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Myocardial Function and Metabolic Energetics in Low Flow Ischemia and with β-Adrenergic Stimulation in Spontaneously Hypertensive Rat Hearts

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Abstract

The effects of cardiac ischemia-reperfusion and β-adrenergic stimulation on metabolic function and energetics were investigated in Langendorff-perfused spontaneously hypertensive rat (SHR) hearts. Sarcoplasmic reticulum Ca^{2+}-dependent ATPase and cardiac lactate dehydrogenase (LDH) are additionally studied. The perfusion medium (1.0 mM Ca^{2+}) contained 5 mM glucose (+5 U/L insulin) and 2 mM pyruvate as substrates. Global ischemia was induced by reducing perfusion pressure of 100 to 40 cmH_2O, followed by 20 min reperfusion. Isoproterenol (ISO, 1 μM) was infused for 10 min. Coronary vascular resistance and myocardial oxygen consumption (MVO_2) of SHR were increased in parallel with enhanced venous lactate during ischemia and reperfusion compared to those of Sprague Dawley (SD) hearts. Although ischemia-induced increase in venous lactate and combined adenosine plus inosine was abolished, coronary vasodilation produced in SD during reperfusion. In SHR, depressed reactive hyperemia was associated with a fall in cardiac ATP and CrP/Pi ratio and a rise in intracellular lactate/pyruvate ratio. On the other hand, ISO produced coronary functional hyperemia and an increase in MVO_2. However, these responses were less than those in SHR hearts. The ATPase activity of SHR was attenuated in free Ca^{2+} concentrations used under basal condition and with ISO compared to that of SD. Venous lactate output and cardiac LDH activity were augmented in SHR as influenced by ISO. These results demonstrate that coronary reactive and functional hyperemia was depressed in SHR, which could be explained by alterations in the cytosolic phosphorylation potential and the cytosolic redox state manipulated by LDH, and by abnormal free calcium handling.

Key words: ischemia, β-adrenergic stimulation, myocardial function, metabolic energetics, spontaneously hypertensive rat heart

INTRODUCTION

The influence of genetic hypertension on arterial muscle excitability and contraction, endothelium-dependent vasorelaxation, and nerve sympathetic activity has been well documented (1-4). However, the genetic factors differ as illustrated by various animal models of hypertension. Enhanced arterial membrane K^+ permeability, alterations in cellular membrane, augmented vascular muscle Ca^{2+} sensitivity, and abnormal Ca^{2+}-handling defects of sarcoplasmic reticulum (SR) have been frequently reported in spontaneously hypertensive rats (SHR) (1,4-7), which may play important roles in the development of hypertension, impairment of relaxation and the limitation of arterial reactivity. The mechanisms responsible for such abnormalities in SHR are still unclear.

The present study compared parameters of coronary circulation and myocardial energy metabolites measured in Sprague Dawley rat (SD) controls and SHR under the conditions of cardiac ischemia and reperfusion. Activities of SR Ca^{2+}-dependent ATPase and lactate dehydrogenase (LDH) were also examined in SD and SHR hearts by relating to isoproterenol (ISO)-induced increases in the myocardial contractility and coronary functional hyperemia. No study has correlated direct changes in the cardiac circulatory and metabolic functions with genetic hypertension, nor examined differential relationships between myocardial oxygen demand, an index metabolic work, and coronary flow regulation of SD and SHR. Such an approach can reveal important differential effects of genetic factors on cardiac function, and provide significance for the study of the pathogenesis of hypertension.

MATERIALS AND METHODS

Langendorff heart perfusion

Hearts were isolated from male SD and SHR of 250~350 g body mass as described elsewhere (8,9) and perfused with modified Krebs-Henseleit bicarbonate buffer (pH 7.40±0.02, 37°C) equilibrated with 95% O_2 : 5% CO_2 and containing 1.0 mM Ca^{2+} and 5 U/L bovine insulin (Sigma, St. Louis, MO). During the experimental period, all hearts were perfused with medium fortified with 5 mM glucose and 2 mM pyruvate as energy substrates.

Hearts were spontaneously beating, and coronary perfusion pressure (CPP) was maintained at 100 cmH_2O. Coronary venous effluent (coronary sinus plus right ventricular thebesian flow, CF) was measured and collected from the cannulated

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pulmonary artery (10). Retrograde aortic inflow and venous effluent fluids were sampled anaerobically; pO_2, pCO_2, and pH were measured in a blood pH/gas analyzer (CIBA-Corning, model 238, Orangeburg, NY) to calculate myocardial oxygen consumption (MVO_2). A silastic tubing was inserted across the incised mitral valve to effect left ventricular drainage (10). Epicardial transudate was also sampled from the cardiac apex as previously described (10).

Experimental protocols

When hearts had achieved hemodynamic steady states at 100 cmH_2O of CPP, hemodynamic and metabolic measurements were collected. Spontaneous heart rate, aortic flow, CF, CPP, and pO_2 were monitored.

Ischemia-reperfusion protocols

The first series of experiments examined coronary circulation and purine releases during ischemia and reperfusion. Hemodynamic measurements were obtained before inducing ischemia. Venous effluent and epicardial transudate were collected during the last 2 min of this period. Subsequently, lowering CPP from 100 to 40 cmH_2O for 10 min induced hypoperfusion ischemia. In the new steady state, all measurements were repeated. Thereafter, hearts were reperfused for the next 20 min after raising CPP to the normal pressure, and measurements obtained between 18 and 20 min.

ISO infusion protocols

A second series of experiments compared coronary circulation and SR Ca^{2+}-dependent ATPase of SD and SHR hearts in absence and presence of ISO. After control basal measurements were obtained, 1 μM ISO was infused for 10 min to induce a coronary functional hyperemia. In a new steady state, hemodynamic and metabolic measurements were collected during the last 2 min of the infusion period. Another set of hearts was perfused for 10 min in the absence of ISO. Hearts were freeze-clamped immediately prior to terminating the experiment (see below). In addition, a separate series of experiments was performed to examine the cardiac LDH in the presence of ISO. The experimental procedures were the same as the above.

Analytical measurements

Experiments were terminated by rapid-freezing the hearts with Wollenberger tongs pre-cooled at a temperature of liquid N_2. Myocardial extraction procedures and enzymatic metabolite measurements were performed as previously detailed (11, 12). Creatine phosphate (CrP), creatine (Cr), inorganic phosphate (Pi), ATP, pyruvate, and lactate were assayed with a UVICON Model 930 spectrophotometer (Kontron Instruments, Tegimenta, Switzerland, measuring at 340 nm wavelength, $\varepsilon = 5.782\ cm^2 \cdot \mu mol^{-1}$). Venous pyruvate and lactate were also enzymatically measured (11,12).

HPLC analyses and measurements of purines

Coronary venous effluents were collected and immediately boiled for 8 min to prevent degradation of purines by released adenosine deaminase. Epicardial transudate was collected in 1.5 mL Eppendorff tubes containing 20 μL 10 N HCl to denature proteins in the transudate. Prior to HPLC separation, 1 mL portions of boiled venous effluent samples was added to 30 μL 1 N HCl, while epicardial transudate samples were adjusted to pH 2~4 with 10 N and 1 N KOH. Purines (adenosine and inosine) in venous effluent and epicardial transudate samples were kept at 4°C and measured using C-18 reverse phase HPLC (Waters Associates, Milford, MA) as previously described (8,9,11). Identification and quantification of purine peaks were accomplished by comparison with calibrated standards with known retention times in combination with measured absorbance characteristics at four different wavelengths (254, 263, 273, 293 nm; Waters model 490 multiple wavelength detector).

Preparation and measurement of SR Ca^{2+}-dependent ATPase

Cardiac SR suspension was prepared from freeze-clamped hearts (13). The ATPase activity was spectrophotometically measured as previously detailed (14,15). Frozen powdered heart tissue (1~2 g) was homogenized in a 15 mL phosphate buffer (pH 7.0) with a Polytron (PT-10 probe) at setting 6. The homogenized mixture was centrifuged for 15 min at 7,700×g at 4°C (Beckman Model J2-20M, Beckman Instruments Inc., Palo Alto, CA), and the supernatant was centrifuged for 30 min at 35,000×g. The pellet (crude SR) was resuspended in an 8.6% sucrose suspension buffer (pH 7.4) containing 30 mM histidine, 10 mM NaF, 10 mM Na_2EDTA. The crude SR suspension was layered on the top of the sucrose gradient (27.1% to 35.2% sucrose buffer, pH 7.4), and centrifuged for 60 min at 65,000 ×g at 4°C (Beckman Model XL-90, rotor SW40). Material at the interface between two sucrose buffers was collected and centrifuged for 30 min at 70,000×g to obtain purified SR. The pellet was resuspended in a 0.5 mL suspension buffer for the measurement of ATPase activity.

The assay for the activity of SR Ca^{2+}-dependent ATPase was performed at various free Ca^{2+} concentrations (pCa 7.68~4.35) in a UVICON spectrophotometer in which absorbance at 340 nm wavelength was monitored over 3.5 min. The ATPase consumes ATP that is coupled to consumption of NADH causing a change in absorbance. The assay buffer contained an ATP regenerating system (phosphoenolpyruvate plus pyruvate kinase), LDH and ionophore A23187 (Boehringer Mannheim, Germany) which prevents accumulation of Ca^{2+} within SR vesicles, thereby ensuring linear reaction rates.

Measurement of cardiac LDH

The cardiac LDH was determined according to the method of Braasch et al. (16). A portion of frozen powdered tissue was homogenized in a 8 mL phosphate buffer (0.1 M KH_2PO_4, 1 mM ADP, 10 mM glutathione, and 10 mM EDTA, pH 7.2). The homogenate was centrifuged for 20 min at 100,000×g (Beckman Model XL-90, rotor SW40). The supernatant was collected, and the pellet suspended and homogenized in a 4 mL phosphate buffer. Centrifugation was repeated as above,

and the yielded supernatant was combined with the first supernatant. The combined supernatant was pipetted in 1.0 mL aliquots. The LDH activity (LDHact) was spectrophotometrically measured in a Tris buffer (pH 7.2) with NADH in absorbance at 340 nm wavelength over 3 min. Pyruvate consumption by the LDH is coupled to consumption of NADH.

Protein contents of the purified SR suspension and the LDH supernatant were determined using Bicinchoninic acid (BCA) protein assay reagent kits (Pierce Co., Rockford, IL) to normalize enzyme activities of the ATPase and LDH. A unit of each enzyme activity was expressed per mg protein.

Data analysis

Data are represented as means ± S.D. Single comparison of mean values was accomplished by Student's *t*-test for unpaired results. For multiple comparisons, analysis of variances (two-tail ANOVA) in combination with Tukey's multiple range test was performed. P values < 0.05 were taken to indicate statistical significance.

RESULTS

Coronary circulation during cardiac ischemia and reperfusion

Table 1 summarizes CF and MVO_2 responses to ischemia and reperfusion. As expected, lowering CPP from the physiological pressure of 100 cmH_2O to the subphysiological pressure of 40 cmH_2O produced significant decreases in CF and MVO_2 in both SD and SHR, while coronary vascular resistance (CVR) increased in both groups (23% in SD vs. 17% in SHR). It should be noted that basal CF and CVR in SHR did not differ from those observed in SD; basal MVO_2, however, increased by 28% compared with that of SD ($p<0.05$). Greater coronary vasodilation was shown with increased MVO_2 in SD during reperfusion (Table 1). On the other hand, a biphasic response of CVR to reperfusion was observed in SHR, and MVO_2 declined with prolonged reperfusion in parallel with a decrease in CF. However, MVO_2 in SHR was still greater than that observed in SD even at the 20 min reperfusion, which was due to increased myocardial oxygen extraction (EO_2, data not shown).

Fig. 1 depicts change curves of arterio-venous pH (ΔpH) and venous-arterial pCO_2 (ΔpCO_2) observed during ischemia and reperfusion. In perfused SHR hearts ΔpH and ΔpCO_2 were markedly enhanced. Ischemia-induced increases in both parameters were much greater in SHR than those of SD; the post-ischemic ΔpH and ΔpCO_2 did not reach the pre-ischemic levels.

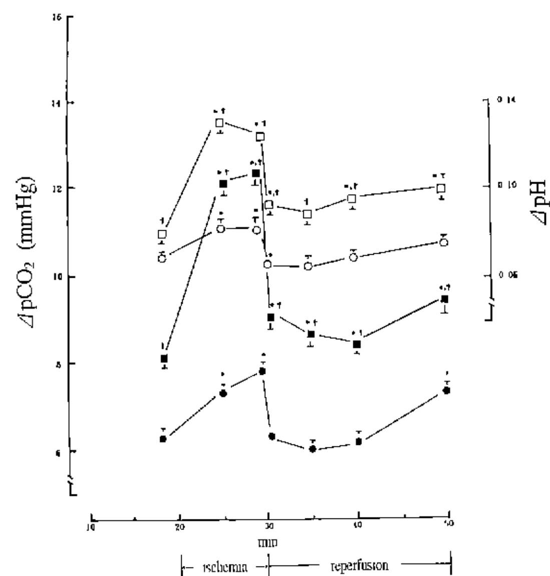

Fig. 1. Graphs showing the changes of coronary venous pH (○, □) and pCO_2 (●, ■) during cardiac ischemia-reperfusion in Sprague Dawley and spontaneously hypertensive rats. Means ± SE, n = 15 SD and 14 SHR. ○ and ●, Sprague Dawley rat: □ and ■, spontaneously hypertensive rat; ΔpH, arterio-venous pH; ΔpCO_2, venous-arterial partial pressure of carbon dioxide. $^{*}p<0.05$, relative to corresponding pre-ischemic values. $^{+}p<0.05$, relative to corresponding SD values.

Cardiac adenylates and glycolytic metabolites

Data on levels of cardiac energy metabolites after the pressure run protocol were shown in Table 2. The ATP pool was depleted by 15% in SHR. However, cardiac CrP and Pi of SHR did not differ from those of SD ($p>0.05$); CrP/Pi ratio decreased by 16%. Marked increases in cardiac lactate and lactate/pyruvate ratio were obtained in SHR despite only a small fall in cardiac pyruvate compared with those of SD (Table 2); this was consistent with an increase in the venous lactate production (Fig. 2). Ischemia-induced increase in venous lactate was

Table 1. Cardiac hemodynamics and myocardial oxygen consumption during cardiac hypoperfusion ischemia and reperfusion in Sprague Dawley and spontaneously hypertensive rats

Protocols	Time (min)	SD			SHR		
		CF	CVR	MVO_2	CF	CVR	MVO_2
Pre-ischemia		5.80±0.11[1)]	15.50±0.23	1.94±0.04	5.97±0.07	15.16±0.13	2.48±0.05^{+}
Ischemia	10	3.39±0.06*	11.86±0.24*	1.38±0.03*	3.16±0.05$^{*,+}$	12.52±0.17$^{*,+}$	1.62±0.03$^{*,+}$
Postischemia	0-1	6.80±0.10*	13.49±0.19*	2.41±0.05*	6.18±0.08$^{*,+}$	14.30±0.19$^{*,+}$	2.84±0.05$^{*,+}$
	5	6.72±0.12*	13.28±0.22*	2.30±0.04*	6.01±0.07^{+}	15.00±0.14^{+}	2.68±0.04$^{*,+}$
	10	6.35±0.11*	14.25±0.21*	2.14±0.04*	5.73±0.07$^{*,+}$	15.56±0.15$^{*,+}$	2.55±0.06^{+}
	20	6.17±0.11*	14.52±0.23*	2.29±0.04*	5.58±0.08$^{*,+}$	16.52±0.17$^{*,+}$	2.48±0.06^{+}

[1)] All values are means ± SE (n = 15 SD and 14 SHR). Cardiac hypoperfusion ischemia was induced by lowering coronary perfusion pressure from 100 to 40 cmH_2O for 10 min, followed by 20 min reperfusion. SD, Sprague Dawley rats; SHR, spontaneously hypertensive rats; CF ($mL \cdot min^{-1} g$ wet wt^{-1}), coronary flow; CVR ($cmH_2O \cdot mL^{-1} min^{-1} g$ wet wt^{-1}), coronary vascular resistance; MVO_2 ($\mu mol\ min^{-1} g$ wet wt^{-1}), myocardial oxygen consumption. $^{*}p<0.05$, relative to corresponding preischemic values. $^{+}p<0.05$, relative to corresponding SD values.

Table 2. Cardiac adenylates and metabolites after global cardiac ischemia-reperfusion in Sprague Dawley and spontaneously hypertensive rats

Rats	pHi	ATP	CrP	Cr	Pi	Lac	Pyr	CrP/Pi	Lac/Pyr
		(μmol·g dry mass^{-1})							
SD	7.21±0.01[1)]	18.0±0.9	34.4±1.3	22.5±1.7	18.8±1.4	2.3±0.2	3.9±0.3	1.88±0.26	0.57±0.04
SHR	7.13±0.03*	15.3±0.9*	32.6±1.5	24.5±2.4	20.6±1.7	2.8±0.2*	3.5±0.2	1.58±0.07*	0.83±0.05*

[1)]All values are means±SE (n = 9 SD and 9 SHR). Hearts were freeze-clamped after 20 min reperfusion for myocardial extraction (see Materials and Methods). SD, Sprague Dawley rats; SHR, spontaneously hypertensive rats; pHi, intracellular pH; CrP, creatine phosphate; Cr, creatine; Pi, inorganic phosphate; Pyr, pyruvate; Lac, lactate. Intracellular pH was calculated from measured coronary venous pCO_2 using an operational equation, $pHi=7.524e^{-0.0008786pCO_2}$ according to Bunger and Soboll (12). *$p<0.05$, relative to corresponding SD values.

abolished in SD during reperfusion, while it was still enhanced in SHR. Comparisons of the cardiac lactate data with the hemodynamic data indicate that increased cardiac and venous lactate in SHR were associated with enhanced MVO_2; cellular acidification was associated with a stimulated oxidative metabolic rate (Table 1 and 2, Fig. 2).

Stimulation of coronary venous purine nucleoside production

Fig. 3 presents data for coronary venous concentration ($[ADO+INO]_v$) and release ($V_{ADO+INO}$) of the combined adenosine plus inosine during ischemia and reperfusion. The basal purine production was not modulated in SHR. Cardiac ischemia markedly enhanced the $[ADO+INO]_v$ in both SD and SHR

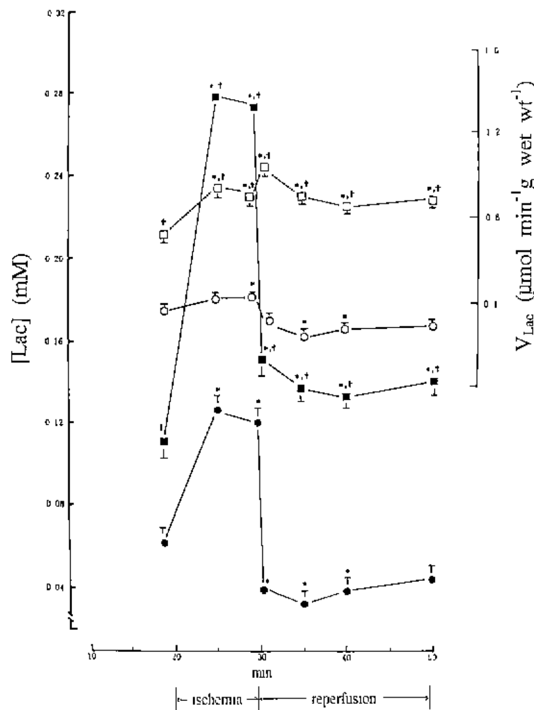

Fig. 2. Graphs showing the concentration (●, ■) and release (○, □) of coronary venous lactate during cardiac ischemia-reperfusion in Sprague Dawley and spontaneously hypertensive rats. Means±SE, n = 15 SD and 14 SHR. ○ and ●, Sprague Dawley rat; □ and ■, spontaneously hypertensive rat; [Lac], coronary venous lactate concentration; V_{Lac}, coronary venous lactate release. *$p<0.05$, relative to corresponding pre-ischemic values. $^{+}p<0.05$, relative to corresponding SD values.

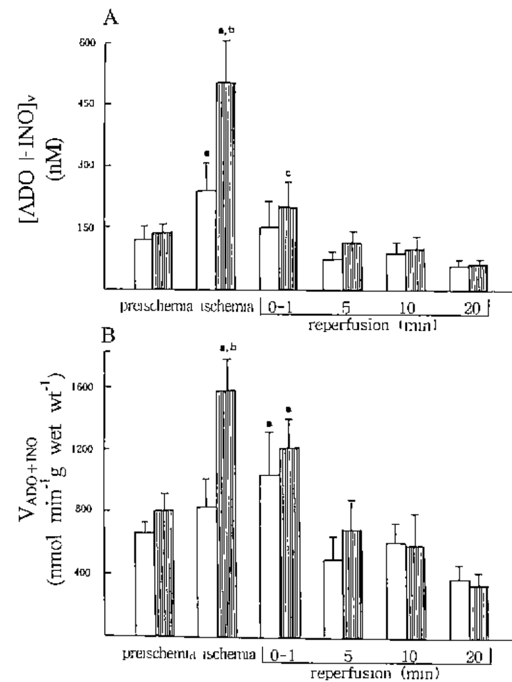

Fig. 3. Coronary venous adenosine plus inosine concentration (A panel, $[ADO+INO]_v$) and release (B panel, $V_{ADO+INO}$) of Sprague Dawley rat (open bars) and spontaneously hypertensive rat (hatched bars) during cardiac ischemia-reperfusion. Each bar represents means ±SE (n = 15 SD and 14 SHR). $^{a}p<0.05$, relative to corresponding preischemic values. $^{b}p<0.05$, relative to corresponding SD values. $^{c}p<0.05$, relative to corresponding ischemic values.

($p<0.05$); $V_{ADO+INO}$ did not increase in SD due to ischemia-induced decrease in CF (Table 1). When CPP was restored to the physiological pressure, $[ADO+INO]_v$ immediately declined to the pre-ischemic levels in both groups; $V_{ADO+INO}$ was still significantly enhanced during 1 min reperfusion, being associated with simultaneous increases in CF.

Isoproterenol-induced coronary functional hyperemia

Coronary hemodynamic responses to ISO are summarized in Table 3. In both groups ISO induced coronary functional hyperemia, and markedly increased MVO_2. However, ISO-induced functional hyperemia was weaker in SHR; CF rose from 5.67±0.58 to 7.40±0.58 mL·min^{-1} g wet wt^{-1} during β-receptor stimulation, while it increased by 66% in SD. Although EO_2 increased to a similar degree in response to

Table 3. Cardiac hemodynamic responses to isoproterenol in Sprague Dawley and spontaneously hypertensive rats

Groups	n	CF (mL · min^{-1} g wet wt^{-1})	EO_2	MVO_2 (μmol · min^{-1} g wet wt^{-1})	⊿pH (units)	⊿pCO_2 (mmHg)
SD -ISO	15	5.45±0.45[1)]	0.56±0.04	1.80±0.15	0.06±0.01	7.00±0.74
+ISO	15	9.03±0.70[a]	0.87±0.01[a]	5.30±0.19[a]	0.16±0.01[a]	20.07±1.35[a]
SHR -ISO	14	5.67±0.58	0.71±0.03[a]	2.61±0.18[a]	0.11±0.01[a]	10.79±0.30[a]
+ISO	13	7.40±0.58[b,c]	0.89±0.01[b]	4.56±0.30[b,c]	0.19±0.01[b,c]	23.46±0.94[b,c]

[1)]All values are means±SE. Cardiac inotropism was increased by infusing isoproterenpol (1 μM) for 10 min. SD, Sprague Dawley rats; SHR, spontaneously hypertensive rats; ISO, isoproterenol; CF, coronary flow; EO_2, myocardial oxygen extraction; MVO_2, myocardial oxygen consumption; ⊿pH, arterio-venous pH; ⊿pCO_2, venous-arterial partial pressure of carbon dioxide. [a]p<0.05, relative to corresponding SD-ISO values. [b]p<0.05, relative to corresponding SHR-ISO values. [c]p<0.05, relative to corresponding SD+ISO values

ISO in both SD and SHR, increased MVO_2 was attenuated in SHR (p<0.05). In SHR ISO-induced increases in ⊿pH and ⊿pCO_2 was less than when SD hearts were perfused (Table 3).

SR Ca^{2+}-dependent ATPase and LDH with β-receptor stimulation

Fig. 4 depicts the activity of SR Ca^{2+}-dependent ATPase as a function of -log free [Ca^{2+}]. A clear-cut bell-curve relationship between pCa and the enzyme activity was found; the maximum stimulation of the activity was observed at about pCa of 5. Under basal conditions, the ATPase activity decreased in SHR regardless of free Ca^{2+} concentrations in a given range. However, the β-receptor stimulation enhanced the ATPase activity in both groups, their curve shapes and positions being altered in SHR.

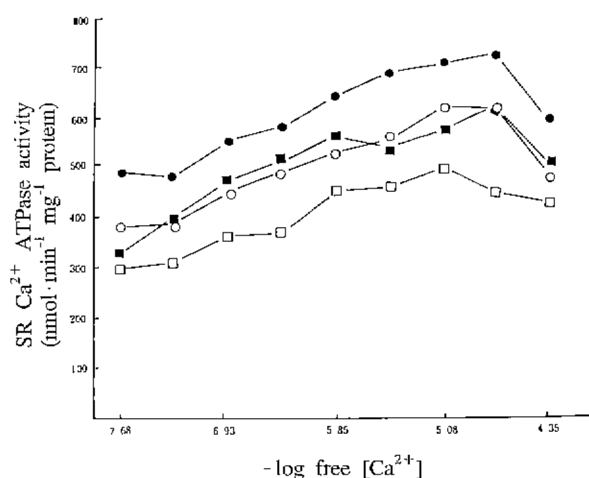

Fig. 4. Curvelinear plots of Sarcoplasmic Reticulum (SR) Ca^{2+}-dependent ATPase activity versus -log free [Ca^{2+}] under basal conditions and during 1 μM isoproterenol infusion. Means±SE, n = 7 SD and 7 SHR. The SR suspension was subjected to solutions at various free Ca^{2+} concentrations. SD, Sprague Dawley rat; SHR, spontaneously hypertensive rat; ISO, isoproterenol. ○, SD-ISO; □, SHR-ISO; ●, SD+ISO; ■, SHR+ISO.

Table 4 summarizes the coronary venous lactate output and cardiac LDHact. Release of venous lactate in SHR was consistently greater than when SD hearts were perfused. A comparison of venous lactate data with the LDHact data suggests that increased venous lactate output in SHR was associated with the stimulated LDHact (Table 4). On the other hand, the lactate output with ISO infusion increased by four-fold in SD compared with a 173% rise in SHR; however, LDHact increased only by 11% in both groups.

DISCUSSION

The present study examined differential effects of cardiac ischemia-reperfusion and -adrenergic stimulation on coronary circulation and myocardial energy metabolites in perfused SD and SHR hearts. The findings of this study demonstrate that genetic hypertension influences coronary vascular reactivity, myocardial oxygen metabolism and metabolic energy states under these conditions. These effects seem to be associated with alterations in the glycolytic process and the SR Ca^{2-} handling. The implication is that in patients with essential hypertension, cardiac function and coronary circulation could be altered under pathopharmacological conditions.

Effects of myocardial ischemia-reperfusion

An increase in coronary vascular smooth muscle tone of

Table 4. Coronary venous lactate production and cardiac lactate dehydrogenase with and without isoproterenol in perfused rat hearts

Groups	[Lac] (μmol · mL^{-1})	V_{Lac} (μmol · min^{-1} g wet wt^{-1})	LDHact (μmol · min^{-1} mg protein^{-1})
SD - ISO	0.18±0.04[1)]	0.77±0.10	2.67±0.24
+ ISO	0.42±0.05[a]	3.63±0.40[a]	2.96±0.36[a]
SHR - ISO	0.33±0.04[a]	1.70±0.16[a]	3.96±0.32[a]
+ ISO	0.63±0.04[b,c]	4.64±0.35[b,c]	4.39±0.35[b,c]

[1)]All values are means±SE (n = 8 SD and 8 SHR). Hearts were freeze-clamped after 10 min infusion of isoproterenol for measurements of the lactate dehydrogenase (see Materials and Methods). SD, Sprague Dawley rats; SHR, spontaneously hypertensive rats; ISO, isoproterenol; [Lac], coronary venous lactate concentration; V_{Lac}, coronary venous lactate release; LDHact, cardiac lactate dehydrogenase activity. [a]p<0.05, relative to corresponding SD-ISO values. [b]p<0.05, relative to corresponding SHR-ISO values. [c]p<0.05, relative to corresponding SD+ISO values

SHR during ischemia and reperfusion produced remarkable effects on the rate of post-ischemic recovery and magnitude of coronary circulation. The temporal recovery of coronary circulation in this study is note worthy. At the end of reperfusion a fully recovered CF response maintained in SD, while CVR was augmented and oxygen uptake reduced in SHR in parallel with a decrease in CF. Although coronary vasoconstriction was elicited in SHR, the oxidative metabolic rate were invariably enhanced; an increase in oxygen demand associated with elevated oxidative metabolic rate appeared to be adjusted by an increase in oxygen extraction. From these results, it should be noted that natural CVR response of SHR must have been partially antagonized due to metabolic vasodilation during ischemia and reperfusion.

Significant alterations in the magnitudes of cardiac adenylates were observed in SHR. The ATP content decreased and the CrP/Pi ratio increased despite relatively unaltered CrP. The depletion of the ATP pool could be explained by increased glycolytic process to favor cardiomyocytic lactate formation when CF and oxygen supply were limited. Changes in coronary function could be partially responsible for an alteration in the cellular energy level of the coronary smooth muscle. On the other hand, it has been reported that CF response and the cardiomyocytic energy state are conversely related in perfused hearts (11,17). Thus, it was hypothesized that adenosine metabolism might be altered in SHR. At the subphysiological perfusion pressure $V_{ADO-INO}$ increased significantly, which was obvious in SHR due to markedly decreased CF. Prolonged reperfusion did not increase $V_{ADO+INO}$ despite decreased myocardial energy levels of SHR. These findings are in agreement with other studies concluding that a major determinant for adenosine formation is an imbalance between oxygen supply and demand but not the metabolic rate *per se* (18,19). All in all, coronary purines could not be assigned to mediate CF response of SHR when the ATP pool becomes depleted.

Metabolic acidosis and enhanced venous lactate release occurred in SHR due to stimulated oxidative metabolic rate during ischemia and the late phase of reperfusion despite decreased CF (Fig. 1 and 2). Noticeable changes in ΔpH and ΔpCO_2 from the experimental intervention were not observed in perfusate gases and pH. This raises the possibility that myocardial glycolytic metabolism was altered and some pathological significance of cardiac LDH assigned in SHR. Alterations in the cellular energy state is most likely linked to the LDH equilibrium in the cytosolic redox state. When pyruvate is converted to lactate, NADH is converted to NAD^+ reducing equivalent, resulting in an overall cellular oxidation. In the current study, hearts were perfused with glucose and pyruvate as energy substrates. The cardiac lactate/pyruvate ratio and LDH-driven lactate flux from the cardiac pyruvate pool in SHR increased in parallel with enhanced coronary lactate output. The increased pyruvate flux to lactate would be associated with enhanced myocardial oxidative rate. An imbalance between oxygen demand and supply would increase anaerobic oxidation and hence cytosolic $NADH/NAD^+$ ratio via the glycolytic process in the cytoplasm. Our data suggest that cardiac LDH is modulated with increased activity in SHR. It has been demonstrated that cardiac LDH is regulated by prevailing energy substrates, i.e., pyruvate and lactate (20).

It is conceivable that pressure-induced vascular stretching releases constrictors in the vicinity of coronary arteriolar smooth muscle of SHR (21). Prostaglandines, short-lived superoxides, and other constricting factors could be released by the endothelium under physical stretching. If these unknown factors were involved, it would be difficult to explain the coronary vasoconstriction in SHR on the basis of the metabolic flow control. Besides, releases of endothelium-derived relaxing factors (EDRFs) that mediate vasorelaxant actions of various endogenous hormones might be reduced in SHR, which leads to an increase in coronary vascular smooth muscle tone. Loss of relaxation to acetylcholine occurs in vessels from individuals with diseases associated with endothelial damage (22-24). It is assumed that blunted release of EDRFs and augmented liberation of endothelium-derived contracting factors may contribute to the increase in coronary resistance characteristic of SHR.

Effects of β-adrenergic stimulation

Hearts perfused with ISO exhibited a functional hyperemia in parallel with an increase in oxygen uptake. However, a vasoconstrictor action seemed to be activated in SHR under this pharmacological condition. This resulted in a less increase in myocardial oxidative metabolic rate by ISO than that observed in SD, where myocardial oxygen-extracting property was intact; EO_2 increased to near maximum (Table 3). Although this study did not examine coronary purines with the ISO intervention, it has been shown that catecholamine-induced functional hyperemia is accompanied by parallel increases in MVO_2 and adenosine release (18,19,25). Alterations in myocardial energy utilization, or in a balance of substrate and/or oxygen supply-to-demand lead to adenosine production to restore the balance and maintain functions. Therefore, small increases in CF and MVO_2 of SHR during ISO infusion suggest that adenosine did not play a major role in the coronary functional hyperemia in SHR, or that adenosine-induced vasodilation was attenuated by unknown vasoconstrictors released during ISO infusion.

On the other hand, depressed functional hyperemia and the inotropic state of SHR may be attributed to alterations in the excitation-contraction coupling process. Such alterations could include the regulatory activity of β-adrenergic receptors of the cell membrane, and derangement of the SR and myofibrils (4,5,7,26). The present study focused on calcium handling of the SR by examining SR Ca^{2+} ATPase and thereby the SR calcium uptake. The SR isolation technique employed is known to exhibit SR physiological function including Ca^{2+} uptake and Ca^{2+} ATPase activity that vary as a function of free Ca^{2+} concentrations (5,27). The ATPase activity in absence and presence of β-adrenergic stimulation was attenuated

in SHR at any free Ca^{2+} concentration used (Fig. 4). Therefore, it seems reasonable to assume that changes in the cytosolic Ca^{2+} dynamics in the cardiac contraction-relaxation cycle may contribute to the depressed β-adrenergic responses in SHR. However, differences between SD and SHR in cardiac responses induced by ISO may arise from intervening factors not studied in the current study; a decrease in calcium influx, an increase in calcium efflux, or a decrease in calcium release from the intracellular pools. Hojo et al. (7) demonstrated that calcium efflux was enhanced and membrane-bound Ca^{2+} reduced in perfused hypertrophic SHR heart. Perez et al. (5) demonstrated in an experiment using papillary muscle of SHR that the SR calcium release was impaired, though calcium uptake remained intact. It has also been reported that cardiac content of intracellular calcium binding proteins is reduced in SHR (28). Furthermore, a study of Moravic et al. (29) has shown that diminished relaxation response to ISO is associated with abnormal functional SR Ca^{2+} cycling in aged SHR. These findings and our results indicate that the behavior of cardiac SR in the calcium sequestration and release is altered in SHR. However, it can not be ruled out the possibility that the depressed responses to β-adrenergic stimulation in SHR are attributed to alterations in the post-receptor mechanism of catecholamines (30).

In summary, the present study demonstrated that depressed reactive and functional hyperemia were observed with enhanced myocardial oxygen uptake, metabolic acidosis, and elevated lactate production in SHR during ischemia-reperfusion and β-adrenergic stimulation. These effects could be due to an alteration in the cytosolic redox state manipulated by cardiac LDH, a decrease in the cytosolic phosphorylation potentials, and due to a SR calcium handling defect for cytosolic free Ca^{2+}, which appear to be involved in eliciting hypertension.

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