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Effect of Hypertension and Hypertension–Glucose Intolerance on Myocardial Ischemic Injury

Mahmood S. Mozaffari, Stephen W. Schaffer

Abstract—Systemic hypertension and type 2 diabetes mellitus are major risk factors for myocardial infarction. Yet, glucose intolerance, a prelude stage to type 2 diabetes, is associated with reduced infarct size. Since chronic hypertension adversely affects the myocardium, we tested the hypothesis that the coexistence of systemic hypertension and glucose intolerance reverses the cardioprotection associated with impaired glucose tolerance. Hearts from 9-month-old animals were subjected to a 40-minute occlusion of the left coronary artery followed by 2 hours of reperfusion. Before ischemia, similar values for the four experimental groups were observed for the coronary flow, heart rate, and maximum ventricular pressure. During the course of the ischemia-reperfusion insult, the two hypertensive groups displayed greater reductions in contractility than their normotensive counterparts. Infarct size was lower in the normotensive glucose-intolerant rat than in the normotensive control rat. Surprisingly, the hypertrophied hearts of the hypertensive and hypertensive glucose-intolerant rats displayed reduced infarct size ($P < 0.05$). However, raising the afterload pressure from 100 to 160 cm H₂O increased infarct size in the two hypertensive groups. This narrowed the differential between the hypertensive glucose-intolerant (160 cm H₂O) and the normotensive control (100 cm H₂O) rats. Nonetheless, at the higher afterload pressure, infarct size was less in the hypertensive glucose-intolerant rats than in their hypertensive counterparts. In conclusion, the impairment in contractile function despite the reduction in infarct size underscores the increased susceptibility of the hypertrophied, hypertensive heart to ischemic injury. Furthermore, exacerbation of cell death at elevated afterload pressure indicates the potential benefit of aggressive antihypertensive therapy. (*Hypertension*. 2003;42:1042-1049.)

Key Words: hypertension, chronic ■ glucose ■ myocardial infarction ■ myocardial contraction

Cardiovascular disease is a major cause of death among diabetic patients. Although this elevated mortality rate has been largely attributed to an accelerated rate of atherosclerosis, diabetic patients have a 2-fold higher risk of dying of a myocardial infarction than their nondiabetic counterparts. While recurring myocardial infarction and enhanced susceptibility to arrhythmias are potential candidates for the observed clinical results, epidemiologists have concluded that the dominant cause for the elevated mortality rate is the development of congestive heart failure.¹ Since the diabetic patient is also at risk of developing congestive heart failure independent of coronary artery disease,² the existence of a preischemic diabetic cardiomyopathy could contribute to the elevated mortality rate. Other likely factors contributing to the high mortality rate are abnormal ventricular remodeling and greater propensity for ischemia-reperfusion injury.

The impact of diabetes on myocardial ischemia-reperfusion injury has been the focus of numerous animal studies. However, as discussed by Feuvray and Lopaschuk³ and Paulson,⁴ there has been considerable debate regarding the sensitivity of the diabetic heart to ischemic injury.

Nonetheless, it is generally found that the ischemic heart from the mildly diabetic animal resists the accumulation of calcium, a major cause of necrosis.³ On the other hand, the severely diabetic heart is more susceptible to ischemic injury because of enhanced oxidative stress and eicosanoid-mediated injury, the accumulation of undesirable metabolic products, vascular dysfunction, and the impairment in glycolytic ATP generation.^{3,4} Moreover, Maddaford et al⁵ have found that with advancing age, the insulin-resistant, obese JCR:LA-cp rat shows increased susceptibility to ischemia, presumably because of augmented glycolysis. Although the diverse experimental results have been attributed to the competing effects of diabetes, the impact of coexisting disorders has received less attention.

A common coexisting disorder in patients with diabetes mellitus is systemic hypertension, which can be manifested either before or after the onset of type 2 diabetes. A number of pathogenic mechanisms could cause the aggravation of ischemic injury by the superimposition of hypertension on type 2 diabetes. First, the coexistence of the two diseases leads to cardiac hypertrophy, a condition that shows enhanced

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Key Features of the Experimental Groups

Group	Body Weight, g	Ventricular Weight, g	Ventricular Weight/Body Weight, mg/g	Blood Glucose, mg/dL	
				Fasting	2-h Postglucose Challenge
Normotensive					
Control (n=8)	468±5	1.80±0.04	3.85±0.09	75.1±1.9	124.6±7.8
Glucose intolerant (n=5)	401±11*	1.60±0.06	4.01±0.17	96.2±5.2*	385.6±32.3*
Hypertensive					
Control (n=6)	438±7	2.27±0.08†	5.17±0.15†	73.0±2.8	146.5±12.5
Glucose intolerant (n=7)	402±6*	2.12±0.08†	5.27±0.20†	94.9±7.9*	267.4±20.3*

* $P < 0.05$ compared with their control counterparts.

† $P < 0.05$ compared with their normotensive counterparts.

susceptibility to ischemic damage. This is due in part to underperfusion of the subendocardial layers of the left ventricle when the coronary circulation is restored after an ischemic episode.⁶ Second, systemic hypertension is associated with activation of neurohumoral mechanisms that are capable of exacerbating myocardial injury after an ischemia-reperfusion insult.^{7–9} Third, the severity of the diabetic cardiomyopathy worsens when hypertension and diabetes coexist.^{10–12} Therefore, we tested the hypothesis that chronic systemic hypertension worsens ischemia-reperfusion injury, reversing the cardioprotection associated with impaired glucose tolerance, a prelude stage to overt type 2 diabetes mellitus.

Methods

Wistar-Kyoto rats, obtained from Harlan Laboratories (Indianapolis, Ind), were bred at the Medical College of Georgia animal facility. To produce impaired glucose tolerance, 3-day-old male neonatal rats were injected intraperitoneally with 90 mg/kg of streptozotocin; control littermates received an injection of citrate buffer (0.1 mol/L; pH 4.5).^{13–18} The animals were weaned at 21 days of age and were maintained at constant humidity (60±5%), temperature (24±1°C), and light cycle (6 AM to 6 PM). Unless otherwise specified, the animals had free access to food and water.

At 4 weeks of age, the streptozotocin-injected and control rats underwent a right nephrectomy during pentobarbital anesthesia (30 mg/kg IP). After a period of 2 weeks to allow for compensatory renal hypertrophy, impaired glucose tolerance was confirmed by the administration of a glucose tolerance test; the test was repeated just before initiating the ischemia-reperfusion protocol at ≈9 months of age. In the glucose tolerance test, blood glucose concentration was measured before and after administration of a glucose load (2 g/kg IP) to a fasted rat.^{13,14,18} Thereafter (ie, 6 weeks of age), the animals were further subdivided and either remained on the basal NaCl diet or were switched to a diet that contained 8% NaCl. We have shown that the combination of unilateral nephrectomy and a high NaCl diet causes significant hypertension in an otherwise normotensive rat.^{13,14,19} Before the rats were killed, tail-cuff blood pressure was measured on 2 consecutive days and the average of the 2 readings recorded for each rat (IITC Inc/Life Science Instruments). The protocol outlined above produced 4 experimental groups with the following designations: normotensive control (n=8), normotensive glucose-intolerant (n=5), hypertensive control (n=6), and hypertensive glucose-intolerant (n=7).

Ischemia-reperfusion injury was determined in 9-month-old animals from each of the 4 experimental groups. After heparinization (1000 U/kg IP, 30 minutes) and decapitation, the hearts were quickly removed and perfused on a Langendorff apparatus with Krebs-Henseleit buffer (37°C) containing 118 mmol/L NaCl, 27.1 mmol/L NaHCO₃, 2.8 mmol/L KCl, 1 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄,

2.5 mmol/L CaCl₂, and 11 mmol/L glucose and equilibrated with 95% O₂–5% CO₂; the afterload pressure was set at 100 cm H₂O (≈67 mm Hg). In additional experiments, hearts from hypertensive (n=7) and hypertensive glucose-intolerant (n=7) rats were perfused at an afterload pressure of 160 cm H₂O (≈107 mm Hg). Each heart was stabilized for 25 minutes, during which time a 2–0 silk suture was loosely placed around the left main coronary artery and passed through a small vinyl tubing to form a snare.¹⁶ Two coronary effluent samples were collected at 19- to 20- and 24- to 25- minute intervals; the average of these samples determined the coronary flow rate before induction of regional ischemia. After the stabilization period, coronary occlusion was affected by pulling the suture through the snare with the use of a customized vascular clamp. The desired period of coronary artery occlusion (40 minutes) was followed by reperfusion for 2 hours. The experiment was terminated by retightening the snare and infusing a 0.5% solution of 1 to 10 μm zinc-cadmium fluorescent particles into the aorta (Duke Scientific). The fluorescent particles were able to delineate the risk zone, which was observed with a 366-nm fluorescent lamp.¹⁶ After administration of the fluorescent particles, each heart was removed from the perfusion apparatus, the atria and great vessels were removed, and the ventricles were weighed.

The hearts were then frozen at least overnight before being cut into 2-mm-thick slices with the use of a heart matrix and a sharp blade. The slices were then incubated for a period of 15 minutes at room temperature in a solution of 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4).¹⁶ The stained slices were stabilized on a glass plate and covered with clear cellulose paper. The area at risk (delineated as nonfluorescent zones) and the infarcted area (lacking staining with tetrazolium) were determined by computerized planimetry. The volumes of the risk and infarcted zones were determined by multiplying the area by the slice thickness. The infarct size was expressed as a percentage of the total risk zone that was infarcted. At specific intervals during the ischemia-reperfusion protocol, the coronary flow rate was measured. Cardiac function was measured with a pressure transducer by inserting a 23-gauge needle through the ventricular wall. The pressure transducer was connected to a computerized heart performance analyzer (MicroMed).

All data are expressed as mean±SEM. Variables that were measured sequentially were analyzed by repeated-measures ANOVA. Furthermore, data were tested for group differences by 1-way ANOVA. Given the significance in ANOVA ($P < 0.05$), means were compared by means of the Duncan post hoc test.

Results

Fasting blood glucose concentration was moderately elevated in the streptozotocin-treated rats (Table; $P < 0.05$). Nonetheless, the streptozotocin-treated rats were severely glucose-intolerant; 2 hours after an intraperitoneal injection of a glucose load, the blood glucose concentration remained significantly elevated in the two streptozotocin-treated groups

(Table). Interestingly, the normotensive glucose-intolerant rats displayed a greater elevation in blood glucose levels after the glucose challenge than did the hypertensive rats. As reported previously, in contrast to the severe hyperglycemic condition that develops in adult rats administered streptozotocin (a type 1 diabetic model), the neonatal streptozotocin model mimics either type 2 diabetes or glucose intolerance.¹³⁻¹⁸

As shown previously, the animals that were treated with the combination of unilateral nephrectomy and dietary NaCl excess had a significant elevation in blood pressure ($P < 0.05$). The systolic blood pressure was 191 ± 6 and 201 ± 3 mm Hg in the hypertensive and hypertensive glucose-intolerant rats, respectively; the corresponding values for their normotensive counterparts were 155 ± 5 and 154 ± 7 mm Hg, respectively. It is noteworthy that an early event in the development of hypertension in this animal model was the increase in systolic pressure.^{14,19} As the animal aged, diastolic blood pressure increased, resulting in a significant elevation in mean arterial pressure.^{13,19} As a result, both the mean arterial pressure and the diastolic blood pressure were significantly higher in the hypertensive (28 and 21 mm Hg) and hypertensive-glucose intolerant (39 and 32 mm Hg) groups, compared with their normotensive counterparts. It is noteworthy that whereas the hypertensive glucose-intolerant group generally displayed higher blood pressure than the hypertensive group, the differential did not reach statistical significance. Heart rate was similar among the two normotensive and the hypertensive glucose-intolerant groups (342 to 362 bpm). However, the hypertensive control group displayed a lower heart rate than the other 3 groups (≈ 30 to 45 bpm; $P < 0.05$).

Body weight was significantly lower in the two glucose-intolerant groups than the normotensive control group (Table). As indicated by the significant increase in both absolute ventricular weight and the ratio of ventricular weight to body weight, systemic hypertension was associated with the development of ventricular hypertrophy (Table). Indeed, in comparison to their normotensive counterparts, the increase in the heart weight to body weight ratio was similar ($\approx 30\%$) in the hypertensive and hypertensive glucose-intolerant rats.

The baseline rates of coronary flow tended to be lower in the two hypertensive groups (4.8 ± 0.4 versus 5.0 ± 0.3 mL/min per gram ventricular wt; hypertensive and hypertensive glucose-intolerant, respectively) than their normotensive counterparts (5.4 ± 0.2 versus 5.6 ± 0.9 mL/min per gram ventricular wt). Occlusion of the left main coronary artery led to a dramatic decrease in coronary flow in all 4 groups of hearts (range, $\approx 57\%$ to 72% by the end of 40 minutes of ischemia). The release of the ligature was associated with a transient elevation in the coronary flow rate ($\approx 10\%$), with the increase in flow seen as early as 2 to 4 minutes of reperfusion, and was followed by a progressive decline in coronary circulation. By the end of the 2-hour reperfusion period, the coronary flow rate was 15% to 18% of baseline values in the 4 experimental groups.

The hypertensive hearts exhibited an increase in the area at risk after 40 minutes of ischemia and 2 hours of reperfusion at an afterload pressure of 100 cm H₂O. However, this

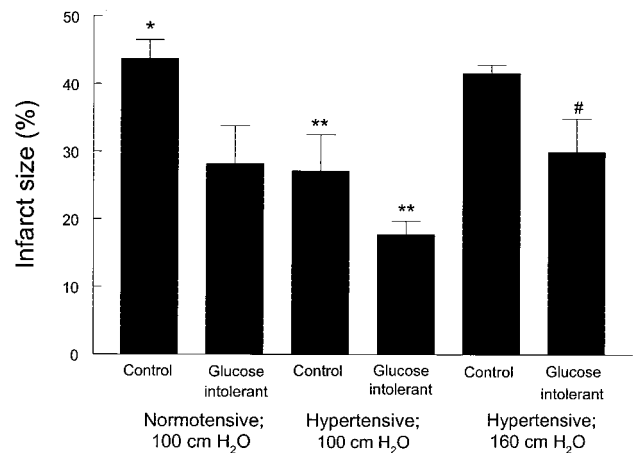


Figure 1. Effect of hypertension and glucose intolerance on infarct size. Hearts were perfused in Langendorff mode at afterload pressure of either 100 cm H₂O (n=5 to 8 rats per group) or 160 cm H₂O (n=7 rats per group). Infarct size is expressed as percentage of infarct zone to risk zone. Data are mean \pm SEM. * $P < 0.05$ compared with the other 3 groups at similar afterload pressure. ** $P < 0.05$ compared with counterparts at the higher afterload pressure. # $P < 0.05$ compared with hypertensive control group at a similar afterload pressure.

difference was only significant when comparing the normotensive glucose-intolerant rats with the two hypertensive groups (data not shown). The infarcted zone was greater in the normotensive control group compared with the other 3 groups, with the differential being significant among the normotensive control and both the normotensive glucose-intolerant and the hypertensive glucose-intolerant groups (data not shown). Surprisingly, at an afterload pressure of 100 cm H₂O, the infarct size of both the hypertensive heart and the normotensive glucose-intolerant heart was significantly reduced relative to that of the normotensive control heart (Figure 1). Whereas both glucose intolerance and hypertension were associated with reduced infarct size, the difference in infarct size among the hypertensive glucose-intolerant heart and either the normotensive glucose-intolerant heart or the hypertensive control heart did not achieve statistical significance (Figure 1).

The observed decrease in infarct size in the hypertensive heart was unexpected. Reasoning that this effect might relate to the low afterload pressure of the perfused heart, infarct size was reevaluated in two additional hypertensive groups at an afterload pressure of 160 cm H₂O. As shown in Figure 1, infarct size of the hypertensive and hypertensive glucose-intolerant rats increased at the higher afterload pressure. As a result, the differential seen between the hypertensive control and the normotensive control group was eliminated. Nonetheless, even at the higher afterload pressure, the hypertensive glucose-intolerant group still displayed a smaller infarct size than its hypertensive counterpart.

Myocardial ischemia is associated with the generation of a significant elevation in the number of irregular beats. To monitor these abnormalities, the number of contractions per minute was determined (Figure 2). In the two normotensive groups, ischemia was associated with a decrease in the number of contractions. Surprisingly, reperfusion did not

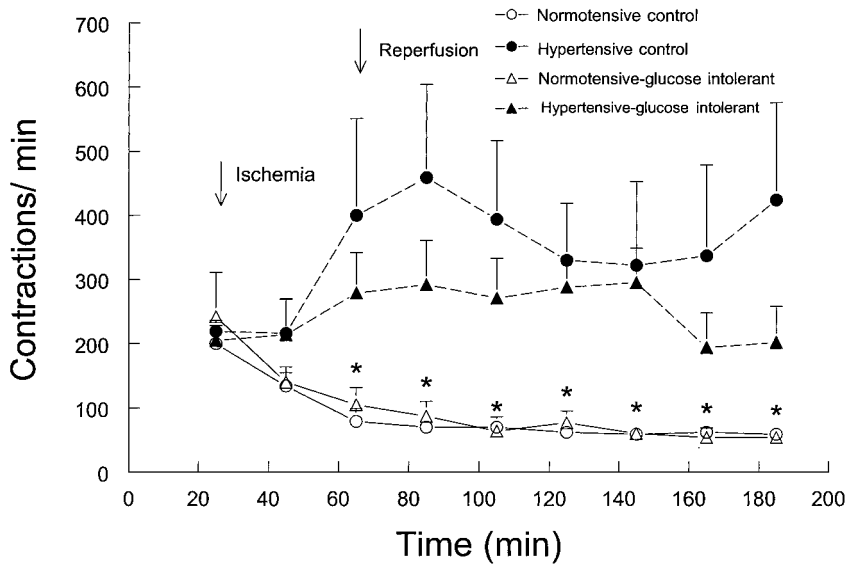


Figure 2. Effect of hypertension and glucose intolerance on contraction frequency. Hearts were perfused in Langendorff mode at afterload of 100 cm H₂O. Contraction frequency was monitored from a pressure transducer connected to a computerized heart performance analyzer. Periods of ischemia and reperfusion are indicated by arrows. Data are mean \pm SEM of $n=5$ to 8 rats per group. * $P < 0.05$ compared with hypertensive counterparts.

increase the frequency of beating in the two normotensive groups. However, the pattern was very different in the hearts from the two hypertensive groups. The number of contractions per minute increased shortly after the onset of ischemia, with the increase in the number of irregular beats remaining throughout the ischemic and reperfusion periods in the two hypertensive groups (Figure 2).

Another defect that develops in the ischemic heart is impaired diastolic function. This defect was most prominent in the two hypertensive groups. As seen in Figure 3, end-diastolic pressure increased after the onset of ischemia and remained elevated throughout the reperfusion period. Because of the development of contracture during the latter part of the ischemic period, end-diastolic pressure values approached those of the maximum ventricular pressure of the hypertensive groups.

Although infarct size was reduced in the two hypertensive groups, both $+dP/dt$ and $-dP/dt$ declined more after the ischemia-reperfusion insult in the two hypertensive groups

than in their normotensive counterparts (Figure 4). During the early part of the reperfusion period, significant differences in $+dP/dt$ were observed among the two hypertensive and two normotensive groups. However, $+dP/dt$ increased slightly during the last 40 minutes of reperfusion in the hypertensive groups, narrowing the differences between the normotensive and hypertensive groups. The hypertensive control group showed slightly higher $-dP/dt$ values than the normotensive control during the early phase of ischemia; however, this difference also disappeared by 40 minutes of ischemia. The abnormalities in heart rate affected the contractility and relaxation patterns of the ischemic and reperfused hearts. Clearly, high rates of contractility and relaxation are difficult to maintain in the face of severe dysrhythmias.

A characteristic feature of the type 1 diabetic, hypertensive rat is impaired contractile function. However, defects in contractility ($+dP/dt$) and relaxation ($-dP/dt$) of the diabetic heart have generally been uncovered only after either a dramatic rise in filling pressure or occlusion of the aor-

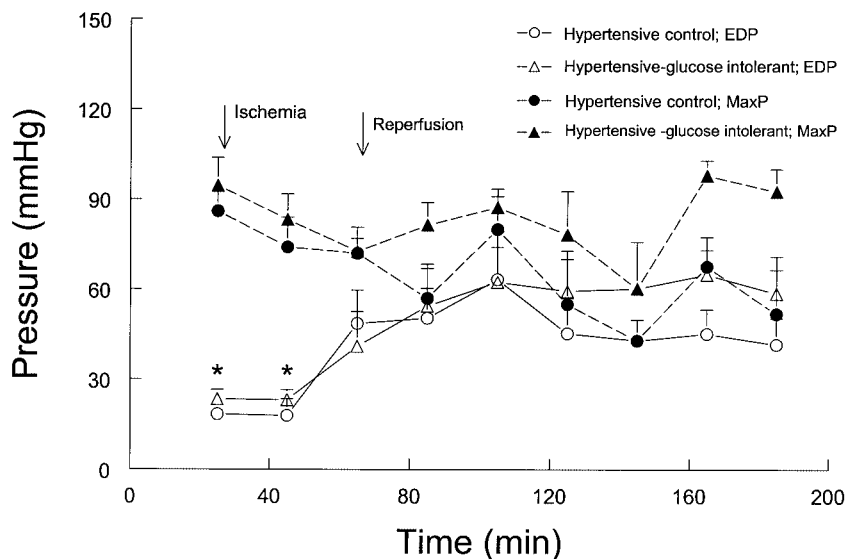


Figure 3. Effect of hypertension on diastolic function. Hearts from hypertensive and hypertensive glucose-intolerant rats were perfused in Langendorff mode at afterload of 100 cm H₂O. End-diastolic pressure (EDP) and maximum ventricular pressure (MaxP) were monitored as in Figure 2. Periods of ischemia and reperfusion are indicated by arrows. Data are mean \pm SEM of $n=5$ to 8 rats per group. * $P < 0.05$ compared with MaxP values.

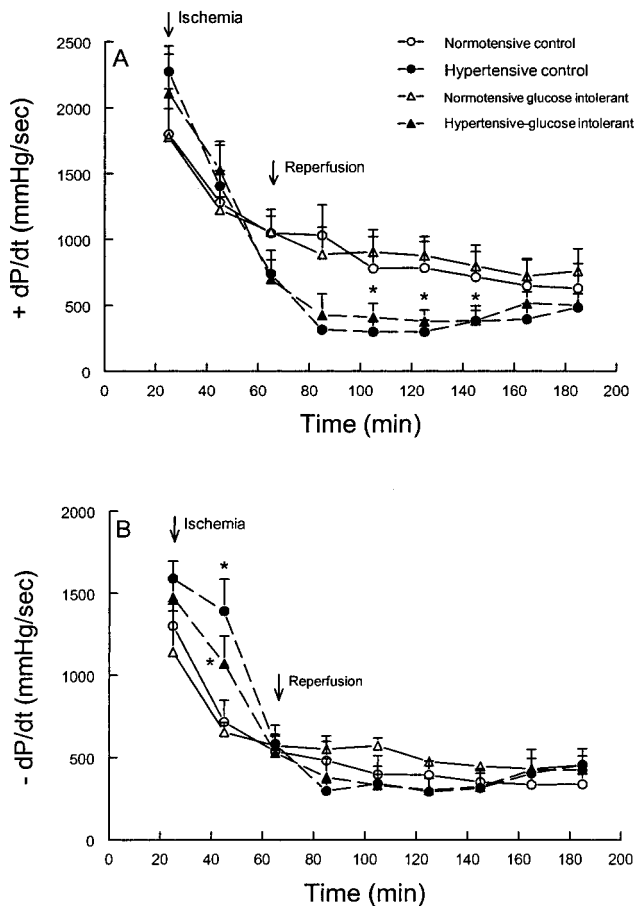


Figure 4. Effect of hypertension and glucose intolerance on myocardial contractility (+dP/dt, A) and relaxation (-dP/dt, B). Hearts from 4 experimental groups were perfused in Langendorff mode at afterload pressure of 100 cm H₂O. Mechanical performance was monitored as in Figure 2. Periods of ischemia and reperfusion are indicated by arrows. Data are mean \pm SEM of $n=5$ to 8 rats per group. * $P<0.05$ compared with normotensive counterparts.

ta.^{10,11,20} In the present study, an isolated perfused Langendorff preparation was used, which did not allow a change in filling pressure. Nonetheless, the baseline values for +dP/dt and -dP/dt tended to be larger, albeit not significantly so, in the two hypertensive groups than in their normotensive counterparts ($\approx 19\%$ to 27% ; Figure 4), an observation consistent with the study of Mathis et al,²¹ using the isolated working heart.

Discussion

The rise in blood pressure that generally accompanies diabetes is frequently salt-sensitive; therefore, the NaCl-induced hypertensive glucose-intolerant rat used in this study represents a rational animal model to investigate cardiac defects. Commonly used techniques were implemented to produce the models of glucose intolerance and hypertension. Streptozotocin was administered to Wistar-Kyoto neonates at 3 days of age, a condition that resulted in a more severe case of glucose intolerance than was produced when streptozotocin was administered at 2 days of age.¹⁸ A right nephrectomy was performed at 4 weeks of age, with high-salt treatment

beginning at 6 weeks of age. We have shown that these rats have a significant elevation in blood pressure as early as 3 months of age.^{13,14,19} Both the hypertensive and the hypertensive glucose-intolerant animals displayed significant myocardial hypertrophy by 9 months of age. Nonetheless, contractile function was not significantly affected in any of the experimental groups. The trend toward higher values of +dP/dt and -dP/dt in the two hypertensive groups was consistent with the study by Mathis et al,²¹ who showed a similar pattern in the spontaneously hypertensive rat. Particularly noteworthy was the observation that the heart of the normotensive glucose-intolerant rat perfused in a Langendorff mode exhibited no change in contractile function relative to the normotensive control. This contrasts with the 1-year-old Wistar rat, which had been given streptozotocin as a neonate. The hearts of these older streptozotocin-treated Wistar rats perfused on a working heart apparatus showed reductions in +dP/dt and -dP/dt, thereby indicating the development of a cardiomyopathy.¹⁷ Thus, these differences may be related to the perfusion technique used, the age of the animal, or the strain, as the Wistar rat is more sensitive to streptozotocin than the Wistar-Kyoto rat.¹⁵

Despite differences in contractile function between the streptozotocin-treated Wistar and Wistar-Kyoto rats, both models render the heart resistant to ischemic injury. This is not surprising because there is a plethora of studies showing that the mildly diabetic heart is also resistant to ischemic injury.³ The mechanism most widely implicated in diabetes-mediated cardioprotection is the reduction in flux through the Na⁺/H⁺ exchanger.³ In the nondiabetic heart, pH_i is significantly reduced during ischemia, but on reperfusion, an exchange occurs between intracellular H⁺ and extracellular Na⁺, leading to an increase in [Na⁺]_i. However, in the diabetic heart, reduced Na⁺/H⁺ exchanger activity, coupled with impaired H⁺ generation from glycolysis, interferes with the accumulation of Na⁺ during reperfusion. Consequently, less Ca²⁺ enters the reperfused, diabetic heart through the Na⁺/Ca²⁺ exchanger. This limits the degree of Ca²⁺ overload, a major cause of ischemic injury.²² A similar mechanism limits hypoxia-induced necrosis in isolated cardiomyocytes pretreated with 25 mmol/L glucose to produce a diabetes-like phenotype.²³

One of the important findings of this study is that mechanical function of the reperfused hypertensive heart is severely impaired. In light of clinical findings, one would have predicted that systemic hypertension would worsen ischemic injury. Indeed, several investigators have reported that post-ischemic recovery of developed pressure, dP/dt, cardiac output, and end-diastolic pressure is worse in the spontaneously hypertensive rat than in the Wistar-Kyoto rat.^{24,25} According to Leenen and Yuan,²⁶ the spontaneously hypertensive rat displays an accelerated mortality rate after occlusion of the coronary artery when compared with the Wistar-Kyoto rat. Moreover, recovery of end-diastolic pressure and cardiac output after 30 minutes of global ischemia was less in the stroke-prone spontaneously hypertensive rat than in the Wistar-Kyoto control.⁹

The surprising observation is that the poor recovery in contractile function was not reflected in the infarct size of the

hypertensive heart. Very few studies have examined the influence of systemic hypertension on infarct size. Koyanagi et al²⁷ found that chronic hypertension in dogs increased infarct size after a 48-hour occlusion of the circumflex coronary artery. Since hypertension was associated with cardiac hypertrophy in their study, it was suggested that perfusion abnormalities associated with the accompanying hypertrophy may have contributed to the larger infarct size. Indeed, Snoeckx et al⁶ showed enhanced sensitivity to ischemia in hearts made hypertrophic by aortic banding. The importance of hypertrophy in ischemic injury was also supported by the work of Bolli et al,²⁸ who found that acute hypertension induced by phenylephrine did not alter either myocyte size or infarct size. Nonetheless, in conflict with the hypertrophic hypothesis, Inou et al²⁹ found that the normalization of blood pressure with nitroprusside prevented infarct enlargement in the hypertensive, hypertrophic heart. Similarly, Speechly-Dick et al³⁰ maintained that the decrease in blood pressure that accompanied their ischemic protocol lowered the vulnerability of the hypertensive, hypertrophic heart to the ischemic insult. The present study provides further support for the notion that afterload pressure is an important determinant of infarct size in the hypertensive, hypertrophied heart. Figure 1 shows that hypertensive hearts perfused at an afterload pressure of 100 cm H₂O exhibited smaller infarct than hearts from the same group that were perfused at an afterload pressure of 160 cm H₂O. Clearly, further studies examining the contributions made by elevated arterial pressure and by cardiac hypertrophy toward infarct size of the hypertensive heart are warranted.

Although our data showed the adverse effect of elevated afterload on infarct size, it did not address the basis for the uncoupling between postischemic mechanical function and infarct size. In agreement with our data, Speechly-Dick et al³⁰ observed that postischemic mechanical function and infarct size were uncoupled in the deoxycorticosterone-acetate salt hypertensive model. Whereas Joyeux et al³¹ found no statistical difference in the recovery of mechanical function and infarct size between control and transgenic [(mREN-2)27] hypertensive rats, heat stress caused a reduction in infarct size without changes in postischemic mechanical function. In contrast to these studies, Nishikimi et al³² described a linear relation between end-diastolic pressure and infarct size in the spontaneously hypertensive rat. The basis for the differences among these studies is unclear. Nonetheless, it is clear that the factors regulating contractile function are different from those affecting infarct size. One of the most important determinants of contractile dysfunction is the status of the viable portion of the infarcted heart. In the case of the hypertensive, hypertrophic heart, the contractile machinery of the viable tissue is weakened by both chronic hypertension and the ischemia-reperfusion insult. By contrast, infarct size depends on the status of signaling pathways that regulate cell survival.

The mechanism underlying improved cell survival in both the hypertensive (afterload, 100 cm H₂O) and the glucose-intolerant animals is presently unclear. In the case of the hypertensive animal, three mechanisms deserve consideration. First, the conditions used in the present study to

produce hypertension, namely, high NaCl feeding coupled with uninephrectomy, should also produce a state of chronic hyperosmotic stress. This hyperosmotic stress should in turn cause cell shrinkage. In response, a volume regulatory increase will be immediately activated and will be followed by a slow accumulation of organic osmolytes, such as taurine.³³ These osmoregulatory events are designed to restore normal cell volume. However, due to the accumulation of lactate, inorganic phosphate, and other osmolytes during ischemia, an osmotic imbalance also develops during the ischemic insult. This imbalance will trigger a regulatory volume decrease. Yet, because the myocyte had already undergone “chronic hyperosmotic preconditioning,” the regulatory volume increase will be prolonged. This prolongation will allow greater levels of K⁺, Cl⁻ and taurine to be lost from the cell, thereby minimizing damage caused by ischemia-mediated cell swelling. Activation of the regulatory volume decrease will also limit the accumulation of Na⁺ and Ca²⁺ through the Na⁺/H⁺ and Na⁺/Ca²⁺ exchangers.³⁴

The second mechanism influencing the status of the ischemic, hypertensive heart is the degree of cell stretching. Figure 1 reveals that an elevation in afterload from 100 to 160 cm H₂O prevented the reduction in infarct size that was observed in the hearts of the hypertensive rats. Interestingly, other investigators have suggested that mild stretching caused by various stimuli (eg, volume loading) preconditions the heart.^{35,36} Thus, both the nature of the stimulus and the extent of stretching could determine whether the stimulus has a beneficial or detrimental effect on the ischemic heart. Apparently, several factors, some of which have opposing effects, are activated by stretching. It is known that cell stretching not only elevates [Ca²⁺]_i but also activates key transporters, such as the Na⁺/H⁺ exchanger.^{37,38} Severe swelling also increases the risk of membrane damage and the initiation of apoptosis.³⁹ Presumably, removal of the heart from the hypertensive animal and its perfusion at the lower afterload pressure of 100 cm H₂O minimizes the undesirable effects of cell stretching. Nonetheless, the worsening of infarct size at the higher afterload pressure raises the possibility that aggressive anti-hypertensive therapy may be beneficial, lessening the severity of myocardial cell death after ischemic episodes.

The reduction in infarct size seen in the hypertensive animal at the lower afterload pressure might also involve neurohumoral factors that contribute to the elevation in blood pressure. Although the renin–angiotensin II system is thought to play an important role in the development of hypertension in many animal models, the salt-induced hypertension model is a low-renin form of hypertension.⁴⁰ Indeed, we have evidence that the renin–angiotensin II system plays only a minor role in both the development of hypertension and the induction of hypertension-induced cardiac hypertrophy in this animal model (unpublished observations). Therefore, despite some evidence that angiotensin II is capable of influencing the outcome of an ischemic event,^{7,8} the renin–angiotensin II system probably does not contribute to the reduction in infarct size seen in the hypertensive rat. In contrast to angiotensin II, enhanced sympathoexcitation is an important contributor to the elevation in blood pressure in the salt-sensitive hypertensive rat.¹³ Interestingly, the cardiomyocyte

can be preconditioned by prior exposure to α_{1b} adrenergic agonists.⁴¹ The agonist can activate protein kinase C, leading to an opening of the mitochondrial K_{ATP} channels, a sequence of events implicated in ischemic preconditioning. However, the activation of protein kinase C also promotes the upregulation in Bcl-2, an important antiapoptotic factor.⁴²

The present data do not support the hypothesis that hypertension, alone or in combination with impaired glucose tolerance, renders the heart more susceptible to ischemia-induced cell death. In fact, even at the higher afterload pressure of 160 cm H₂O, infarct size remains smaller in the hypertensive glucose-intolerant heart than in the hypertensive control heart. A plausible mechanism for this interaction between salt-sensitive hypertension and glucose intolerance is the modulation in Na⁺ and Ca²⁺ homeostasis; both conditions should reduce [Ca²⁺]_i during the ischemia-reperfusion insult. Despite the reduction in infarct size, postischemic contractile function is more impaired in the hypertensive glucose-intolerant heart than in the normotensive glucose intolerant heart. Thus, superimposing hypertension on the glucose intolerant rat renders the heart susceptible to undesirable contractile defects.

Perspectives

This study indicates that afterload pressure is an important determinant of myocardial ischemia-reperfusion injury. One implication of this finding is that aggressive antihypertensive therapy could potentially limit cell death after an ischemia-reperfusion insult. More importantly, however, is the potential relevance of this finding to cell survival. It is now recognized that initiation of certain survival pathways protects the cell against necrosis and apoptosis. A variety of stimuli are capable of activating these pathways, including short bouts of ischemia or hypoxia, rapid cardiac pacing, thermal stress, mild stretch induced by volume loading, osmotic stress, and various pharmacological agents.³⁶ Diabetes and glucose intolerance may interact with these pathways through the activation of protein kinase C and the downregulation of the Na⁺/H⁺ exchanger. Salt-sensitive hypertension may also interact with these pathways through α -adrenergic-induced activation of protein kinase C and salt-induced alterations in the Na⁺/H⁺ exchanger. Elevations in afterload appear to interfere with the ability of the glucose-intolerant and the salt-sensitive hypertensive rat to activate these survival pathways. It is likely that increased afterload will similarly affect the survival pathways activated by other stimuli. Left unanswered is the mechanism(s) underlying the effect of elevated afterload. Nonetheless, the data reveal the potential importance of hypertension in adversely affecting cell survival.

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