SUMMARY. We tested the hypothesis that there is an enhanced susceptibility in hypertrophied cardiac muscle to develop decreased diastolic distensibility of the left ventricle in response to hypoxia. The effects of brief hypoxia (3 minutes) were studied in rats with and without chronic left ventricular pressure overload hypertrophy using an isolated buffer-perfused and isovolumic (balloon-in-left ventricle) heart preparation with excised pericardium and vented right ventricle. We compared hypertrophied hearts from hearts from hypertensive uninephrectomized Wistar-Kyoto rats (n = 12) with normotensive uninephrectomized age-matched controls (n = 13). Coronary flow was held constant and adjusted so that an identical flow per gram left ventricular weight was achieved in both groups. The left ventricular balloon volume was adjusted to produce an initial left ventricular end-diastolic pressure of 10 mm Hg in both groups and was held constant thereafter so that changes in left ventricular end-diastolic pressure during hypoxia represented changes in diastolic chamber distensibility. Under aerobic conditions, left ventricular systolic pressure was 66% higher in the hypertrophied hearts than in the controls, but there was no difference in the rate or extent of left ventricular relaxation as estimated by the exponential time constant of pressure decay and the asymptote to which pressure decayed. In response to hypoxia, left ventricular end-diastolic pressure was significantly higher in the hypertrophied hearts than in the controls (37 ± 5 vs. 22 ± 5 mm Hg, P < 0.001). In response to hypoxia, the rate of left ventricular relaxation was depressed to a comparable degree in both groups, but there was a greater upward shift in the asymptote to which pressure decayed in the hypertrophied hearts. Hypoxia-induced coronary vasodilation as assessed by the change in coronary vascular resistance was similar in the hypertrophied and control hearts (2.9 ± 0.5 vs. 2.3 ± 0.9 mm Hg/[(ml/min)/g], NS). The degree of hypoxia-induced anaerobic metabolism as estimated by the coronary arterial-venous lactate concentration difference was also similar in both groups (—0.72 ± 0.23 vs. —0.73 ± 0.16 mm/liter, NS). It is concluded that brief hypoxia results in a greater decrease in diastolic distensibility of the left ventricle in the presence of chronic pressure overload hypertrophy than in its absence. (Circ Res 58: 653-663, 1986)
ceptibility to the development of diastolic dysfunction in response to any degree of ischemia or hypoxia compared with nonhypertrophied myocardium. In this regard, prior observations suggest that chronically hypertrophied cardiac muscle exhibits intrinsic prolongation of the duration of contraction and relaxation and an increased duration of the intracellular calcium transient (Morgan and Morgan, 1984). Therefore, it is plausible that the hypertrophied left ventricle may be especially vulnerable to the development of a decreased rate or extent of left ventricular relaxation in response to ischemia or hypoxia.

Consequently, our study was carried out to test the hypothesis that a more profound impairment of the rate and extent of left ventricular relaxation occurs in response to hypoxia in the presence of pressure overload hypertrophy than in its absence. To test this hypothesis, the effects of brief hypoxia were studied in an isolated buffer-perfused rat heart preparation. We compared hearts from hypertensive uninephrectomized Wistar-Kyoto (WKY) rats treated with deoxycorticosterone and increased salt intake with hearts from age-matched normotensive control rats treated with uninephrectomy alone. To assess changes in left ventricular diastolic pressure and relaxation in the absence of changes in left ventricular volume or the influence of right ventricular-pericardial interaction, the left ventricle contracted isovolumically with absent pericardium and vented right ventricle. Coronary flow was held constant at perfusion pressure levels chosen to produce identical flow per gram of left ventricular wet weight in both groups of animals to minimize the influence of differences in coronary vascular reserve and myocardial perfusion between hypertrophied and nonhypertrophied hearts. Thus, our study was carried out to examine and compare diastolic behavior in response to an identical hypoxic stress in hearts with and without chronic pressure-overloaded left ventricular hypertrophy.

Methods

Eight-week-old male Wistar-Kyoto rats from the Okamoto-Aoki strain were supplied by the Charles River Breeding Laboratories, and upon arrival, the rats were handled by the same person, and fed normal rat chow (Purina). Blood pressure was measured by a standard tail cuff technique (Narco Bio Systems, Inc.) on unanesthetized rats maintained quietly at 35°C for 5–10 minutes in a specially constructed chamber (Brecher et al., 1978). The preparation of the hypertensive and control groups followed methods previously described by Brecher et al. (1978). All rats were uninephrectomized during the first week after arrival and were randomized to hypertensive treatment (LVH group) or to control treatment (control group). For the LVH group, 7 days after uninephrectomy, deoxycorticosterone pivalate (Percoten, Ciba) was administered subcutaneously (1.5 mg/100 g body weight) twice weekly for a 7-week treatment period beginning 7 days after nephrectomy, and 1% saline was substituted for the drinking water. Control rats were uninephrectomized and maintained on the same diet as treated animals, but received no saline or deoxycorticosterone injections during the 7-week treatment period. Weight and tail cuff blood pressure were recorded weekly during the 7-week treatment period.

Perfusion Technique

Figure 1 illustrates the isolated isovolumic working rat heart preparation which has been previously described in detail (Fallen et al., 1967b; Apstein et al., 1977). Rats were injected intraperitoneally with 1–1.5 ml pentobarbital (15 mg/ml) and the thorax was rapidly opened. The heart was quickly removed from the thorax and placed in a water-jacketed constant temperature chamber (37.0°C). The coronary arteries were perfused retrograde by a constant flow circulating pump (Masterflex, Cole-Parmer, Inc.) through a short cannula inserted into the aortic root. Less than 15 seconds elapsed between opening the thorax and the onset of coronary perfusion in all experiments. The perfusate consisted of modified Krebs-Henseleit buffer: 118 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 0.4 mM Na2EDTA, 5.5 mM glucose, and 1.0 mM lactate. Lactic acid, which was neutralized with NaOH before being added to the buffer, was added to the perfusate so that aerobic myocardial lactate extraction could be measured. The oxygenated perfusate was equilibrated with a 5% CO2-95% O2 gas mixture such that the perfusate Po2 was approximately 550 mm Hg. The buffer for the hypoxia intervention was equilibrated with a 5% CO2-95% gas mixture and had a Po2 of 10–20 mm Hg.

After coronary perfusion was initiated, the left ventricle was immediately decompressed by an apical puncture and the insertion of a short apical drain to vent left ventricular Thesbian drainage. A polyethylene cannula was inserted via the pulmonary artery into the right ventricle. The distal main pulmonary artery and vena caval stumps were ligated to permit complete collection of coronary sinus venous drainage via the pulmonary artery cannula. A collapsed latex balloon, slightly larger than the left ventricular chamber, was inserted into the left ventricle via a left atrial incision. The balloon was filled with bubble-free saline and attached to both a Statham P23Db pressure transducer (Statham Instruments, Inc.) via a 15 cm length of polyethylene tubing, and to a high-fidelity 3F micro-manometer catheter (Millar Instruments). The high-fidelity catheter was calibrated with 100 mm Hg, and each high-fidelity recording of left ventricular pressure was matched with the pressure recording through the fluid-filled system before and after each record (Grossman et al., 1980). Coronary perfusion pressure was measured via a Y-shaped connector attached to the aortic infusion cannula. A thermistor and a bipolar pacing electrode were inserted in the right ventricle, and the heart was stimulated by a Grass model 55 stimulator (Grass Instrument Co.). Heart temperature was maintained constant (37°C).

Measurement of Mechanical Function

Pressure signals were recorded on a photographic recorder (Electronics for Medicine, Inc.). The first derivative of left ventricular pressure was obtained by electronic differentiation of the high-fidelity pressure signal. Left ventricular relaxation was estimated by the calculation of T1, the time constant of left ventricular pressure decay from the linear regression of ln P vs. t, assuming exponential pressure decay to a fixed zero asymptote (Weiss et al., 1976). The isovolumic relaxation period was defined as the time of peak −dp/dt to the time when left ventricular pressure fell to 5 mm Hg above end-diastolic pressure.
of the next beat. This approach has the advantage of simplicity, but it neglects any influences that may cause baseline shifts in pressure, such as fluctuations in intracavity pressure in the intact animal or actual changes in resting left ventricular diastolic tone or the extent of relaxation (Raff and Glantz, 1981). Therefore, the exponential time constant of left ventricular relaxation ($T_D$) was also calculated from a linear regression of $-dP/dt$ vs. $P$ using the equation, $dP/dt = -1/T(P-P_B)$ (Raff and Glantz, 1981; Eichhorn et al., 1982). $T$ calculated from the derivative of pressure is denoted as $T_D$, and is an estimation of the relaxation rate. The extrapolated baseline pressure ($P_B$), calculated at $dP/dt = 0$, represents the baseline asymptote to which pressure would exponentially fall if decay continued indefinitely, and is an estimate of the extent of relaxation (Eichhorn et al., 1982; Carroll et al., 1983). The fit of actual data points to this model was excellent such that the mean correlation coefficient was 0.994 (range 0.950–0.999).

Left ventricular diastolic chamber distensibility should ideally be evaluated from a plot of the relationship between diastolic pressure and volume over a wide range. However, in the present study, the rapid changes in diastolic and systolic function precluded serial measurements of multiple diastolic pressure-volume points. Instead, 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 used previously in similar animal models (Apstein et al., 1977; Henry et al., 1977; Serizawa et al., 1981).

Comparison of LV pressures in the hypertrophied and control hearts was done by expressing systolic and diastolic pressure per gram of LV, to estimate wall tension per unit of myocardium. Ideally, one would like to calculate systolic and diastolic wall stresses precisely. To approximate the load per unit of myocardium, we assumed a spherical LV shape for both groups of hearts; thus wall stress would be proportional to LV pressure × radius/thickness (law of Laplace). The LV volumes were comparable in both groups (see p. 656). Therefore, the radii were comparable and wall stress was proportional to thickness. Furthermore, because the LV volumes were comparable, wall thickness is directly related to LV mass. Thus, LV pressure per gram of LV becomes an approximation of wall stress under our experimental conditions.

Measurements of Coronary Resistance and Metabolism

Coronary flow (CF) was divided by left ventricular wet weight and expressed as milliliter per minute per gram of myocardium (ml/min per g). Coronary vascular resistance (CVR) was calculated as mean coronary perfusion pressure (CPP) minus left ventricular end-diastolic pressure, divided by CF, and expressed as mm Hg/[(ml/min per g)] (Bache et al., 1984b). Arterial and venous perfusate samples were collected and analyzed for lactate concentration by the specific enzymatic method of Apstein et al. (1970). Lactate samples were immediately mixed with iced 5% trichloroacetic acid and kept under refrigeration until chemical analysis. Lactate data are expressed as arterial minus coronary venous lactate concentration difference in units of mmol/g.

Experimental Protocol

Before each experiment, the heart was perfused for 30 minutes and paced at a heart rate of 4 Hz to allow performance to stabilize. During the stabilization period in the aerobic state, the left ventricular pressure-volume

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**Figure 1.** Isovolumic working rat heart perfusion apparatus. The perfusate is delivered by a constant flow pump to the coronary arteries through a cannula in the aortic stump. Either oxygenated buffer or buffer equilibrated with a 5% CO$_2$-95% gas mixture is delivered. Since the rate of coronary flow is constant, mean aortic (coronary perfusion) pressure is proportional to the coronary vascular resistance. A thin-walled latex balloon fills the left ventricular cavity and is attached to a Millar micromanometer catheter and to a fluid-filled pressure transducer. Left ventricular balloon volume determines left ventricular volume, and was held constant throughout the experiment. Left ventricular Thebesian drainage is vented by an apical drain which ensures that left ventricular volume is determined by the volume of the balloon. All coronary venous efflux is collected via a drain in the pulmonary artery. Heart rate is held constant by a right ventricular pacer, and temperature is held constant at 37°C and monitored with an intraventricular temperature probe (not shown).
relationships were determined for the hypertrophied and nonhypertrophied hearts under the conditions of coronary perfusion described below. Left ventricular balloon volume was then adjusted so that left ventricular end-diastolic pressure was 10 mm Hg under aerobic (95% O₂-5% CO₂) conditions in both the LVH and control groups, and this balloon volume was maintained unchanged throughout the subsequent experiment. At this level of left ventricular end-diastolic pressure under aerobic baseline conditions, the hearts were studied at a point on their pressure-volume curves where left ventricular balloon volume was comparable in the hypertrophied and control hearts (0.35 ± 0.06 vs. 0.39 ± 0.06 ml, NS). In the nonhypertensive control group (n = 13), coronary flow was adjusted to achieve a mean coronary perfusion pressure of 100 mm Hg under aerobic conditions and was fixed at that level of flow throughout the subsequent experiment. In the chronically hypertensive LVH group (n = 12), coronary flow was adjusted to achieve a mean coronary perfusion pressure of 150 mm Hg under aerobic conditions and was fixed at that level of flow throughout the subsequent experiment. These differing levels of coronary flow and initial coronary perfusion pressures were selected in an effort to approximate the in vivo mean coronary perfusion pressures to which the control and LVH groups were chronically exposed, and because preliminary pilot studies suggested that this approach would achieve comparable coronary flow per gram of left ventricular wet weight in both the LVH and control groups. As described in Results, this experimental goal was achieved.

Control Measurements under Aerobic Conditions

At the end of the 30-minute stabilization period, measurements of left ventricular pressure, CPP, CF, and arterial-coronary venous lactate concentration difference were made at a heart rate of 4 Hz.

Effects of Hypoxia

After baseline measurements, the coronary perfusion was switched to the Krebs-Henseleit buffer of the same composition as the control perfusate, but was equilibrated with a 5% CO₂-95%N₂ gas mixture. Recordings and measurements of left ventricular pressure and CPP were made continuously during 3 minutes of hypoxia, and arterial and coronary venous lactate samples were obtained during the final 15 seconds of the 3-minute period of hypoxia. Heart rate (4 Hz), temperature, left ventricular balloon volume, and coronary flow rate were identical during aerobic and hypoxic conditions.

Heart weight was measured after recovery under aerobic conditions for 15 minutes. The heart was removed from the perfusion cannula and its great vessels were excised, lightly blotted, and weighed to determine heart wet weight. The free wall of the right ventricle and the atria were removed, and the left ventricle was weighed to determine its wet weight. The left ventricle was then allowed to dry to constant weight, and the wet:dry weight ratio was determined.

Data Analysis

Statistical comparisons between the control group and the hypertensive LVH group under aerobic and hypoxic conditions were made by Student's t-test (Wallenstein et al., 1980). All data are reported as the mean value ± so.

Results

Baseline data characterizing the magnitude of hypertension and left ventricular hypertrophy for 13 control rats (control group) and 12 hypertensive rats (LVH group) are shown in Table 1. The in vivo systolic arterial pressure assessed by tail cuff of the LVH group was significantly greater than the control group at age 15 weeks, indicating that hypertension was successfully produced in the uninephrectomized deoxycorticosterone-saline-treated rats in comparison with age-matched control animals (Fig. 2).

All rats in the LVH group had moderate left ventricular hypertrophy relative to the control group, with the mean left ventricular wet weight increased 42% above control. Because mean body weight was lower in the LVH group, the mean left ventricular:body weight ratio was increased 69% above control (Fig. 2).

Left ventricular and coronary hemodynamic parameters during baseline aerobic conditions are shown in Table 2. At a pacing rate of 4 Hz and at an identical left ventricular end-diastolic pressure, left ventricular systolic pressure in the LVH group was 67 mm Hg (66%) higher than in the control group, and left ventricular developed pressure per unit of left ventricular mass was greater in the LVH group than the control group (134 ± 23 vs. 109 ± 22 mm Hg/g, P < 0.05). Left ventricular maximum +dP/dt/P was comparable for both groups.

By study design, coronary flow was adjusted at baseline to achieve a mean coronary perfusion pressure of 100 mm Hg (99 ± 5.7 mm Hg) in the control group, and a mean coronary perfusion pressure of 150 mm Hg (151 ± 7.6 mm Hg) in the LVH group. This resulted in a myocardial perfusion rate per gram left ventricular weight of 20 ± 5 (ml/min) per g in the control group and 18 ± 4 (ml/min) per g in the

<table>
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<th>TABLE 1</th>
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<td>Magnitude of Left Ventricular Hypertrophy (LVH) and Chronic Hypertension</td>
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<tr>
<td>Control group</td>
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<td>LVH group</td>
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SAP = mean systolic arterial tail cuff pressure averaged over 3 weeks prior to experiment; body wt = body weight; h wt = wet heart weight; LV wt = left ventricular wet weight including interventricular septum.
LVH group, \( P = \text{NS} \). At comparable levels of myocardial perfusion per gram left ventricular weight, baseline coronary vascular resistance was higher in the LVH group. Neither group had metabolic evidence of ischemia at baseline, and myocardial lactate extraction was comparable.

The rate of left ventricular relaxation was estimated by calculation of left ventricular maximum \(-\frac{dP}{dt}/P\), RT\(_V\), and TD, the relaxation time constant derived from the logarithm of pressure with exponential pressure decay to a zero asymptote, and \( T_D \), the relaxation time constant derived from the derivative of pressure with pressure decay to a non-zero asymptote, \( P_A \). The extent of left ventricular relaxation was estimated by the asymptote, \( P_B \), to which left ventricular pressure would fall if left ventricular pressure decayed indefinitely and was not influenced by diastolic myocardial blood flow. There were no differences between the LVH group and control group in any indices of either the rate or extent of left ventricular relaxation during aerobic conditions.

Figure 3 shows the response to hypoxia of a typical experimental animal from the control group and from the LVH group. The effects of 3 minutes of hypoxia at constant coronary perfusion, balloon volume, and heart rate of 4 Hz for both groups are shown in Table 2. Hypoxia resulted in the expected fall in left ventricular systolic pressure in both groups, but mean left ventricular systolic pressure continued to be significantly higher (75%) in the LVH group than in the control group. Left ventricular contractile state as assessed by left ventricular maximum \(+\frac{dP}{dt}/P\) was depressed to a comparable extent. Left ventricular developed pressure per unit of left ventricular mass during hypoxia was similar in the LVH and control groups (24 ± 7 vs. 19 ± 5 mm Hg/g; \( P = \text{NS} \)). Coronary vasodilation occurred in both groups in response to hypoxia, resulting in a fall in coronary perfusion pressure from 99 ± 5.7 to 68 ± 15.5 mm Hg in the control group and from 151 ± 7.6 to 126 ± 17.4 mm Hg in the LVH group. As in baseline conditions, during hypoxia coronary vascular resistance was higher in the LVH group, compared with the control group, but the extent of

**FIGURE 2.** Changes produced by deoxycorticosterone-saline treatment for 7 weeks in uninephrectomized Wistar-Kyoto rats (LVH, \( n = 12 \)) are compared with those in normotensive age-matched uninephrectomized control (C, \( n = 13 \)) rats. The mean left ventricular (LV):body weight ratio was 69% higher in the LVH group, compared with the control group. The magnitude of chronic pressure overload, as estimated by the systolic tailcuff blood pressures averaged over the 3-week period prior to the experiment, was significantly higher in the LVH group, compared with the control group.

**TABLE 2**

<table>
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<tr>
<th>Measurements during Aerobic Conditions and Brief Hypoxia (3 Min)</th>
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<tr>
<td>( \text{LVSP} ) +( \frac{dP}{dt}/P )</td>
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<tr>
<td>(mm Hg) (per sec)</td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td><strong>Acrobic</strong></td>
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<td>Control</td>
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<td>( P )</td>
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**LVSP** = left ventricular systolic pressure; +\( \frac{dP}{dt}/P \) = peak positive left ventricular \( \frac{dP}{dt}/P \) instantaneous pressure; \( \text{LVEDP} \) = left ventricular end-diastolic pressure; –\( \frac{dP}{dt}/P \) = peak negative left ventricular \( \frac{dP}{dt}/P \) instantaneous pressure; \( \text{RTI} \) = half time left ventricular pressure decay; \( T_D \) = time constant left ventricular pressure decay calculated by derivative method; \( P_A \) = non-zero asymptote of left ventricular pressure decay; CPP = coronary perfusion pressure; CF = coronary flow per gram left ventricular wet weight; A-V lactate = coronary arterial-venous lactate concentration difference; CVR = coronary vascular resistance; \( \Delta \text{CVR} \) = reduction in coronary vascular resistance in response to hypoxia.
coronary vasodilation induced by the hypoxia as assessed by the change in coronary vascular resistance was identical in both groups. Alternatively, a decrease in extravascular compression due to depressed contractility in response to hypoxia might have contributed to the apparent vasodilation which occurred in both groups.

However, as illustrated in Figure 4, in response to hypoxia, left ventricular end-diastolic pressure increased significantly more in the LVH group in comparison with the control group (37 ± 5 vs. 22 ± 5 mm Hg, P < 0.001). The increase in left ventricular end-diastolic pressure during hypoxia per unit of left ventricular mass was also significantly greater in the LVH group than in the control group (22 ± 5 vs. 14 ± 6 mm Hg/g, P < 0.005). The rate of rise of left ventricular end-diastolic pressure was also more rapid in the LVH group than in the control group, such that an increase of 5 mm Hg occurred in 24 ± 5 seconds in the LVH group and in 79 ± 11 seconds (P < 0.001) in the control group. Indices of the rate of left ventricular relaxation, including \(-\frac{dP}{dt}/P\), RTV/2, TL, and TD, were depressed during hypoxia, but did not differ between the LVH and control groups. The extent of left ventricular relaxation during hypoxia, as assessed by the asymptote P0, to which left ventricular pressure exponentially declined, was significantly more impaired in the LVH group than in the control group.

Both groups showed evidence of anerobic metab-
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Discussion

A decrease in left ventricular diastolic distensibility has repeatedly been observed during demand ischemia in humans (Mann et al., 1979; Grossman and Barry, 1980; Bourdillon et al., 1983), in dogs (Serizawa et al., 1980) with coronary stenoses, and in isolated blood-perfused hearts subjected to low-flow ischemia and pacing tachycardia (Isoyama et al., 1985). This decrease in left ventricular diastolic distensibility appears to be related in part to ischemia-induced changes in the rate and extent of left ventricular relaxation (McLaurin et al., 1973; Mann et al., 1979; Serizawa, 1980; Bourdillon et al., 1983; Carroll et al., 1983). Brief hypoxia during constant coronary perfusion has also been shown to cause an increase in left ventricular diastolic pressure relative to volume accompanied by marked slowing of left ventricular relaxation in buffer-perfused rabbit hearts contracting isovolumically (Serizawa et al., 1981).

An increase in left ventricular diastolic pressure during demand ischemia has also been described in patients with chronic pressure overload hypertension without coronary stenoses (Linhart, 1972; Lorell et al., 1983). Similarly, pacing-induced demand ischemia in dogs with chronic left ventricular hypertrophy without coronary stenoses also causes a marked increase in left ventricular end-diastolic pressure (Bache et al., 1984a). In patients and animals with chronic pressure overload left ventricular hypertrophy, there is evidence that ischemia may be caused by subendocardial hypoperfusion and impaired coronary vascular reserve (Fallen et al., 1967a; Trenouth et al., 1976; Vrobel, 1980; Pichard, 1981; Bertrand, 1981; Marcus et al., 1982; Bache et al., 1984a). Thus, any changes in left ventricular diastolic distensibility during ischemia in the presence of chronic pressure-overload hypertension usually have been attributed to the influence of ischemia per se (Grossman and Lorell, 1983; Bache et al., 1984a). However, to date, no study has addressed the possibility that diastolic behavior of the hypertrophied and non-hypertrophied left ventricle may differ in response to the same degree of ischemic or hypoxic stress.

In this study, we attempted to perform a direct comparison of left ventricular diastolic function during aerobic conditions and during brief hypoxia (3 minutes) in isovolumically contracting hearts from
age-matched control rats and hypertensive rats with compensatory pressure overload left ventricular hypertrophy. At identical coronary flow rates per gram of left ventricular weight, left ventricular systolic developed pressure was significantly higher in the LVH group compared with the control group during both aerobic and hypoxic conditions. During aerobic conditions at an identical left ventricular end-diastolic pressure, the LVH and control groups did not differ with respect to the rate or extent of left ventricular diastolic relaxation.

Left ventricular end-diastolic pressure at constant left ventricular volume rose progressively in both groups during hypoxia, indicating the development of decreased left ventricular diastolic distensibility. However, the LVH group showed a much higher and more rapid rise in left ventricular end-diastolic pressure at constant left ventricular volume compared with the control group. The rise in left ventricular end-diastolic pressure was also greater in the LVH group when normalized for left ventricular mass compared with the control group. This observation suggests that the greater decrease in left ventricular diastolic distensibility during hypoxia in the LVH group cannot be explained simply by the presence of more myocardial units having an additive effect on the total left ventricular diastolic pressure increase in the LVH group.

The LVH and control groups had similar depressions of the rate of relaxation during hypoxia as measured by $-\frac{dP}{dt}/P$, T, and RT%. However, the LVH group exhibited a greater upward shift of the asymptote $P_\infty$ to which left ventricular pressure decayed, suggesting a greater increase in resting diastolic tone during hypoxia in the hypertrophied hearts in comparison with controls. Thus, the identical hypoxic stress appeared to be associated with a greater loss of left ventricular diastolic distensibility and a lesser extent of relaxation in hypertrophied hearts, compared with controls, without a difference in the rate of relaxation between the two groups.

**Limitations of the Experimental Model**

Several comments are warranted regarding the model of hypertrophy, and the experimental preparation used in this study. The hypertensive model of uninephrectomized deoxycorticosterone-saline-treated rats has been shown by others to result in a rise in systolic blood pressure beginning as early as the second week of treatment, from a level of approximately 170 mm Hg to 220 mm Hg from 4 to 8 weeks of treatment (Brecher et al., 1978). Our hypertensive LVH group achieved a similar level of systolic arterial hypertension, averaging 201 mm Hg for weeks 5–7 of treatment, in contrast to a mean normotensive level of 129 mm Hg in the control rats. This level of chronic arterial hypertension resulted in a 42% increase in mean wet left ventricular weight and a 69% increase in mean left ventricular:body weight ratio. Mean body weight was lower in the LVH group than in the controls at the end of the treatment period. This degree of left ventricular hypertrophy is less than that which can be achieved in the spontaneous hypertensive rat (SHR) model (Brecher et al., 1978). However, it is comparable to the degree of hypertrophy achieved in the animal model of aortic banding recently reported by Bache et al. (1984a) in which there was also a tendency for mean body weight to be lower than in controls, and in which a striking rise in left ventricular diastolic pressure was noted during ischemia.

There are also limitations in the use of the crystalloid-perfused rat heart preparation as a model of hypoxia. Although ischemia and hypoxia-induced changes in diastolic tone have been detected in many mammalian species, rat myocardium may function at near-maximally saturated myocardial calcium levels (Nayler et al., 1975), and it appears to be particularly sensitive to the development of hypoxia-induced contracture relative to other species (Greene and Weisfeldt, 1977).

In addition, the extent of filling of the coronary reservoir and its contribution to cardiac muscle turgor were potentially important factors in our study, since all buffer-perfused heart preparations required a rate of coronary flow in excess of that required in vivo, and because coronary perfusion pressure usually exceeded both systolic and diastolic pressure in this preparation during hypoxia and constant coronary flow. Vogel et al. (1982) and Serizawa et al. (1981) have previously demonstrated an "erectile" or "hydraulic effect" of the coronary vasculature on diastolic properties in both buffer and blood-perfused isovolumically contracting rabbit hearts. The levels of coronary flow chosen in this experiment resulted in comparable flow per gram of left ventricular weight in both groups during aerobic and hypoxic conditions, associated with a higher coronary vascular resistance in the LVH group. However, because CPP was significantly higher in the LVH group than in the controls during hypoxia, we cannot exclude a greater turgor contribution to the hypoxia-induced decrease in diastolic distensibility in the LVH group. The possible contribution of differing degrees of hypoxia-induced edema in the LVH and control groups was also considered. There was no difference in the left ventricular wet:dry weight ratios at the conclusion of the experiment following reoxygenation between the hypertrophied and control hearts.

There are several other mechanisms which might account for the differences in the diastolic response to hypoxia seen in hypertrophied and control hearts in our study. Abnormalities of diastolic relaxation may be related to the presence of chronic ischemic damage and/or fibrosis (Bailey et al., 1977; Lund et al., 1979; Yonekura et al., 1985) or to systolic myocardial failure which may supervene during advanced cardiac hypertrophy (Sordahl et al., 1973; Krayenbuehl et al., 1983; Grossman and Lorell, 1983). It is unlikely that the differences in diastolic
behavior seen during hypoxia in the two groups in this study were related to overt end-stage heart failure or chronic ischemia in the hypertensive rats. First, the marked elevation in systolic tail cuff pressure was stable without decompensation prior to the experiments in the LVH group. Second, in the isovolumetrically contracting hearts at a normal physiological LVEDP of 10–11 mm Hg, the LVH group generated a mean left ventricular systolic pressure similar to that measured in vivo, and 66% higher than the controls. Third, there were no differences in relaxation indices in the baseline aerobic condition between the LVH and control groups. Fourth, there was no difference in baseline lactate extraction between the two groups. These considerations suggest that the exaggerated decrease in left ventricular diastolic distensibility noted in the LVH group during hypoxia is not likely to result from overt end-stage myocardial failure or chronic ischemic damage.

**Influences of Load on Relaxation**

The effect of load per se on the rate and extent of relaxation in the control and hypertrophied hearts should be considered. Diastolic relaxation in both isolated cardiac muscle and the intact heart is currently hypothesized to be controlled by the complex interaction of (1) load, (2) inactivation of force-generating sites, and (3) any spatial or temporal nonuniformity of the distribution of load and inactivation (LeCarpentier et al., 1979; Housmans et al., 1983; Brutsaert et al., 1984). The imposition of a greater systolic load throughout the period of tension development in the intact heart would be expected to modify the time course of relaxation (Raff and Glantz, 1981; LeCarpentier et al., 1982; Housmans et al., 1983; Brutsaert et al., 1984). Although left ventricular systolic pressure clearly differed in the control and LVH groups in our experiments, the observation that both the rate and extent of relaxation were identical in the hypertrophied and control hearts during the pre-hypoxic well-oxygenated baseline stage indirectly suggests that “after-load mismatch” with excessively high systolic wall stress was not present in the LVH group relative to the controls during the aerobic experimental condition. Furthermore, in the isovolumically contracting hearts in this experiment, the influence of pericardial/right ventricular constraints (Ross, 1979) and impedance changes during ejection and viscoelastic factors during early diastolic ventricular filling were clearly not operative.

**Differences in Force Inactivation**

In isolated heart muscle experiments (Chuck et al., 1981), myocardial muscle relaxation during hypoxia appears to be load independent, and appears to be related primarily to differences in force inactivation itself; that is, the processes influencing the dissipation of force-generating crossbridge attachment (Chuck et al., 1981; LeCarpentier et al., 1982; Brutsaert et al., 1984). The biochemical processes that influence the rate and extent of force inactivation and final resting cardiac tone are incompletely understood, but appear to depend in part on the ATP-dependent rate and capacity of calcium sequestration by the sarcoplasmic reticulum (Nayler et al., 1979; Lewis et al., 1979; LeCarpentier et al., 1979, 1982; Grossman and Barry, 1980; Brutsaert et al., 1984). In our experiments, the extent of high energy phosphate depletion during hypoxia was probably comparable in the LVH and control groups as indirectly assessed by the comparable degrees of myocardial lactate production (i.e., glycolytic flux, which is the sole source of ATP synthesis during hypoxia) and the comparable degrees of contractile work done during hypoxia (i.e., developed pressure per unit left ventricular mass), which reflects the amount of ATP utilization. Nonetheless, it would be of great interest to measure directly intracellular high energy phosphates and intracellular pH during hypoxia, which was not done in these experiments.

In our experiments, the rate of relaxation during hypoxia appeared to be slowed to a comparable degree in the hypertrophied and control hearts, whereas the extent of relaxation appeared to be depressed to a greater degree in the hypertrophied hearts. Prior observations that caffeine modulates the development of ischemic or hypoxic contracture (Lewis et al., 1979; Paulus et al., 1982) may lend insight into our findings. Paulus et al. (1982) showed that caffeine enhances the demand ischemia-induced rise in left ventricular diastolic pressure relative to volume in dogs with coronary stenoses, with a marked dissociation between the behavior of the rate and extent of myocardial relaxation similar to that seen in our experiments. The addition of caffeine during demand ischemia resulted in a doubling of left ventricular diastolic pressure during ischemia and a further upward shift in the diastolic pressure-volume relation with no further change in the rate of relaxation. They postulated that this apparent dissociation between the rate and extent of relaxation by caffeine may be related to saturation of the capacity of the sarcoplasmic reticulum such that excess calcium availability results in a persistent incomplete inactivation of crossbridges, which imposes an “active stiffness” on cardiac muscle throughout diastole.

In our experiments, differences between the hypertrophied and nonhypertrophied hearts in myocardial calcium availability for diastolic crossbridge interaction could account for the greater reduction in left ventricular diastolic distensibility and extent of relaxation observed in hypertrophied hearts relative to controls during hypoxia. In this regard, there is evidence that pressure-overloaded hypertrophied cardiac muscle may have intrinsic changes in the duration of the active state and intracellular calcium availability. Alpert and Mulieri (1983) have examined the thermomechanical properties of control and chronically hypertrophied rabbit hearts with pul-
monary artery banding. Their results suggest that compensatory pressure overload hypertrophy is associated with the appearance of a slower rate of crossbridge cycling but a longer duration of crossbridge force generation relative to controls. Similarly, Morgan and coworkers (Morgan and Morgan, 1984; Gwathmey and Morgan, 1985) have studied cardiac hypertrophy both in humans and in a ferret model of chronic pressure-overloaded hypertrophy using the aequorin injection technique, and observed a significant prolongation in the time course of both tension development and relaxation, and a prolonged time course of the intracellular calcium transient. These adaptations may reflect changes in both myosin isozymes (Alpert and Mulieri, 1983) and sarcoplasmic reticulum function (LeCarpentier et al., 1982) during development of hypertrophy. Such changes may reflect a salutary adaptation of hypertrophied muscle which permits the development of high systolic pressure with a lower rate of pressure development (dP/dt) resulting in maximum metabolic efficiency during aerobic conditions (Alpert and Mulieri, 1983; Swynghedauw et al., 1984). However, we hypothesize that these adaptations may make hypertrophied myocardium more sensitive to the development of an impaired extent of diastolic crossbridge inactivation with an increase in diastolic tone during interventions such as hypoxia or ischemia which reduce the calcium-binding capacity of the sarcoplasmic reticulum. To address this hypothesis directly, it will be necessary to have the capability to measure directly rapid changes in intracellular calcium in nonhypertrophied and hypertrophied cardiac muscle under aerobic and hypoxic conditions.

In summary, brief hypoxia results in a greater reduction in left ventricular diastolic distensibility in isovolumically contracting hypertrophied rat hearts than in nonhypertrophied controls. Although the mechanisms responsible for this observation are not known, it is not likely to be due to either differences in left ventricular mass per se, systolic contractile state, or systolic loading. Our observations may be explained by a difference in the coronary turor contribution to diastolic stiffness between the two groups. Alternatively, the rise in left ventricular diastolic pressure observed with pressure overload hypertrophy during an ischemic or hypoxic stress may be related in part to a greater impairment of diastolic muscle inactivation relative to high energy phosphate depletion than is seen in the absence of hypertrophy.

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