perhaps actin-myosin rigor bond formation. Whether this late "contracture" development of the isolated heart is mechanistically similar to or distinct from diastolic dysfunction during angina pectoris in man is unclear.

In our experiments, the use of hypoxic perfusates in the isolated heart produced a much earlier change in diastolic function than did global ischemia. This temporal sequence is more analogous to the changes seen in man and in the dog coronary stenosis...
WALL THICKNESS (mm)

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FIGURE 5. The effect of global ischemia on left ventricular end-diastolic distensibility and wall thickness. ○, control; ▲, 1 minute of global ischemia; □, 30 minutes; ▲, 60 minutes; ○, 75 minutes. Aortic cross-clamping produces an early increase in distensibility (downward shift of the pressure-volume relation) and decrease in wall thickness (end diastolic, measured at 100% volume, to right of each curve). A decrease in distensibility occurs only after 60 minutes of global ischemia ("ischemic contracture") and is not associated with a significant increase in wall thickness. (*p<0.05 vs. control).

model, wherein LVEDP is seen to rise virtually coincident with the onset of angina and ischemia. While hypoxia did not produce this immediate (i.e., within several beats) rise in LVEDP, there was a gradual and sustained increase to the physiologically and statistically significant level noted at 5 minutes. The reason for this less abrupt change may be related to the differences in oxygen supply and demand. During pacing-induced angina, and in the dog coronary stenosis-pacing model, there is both a decrease in myocardial oxygen supply and an increase in myocardial oxygen demand. During global hypoxia, there is a decreased oxygen supply, yet there is no increase (and perhaps even a decrease, due to a fall in left ventricular systolic pressure) in demand. This may explain the more gradual time course of changes in distensibility.

Other investigators have demonstrated the relatively rapid effects of hypoxia on diastolic distensibility as well. While a hypothetical mechanism for this effect involves decreased sequestration of intracellular calcium by the SR, and thus slow and/or incomplete relaxation, there are again other complicating factors involved, most notably that of coronary turgor. The effects of variations in coronary pressure and flow on diastolic chamber distensibility (the "erectile effect") have been studied in some detail. Specifically, the increase in coronary turgor associated with hypoxia-induced vasodilation plays a significant role in compliance changes. Global ischemia, by definition, involves the loss of such turgor, thus providing at least partial explanation for the observed acute increase in distensibility. Yet another factor to be considered in these preparations is that of intracellular hydrogen ion accumulation. The increase in intracellular H+ associated with low-flow ischemia could, via effects on myofilament-Ca2+ binding sensitivity, lead to a blunting of the diastolic stiffening seen with hypoxia alone, since flow is maintained during hypoxic perfusion and H+ is washed out.

Thus, intracellular calcium changes, coronary turgor, and pH are all potential factors that might explain the differences between hypoxia and ischemic contracture. However, another significant factor in diastolic functional abnormalities that has received little attention in the analysis of hypoxic versus ischemic alterations is left ventricular wall thickness. Wall thickness has been shown to be an important determinant of left ventricular diastolic stiffness and pressure, independent of LV mass or the presence of LV hypertrophy. While wall thickness is known to decrease acutely with global ischemia, the extent and duration of this effect are not well understood. Further studies are needed to clarify the role of wall thickness in the development of diastolic dysfunction following global ischemia.

FIGURE 6. Changes in intramyocardial pH during hypoxia and global ischemia (n=8). Intramyocardial pH falls slightly during 15 minutes of hypoxia at both constant coronary perfusion pressure (▲) and flow (●), while at 15 minutes of global ischemia (○) there is a marked drop in pH, with a further decline during more prolonged ischemia. (*p<0.05 vs. control).
ischemia, direct measurements of thickness with hypoxia at varying coronary flows (including transient vascular collapse), and the comparison of this with prolonged ischemia, have not been reported previously.

In our studies hypoxic perfusion led to an early and sustained decrease in LV diastolic chamber distensibility at both constant coronary pressure and flow. Wall thickness increased significantly and simultaneously with the decrease in distensibility. Direct measurement of LV wall thickness in the presence and absence of coronary tumor demonstrated that the increase in diastolic wall thickness during hypoxia, at both constant pressure and flow, was independent of hypoxia-induced changes in the vascular compartment; that is, the increase in wall thickness was due primarily to a "residual" myocardial effect. In addition, wall thickness changes were reversible upon reoxygenation.

Global ischemia in our model produced a fall in LVEDP at 100% volume, with increases in LVEDP developing only after prolonged (>60 minutes) global ischemia. Wall thickness, similarly, decreased immediately upon aortic cross-clamping. However, increases in wall thickness did not parallel the subsequent increases in LVEDP, as had been the case with hypoxia. At 75 minutes of ischemia, when diastolic contracture had produced marked stiffening of the ventricle, wall thickness had not increased to control values.

Thus, in addition to coronary tumor and pH, wall thickness, a significant determinant of diastolic function, is affected quite differently by acute hypoxia and global ischemia. The mechanism of these differences is not entirely clear. Wall thickness in the absence of the pericardium is determined by vascular, myocardial cellular, and extracellular components. We have demonstrated that hypoxia-induced changes in the vascular compartment, while significant, do not effect the changes in wall thickness. Myocardial cellular changes in hypoxia and ischemia could conceivably be due to cell swelling and/or interstitial edema or to incomplete inactivation of systolic tension development. As noted previously, persistent actin-myosin interaction into diastole may account for the changes in relaxation and chamber compliance seen during acute hypoxia and prolonged ischemia. How might wall thickness differences between the two be explained in accordance with this hypothesis? The mechanism of rigor formation during prolonged ischemia is presumably due to severe ATP depletion, but whether this results in decreased removal of calcium from the troponin-tropomysin complex or in direct binding of actin to myosin is unclear. The latter has been documented in skeletal muscle and isolated heart experiments employing a "quick stretch" to temporarily relieve ischemic contracture have suggested this mechanism as well. The cause of diastolic stiffening in hypoxia, on the other hand, has been attributed to impairment of calcium uptake by the SR, resulting in increased availability of calcium to the myofilament apparatus, with persistent cross-bridge cycling. These differences in intracellular mechanisms of contracture could conceivably lead to differences in wall thickness, with continued cross-bridge cycling causing a prominent increase in wall thickness (analogous to systolic wall thickening). On the other hand, direct actin-myosin bond formation, without the physiological cross-bridge cycling that is associated with normal tension development, might result in a static wall thickness even with markedly increased myocardial stiffness. Additional support for this concept comes from studies on papillary muscle contracture, in which changes in the stiffness-tension relation were shown to be linearly related to resting force, suggesting that the type of stiffness characterized by this contracture was different from that participating in active force development.

Changes in the extracellular matrix could also account for wall thickness differences. Although the role of edema in the physiological setting of ischemia and hypoxia is controversial, interstitial edema could conceivably result in increased wall thickness during hypoxia (especially in the setting of a perfusate with decreased oncotic pressure). However, as noted above, whether the rapid inception and resolution of compliance changes in this and other studies could be the result of edema formation is unclear. Of interest, other changes in the extracellular matrix (primarily collagen structure and cross-linking) have recently been noted in association with repetitive bursts of ischemia and reperfusion although it is uncertain whether changes of this nature could occur with brief episodes of hypoxia, as such the 5-15-minute hypoxic perfusion used in this study.

In summary, our studies on diastolic function in the isolated heart model have demonstrated that the hypoxia-induced decreases in left ventricular chamber distensibility are associated with a substantial increase in end-diastolic wall thickness that is independent of changes in coronary tumor. In addition, there are important differences in the time course and degree of wall thickening during acute hypoxia and prolonged ischemia. These results support the concept that there is a fundamental mechanistic difference between the diastolic dysfunction of acute hypoxia and prolonged ischemia in this preparation, despite their similar effects on LV diastolic pressure relative to volume.

**Acknowledgment**

The authors wish to acknowledge Alvin Franklin for his expert technical assistance in the performance of this experimental protocol.

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KEY WORDS • diastole • isolated heart • hypoxia • ischemia
Comparative effects of hypoxia and ischemia in the isolated, blood-perfused dog heart: evaluation of left ventricular diastolic chamber distensibility and wall thickness.
R M Wyman, E R Farhi, O H Bing, R G Johnson, R M Weintraub and W Grossman

Circ Res. 1989;64:121-128
doi: 10.1161/01.RES.64.1.121

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