Influence of Hypertension With Minimal Hypertrophy on Diastolic Function During Demand Ischemia

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Hearts with advanced pressure-overload hypertrophy from systemic hypertension have been shown to have an increased susceptibility to the development of diastolic dysfunction in response to tissue hypoxia and ischemia. It is not known if this propensity to develop diastolic dysfunction in response to ischemia is dependent on the presence of a substantial increase in left ventricular mass, or alternatively, is characteristic of hearts subjected to mild chronic hypertension early in the development of cardiac hypertrophy. We tested the hypothesis that systemic hypertension associated with mild left ventricular hypertrophy increases the susceptibility to the development of diastolic dysfunction in response to demand ischemia. The effects of demand ischemia (6 minutes) were studied in hearts from New Zealand white rabbits with chronic systemic hypertension produced by the one-kidney, one-wrap method (n=15) and compared with age-matched, sham-operated control rabbits (n=11) with similar left ventricular mass (5.4±0.2 vs. 5.4±0.3 g, respectively). The hearts were studied using an isolated, isovolumic (balloon in left ventricle) preparation with absent pericardium that was perfused with fresh whole blood. At baseline, coronary perfusion pressure was 100 mm Hg with comparable coronary flow per gram left ventricular weight; the hearts were paced at a physiological rate of 3 Hz, and the left ventricular balloon volume was adjusted to achieve a left ventricular end-diastolic pressure of 15 mm Hg in both groups. Left ventricular balloon volume was similar in both groups and volume was thereafter held constant. At baseline, left ventricular systolic pressure (114±4 vs. 95±3 mm Hg, p<0.001) and developed pressure (18.9±1.2 vs. 15.1±0.9 mm Hg/g, p<0.05) were higher in the hearts from the hypertensive group in comparison with the control group. During the first minute of global ischemia produced by reducing coronary perfusion pressure from 100 to 20 mm Hg, there was an immediate fall in left ventricular systolic pressure in both groups without an increase in diastolic pressure. In response to the superimposition of pacing tachycardia (heart rate, 6 Hz) during the remaining 5 minutes of the period of ischemia, left ventricular developed pressure was comparable. However, isovolumic left ventricular end-diastolic pressure (measured during long diastoles obtained with transient cessation of pacing) rose to a significantly higher level in the hearts from hypertensive rabbits than in those from the control rabbits (29±3 vs. 18±2 mm Hg, p<0.01). During ischemia, coronary flow rate per gram left ventricular weight was severely depressed to 10% of baseline and was similar in the hypertensive and control groups (0.2±0.03 vs. 0.2±0.03 ml/min/g, NS). The severity of ischemia-induced anaerobic metabolism, as estimated by the venous minus arterial lactate concentration difference, was also comparable. We conclude that the presence of chronic systemic hypertension results in an enhanced susceptibility to the development of impaired left ventricular diastolic function in response to demand ischemia, and that this impaired extent of force inactivation in response to ischemia is evident even before the development of a substantial increase in left ventricular mass. (Hypertension 1989;13:361–370)
Hears with chronic left ventricular hypertrophy due to severe systemic hypertension have been shown to have an enhanced susceptibility to the development of diastolic dysfunction in response to myocardial hypoxia or ischemia. We have previously shown that brief hypoxia results in a greater impairment of diastolic distensibility and relaxation in hypertrophied hearts from rats with severe hypertension induced by the deoxycorticosterone (DOC)-salt method compared with hearts from control rats studied in an anovolumic, buffer-perfused preparation.\textsuperscript{1,2} The presence of substantial left ventricular hypertrophy has also been shown to have an adverse effect on diastolic function in studies that have compared hypertrophied hearts with impaired coronary reserve with normal hearts\textsuperscript{3} and in studies that have examined the influence of prolonged no-flow ischemia.\textsuperscript{4} However, the influence of chronic mild hypertension before the development of a substantial increase in left ventricular mass on the diastolic response to transient ischemia is not known. The issue is of potential clinical relevance because patients with coronary artery disease commonly have coexisting systemic hypertension with mild cardiac hypertrophy.

The abrupt reversible impairment of diastolic function that occurs in response to demand ischemia in normal hearts\textsuperscript{5,6} appears to be related to a depressed rate and extent of myofilament force inactivation associated with several factors including high energy phosphate depletion and cytosolic calcium overload.\textsuperscript{7-10} Hearts adapting to chronic pressure overload may be susceptible to the development of impaired force inactivation during ischemia since altered calcium reuptake of the sarcoplasmic reticulum\textsuperscript{11-14} in association with intrinsic prolongation of the calcium transient\textsuperscript{15,16} appear to be subcellular adaptations that occur as early compensatory responses to chronic pressure overload. Thus, it is not known whether the susceptibility of the heart subjected to chronic pressure overload to the development of ischemic diastolic dysfunction is dependent on the presence of substantial changes in left ventricular mass, or alternatively, is related to early subcellular adaptations to chronic pressure overload that are independent of changes in left ventricular mass.

The aim of this study was to test the hypothesis that the presence of systemic hypertension with very mild left ventricular hypertrophy results in an increased sensitivity to the development of impaired diastolic function in response to demand ischemia. To test this hypothesis, the effects of brief demand ischemia were studied in an isolated and isovolumic blood-perfused rabbit heart preparation in response to a 6-minute period of global low-flow ischemia with the superimposition of pacing tachycardia. We have previously shown in normal rabbit hearts that this demand ischemia protocol results in a rapid and reversible decrease in diastolic distensibility similar to that which occurs during exertional or pacing-induced angina in humans.\textsuperscript{17} In the current study, we used this model of demand ischemia to compare hearts from New Zealand white rabbits with hypertension induced by the one-kidney, one-wrap method with hearts from age-matched, sham-operated control rabbits. The hearts from the hypertensive and control rabbits were characterized by in vivo sysolic arterial pressures of 118±7 vs. 98±3 mm Hg (p<0.05) and left ventricular weights of 5.4±0.2 vs. 5.4±0.3 g (NS). The left ventricular balloon volume was comparable in the two groups and was held constant during the experiment so that changes in left ventricular diastolic pressure reflected changes in diastolic chamber distensibility. Coronary blood perfusion was reduced to achieve a similar and severe reduction of flow per gram left ventricular weight in both groups to minimize the influence of differences in coronary reserve and to avoid the exaggerated coronary turgor effect that may confound studies of hypoxic buffer perfusion.

Thus, our experiment was done to examine and compare diastolic function in response to brief demand ischemia in blood-perfused hearts with and without chronic left ventricular pressure overload before the development of left ventricular hypertrophy.

Materials and Methods

Male albino New Zealand rabbits weighing 1–2 kg were used. The preparation of the hypertensive and control groups followed methods previously described by Fletcher.\textsuperscript{18} Animals were anesthetized with intravenous sodium pentobarbital (40–50 mg/kg). One-kidney, one-wrap hypertension was produced by exposing and wrapping the left kidney with sterile cellophane dialysis membrane followed by right nephrectomy at the same operation. Care was taken not to bind the renal pedicle during the wrapping procedure. The age-and weight-matched control group rabbits underwent sham operation under anesthesia. After the operation, the animals were fed normal rabbit chow and water ad libitum. Ten weeks after operation, on the day before the experiment, the animals were maintained quietly for 10 minutes in a specially constructed chamber and blood pressure was measured by a standard tail-cuff technique (Narco Bio-Systems, Houston, Texas).

Perfusion Technique

An isolated blood-perfused rabbit heart model that simulates the physiology of angina was used.\textsuperscript{17} Male albino New Zealand rabbits weighing 1–2 kg served as blood donor rabbits. Both the blood donor rabbits and the experimental rabbits were treated with heparin (500 units/kg) and dexamethasone (1 mg/kg) to prevent thrombosis and blood-blood interactions in the perfusion apparatus and were anesthetized with sodium pentobarbital (50 mg/kg) intravenously. The blood donor rabbit contributed 100–125 ml of fresh whole heparinized blood. The thorax of the heart donor rabbit was opened and the heart was isolated, removed from
the pericardium, and placed in a water-jacketed, constant-temperature chamber (37° C). A short perfusion cannula was inserted into the ascending aorta above the aortic valve. The interval between isolation of the heart and initiation of coronary perfusion was less than 20 seconds in all experiments.

The perfusion system consisted of a "venous" reservoir, a variable-flow pump, an oxygenator, a water-jacketed "arterial" reservoir, and a filter (Figure 1). In this system, the arterial reservoir was pressurized such that coronary perfusion pressure was controlled by a valve that adjusted the pressure of the reservoir; coronary blood flow was allowed to vary and depended on coronary vasomotor regulation. After being pumped from the venous reservoir, the blood passed through an oxygenator into the pressurized arterial reservoir and then through a 40-μm pore size filter into the aortic cannula. The oxygenator was manufactured by coiling approximately 7.5 m of silastic tubing (0.58 mm i.d. by 0.77 mm o.d., Dow-Corning Corporation Medical Products, Midland, Michigan, catalog no. 602-235) as has been previously reported from this laboratory.17,19 The silastic tubing was placed inside a large beaker, which was covered and equilibrated with a gas mixture of 20% O2, 3% CO2, and the balance N2, to achieve an arterial pO2 of 90–110 mm Hg and pH of 7.38–7.42. Glucose was added to maintain the arterial glucose concentration within a range of 80–120 mg/dl throughout the experiment.

After initiation of coronary perfusion, the pulmonary artery was cut and a cannula was inserted into the right ventricle to empty the right ventricle and completely collect coronary venous effluent. A thermistor (Yellow Springs Instr. Co., Yellow Springs, Ohio) and a pacing electrode were inserted into the right ventricle via the right atrium and the venae cavae were ligated. Myocardial temperature was continuously monitored and maintained at 37° C. A drainage cannula was placed in the left ventricular apex to decompress any Thebesian drainage. A collapsed latex balloon was placed in the left ventricle via the left atrium. The balloon was sufficiently large that no measurable pressure was generated by the balloon itself over the range of left ventricular volumes used in the experiment.

Measurement of Mechanical Function

The left ventricular balloon was filled with bubble-free saline solution and attached to a high-fidelity micromanometer catheter (Millar Instruments, Houston, Texas). Coronary perfusion pressure was measured from a side arm of the perfusion cannula connected to a Statham (Oxnard, California) P23Db pressure transducer. Pressure signals were recorded on a multichannel recorder. In this study, the rapid changes in diastolic and systolic function that occurred during ischemia precluded the evaluation of left ventricular diastolic chamber distensibility by serial measurements of a range of multiple diastolic pressure-volume points. To assess left ventricular chamber distensibility, left ventricular balloon volume was kept constant so that an increase in left ventricular end-diastolic pressure signified a decrease in diastolic chamber distensibility. This approach has been applied in similar isolated heart animal models.1,17,20,21

To compare pressures in the hearts from hypertensive and control groups, one would ideally like to calculate systolic and diastolic wall stresses precisely. To estimate the load per unit of myocardium, we assumed a spherical left ventricular shape for both groups of hearts; therefore, wall stress would be proportional to pressure x radius/wall thickness by the Law of LaPlace. The balloon volumes were comparable in both groups (see below). Thus, the left ventricular radii were comparable, and wall stress was inversely proportional to thickness, which is directly related to left ventricular mass. Therefore,
left ventricular pressure per gram is an approximation of wall stress under our experimental conditions.¹

**Measurements of Coronary Flow and Metabolism**

Coronary flow was divided by left ventricular wet weight and expressed as milliliters per minute per gram. Arterial and venous perfusate samples were collected and analyzed for lactate concentration by the specific enzymatic method of Apstein et al.²² Lactate samples were immediately mixed with iced 5% trichloroacetic acid and kept under refrigeration until analysis. Lactate data are expressed as arterial minus coronary venous lactate concentration differences in units of millimolar per liter.

**Experimental Protocol**

Each heart was perfused for 30 minutes and paced at a physiological heart rate of 3 Hz to allow performance to stabilize. In both groups, the coronary perfusion pressure was adjusted to a level of 100 mm Hg. Left ventricular balloon volume was adjusted so that end-diastolic pressure was 15 mm Hg in both the hypertensive and control groups, and this balloon volume was maintained unchanged throughout the experiment. At this level of left ventricular end-diastolic pressure, balloon volume was comparable in the hypertensive and control groups (1.8±0.2 vs. 2.2±0.2 ml, NS).

At the end of the 30-minute stabilization period, measurements of left ventricular pressure, coronary perfusion pressure, and coronary flow were made, and arterial-coronary venous blood samples were obtained for analysis of lactate content. After baseline measurements, coronary perfusion pressure was reduced to 20 mm Hg for 6 minutes. After the first minute of global ischemia, the paced heart rate was increased from 3 to 6 Hz and maintained at this level for the last 5 minutes of ischemia. Measurements of left ventricular pressure were made at the end of the 6-minute period of ischemia during 5 seconds of transient discontinuation of pacing to permit measurement of diastolic pressure after it reached its nadir during prolonged diastoles. Coronary venous effluent was completely collected during the last 5 minutes of ischemia, and this total collection volume was used to sample coronary venous lactate. The arterial lactate sample was obtained during the last 15 seconds of the ischemic period.

To observe the recovery of systolic and diastolic function, the coronary perfusion pressure was returned to 100 mm Hg and paced heart rate was returned to 3 Hz. Hemodynamic and metabolic measurements were made at 30 minutes of recovery, and the wet weights of the heart and left ventricle were determined.

**Statistical Analysis**

Statistical comparisons between the control group and the hypertensive group were made by Student’s t test.²³ All data are reported as the mean±SEM.

**Results**

Baseline data characterizing the extent of hypertension and left ventricular hypertrophy for 11 control rabbits and 15 hypertensive rabbits are obtained for analysis of lactate content.
shown in Table 1. The in vivo systolic arterial pressure, assessed by tail-cuff method, of the hypertensive group was 20% higher than in the control group. In the hypertensive group, there was mild left ventricular hypertrophy evident as a 18% increase in the left ventricular/body weight ratio with no difference in absolute left ventricular weight relative to the control group.

Left ventricular and coronary hemodynamic measurements during baseline conditions before the imposition of demand ischemia are shown in Table 2. At a pacing rate of 3 Hz and at the same left ventricular end-diastolic pressure, left ventricular systolic pressure was 20 mm Hg (21%) higher and left ventricular developed pressure per unit left ventricular mass was higher in the hypertensive group than the control group (18.9±1.2 vs. 15.1±0.9 mm Hg/g, p<0.05).

By experimental design, coronary perfusion pressure was regulated at baseline to achieve a comparable and physiological level of 100 mm Hg in the hypertensive and control groups (102±2 vs. 97±3 mm Hg, NS). At this level of coronary perfusion pressure, the myocardial perfusion rate per gram left ventricular weight was similar in the hypertensive and control groups (1.8±0.1 vs. 1.9±0.2 ml/min/g, NS).

Figure 2 shows the response to demand ischemia of a typical experimental heart from the control group and from the hypertensive group. The effects of 6 minutes of demand ischemia (constant coronary perfusion pressure of 20 mm Hg with the superimposition of pacing tachycardia at 6 Hz) at constant balloon volume are shown in Table 2. Demand ischemia resulted in a prompt and progressive fall in left ventricular systolic pressure in both groups. At 6 minutes of ischemia, left ventricular systolic pressure was higher in the hypertensive group than in the control group (34±3 vs. 25±2 mm Hg, p<0.05), while left ventricular developed pressure per unit of left ventricular mass was comparable (Figure 3).

During the first minute of low-flow ischemia before the imposition of pacing tachycardia, there was no increase in left ventricular end-diastolic pressure in either the hypertensive or the control group. In response to the imposition of pacing tachycardia during the remaining 5 minutes of the 6-minute period of low-flow ischemia, both groups showed a gradual and progressive rise in left ventricular end-diastolic pressure at constant left ventricular volume. As illustrated in Figure 4, left ventricular end-diastolic pressure increased to a significantly higher level in the hypertensive group than in the control group (29±3 vs. 18±2 mm Hg, p<0.05). The increase in left ventricular end-diastolic pressure per unit of left ventricular mass was also greater in the hypertensive group in comparison with the control group (2.8±0.7 vs. 0.9±0.3 mm Hg/g, p<0.05). It should be emphasized that left ventricular end-diastolic pressure was always measured after it reached its nadir during a long diastole.
after abrupt cessation of pacing. In all experiments, the elevation of left ventricular diastolic pressure persisted and did not decay during a long diastole (Figure 2), which is consistent with an impairment of the extent of relaxation and excludes an artifactual increase in diastolic pressure from incomplete relaxation due to tachycardia per se.

During demand ischemia at a coronary perfusion pressure of 20 mm Hg, coronary flow rate was depressed to 10% of baseline, and the myocardial flow rate per gram left ventricular weight was comparable in the hypertensive and control groups (0.2±0.03 vs. 0.2±0.03 ml/min/g, NS) (Figure 5). Both the hypertensive and control groups showed evidence of anaerobic metabolism during demand ischemia, and the venous-arterial lactate difference across the coronary bed was comparable (1.1±0.2 vs. 1.2±0.2 mM/l, NS).

During the 30-minute recovery period with restoration of coronary perfusion pressure to 100 mm Hg and heart rate to 3 Hz, the hypertensive and control groups showed a similar recovery of left ventricular systolic and diastolic function. Left ventricular systolic pressure returned to 79% and 81% of baseline values in the hypertensive and control groups, respectively. Left ventricular end-diastolic pressure in the hypertensive group was identical to that of the control group in response to recovery (20±2 vs. 20±2 mm Hg, NS). The coronary flow rate per gram left ventricular weight returned to baseline and was comparable in both groups.

Discussion
We have previously shown that rat hearts with chronic pressure overload hypertrophy due to severe systemic hypertension show an enhanced susceptibility to the development of impaired diastolic function in response to tissue hypoxia in comparison with hearts from age-matched control rats. Other experimental studies have also shown that the presence of substantial left ventricular pressure-overload hypertrophy adversely modifies diastolic function during ischemia. It is controversial as to whether this sensitivity of the pressure-overloaded heart to the development of ischemic diastolic dysfunction is related to subcellular changes in force

**Figure 3.** Bar graph showing left ventricular (LV) developed pressure at baseline and in response to demand ischemia. LV developed pressure was higher in the hearts from hypertensive (HTN) rabbits at baseline, but was comparable with that of the control group (C) in response to ischemia.

**Figure 4.** Bar graphs showing left ventricular (LV) diastolic pressure measured at end diastole under isovolumic conditions at baseline and in response to ischemia. LV end-diastolic pressure was comparable in the hypertensive (HTN) and control (C) groups at baseline. In response to demand ischemia, LV end-diastolic pressure increased to a significantly higher level in the HTN hearts compared with the control hearts. The magnitude of the increase in LV end-diastolic pressure in response to ischemia was also greater in the HTN hearts compared with the control hearts when normalized per unit of LV mass. See text.
inactivation or can be simply explained by an increase in left ventricular mass per se, with the presence of more myocardial units having an additive effect on left ventricular diastolic pressure generation. In this study, we showed that isovolumic blood-perfused hearts from rabbits with mild systemic hypertension induced by the one-kidney, one-wrap method developed a more severe impairment of diastolic function, which did not depend on the presence of a substantial increase in left ventricular mass, in response to demand ischemia compared with control hearts.

The aim of this experiment was to compare diastolic function at baseline and in response to 6 minutes of demand ischemia (reduction of coronary perfusion pressure to 20 mm Hg with superimposed pacing tachycardia) in blood-perfused isovolumically contracting hearts from hypertensive rabbits with minimal hypertrophy and from age-matched, sham-operated control rabbits. At similar coronary flow rates per gram of left ventricular weight and at comparable left ventricular volumes, the baseline left ventricular systolic pressure and left ventricular developed pressure per gram left ventricular weight were significantly higher in the hearts from the hypertensive rabbits compared with hearts from the control group. These baseline perfusion conditions successfully simulated the magnitude of systemic hypertension detected in vivo in the hypertensive rabbits compared with the control rabbits. Demand ischemia was imposed by reducing coronary perfusion pressure to 20 mm Hg for 6 minutes and imposing pacing tachycardia of 6 Hz during the last 5 minutes of ischemia. Coronary flow rate per gram was reduced to 10% of baseline and was comparable in both groups, as was myocardial lactate production. Left ventricular systolic function, as assessed by developed pressure per gram, was depressed to a similar extent in both groups in response to demand ischemia.

The diastolic response to demand ischemia differed in the hearts from the hypertensive and control rabbits. In both groups, left ventricular diastolic pressure began to progressively rise within 30–90 seconds of the superimposition of pacing tachycardia during global low-flow ischemia. At the end of 6 minutes of ischemia, the hearts from the hypertensive rabbits showed a significantly greater rise in left ventricular end-diastolic pressure in comparison with hearts from the control group. In every instance, the elevation of left ventricular diastolic pressure persisted without decay during a long diastole when the pacemaker was transiently turned off, which excludes an artificial rise in diastolic pressure from abbreviation of diastole during tachycardia. This finding is consistent with an impairment in the extent or completeness of left ventricular relaxation, and is similar to our previous observations in rat hearts with advanced hypertrophy, which showed an impaired extent of diastolic relaxation in response to hypoxia.1,2 Absolute left ventricular weight was similar in both groups, and the rise in left ventricular end-diastolic pressure in the hearts from the hypertensive group was also more marked when normalized for left ventricular mass in comparison with hearts from the control group. Thus, the greater sensitivity of the hearts from the hypertensive rabbits to the development of impaired diastolic function during ischemia cannot be simply explained by the presence of more contractile units having a simple additive effect on total left ventricular pressure generation1 and implicates an alteration of diastolic behavior at the level of myocyte per se. These data suggest that the vulnerability of myocardium subjected to chronic hypertension to the development of diastolic dysfunction in response to tissue hypoxia is not limited to advanced hypertrophy, but may occur very early in response to mild left ventricular pressure overload in the absence of a substantial increase in left ventricular mass.

Merits and Limitations of the Model

We chose this rabbit model of mild hypertension to contrast with that of our previous studies in which we showed that hearts from rats with severe DOC-salt–induced hypertension developed a greater
impairment of diastolic relaxation in response to hypoxic buffer perfusion.\(^1\)\(^2\) This cohort of rabbits was characterized by moderate systemic hypertension induced by the one-kidney, one-wrap method in vivo, enhanced left ventricular systolic function under baseline isovolumic perfusion conditions in association with minimal left ventricular hypertrophy evident as an 18% increase in left ventricular body weight ratio, and similar absolute left ventricular weight in comparison with age-matched, sham-operated control rabbits. This contrasts with the magnitude of hypertrophy found in our studies of DOC-salt-induced hypertensive rats that were characterized by a 42% increase in left ventricular weight and a 69% increase in left ventricular body weight ratio. Furthermore, in those studies, the rat hearts were exposed to constant high-flow hypoxic buffer perfusion in which there may be a substantial erectile effect of the distended coronary vasculature on left ventricular diastolic pressure.\(^19\)\(^20\) In the present study, the use of the more physiological perfusate of fresh whole blood rather than buffer and the study of ischemia produced by a profound reduction of coronary flow rather than hypoxia exclude any confounding influence of an exaggerated coronary turgor effect on the greater rise in isovolumic left ventricular diastolic pressure seen in the hearts from hypertensive rabbits.

The demand ischemia model used in this study has been shown in normal rabbit hearts\(^17\) to elicit the transient upward shift in left ventricular diastolic pressure relative to volume that is the hallmark of the diastolic physiology observed in patients during angina provoked by exertion or pacing tachycardia.\(^2\)\(^24\)\(^25\) Critical components are the presence of both the superimposition of increased energy demand and a severe reduction of coronary perfusion pressure to the range of 20 mm Hg, which is comparable to the levels of coronary perfusion pressure seen distal to a subtotal coronary stenosis in patients with coronary artery disease.\(^26\)\(^27\) We have previously shown that a reduction in coronary perfusion pressure to this level for a period of 6 minutes in the blood-perfused rabbit heart results in a fall in systolic pressure with no increase in diastolic pressure unless an increase in energy demand is superimposed.\(^17\)\(^28\) It must be emphasized that the diastolic physiology of this model differs from prolonged global no-flow ischemia, which is characterized by a profound reduction of energy demand, severe acidosis, and an initial fall in diastolic pressure followed by the delayed development of incompletely reversible contracture.\(^4\)\(^7\)\(^25\) This demand ischemia model also differs from prior studies of severe pressure overload hypertrophy in which a rise in left ventricular diastolic pressure occurred in response to pacing tachycardia in the absence of restricted coronary inflow.\(^5\)\(^29\) In such studies, changes in diastolic function in the hypertrophied hearts are related not only to the influence of ischemia on the myocyte but also to differences in coronary reserve available to be recruited when compared with normal hearts.\(^30\)\(^33\) In the present experiment, diastolic function was studied in hearts from the hypertensive and control rabbits during a profound and comparable reduction in coronary flow per gram and in the presence of a similar degree of myocardial lactate production. Endocardial/epicardial flow measurements with microspheres were not performed, and the possibility cannot be excluded that differences in the exhaustion of subendocardial flow reserve during global low coronary flow were present in the hearts from hypertensive and control groups.

An important advantage of this model of low-flow global demand ischemia is that it permits the study of the effects of myocardial ischemia on diastolic properties without the confounding influence of dysynchronous contraction of ischemic and non-ischemic segments. In the clinical setting of ischemia related to epicardial coronary stenoses, regional dysynchrony of contraction of ischemic and non-ischemic segments may contribute to the rise in left ventricular diastolic pressure.\(^25\)\(^24\) Thus, our experiments address the interaction of hypertrophy and demand ischemia per se on diastolic function, and future studies will be required to study any additive or enhancing influence of segmental dysynchrony from regional coronary stenoses in hearts with chronic pressure overload.

**Possible Subcellular Mechanisms**

Cardiac muscle relaxation during ischemia is independent of load and is primarily determined by the intracellular processes that influence the rate and extent of force inactivation, which includes the adenosine triphosphate–dependent rate and capacity of calcium sequestration by the sarcoplasmic reticulum.\(^7\)\(^10\)\(^25\)\(^34\)\(^37\) Tissue levels of high energy phosphates were not measured in this study. However, we have previously shown in experiments using P-31 nuclear magnetic resonance spectroscopy that myocardial hypoxia is associated with a greater impairment of diastolic function in hypertrophied rat hearts in comparison with control hearts despite a comparable rate and extent of depletion of myocardial high energy phosphate levels and degree of intracellular acidosis.\(^2\) These data suggest that the pressure-overloaded heart is more susceptible to the development of impaired relaxation for any level of high energy phosphate depletion in comparison with control hearts.

Differences in cytosolic calcium available for diastolic crossbridge attachment could account for the sensitivity of the pressure-overloaded heart to the development of impaired force inactivation during demand ischemia or hypoxia. There is evidence from experiments using the calcium indicator aequorin that hearts with chronic pressure overload are characterized by intrinsic alterations in the duration of the intracellular calcium transient.\(^15\)\(^16\) This may be related in part to alterations in sarcoplasmic reticulum function\(^11\)\(^14\)\(^38\) as well as the
capacity to maintain normal transsarcolemmal cation gradients.\(^{39-41}\)

These changes in cytosolic calcium regulation in pressure-overloaded hearts may be an adaptation distinct from an increase in pressure-generating units per se and thus facilitate the development of a high and sustained level of systolic tension with greater efficiency under well-oxygenated conditions.\(^{42}\) In this regard, it is of interest in this study that systolic pressure generation at a comparable isovolumic preload was enhanced under non-ischemic baseline conditions in the hearts from hypertensive rabbits relative to hearts from control rabbits in the absence of an increase in left ventricular mass. However, the pressure-overloaded heart may be at risk for an adverse interaction between an intrinsic and adaptive slowing of diastolic calcium sequestration and the superimposition of ischemia that would further decrease calcium transport by the sarcoplasmic reticulum and sarcosome.\(^{43}\) In comparison with normal hearts, a comparable level of high energy phosphate depletion would be expected to cause a more profound impairment of diastolic cytosolic calcium regulation and force inactivation in the pressure-overloaded heart. The present study lends support to the hypothesis that such subcellular alterations that increase the vulnerability to ischemia may occur in response to mild chronic pressure overload and do not depend on the presence of a substantial increase in left ventricular mass.

Downstream mechanisms that modify calcium sensitivity of the myofilaments could also account for the findings in this study. In this regard, a reduced responsiveness to catecholamines at the receptor level or a blunted generation of cyclic adenosine monophosphate\(^{44,45}\) could potentially contribute to impaired diastolic function in the hearts with hypertensive pressure overload. This potential mechanism is unlikely to account for our findings since it is inconsistent with the observed increase in systolic performance in the hypertensive group at baseline.

In summary, brief demand ischemia resulted in a greater impairment of left ventricular diastolic function in isovolumically contracting hearts from hypertensive rabbits in comparison with hearts from control rabbits. These observations in hearts from rabbits with mild chronic hypertension and minimal hypertrophy suggest that even mild chronic pressure overload is characterized by an increase in systolic force generation per unit of myocardium under nonischemic conditions and an increased sensitivity to the development of impaired diastolic force inactivation during tissue hypoxia or ischemia, similar to that previously observed in hearts with substantial hypertrophy secondary to severe hypertension. These observations suggest that the sensitivity of myocardium exposed to chronic pressure overload to the development of ischemic diastolic dysfunction does not depend on the presence of a substantial increase in left ventricular mass. The responsible mechanisms are not yet known, but may be related to adaptations in cytosolic calcium regulation. Future studies that directly measure changes in cytosolic calcium in isolated working hearts from hearts with mild versus severe degrees of pressure overload and hypertrophy will be needed to address this hypothesis.

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