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Article

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Preservation of canine myocardial high-energy phosphates during low-flow ischemia with modification of hemoglobin–oxygen affinity

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Introduction

Myocardial ischemia is classically defined as an imbalance between oxygen supply and demand, and it is this primary metabolic abnormality that results in the electrocardiographic, contractile, and arrhythmic consequences of ischemia. During ischemia, oxidative metabolism is restricted and myocardial high-energy phosphate levels decline. Creatine phosphate (PCr) levels fall rapidly, with a concomitant increase in inorganic phosphate and H^+ concentrations. If ischemia is severe or sufficiently prolonged, PCr is depleted, adenosine triphosphate (ATP) levels decrease, and acidosis worsens (1–4). The magnitude of the decline in cardiac high-energy phosphates and intracellular pH directly indicates the severity of the imbalance of oxygen supply and demand in the myocardium, as well as the effectiveness of anti-ischemic interventions (1, 5, 6).

Traditional treatment strategies for myocardial ischemia either augment myocardial oxygen supply or lessen oxygen demand. Oxygen supply can be increased in the setting of fixed disease with revascularization interventions (coronary artery bypass surgery or percutaneous transluminal coronary angioplasty) and in the setting of coronary spasm with vasodilators (nitrates and calcium channel blockers). β-adrenergic blocking agents are a mainstay of anti-ischemic therapy and primarily act by reducing myocardial oxygen demand. A novel approach to increasing oxygen supply to ischemic myocardial tissue is modification of the hemoglobin–oxygen (Hb–O$_2$) interaction in a manner that would decrease the affinity of hemoglobin for oxygen and result in more oxygen unloading in ischemic tissue.

The allosteric characteristics of Hb–O$_2$ binding are well known and lead to the sigmoidal shape of the Hb–O$_2$ dissociation curve. The relative affinity of hemoglobin is expressed as the p50, the oxygen tension that produces 50% saturation of the hemoglobin–oxygen binding sites at 37°C and pH 7.4. There are several naturally occurring modifiers of hemoglobin affinity, including 2,3-diphosphoglycerate (DPG), CO$_2$, and H$^+$. A rightward shift in the Hb–O$_2$ dissociation curve is observed in patients with angina, congestive heart failure with shock, and acute myocardial infarction, and is believed to be an adaptive response that increases myocardial oxygen delivery (7–13). Clofibrate and some other synthetic compounds also modestly alter the affinity of hemoglo-
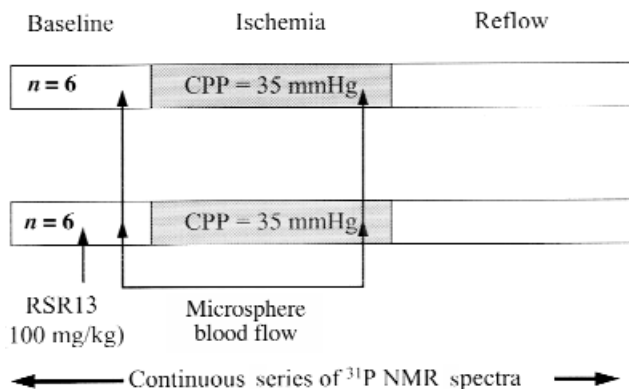
Figure 1

First experimental protocol: RSR13 administration before ischemia. *NMR,* nuclear magnetic resonance.

bin for oxygen (14–18). A 2-[4-[[(3,5-disubstituted anilino)carbonyl]methyl] phenoxy] -2-methylproprionic acid derivative known as RSR13 is one of the most potent of the recently discovered compounds. This agent markedly shifts the Hb–O_2 dissociation curve rightward in the *in vitro* and *in vivo* rodent models (19, 20). Although theoretically attractive, the acute effects of a relatively rapid and significant shift in the Hb–O_2 curve on the severity of myocardial ischemia are unknown. Therefore we used ^{31}P nuclear magnetic resonance (NMR) spectroscopy, the only method that can nondestructively quantify and index serial changes in high-energy phosphates during ischemia, to test the hypothesis that a rightward shift of the Hb–O_2 dissociation curve induced by RSR13, a potent synthetic allosteric modifier of hemoglobin affinity, decreases the decline in myocardial high-energy phosphates and intracellular pH during low-flow ischemia in *in vivo* canine hearts.

Methods

Animal surgery. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals,* published by the US National Institutes of Health (NIH publication no. 85-23; revised 1985). Mongrel dogs weighing 15–22 kg were studied under sodium pentobarbital anesthesia dosed initially at 25–30 mg/kg and repeated as needed. Dogs were orally intubated using a cuffed endotracheal tube, and respiration was maintained with a ventilator. Supplemental administration of oxygen maintained arterial blood pO_2 150 mmHg. The left femoral vein and artery were cannulated for the administration of intravenous fluids as well as arterial blood sampling during microsphere injections, respectively. A left lateral thoracotomy was performed in the fifth intercostal space, the pericardium was opened anteriorly, and a cradle created. A catheter was placed in the left atrium for injection of microspheres to measure myocardial blood flow. The proximal portion of the left anterior descending (LAD) coronary artery was dissected free. The left common carotid artery was dissected free and the most distal portion ligated. Plastic tubing with a variable flow resistor was inserted between the proximal carotid and the LAD artery, which was ligated proximally. This created a vascular shunt that allowed graded control of LAD coronary artery blood flow. A fluid-filled tube was inserted via a side port within the vascular shunt just above the LAD insertion; its distal attachment to a Gould pressure transducer allowed continuous measurement

of coronary artery perfusion pressure on a Gould strip-chart recorder. Before the vascular shunt was placed a bolus dose of 9,000 U of heparin was given intravenously, and repeat doses of 2,000 U were given every hour. An NMR surface coil was placed over the LAD territory, and the coil and surrounding structures were covered with plastic wrap to reduce fluid loss. The dog was then placed in a 4.7 T NMR magnet/spectrometer.

Experimental protocol. The first experimental protocol aimed to determine whether RSR13 administered before ischemia attenuated the decline in high-energy phosphates during low-flow ischemia. It is summarized in Figure 1. Fourteen dogs were initially studied, of which 12 dogs met and completed the protocol with one exclusion from each group. Under baseline conditions, RSR13 (100 mg/kg; 20% in volumes ranging 88–103 ml) was administered intravenously by pump over 15 min in six of the animals, while the six others that received 0.9% NaCl solution served as controls. Myocardial ischemia was produced 10 min later by reducing coronary perfusion pressure to, and maintaining it at, 35 mmHg for 20 min, which in preliminary experiments reduced PCr content to a steady level. ^{31}P NMR spectra were collected before, during, and after ischemia to determine relative cardiac high-energy phosphate content and intracellular pH. Hemodynamic data, including blood pressure, coronary perfusion pressure, and heart rate, were collected at 5-min intervals throughout the experiment. Radioactive microspheres were injected after RSR13 or vehicle administration, but before ischemia, and again 5 min before the end of the ischemic period for determination of myocardial blood flow (technique outlined below). Perfusion was restored after 20 min of ischemia by removing the partial occlusion on the vascular shunt. At the conclusion of the study, monastral blue was injected into the vascular shunt to demarcate the LAD bed supplied by shunt flow and to confirm the position of the NMR coil over that bed. The animals were then euthanized with an overdose of potassium chloride. Hearts were removed immediately for determination of microsphere blood flow.

The aim of the second experimental protocol was to determine whether RSR13 administered during ischemia would improve the metabolic and contractile consequences of low-flow ischemia. It is summarized in Figure 2. Metabolic param-
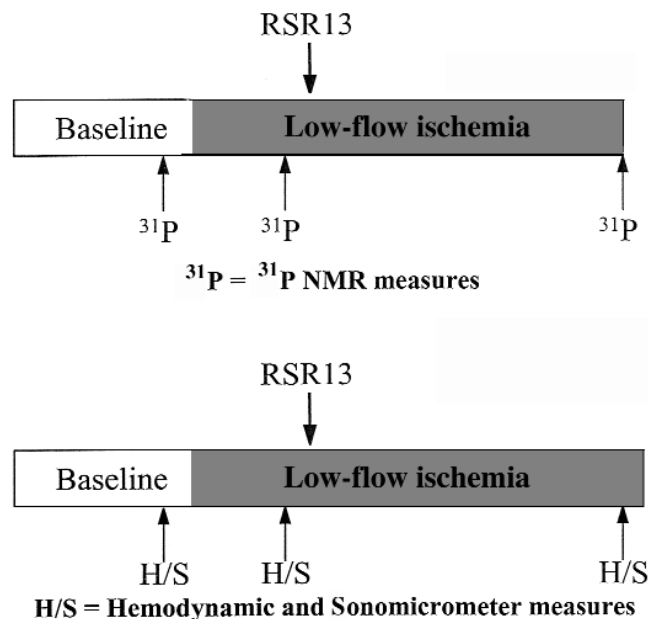

Figure 2

Second experimental protocol: RSR13 administration during ischemia.

eters were studied with ^{31}P NMR in one series of dogs, and contractile parameters were studied with pairs of regional sonomicrometer crystals and a left ventricular pressure catheter (Millar) before and during inferior vena cava (IVC) occlusion in other animals under similar conditions. After a stabilization period, baseline measures were obtained. Myocardial ischemia was produced by reducing coronary perfusion pressure to 35–45 mmHg and maintaining it at that level in each heart for 30–40 min. Contractile and metabolic parameters were measured between 5 and 10 min of low-flow ischemia. RSR13 (100mg/kg) was then given intravenously by pump over 10 min to all animals. Contractile and metabolic parameters were measured ~7–12 min after completion of the RSR13 infusion or ~30 min after the onset of low-flow ischemia. Hemodynamic data, including blood pressure, coronary perfusion pressure, and heart rate were also recorded in all animals. The animals were euthanized with an overdose of potassium chloride.

^{31}P NMR methods. A custom-built, double-tuned hydrogen-1 and ^{31}P surface coil (4 cm in diameter) was carefully positioned over the LAD territory before placement of each animal in a 4.7 T NMR magnet interfaced to a GE Omega® imaging/spectroscopy system (General Electric, Milwaukee, Wisconsin, USA). Magnetic field homogeneity was optimized by shimming on the water proton signal. Nongated ^{31}P NMR acquisitions were collected over 2 min (2-s interpulse delay, 60 pulses, ~30° flip angle) in a series before, during, and after ischemia. Peak areas were determined by a semiautomated time-domain fitting program (21). Metabolite results were expressed as percent of preischemic values. PCr/ATP ratios were not corrected for partial saturation effects considered minimal under these experimental conditions. Intracellular pH was determined from the relative chemical shift of the inorganic phosphate peak (22).

Myocardial blood flow by radioactive microspheres. Myocardial blood flow was determined using an established radioactive microsphere technique (23). Briefly, a known amount of suspended microsphere particles labeled with the radionuclides ^{153}Gd or ^{113}Sn is injected into the left atrium while blood is continuously withdrawn from the femoral artery catheter by a Harvard pump. Gadolinium-153 is used for the determination of blood flow before ischemia, and tin-113 is used for calculation of blood flow during ischemia. At the end of the experiment, myocardial tissue is sectioned and labeled as ischemic (stained with monastral blue) or nonischemic samples. Each of the samples is further sectioned into epicardial, mid-wall, and endocardial layers. The collected blood and myocardial tissue samples are then placed in a Cobra auto-gamma counting system (Packard Instrument Co., Meriden, Connecticut, USA), and a quantitative measurement of gamma radiation is obtained. Because the number of microspheres trapped in a particular tissue region is directly proportional to the blood flow to that region, and because it can be estimated by measuring the gamma activity of a postmortem tissue sample, this measurement, coupled with the calibrated withdrawal of the sample of arterial blood containing microspheres, permits calculation of the regional blood flow. The mathematical relationship for determining blood flow with this technique is $Q_{ref}/Q_{tis} = C_{ref}/C_{tis}$ where Q_{ref} and Q_{tis} represent the rate of withdrawal of the reference blood samples and the regional blood flow of the tissue, respectively, and C_{ref} and C_{tis} represent the number of counts in the reference blood samples and in the tissue sample, respectively.

Hemoglobin p50 was determined on arterial samples obtained before and 5 min after completion of the RSR13 infusion in the first experimental protocol by three-point tonometry using a Hemoscan instrument.

Measures of regional contractile function. The pairs of sonomicrometer crystals provided anterior- and lateral-wall segment length from the LAD and circumflex territories, respectively. Fractional shortening was equal to (end-diastolic length – endsystolic length) / end-diastolic length × 100. Segment length was also plotted vs. simultaneous left ventricular pressure to derive pressure-length loops. Regional work was derived from the area circumscribed by each loop, calculated by digital integration. End-diastolic pressure was taken as the pressure when dP/dt exceeded 10% of its maximal value after the onset of electrical activation. Pressure-dimension relations were also measured by transiently reducing preload volume by occlusion of inferior vena caval inflow. The end-systolic pressure segment-length relation provides a useful assessment of regional contractile function (24).

Statistical analysis. Results of continuous variables are reported as mean ± SEM. For the first experimental protocol, the data were found to be normally distributed and therefore allowed for parametric statistical comparisons of the two groups (control and RSR13). Two variables, intracellular pH and the PCr/ATP ratio, had initial non-normalized values and were therefore examined separately at baseline with one-way ANOVA. For these variables over time and for other variables repeated measures, two-way ANOVA was performed separately over the ischemic and reperfusion intervals for comparison of the two groups. For two-way analyses, group x time interaction was also examined. For the second protocol, comparisons of contractile, hemodynamic, and metabolic parameters before RSR13 during ischemia were compared with the same parameters in the same animals after RSR13 administration by paired *t* tests. Two-tailed *P* < 0.05 was considered significant.
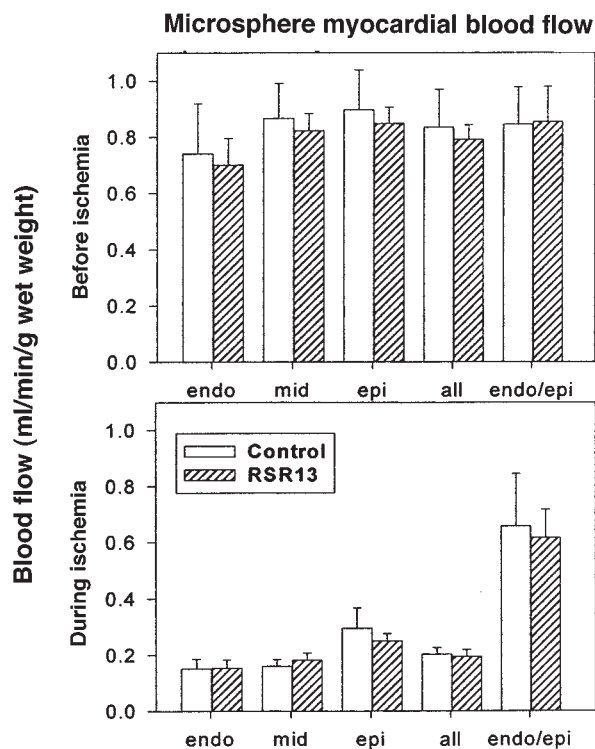
Microsphere myocardial blood flow

Figure 3

Myocardial blood flow measurements by radioactive microspheres before ischemia (*top*) and during ischemia (*bottom*), in control (*open bars*) and RSR13-treated animals (*hatched bars*). There is no difference in myocardial blood flow between the control and RSR13 animals for any of the comparisons. *RSR13,* 2-[4-[[(3,5-disubstituted anilino)carbonyl]methyl] phenoxy] -2-methylproprionic acid derivative.
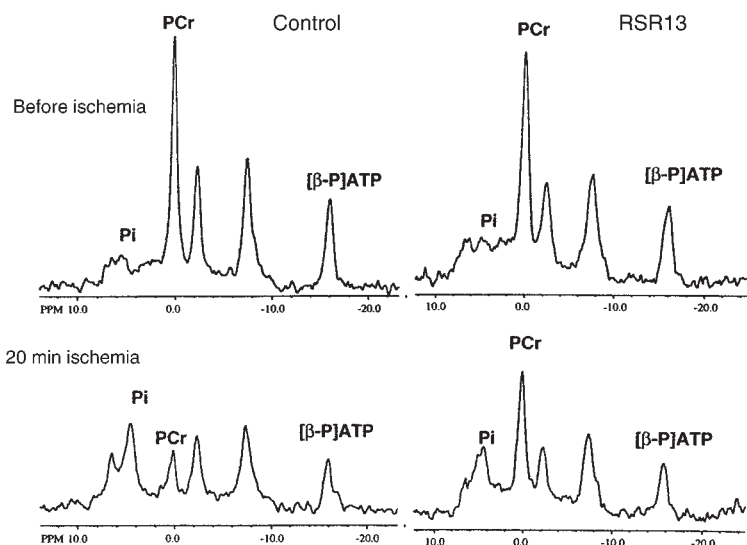
Results

RSR13 administration before ischemia. Before the start of the ischemic period, there were no statistically significant differences in hemodynamic, blood flow, or metabolic parameters between the RSR13-treated group and the control group. For the control vs. RSR13 groups, respectively, mean baseline systolic blood pressure (109 ± 5 vs. 115 ± 4 mmHg), diastolic blood pressure (75 ± 5 vs. 76 ± 4 mmHg), heart rate (144 ± 7 vs. 139 ± 9/min), coronary perfusion pressure (76 ± 5 vs. 75 ± 4 mmHg), and rate pressure product (15.8 ± 0.1 vs. 15.9 ± 0.1 mmHgK·beats/min) did not differ between the groups All parameters were mean ± SEM; P = not significant (NS). Myocardial blood flow was also comparable between control and RSR13 animals before ischemia (Fig. 3). Likewise, there were no significant differences in the mean myocardial PCr/ATP ratio (2.20 ± 0.12 vs. 2.19 ± 0.13) or the intracellular pH (7.20 ± 0.03 vs. 7.19 ± 0.04) between control and RSR13 animals, respectively, before ischemia (Figs. 4 and 5; see Fig. 7).

Intravenous administration of RSR13 (100 mg/kg) over a 15-minute period resulted in a substantial 17.9 ± 2.2 mmHg (mean ± SEM) increase in the mean p50, from a baseline of 33.2 ± 1.0 mmHg to a final p50 of 51.2 ± 2.7 mmHg. Systolic blood pressure (116 ± 3 vs. 115 ± 4 mmHg), diastolic blood pressure (75 ± 4 vs. 76 ± 4 mmHg), heart rate (134 ± 8 vs. 139 ± 9/min), and coronary perfusion pressure (77 ± 4 vs. 75 ± 4 mmHg) were unchanged five minutes after the RSR13 infusion, as compared with values obtained before infusion. Mean myocardial blood flows in the unshunted regions were similar in control and RSR13 animals (1.10 ± 0.15 vs. 1.03 ± 0.07 ml/min/g wet weight, respectively; P = NS), as were endocardial/epicardial blood flow ratios (1.2 ± 0.15 vs. 1.1 ± 0.19 for control and RSR13, respectively; P = NS).

During reduction of coronary perfusion pressure to 35 mmHg, there were no significant differences in systolic blood pressure (100 ± 12 vs. 106 ± 5 mmHg, control vs. RSR13; P = NS), diastolic blood pressure (65 ± 9 vs. 71 ± 5 mmHg), heart rate (146 ± 9 vs. 136 ± 9/min), coronary perfusion pressure (36 ± 1 vs. 35 ± 1 mmHg), or the rate pressure product (14.6 ± 0.1 vs. 14.7 ± 0.2 mmHgK·beats/min)

between control and RSR13 animals, respectively. Likewise, microsphere blood flows during ischemia were similar in RSR13 and control hearts in the endocardium (0.15 ± 0.07 vs. 0.15 ± 0.03), midwall (0.16 ± 0.02 vs. 0.18 ± 0.03), epicardium (0.29 ± 0.07 vs. 0.25 ± 0.03 ml/min/g; P = NS), and across the entire wall (0.20 ± 0.02 vs. 0.19 ± 0.02 ml/min/g; P = NS) of the ischemic region (Fig. 3), as well as in the same sections of the nonischemic vascular territories (endocardium: 1.00 ± 0.12 vs. 1.07 ± 0.08; midwall: 0.94 ± 0.11 vs. 1.0 ± 0.08; and epicardium: 0.98 ± 0.11 vs. 0.97 ± 0.08 ml/min/g wet weight, for control and RSR13, respectively; P = NS).

Low-flow ischemia caused a rapid decline in PCr to a stable level that was lower in control than in RSR13 animals (Figs. 4 and 6). Myocardial PCr was significantly better preserved during the ischemic interval in RSR13 than in control animals and was 62 ± 2% of preischemic values in RSR13 and 44 ± 3% in control animals; P < 0.001 at the end of ischemia (Fig. 6). The PCr/ATP ratio was also significantly higher during the 20-minute ischemic interval in RSR13 than in control hearts (P < 0.001 by repeated measures ANOVA) and at the end of 20 minutes of myocardial ischemia was 1.8 ± 0.2 in RSR13 animals and 1.1 ± 0.1 in control animals (P < 0.001, Fig. 5). Mean intracellular pH during the ischemic period was also significantly higher in RSR13 than in control animals (6.99 ± 0.02 vs. 6.80 ± 0.04, respectively, at the end of ischemia; P < 0.01, Fig. 7). ATP, as a percent of the initial value, was unchanged during this low-flow ischemia protocol and did not differ between the two groups (Fig. 8). Therefore, although RSR13 did not have an effect on myocardial blood flow, heart rate, or blood pressure, it did significantly attenuate the decline in PCr and intracellular pH during low-flow ischemia.

During postischemic reperfusion, metabolic recovery was observed in all hearts, with rapid restoration of PCr and normalization of intracellular pH. By the end of the reperfusion period, the two groups had comparable PCr/ATP ratios (3.3 ± 0.4 vs. 2.8 ± 0.2, control vs. RSR13; P = NS), percent PCr (101 ± 4% vs. 101 ± 3%), and intracellular pH (7.24 ± 0.01 vs. 7.21 ± 0.02).
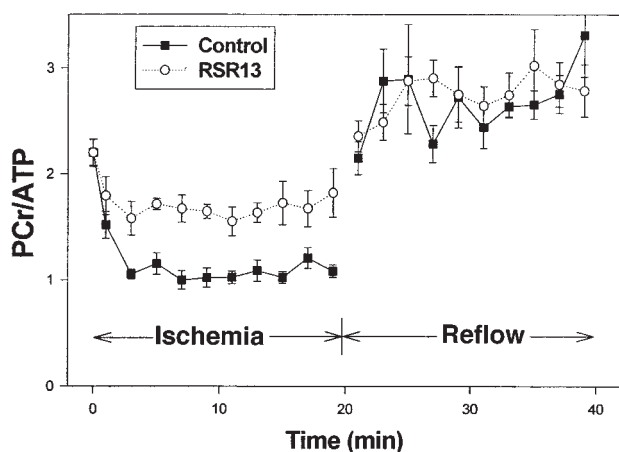
Figure 5
Myocardial PCr/ATP ratios vs. time for control (*closed squares*, *solid line*) and RSR13 (*open circles*, *dotted line*) animals. Time 0 indicates baseline readings just before the onset of low-flow ischemia. The myocardial PCr/ATP ratio is higher in the RSR13-treated animals throughout the ischemic period.

RSR13 administration during ischemia: contractile effects. In the second series of experiments, RSR13 was administered after the onset of ischemia, and each animal served as its own control. In the animals studied with ^{31}P NMR spectroscopy, baseline systolic blood pressure (106 ± 4.6 mmHg), diastolic pressure (69 ± 6.6 mmHg), heart rate (118 ± 8 beats/min), mean coronary perfusion pressure (75 ± 4.6 mmHg), as well as myocardial PCr/ATP ratio (1.92 ± 0.24) and intracellular pH (7.23 ± 0.04), were comparable to those observed at baseline in the first experimental protocol. The mean myocardial PCr/ATP ratio declined during early ischemia before RSR13 to 1.2 ± 0.1 but increased significantly after RSR13 administration to 1.6 ± 0.2 (P = 0.038) at 30 minutes of ischemia. The former value is similar to that of control hearts during early ischemia in the first protocol (Fig. 4), while the latter is similar to that of RSR13-administered hearts at the end of ischemia (Fig. 4). Mean intracellular pH fell to 6.97 ± 0.03 during early ischemia before RSR13 but fell no further by 30 minutes of ischemia. Intracellular pH at the end of ischemia in hearts given RSR13 after the onset of ischemia (pH of 6.98 ± 0.03) was the same as that of the RSR13-treated animals (pH of 6.99 ± 0.02) and higher than that of control animals (pH of 6.79 ± 0.04) at similar times. These observations demonstrate that RSR13 given during low-flow ischemia increases the myocardial PCr/ATP ratio and halts the decline in intracellular pH during ischemia.

In the studies of the effects of RSR13 on ischemic regional contractile function, baseline mean systolic blood pressure was 101 ± 6 mmHg, left ventricular diastolic pressure was 6 ± 2 mmHg, heart rate was 138 ± 6 beats/min, mean coronary perfusion pressure was 83 ± 7 mmHg, mean fractional shortening was $16 \pm 2\%$, and regional work was 237 ± 35 mmHg × mm. Representative pressure-dimension loops from an LAD region are presented in Figure 9a. During the reduction in LAD blood flow, there was regional dysfunction with an increase in diastolic and end-systolic dimensions, a rightward shift of the pressure-dimension loops and relations, and reductions in fractional shortening and regional work. There were no significant changes in the circumflex territory (Fig. 9b). Ten minutes after administration of intravenous RSR13 with unchanged coronary perfusion pressure, there was significant improvement in function, evidenced in part by a leftward shift of the pressure-dimension curves to lower-end systolic and end-diastolic dimensions (Fig. 9a). Mean fractional shortening in the LAD territory improved from $2 \pm 2\%$ before RSR13 to $6 \pm 3\%$ after RSR13 (P = 0.028), while LAD regional work also increased significantly, from 39 ± 25 mmHg × mm before RSR13 to 91 ± 40 mmHg × mm after RSR13 (P = 0.028). Thus, RSR13 administration during low-flow ischemia improved contractile function in the ischemic region as measured by fractional shortening and regional work, but had no effect on regional function within the nonischemic territory.

Discussion

The administration of a potent synthetic allosteric modifier of Hb–O_2 affinity (RSR13) to *in vivo* dogs, results in a dramatic rightward shift in the Hb–O_2 dissociation curve (*i.e.*, increased Hb p50) and a significant attenuation of acidosis and of the decline in myocardial PCr during subsequent low-flow ischemia. This reduction in the metabolic consequences of myocellular ischemia is seen without an increase in coronary blood flow or a reduction in the determinants of hemodynamic demand. In addition, administration of this agent during low-flow ischemia improves the cardiac PCr/ATP ratio, halts the decline in intracellular pH, and improves regional contractile function in the ischemic zone. Taken together, these data suggest that agents that shift the Hb–O_2 dissociation curve rightward may represent a novel anti-ischemic therapeutic strategy.

A rightward shift in Hb–O_2 dissociation is observed clinically in subjects with heart disease and is thought to be an adaptive response that enhances O_2 unloading to
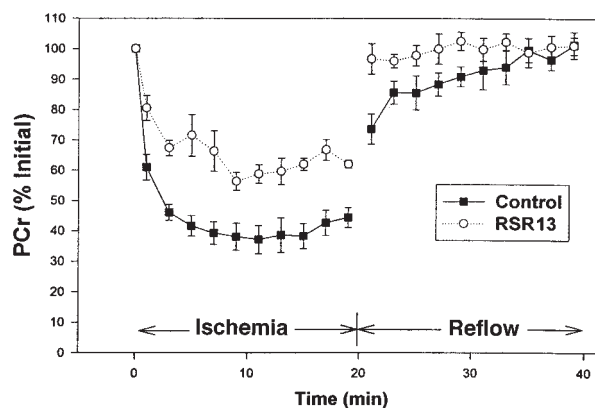

Figure 6
Myocardial PCr as a percent of initial values (*PCr % initial*) vs. time for control (*closed squares*, *solid line*) and RSR13 (*open circles*, *dotted line*) animals. Time 0 indicates baseline readings just before the onset of low-flow ischemia. PCr is significantly higher in the RSR13-treated animals throughout the ischemic period.
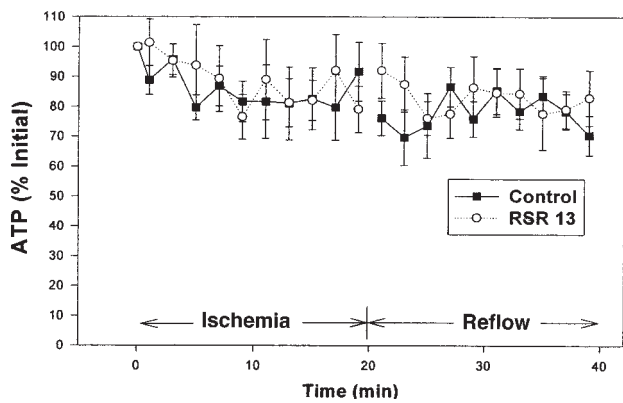
Figure 7

Myocardial ATP as a percent of initial values (*ATP % initial*) vs. time for control (*closed squares, solid line*) and RSR13 (*open circles, dotted line*) animals. Time 0 indicates baseline readings just before the onset of low-flow ischemia. ATP does not change during the mild–moderate ischemic period in either group.

tissues. Increased hemoglobin p50 is reported in patients during angina (7), after acute myocardial infarction (8–10), with congestive heart failure and cardiogenic shock (12, 13, 25), as well as in patients with congenital heart disease (26). Higher 2,3-diphosphoglycerate (DPG) levels contribute to the increased p50 in these clinical settings (11) and is typically on the order of ~2–3 mmHg. A similar order-of-magnitude increase in Hb-p50 occurs in the coronary bypass surgery setting after DPG-enriched blood replacement and is associated with improved cardiac performance (27).

Some commonly used antianginal medications, such as propranolol (28, 29) and nitroglycerin (30), also cause a rightward shift in the Hb–O_2 dissociation curve. The modest increase in p50 (~2 mmHg) associated with their use is estimated to increase O_2 delivery by 15%–30% in some settings (29, 30). Whether this actually provides anti-ischemic protection, in addition to their well-known negative inotropic and vasodilatory properties, remains undemonstrated in experimental and clinical settings.

Prior experimental interventions designed to produce a rightward shift of the Hb–O_2 dissociation curve typically increase p50 only modestly and have other confounding effects on myocardial energy demand and production. An infusion of dihydroxyacetone, phosphate, and pyruvate, which increases red blood cell DPG content, increased p50 by a mean of 2 torr (*P* < 0.01) and reduced infarct size after canine coronary occlusion in regions where myocardial blood flow was reduced by >40% (31). However, the negative inotropic effects, hypertonicity, and glycolytic substrates of the solution, were also acknowledged as potential contributors to the ischemic protection observed. Clofibrate was one of the first synthetic compounds recognized to cause a rightward shift in the Hb–O_2 curve (14), and others have been studied in experimental myocardial ischemia. The effects of ortho-iodo sodium benzoate (OISB), a benzoic acid derivative that decreases the affinity of hemoglobin for oxygen independent of DPG (32), on ischemic injury were studied in isolated and intact canine hearts. Intra-

coronary infusion of OISB in isolated canine hearts increased coronary sinus p50 and pO_2, and decreased heart rate and left ventricular systolic pressure (32). During paced, low-flow ischemia in the same model, OISB (200 mg/min) increased p50 (~4 mmHg) and left ventricular systolic pressure, but this was confounded by significant increases in coronary perfusion pressure and relative flow (32). Intravenous OISB (500 mg/kg) administration in intact dogs increased p50 by a mean ~4 mmHg, reduced electrocardiogram ST segment elevation 15 minutes after coronary occlusion, and reduced myocardial infarction size by 29% (33). In this setting, OISB also significantly decreased heart rate and contractility (dP/dt), which may have affected the reduction in acute ischemic injury. In summary, prior experimental interventions modestly decreased the affinity of hemoglobin for oxygen and afforded some protection from acute myocardial ischemia. Because all of these interventions had other potentially beneficial effects (*i.e.*, reduction in myocardial contractility or increase in coronary perfusion), the reduction in ischemic consequences observed could not be attributed unambiguously to increased tissue oxygen delivery by the increased p50.

This study provides novel information concerning the acute effects of a rightward shift in p50 that is three to five times more marked (~18 mmHg) than those observed with other agents (2–6 mmHg). In addition, this study generates important novel observations regarding a rightward shift in p50 in the absence of significant hemodynamic or blood flow effects to lessen the consequences of low-flow ischemia in the *in vivo* setting. The circumflex sonomicrometer data (Fig. 9) directly demonstrate that RSR13 does not reduce regional contractility, eliminating negative inotropy as a potential mechanism contributing to the metabolic protection afforded during ischemia by RSR13. In fact, the improvement in ischemic LAD fractional shortening and regional work after RSR13 administration is consistent with the metabolic protection demonstrated in the first series of experiments and provides the first evidence that a
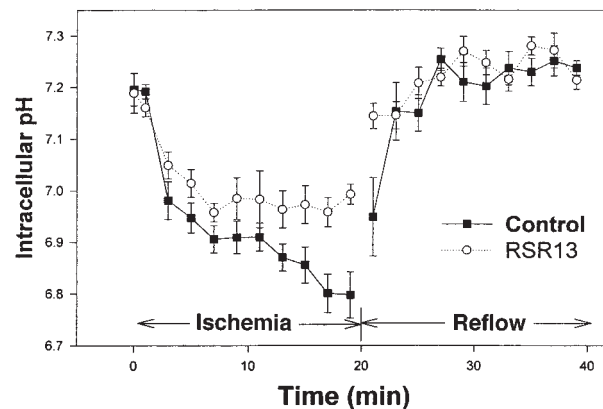

Figure 8

Myocardial intracellular pH vs. time for control (*closed squares, solid line*) and RSR13 (*open circles, dotted line*) animals. Time 0 indicates baseline readings just before the onset of low-flow ischemia. Myocardial pH is significantly higher in the RSR13-treated animals throughout the ischemic period.
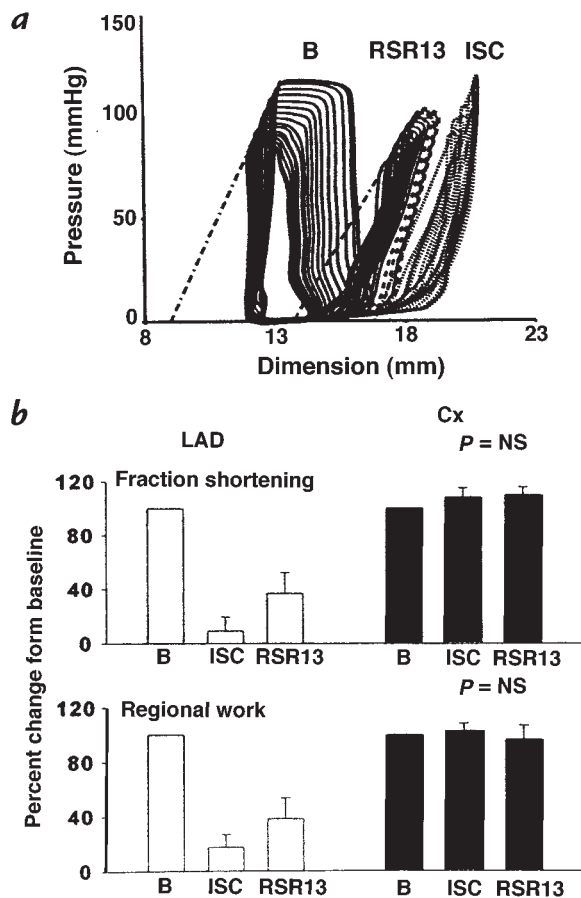
Figure 9
(*a*) Representative examples of pressure-dimension relationships in the LAD territory in a single animal before ischemia, during subsequent low-flow ischemia (*ISC*), and later during identical ischemia, but 10 min after completion of RSR13 infusion (*RSR13*). Note that the effect of RSR13 is to decrease the ischemia-induced increase in ventricular dimensions and to somewhat increase the area circumscribed in each curve. (*b*) Summary fractional shortening and regional work data for both territories for all six animals. LAD ischemia markedly reduced fractional shortening and regional work (*LAD, ISC*), but RSR13 administration during ischemia significantly improved both fractional shortening and regional work (*LAD, RSR13*). RSR13 had no effect of regional function in the nonischemic circumflex territory (*Cx*). *LAD*, left anterior descending.

rightward shift in the Hb–O$_2$ curve during ischemia improves ischemic dysfunction.

In future work it will be important to investigate longer periods of ischemia to determine whether RSR13 attenuates ischemic injury or only delays its onset. Likewise, studies of more severe reductions in coronary flow and studies of the absence of supplemental oxygen are required to determine whether protection is afforded by RSR13 during severe ischemia and infarction and the extent to which O$_2$ supplementation is required.

Finally, noninvasive serial measurements of *in vivo* myocardial high-energy phosphates with ^{31}P NMR spectroscopy probably provide some of the best indicators of the metabolic consequences of myocardial ischemia; they demonstrate here that RSR13 reduces the decline or improves high-energy phosphates and intracellular

pH during ischemia. The fact that the reduction in relative myocardial high-energy phosphates in this canine model of decreased LAD flow is similar to that reproducibly observed in patients with coronary artery disease during exercise (5, 6) suggests that the degree of experimental ischemia studied here is clinically relevant and that these data provide a rationale for future clinical studies. Taken together, these metabolic and contractile data provide compelling *in vivo* evidence that a rightward shift in the Hb–O$_2$ curve produced by potent synthetic allosteric modifiers of Hb–O$_2$ affinity may provide an important and complementary approach for reducing myocardial ischemia.

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