Improvement in myocardial performance without a decrease in high-energy phosphate metabolites after isoproterenol in Syrian cardiomyopathic hamsters

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ABSTRACT To determine the effect of isoproterenol on cardiac energetics and function in an animal preparation of cardiomyopathy, we studied Langendorff perfused hearts from Syrian cardiomyopathic hamsters. High-energy phosphate metabolites (phosphocreatine [PCr], ATP, inorganic phosphate [Pi]) and intracellular pH (pH_i) were measured by ^{31}P nuclear magnetic resonance spectroscopy and correlated with left ventricular developed pressure, coronary flow, and O_2 consumption before and during a 10^{-6}M infusion of isoproterenol. Total intracellular calcium was also determined by atomic absorption spectroscopy with the use of potassium ethylenediamine tetra-acetate cobaltate as a marker for extracellular space. In cardiomyopathic hamsters, isoproterenol infusion increased mean developed pressure by 300% ($p < .005$ compared with control; $n = 5$), O_2 consumption eightfold ($p < .0005$), and PCr by 40% ($p < .05$). PCr/Pi ratio, which is analogous to phosphorylation potential, improved 100% ($p = .05$). In normal hamsters, isoproterenol infusion resulted in an 83% increase in developed pressure ($p < .001$) and a 25% increase in O_2 consumption (NS). However, mean PCr and PCr/Pi decreased by 30% and 50%, respectively ($p < .05$ for both), during isoproterenol infusion. pH_i decreased in normal animals ($p < .01$), but tended to improve in diseased animals (NS) during isoproterenol infusion. Freeze-clamp measurements of phosphate metabolites correlated well with the nuclear magnetic resonance data. Intracellular calcium increased from 0.0102 ± 0.002 to 0.144 ± 0.030 μmol/ml heart water in normal hamsters during isoproterenol infusion. Cardiomyopathic hamsters had a markedly elevated baseline calcium content of 60.82 ± 5.85 μmol/ml heart water due to the presence of dystrophic calcification. Isoproterenol did not significantly alter this calcium content. We conclude that in cardiomyopathic hamsters, isoproterenol markedly increases contractile function and energy demand without an associated deterioration in the high-energy phosphate profile. In contrast, normal hamsters are unable to synthesize sufficient ATP to replenish the amount used in meeting the increased workload during isoproterenol infusion.
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THE ROLE of catecholamines in cardiac disease is complex and poorly understood. Adrenergic dysfunction[1–5] has been implicated in the pathogenesis of the hereditary polymyopathy seen in a strain of Syrian hamsters designated UM-X7.1, a derivative of the Bio 14.6 strain.[6] These animals develop a cardiomyopathy with distinct phases of heart failure that has been extensively studied as a model of dilated cardiomyopathy.[6, 7] Cardiac necrosis and myolysis are noted by 30 to 40 days of age. By 90 to 100 days of age the degenerative changes subside and myocardial hypertrophy, dilatation, and fibrosis develop. Congestive heart failure, manifested by tachypnea, ascites, hepatomegaly, and peripheral edema, is evident by 180 to 200 days of age.[6] During the necrotic phase, increased numbers of adrenergic nerve terminals,[3] increased norepinephrine uptake,[4] and elevations of urinary norepinephrine levels[2] have been documented, suggesting catecholamine dysfunction. Isoproterenol (a synthetic catecholamine), when given in 1 to 20 mg/kg doses, has been shown to increase total myocardial calcium content in these animals.[8] This exacerbates the intracellular cal-

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cium overload that is a prominent feature of this myopathy.[9–12] The pathologic changes observed in hearts from this myopathic strain are also reminiscent of the cardiac necrosis seen in isoproterenol overdose.[5]

Unexpectedly, Jasmin and Proschek[13] found that long-term isoproterenol (0.05 mg/kg injection twice daily) prevented development of the myocardial necrotizing process in young cardiomyopathic hamsters. Isoproterenol-treated diseased hamsters also had a 60% reduction in serum creatine kinase and a 75% reduction in myocardial calcium when compared with untreated myopathic hamsters. This study suggests that isoproterenol, when given in appropriate doses, may have beneficial effects in this cardiomyopathic preparation.

Isolated Langendorff heart preparations from cardiomyopathic hamsters in the postnecrotic stage were therefore studied to: (1) determine the hemodynamic effect of adding isoproterenol to the perfusate, (2) correlate myocardial performance with energy metabolism as measured by ^{31}P nuclear magnetic resonance (NMR) spectroscopy, and (3) compare the effect of isoproterenol on cardiomyopathic and healthy control hamsters. Left ventricular developed pressure (developed pressure) coronary flow, and O_2 consumption were monitored simultaneously with measurements of ATP, phosphocreatine (PCr), inorganic phosphate (Pi), and intracellular pH (pH_i) obtained by ^{31}P NMR spectroscopy. Traditional freeze-clamp biochemical methods were used to confirm the ^{31}P NMR data. In addition, the effect of short-term isoproterenol administration on total intracellular calcium was evaluated by atomic absorption spectroscopy with the use of potassium ethylenediamine tetra-acetate cobaltate (KCoEDTA) as an extracellular marker.

Methods

Animals. Syrian hamsters of the UM-X7.1 strain[6] (a derivative of the Bio 14.6 strain) between 150 and 200 days of age were used as experimental animals. Healthy age-matched Syrian hamsters from an unrelated strain served as controls. All the hamsters were maintained under identical conditions with free access to laboratory chow and water.

Perfused heart preparation and experimental protocol. Hamsters were anesthetized with ether before midline sternotomy. The hearts were rapidly excised and perfused by the Langendorff method as previously described[14] with a modified Krebs-Henseleit solution containing 117 mM NaCl, 4.3 mM KCl, 2.4 mM $MgCl_2$, 0.1 mM K_2HPO_4, 25 mM $NaHCO_3$, 3.5 mM $CaCl_2$, 0.5 mM NaEDTA, 15 mM glucose, and 100 units/liter of insulin. Coronary perfusion pressure was 140 cm H_2O. Perfusate temperature was maintained constant at 35° C with counter-current heat exchangers[15] and a thermostat-regulated circulating water bath. To maintain a constant heart rate, pacing leads were inserted into the right ventricular base and connected to a Medtronic model 5320 pulse generator. Hearts were paced at a rate just above the intrinsic heart rate observed during isoproterenol infusion.

Measurements of left ventricular pressure were obtained from a cannula inserted through the left atrium and mitral valve into the left ventricular cavity. The cannula was sutured firmly into place at the level of the atrial appendage to provide a tight seal and was then connected to a pressure transducer. Pressures were recorded on a four-channel Beckman dynagraph.

Coronary flow was measured by continuously siphoning the effluent from the right ventricular outflow tract out of the NMR chamber. Aortic perfusate (arterial) and right ventricular outflow tract (venous) samples were obtained before and after placing the heart in the magnet and immediately measured for partial pressure of oxygen with a Corning gas analyzer. Myocardial oxygen consumption was calculated as the product of coronary flow and the arterial-venous O_2 difference across the heart.[16]

The hearts were perfused with Krebs-Henseleit solution for 20 min before data collection. This allowed the heart preparation to reach a steady state.[16] Hemodynamic and ^{31}P NMR spectroscopic measurements were then taken. The perfusate was then changed to Krebs-Henseleit solution containing 10^{-6}M isoproterenol. After a 20 min reequilibration period hemodynamic measurements and ^{31}P NMR spectra were repeated.

^{31}P NMR spectroscopy. ^{31}P NMR spectra of the beating isolated perfused heart were obtained on a 5.6 tesla vertical 76 mm bore magnet. The home-built spectrometer was connected to a 1180 Nicolet computer, a pulse programmer, and a high-resolution 20 mm broad-band probe. Undecoupled ^{31}P spectra were obtained at 97.3 MHz. Pulse angle was 75 degrees, recycle time 2.25 sec, and spectral width was ±4000 Hz. Transients were accumulated for 20 min. To correct for partial saturation, fully relaxed spectra were obtained at a 15 sec recycle time and correction factors for PCr and Pi were determined (3% and 5%, respectively). The relaxation rates expressed as T_1 under these conditions are 0.5 to 1.0 sec for ATP, 1.5 to 2.0 sec for PCr, and 2.5 to 3.0 sec for Pi. These values were not significantly different in normal vs myopathic hearts and did not change with isoproterenol administration. Zero parts per million was assigned to the resonance position of PCr. For each spectrum, the characteristic Pi, PCr, and β-ATP peaks were identified.[17–19] The area under each peak was calculated with a computerized hand-regulated electronic planimeter and assigned an arbitrary value. No electronic baseline correction was required. Figure 1

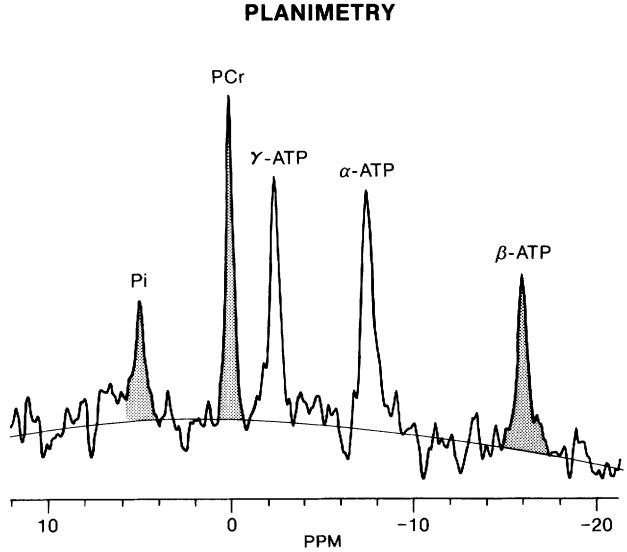

FIGURE 1. A representative ^{31}P NMR spectrum illustrating the technique used to calculate the area under the Pi, PCr, and β-ATP peaks. The shaded areas show the boundaries of each peak, which were planimetered to determine the respective peak area.

shows a sample spectrum and the baseline used to determine the respective peak areas. A "phosphate percent" was calculated for Pi, PCr, and ATP by dividing each individual peak area by the sum of the areas for all three peaks. The phosphate percent does not give an absolute value and is not normalized for tissue weight. The PCr/Pi ratio, an index of mitochondrial function,[20, 21] was calculated from the respective phosphate percents. pH_i was estimated from the chemical shift of the pH-dependent Pi peak relative to the PCr peak.[22]

Biochemical assays. In a parallel series of experiments, normal and myopathic hearts were frozen during control and isoproterenol perfusion with a stimulator-triggered freeze clamp. This instrument decreases the temperature of the heart from 37° to −80° C in 5 msec.[23] Heart tissue extracts were prepared as described previously.[16] By use of the neutralized extract, samples were analyzed by high-pressure liquid chromatography. For separation of nucleotides, a Beckman chromatograph with a C-18 reverse-phase column was used. For the mobile phase, 19% acetonitrile in 0.03M KH_2PO_4 with 0.01M tetrabutylammonium (TBA) phosphate, pH 2.65, was used. Elution was 1 ml/min with detection by a Beckman ultraviolet spectrophotometer at 254 nm. PCr and creatine were analyzed on the same column, but elution was 1.5 ml/min with 0.3% KH_2PO_4 and 0.1% TBA (pH 3.0). Detection was at 210 nm. PCr and creatine and nucleotides were quantified by integration of defined peaks of unknown in relation to integration of defined peaks of known standard concentrations. All were analyzed within the linear range. The concentrations of adenosine, inosine, and hypoxanthine in the coronary effluent were measured by injecting 40 μl on the Beckman C-18 reverse-phase column with use of a 10 min gradient of 0 to 20% methanol in water followed by a 10 min elution time at 20% methanol.

Total intracellular calcium determination. In a parallel series of experiments, total intracellular calcium concentration was determined by measurement of total myocardial calcium content and subtraction of the extracellular fraction determined with KCoEDTA as an extracellular marker.[24–26] KCoEDTA was synthesized by the method of Dwyer et al.[27] and added to the Krebs-Henseleit perfusate at a concentration of 0.6 mM. At the end of the control or isoproterenol perfusion period, the hearts were removed from the perfusion apparatus, weighed, and minced. A dry weight was obtained after 72 hr at 110° C. The difference between wet and dry heart weight yields the volume of heart water assuming 1 g = 1 ml H_2O. Total myocardial calcium and cobalt content were measured by flame atomic absorption spectroscopy on a Perkin-Elmer 2380 spectrophotometer after acid extraction with a solution of 1N HCl and 1% lanthanum chloride. Measurements were taken at 422.7 and 240.7 nm for calcium and cobalt, respectively. Samples were compared with standards obtained from the U.S. Department of Commerce, National Bureau of Standards, and analyzed over the linear range.

The extracellular space was determined by the following formula:

$$ECS = \frac{Co_t}{Co_p}$$

where ECS is the fraction of extracellular space, Co_t is the total myocardial cobalt content per milliliter of heart water, and Co_p is the mean perfusate and coronary effluent cobalt content per milliliter as measured by atomic absorption spectroscopy.

Total intracellular calcium content was then calculated by the following formula:

$$Ca_i = Ca_t - [(Ca_p)(ECS)]$$

where Ca_i is the total intracellular calcium content and Ca_p is the mean perfusate and coronary effluent calcium content per milliliter as measured by atomic absorption spectroscopy. Total intracellular calcium content was expressed as micromoles per milliliter of heart water.

Statistical analysis. Values are reported as mean ± SE. The differential effect of isoproterenol-containing perfusate versus control perfusate in the same animal was analyzed by Student's paired t test. Differences between cardiomyopathic and control hamsters were analyzed with Student's unpaired t test. The null hypothesis was rejected at the 5% confidence limit.

Results

Effect of isoproterenol on normal hamster hearts. Table 1 summarizes the hemodynamic changes associated with isoproterenol infusion in normal and myopathic hearts. Normal hamsters had an 83% increase in left ventricular developed pressure after addition of isoproterenol to the perfusate ($p < .001$ compared with control). Oxygen consumption showed a 25% increase that did not reach statistical significance. Coronary flow, end-diastolic pressure, and heart rate were unchanged.

Representative ^{31}P NMR spectra are shown in figure 2. The improved mechanical performance during isoproterenol infusion was accompanied by a deterioration of the metabolic variables measured by ^{31}P NMR spec-

TABLE 1
Hemodynamic variables in normal and myopathic hamsters

	Coronary flow (ml/min)	MVO_2 (μmol/g dry wt/min)	Developed pressure (mm Hg)	EDP (mm Hg)	Heart rate (beats/min)
Normal hamsters (n = 5)					
− isoproterenol	8.5 ± 0.9	37 ± 7	128 ± 16	2 ± 1	238 ± 2
+ isoproterenol	9.5 ± 0.9	46 ± 7	234 ± 17	2 ± 1	242 ± 5
Myopathic hamsters (n = 5)					
− isoproterenol	6.8 ± 0.4	9 ± 2	65 ± 6	28 ± 3	236 ± 2
+ isoproterenol	8.2 ± 0.4	73 ± 6	261 ± 35	4 ± 1	236 ± 2

Values are mean ± SE.
MVO_2 = myocardial oxygen consumption; EDP = end-diastolic pressure.
− isoproterenol = control perfusate; + isoproterenol = isoproterenol-containing perfusate.

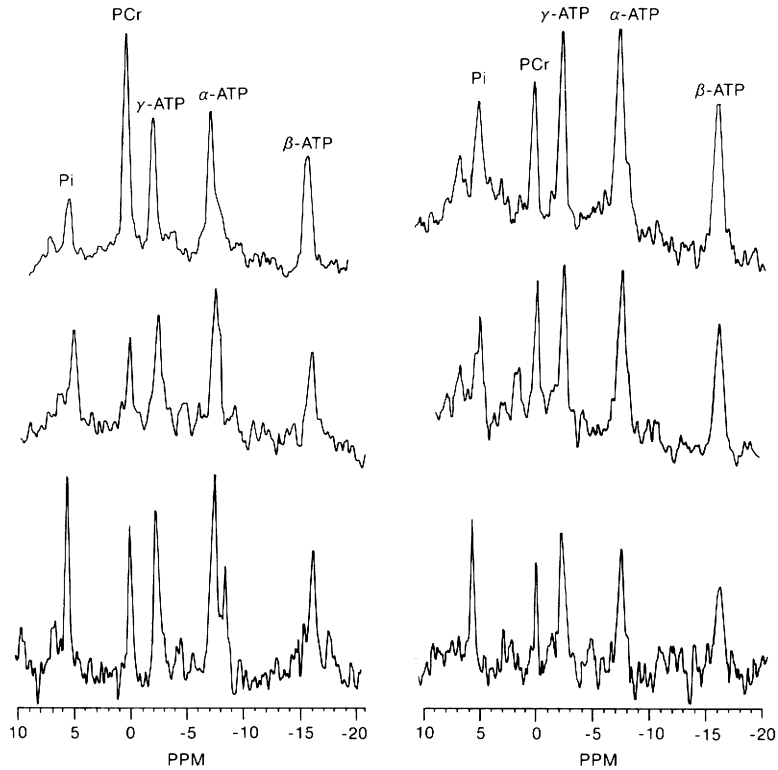

FIGURE 2. Representative ^{31}P NMR spectra of normal (first column) and myopathic (second column) hamster hearts. The spectra in the first row are from Langendorff-perfused hearts under control conditions. The spectra in the second row are from Langendorff-perfused hearts during isoproterenol infusion. The spectra in the third row are from tissue extracts used for freeze-clamp biochemical analysis.

troscopy (figure 3). The Pi phosphate fraction increased from 30 ± 4% to 46 ± 6% ($p = .01$) and PCr phosphate fraction decreased from 36 ± 4% to 26 ± 4% ($p < .005$). The ATP phosphate fraction was unchanged. The PCr/Pi was also significantly lower after perfusion with isoproterenol ($p < .05$). pH_i decreased from 7.14 ± 0.02 to 7.06 ± 0.02 ($p < .01$). To determine whether there was ischemia or a significant energy contribution via the glycolytic pathway, measurements of inosine, hypoxanthine, and lactate in coronary effluent were made. As noted in table 2, there was no significant difference in these variables before and during isoproterenol administration.

Baseline total intracellular calcium content in normal hamster hearts as measured by atomic absorption spectroscopy was 0.0102 ± 0.002 μmol/ml of heart water ($n = 5$). This value increased significantly to 0.144 ± 0.030 μmol/ml of heart water ($n = 6$) during perfusion with isoproterenol.

Effect of isoproterenol on cardiomyopathic hamster hearts (figure 2). When perfused with control buffer, cardiomyopathic hamsters had significantly lower mean developed pressure and O_2 consumption in comparison with normal hamsters ($p < .005$ and $p < .05$, respectively) (see table 1). This agrees with data from 180- to 200-day-old cardiomyopathic hamsters studied

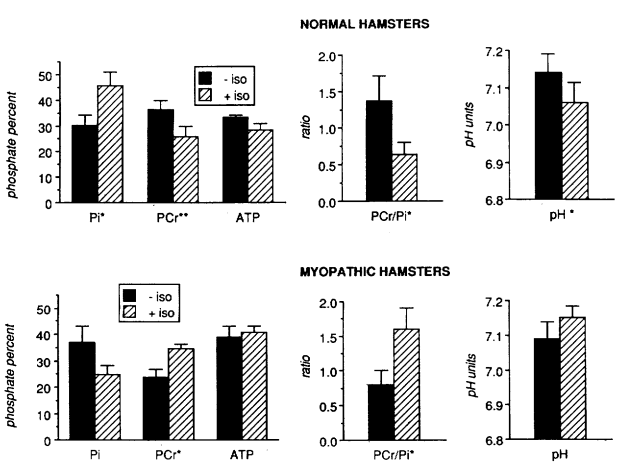

FIGURE 3. The effect on phosphate metabolites and pH_i of adding isoproterenol to the perfusate of normal ($n = 5$) and cardiomyopathic ($n = 7$) Langendorff-perfused hamster hearts. Values are mean ± SEM. *$p \leq .05$; **$p < .005$.

TABLE 2
Coronary effluent analysis in normal and myopathic hamsters

	Inosine + hypoxanthine (nM/ml)	Lactate (mg/dl)
Normal hamsters		
− isoproterenol (n = 3)	0.078 ± 0.02	3.3 ± 0.7
+ isoproterenol (n = 5)	0.177 ± 0.06	3.0 ± 0.03
Myopathic hamsters		
− isoproterenol (n = 6)	0.294 ± 0.09	2.5 ± 0.3
+ isoproterenol (n = 6)	0.132 ± 0.05	3.3 ± 0.3

Values are mean ± SE. There were no significant differences between groups with and without isoproterenol.

− isoproterenol = control perfusate; + isoproterenol = perfusate containing isoproterenol.

TABLE 3
Metabolic variables in normal and cardiomyopathic hamsters as determined by freeze-clamp analysis and ^{31}P NMR

	ATP		PCr		Creatine	
	− iso	+ iso	− iso	+ iso	− iso	+ iso
Normal hamsters						
Freeze clamp (n = 3)	9.9 ± 0.4	7.0 ± 0.8	12.1 ± 1.3	6.8 ± 1.1	11.9 ± 1.3	17.2 ± 1.8
^{31}P NMR (n = 5)	10.4 ± 1.3	7.5 ± 1.4	11.0 ± 1.2	7.0 ± 1.2	13.0 ± 1.1	17.0 ± 1.2
Myopathic hamsters						
Freeze clamp (n = 3)	5.7 ± 0.4	6.5 ± 0.7	5.5 ± 0.9	7.5 ± 1.0	16.5 ± 0.9	14.5 ± 1.4
^{31}P NMR (n = 6)	6.1 ± 1.7	6.5 ± 1.7	3.8 ± 1.5	7.3 ± 1.5	18.2 ± 1.4	14.7 ± 1.3

Values are mean ± SD in mM free cytosolic fraction. ^{31}P NMR data were standardized by use of baseline freeze-clamp values to allow comparison between ^{31}P NMR data and the freeze-clamp data. All data (^{31}P NMR and freeze clamp) are corrected for free cytosolic fraction as described previously[14] with 40% wet weight as cytosolic. No correction was made for assumed bound ATP, PCr, or creatine, as described in earlier studies.[14] Pi was corrected for 8% bound in cytosolic fraction as described previously.[14] PCr + creatine in normal hamster as determined by freeze-clamp analysis is 24 mM free cytosolic or 65 μM/g dry weight. PCr + creatine in myopathic hamsters as determined by freeze-clamp analysis is 22 mM free cytosolic or 58 μM/g dry weight. For the ^{31}P NMR data, creatine concentration was determined by subtracting the PCr concentration from the PCr + creatine total determined by freeze-clamp analysis. Free ADP was calculated from the creatine kinase equilibrium with 2.36×10^9 as the equilibrium constant aand the following equation: $(ADP)_{free} = [(ATP)(Cr)]/[(K_{eq})(H^+)(PCr)]$. − iso = control perfusate; + iso = isoproterenol-containing perfusate.

in our laboratory.[28] During perfusion with isoproterenol-containing buffer, mean developed pressure in cardiomyopathic hamsters increased 300% ($p < .005$) to a level similar to that attained in isoproterenol-perfused normal hamsters. Oxygen consumption increased from 9 ± 2 to 73 ± 6 μmol/g dry weight/min ($p < .0005$) to a value significantly higher than in isoproterenol perfused normal hamsters ($p < .05$). End-diastolic pressure returned to normal after isoproterenol treatment.

The increased mechanical performance after isoproterenol was associated with a stable and in some cases improved high-energy phosphate profile in cardiomyopathic hamsters. The mean PCr phosphate fraction, which was significantly lower in cardiomyopathic vs normal hamsters ($p < .01$) during control perfusion, increased from 24 ± 3% to 35 ± 2% ($p < .05$) during isoproterenol perfusion (figure 3). Mean PCr/Pi ratio also increased a small but significant amount ($p = .05$). Mean ATP and Pi phosphate fractions and pH_i did not significantly change with isoproterenol perfusion. Table 2 shows that there were no significant differences in the concentrations of adenine nucleotide degradation products (inosine and hypoxanthine) or lactate in the coronary effluent.

Total intracellular calcium concentration during control perfusion was markedly elevated at 60.82 ± 5.85 μmol/ml of heart water (n = 4). During isoproterenol treatment there was a mean decrease to 56.02 ± 6.35 μmol/ml of heart water (n = 7), which was not statistically significant.

Correlation of ^{31}P NMR and freeze-clamp measurements. Table 3 shows the absolute concentrations of metabolites measured by the rapid freeze-clamp technique in normal and cardiomyopathic hamsters. In addition, the ^{31}P NMR data were standardized by use of baseline freeze-clamp values to provide NMR-derived metabolite concentrations. There was an excellent correlation between the ^{31}P NMR data and results of biochemical analysis of tissue extract metabolites when correction was made for the cytosolic, bound Pi fraction[14] not seen by NMR. The greatest discrepancy between NMR and freeze-clamp data was seen in the Pi measurements in cardiomyopathic hamsters. This was most likely due to the phosphate present in the dystrophic calcification of myopathic hearts,[7] which was measured by the freeze-clamp technique but not by ^{31}P NMR.

It is important to note that normal perfused hamster hearts have higher Pi values when compared with normal rat hearts perfused with the same Krebs-Henseleit buffer. This is in agreement with previously published reports.[14, 29] The baseline PCr/Pi ratio in normal hamsters is therefore lower than the value measured in normal rat hearts.[16, 29]

Discussion

^{31}P NMR spectroscopy is uniquely suited for the study of cardiac energy metabolism because of its ability to provide nondestructive, repetitive, and quantitative measurements of bioenergetics in the functioning, intact heart.[17–19] The hemodynamic response to therapeutic interventions can therefore be correlated with changes in the high-energy phosphate profile. In this study, ^{31}P NMR spectroscopy was used to evaluate the effects of isoproterenol in a preparation of cardiomyopathy. Traditional biochemical assays were used to confirm the ^{31}P NMR spectroscopy data. In addition, intracellular calcium was measured to pro-

TABLE 3
(Continued)

Pi		free ADP	
−iso	+iso	−iso	+iso
6.7±1.0	16.0±1.2	0.046±0.003	0.075±0.003
7.2±4.4	16.8±3.9	0.054±0.004	0.075±0.004
12.7±2.6	8.3±1.6	0.072±0.002	0.052±0.004
8.2±3.4	4.8±2.4	0.12±0.04	0.056±0.006

vide insights into the possible mechanism of isoproterenol's effect.

Catecholamines are important in the normal regulation of myocardial contractility and metabolism but they may also have deleterious effects. Isoproterenol, when given above physiologic doses (5 to 85 mg/kg body weight), produces disseminated myocardial necrosis in laboratory animals.[30–32] It has been postulated that these high doses deplete energy reserves, leading to biochemical alterations and subsequent structural damage.[32] Intracellular calcium overload is a crucial factor in the development of this injury.[32] Several features of this isoproterenol-induced cardiotoxicity are similar to those of the cardiomyopathy in Syrian hamsters.[11] Loss of calcium homeostasis is a prominent feature of this polymyopathy,[9–12] and Lossnitzer et al.[8] have shown that a single high dose of isoproterenol increases the total myocardial calcium content in these animals. In view of these observations, it is remarkable that isoproterenol improved mechanical function without inducing a deterioration in the high-energy phosphate profile when it was added to the perfusate of cardiomyopathic hamster hearts.

Abnormal cardiac performance is a major feature of the Syrian hamster cardiomyopathy. The baseline hemodynamic abnormalities noted in this study are consistent with previous reports documenting systolic and diastolic contractile dysfunction in perfused[14, 33] and in vivo cardiomyopathic hamsters.[34] In cardiomyopathic hamsters perfused by the Langendorff method perfusion pressure and heart rate are major determinants of left ventricular diastolic and systolic pressure. Our laboratory has reported[33] elevated diastolic and reduced systolic pressures in 155- to 170-day-old Langendorff perfused cardiomyopathic hamsters (perfusion pressure 140 mm Hg) when compared with healthy age-matched controls. In cardiomyopathic hamsters, increasing heart rate from 170 to 270 beats/min resulted in a 63 mm Hg elevation in mean diastolic pressure and a 25 mm Hg reduction in systolic pressure. However, normal hamsters showed no significant changes in systolic or diastolic pressures at different heart rates. It therefore appears that at 140 mm Hg perfusion pressure, thebesian flow is sufficient to significantly elevate diastolic pressure in failing myopathic hearts. In the present study, all hearts were paced at the same rate (just above the intrinsic rate observed during isoproterenol administration) to control for isoproterenol's chronotropic effect. It is important to note that in cardiomyopathic hamsters, isoproterenol administration not only improved systolic function but also resulted in reduction of the elevated diastolic pressure, suggesting improved diastolic relaxation.

Although high-dose isoproterenol has been reported to have toxic affects in cardiomyopathic hamsters,[8] the lower dose used in our study did not result in deterioration of the metabolic variables we evaluated. Although mitochondrial dysfunction is a hallmark of this polymyopathy,[35] isoproterenol infusion was associated with a stable and in several cases increased PCr/Pi ratio in the presence of a markedly increased energy demand. PCr/Pi is an energetic index that has been shown to correlate with the phosphorylation potential ([ATP]/[ADP] [Pi]),[20, 21] which is believed to play a role in regulating mitochondrial respiration and cardiac contractility.[36] ATP levels also remained unchanged during isoproterenol infusion. This suggests that in cardiomyopathic hamsters, oxidative phosphorylation was sufficient to meet the increased energy demands imposed by isoproterenol. However, mitochondrial activity remained inefficient in the myopathic hearts since oxygen consumption was 1.8 times higher than in normal hearts at a similar level of increased cardiac performance. To exclude the possibility of ischemia or a significant energy contribution from glycolysis during isoproterenol administration, determinations of lactate and adenine nucleotide degradation products in coronary effluent were made. Since there was no increase in coronary efflux of either lactate or xanthine and hypoxanthine during isoproterenol treatment, it is unlikely that ischemia or a significant energy contribution via the glycolytic pathway occurred.

Our study also suggests that normal hamster hearts are more sensitive to isoproterenol's deleterious effects since they showed a deterioration in the metabolic profile at the dose that had no adverse effect on the energy profile in diseased hamsters. The reduction in PCr/Pi ratio with a trend toward increasing O_2 consumption observed in normal hamsters during isoproterenol infusion suggests that mitochondrial activity was inadequate to replenish the ATP hydrolyzed in meeting the increased energy demand. A similar

inverse relationship between O_2 consumption and PCr/Pi has been observed in our laboratory with perfused rat hearts during high workload conditions.* Our results are also consistent with a recent report by Bittl et al.[37] that documented a 43% reduction in PCr during norepinephrine infusion in living, anesthetized normal rats.

The basic mechanism for cell injury in the Syrian cardiomyopathic hamster is unknown, but the reported defects include: abnormal membrane function leading to intracellular calcium accumulation,[12, 38] decreased cyclic AMP levels,[39] impaired mitochondrial activity,[35] and intracellular acidity.[28] The mechanism for isoproterenol's beneficial effect on this cardiomyopathic preparation is difficult to explain since isoproterenol is known to promote calcium ion influx in normal myocytes via the slow calcium channels.[40] We therefore measured the effect of the short-term infusion of isoproterenol on total intracellular calcium content in the functioning, intact heart. In this study, total intracellular calcium is defined as the amount of calcium not present in the extracellular space as determined by KCoEDTA. Cobaltic EDTA does not enter myocardial cells, exhibits no tissue binding, and has a volume of distribution nearly identical to that of sucrose.[24] These characteristics make it a useful marker for myocardial extracellular space.[25, 26]

The control value for total intracellular calcium reported in this study for normal hamsters correlates well with previously published reports.[26, 41] The short-term infusion of isoproterenol increases total intracellular calcium 14-fold in normal hamsters. This agrees with a recent perfused rabbit heart study[42] that documented a 10-fold increase in intracellular calcium after treatment with norepinephrine, a catecholamine similar to isoproterenol. Total intracellular calcium content measured in the cardiomyopathic hamsters was markedly elevated, primarily due to the dystrophic calcification present in these animals.[6, 7] These calcified deposits are included in the total intracellular calcium measurement obtained by the technique used in this study. In spite of this limitation, it is noteworthy that isoproterenol did not increase the elevated calcium level in these diseased hamsters.

Our findings are consistent with Jasmin and Proschek's report[13] that isoproterenol (0.05 mg/kg twice daily), when given from 30 to 60 days of age to Syrian hamsters from a myopathic strain, essentially prevented expression of the cardiomyopathic state. The typical cardiac necrosis and myolysis observed in this cardiomyopathic strain was markedly diminished in the treated group. In addition, isoproterenol-treated hamsters had a 75% reduction in total myocardial calcium when compared with untreated cardiomyopathic hamsters. This demonstrates that isoproterenol, through an undefined mechanism, can prevent the accumulation of myocardial calcium in young cardiomyopathic hamsters. The results of this long-term study, coupled with our finding that the short-term infusion of isoproterenol does not exacerbate the intracellular calcium overload seen in cardiomyopathic hamsters, suggests that appropriate doses of isoproterenol may exert a beneficial effect on calcium homeostasis in this disease state.

The salutary effect of isoproterenol noted in this cardiomyopathic preparation has an important parallel in the clinical setting. In patients with idiopathic dilated cardiomyopathy, the short-term infusion of dobutamine (another synthetic catecholamine) results in improved cardiac function that is sustained over several weeks.[43] Unverferth et al.[44, 45] documented normalization of mitochondrial structure and an increased ATP/creatine ratio in endomyocardial biopsy samples obtained from these patients after dobutamine infusion. This suggests that the hemodynamic benefit results from improved mitochondrial function and energetics. The beneficial action of isoproterenol on cardiomyopathic hamsters therefore appears to have a corollary in dobutamine-treated patients with dilated cardiomyopathy. Despite obvious differences between this animal preparation and human disease, elucidation of the mechanisms involved in isoproterenol's beneficial effect may provide insight into the role of catecholamines in the pathogenesis and treatment of idiopathic dilated cardiomyopathy.

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