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ATP sensitive K^+ channels are critical for maintaining myocardial perfusion and high energy phosphates in the failing heart

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Abstract

Congestive heart failure (CHF) is associated with intrinsic alterations of mitochondrial oxidative phosphorylation which lead to increased myocardial cytosolic free ADP. ATP sensitive K^+ channels (K_{ATP}) act as metabolic sensors that are important for maintaining coronary blood flow (MBF) and in mediating the response of the myocardium to stress. Coronary adenosine receptors (AdR) are not normally active but cause vasodilation during myocardial ischemia. This study examined the myocardial energetic response to inhibition of K_{ATP} and AdR in CHF. CHF (as evidenced by LVEDP > 20 mmHg) was produced in adult mongrel dogs (n=12) by rapid ventricular pacing for 4 weeks. MBF was measured with radiolabeled microspheres during baseline (BL), AdR blockade with 8-Phenyltheophylline (8-PT; 5 mg/kg iv), and K_{ATP} blockade with Glibenclamide (GLB; 20 μg/kg/min ic). High energy phosphates were examined with ^{31}P magnetic resonance spectroscopy (MRS) while myocardial oxygenation was assessed from the deoxymyoglobin signal (Mb-δ) using ^{1}H MRS. During basal conditions the phosphocreatine (PCr)/ATP ratio (1.73±0.15) was significantly lower than in previously studied normal dogs (2.42±0.11) although Mb-δ was undetectable. 8-PT caused ≈ 21% increase in MBF with no change in PCr/ATP. GLB caused a 33±0.1% decrease in MBF with a decrease in PCr/ATP from 1.65±0.17 to 1.11±0.11 ($p<0.0001$). GLB did not change the pseudo-first-order rate constant of ATP production via CK (k_f), but the ATP production rate via CK was reduced by 35±0.08%; this was accompanied by an increase in P_i/PCr and appearance of a Mb-δ signal indicating tissue hypoxia. Thus, in the failing heart the balance between myocardial ATP demands and oxygen delivery is critically dependent on functioning K_{ATP} channels.

Keywords

Heart Failure; K_{ATP} channels; Myocardial Blood Flow

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INTRODUCTION

ATP sensitive potassium (K_{ATP}) channels are located on the inner membrane of the mitochondria and on the sarcolemma of coronary vascular smooth muscle cells and cardiac myocytes[1–5]. K_{ATP} channels are activated by metabolic signals such as an increase in ADP/ATP ratio near the sarcolemma, acidosis and hypoxia [6]. When K_{ATP} channels on vascular smooth muscle cells open, potassium exits the cells and hyperpolarizes the sarcolemma, thereby closing voltage-dependent calcium channels, reducing calcium influx, and promoting vasodilation [7]. Consequently, blocking K_{ATP} channels leads to vasoconstriction and a decrease in coronary blood flow (MBF) that can result in a decrease in regional systolic wall thickening [6–8].

Glibenclamide is a K_{ATP} channel inhibitor. When administered into the coronary arteries of open-chest dogs or awake-resting dogs, doses of 10–50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ caused vasoconstriction with a 20–55% decrease in MBF that was associated with a decline in coronary venous oxygen tension for any given level of myocardial oxygen consumption (MVO_2) [6–9]. We have previously shown that the decrease in MBF produced by glibenclamide also reduced the ratio of phosphocreatine (PCr) to ATP (PCr/ATP), increased inorganic phosphate (Pi) levels, and led to the appearance of a prominent deoxymyoglobin peak in the magnetic resonance spectrum. Furthermore, these changes in high energy phosphate (HEP) levels were substantially greater than the changes observed when coronary flow was reduced to a similar extent by stenosis [10]. Collectively, our findings suggested that the decrease in coronary blood flow produced by an arterial stenosis was accompanied by a decrease in myocardial energy demand, and that this response to hypoperfusion was inhibited by K_{ATP} channel blockade [10]. Mitochondrial K_{ATP} channel activity can also be cardioprotective, presumably because the influx of K^+ across the mitochondrial inner membrane reduces the mitochondrial membrane potential ($\Delta\psi m$), matrix swelling, and the production of reactive oxygen species (ROS) [11]. Nevertheless, we have shown that the decrease in MVO_2 produced by glibenclamide administration is caused by coronary vasoconstriction and the accompanying decline in oxygen availability, rather than by a direct effect on mitochondrial respiration [10, 12].

Congestive Heart Failure (CHF) is associated with a decrease in coronary blood flow that is matched by a parallel reduction in MVO_2, which suggests that energy utilization has been down-regulated [13]. K_{ATP} channel blockade with intracoronary glibenclamide reduced both coronary blood flow and MVO_2, but did not change the relationship between coronary flow and MVO_2 in animals with pacing induced CHF [14]. However, blockade of adenosine receptors in animals with CHF was associated with a significant increase in coronary blood flow that occurred secondary to an increase in MVO_2 [14]. Adenosine, a potent coronary vasodilator, does not regulate myocardial blood flow under physiological conditions [15] but does contribute to coronary vasodilation when the supply of oxygen is insufficient [16]. Thus, intravenous administration of 8-phenyltheophylline, which blocks adenosine receptors, reduced the total excess volume of blood flow during the reactive hyperemia that followed a brief total coronary artery occlusion [15].

Our group has used in vivo ^{31}P NMR spectroscopy to monitor HEP levels and have identified bioenergetic abnormalities in animals with CHF that are characterized by decreases in ATP, PCr, and the PCr/ATP ratio [17–19]. We have shown that myocardial free ADP levels are significantly increased in CHF hearts [17–19], and that this increase is associated with reductions in mitochondrial F0F1-ATPase protein expression [18]. Protein levels of CK-M and CK-mito, as well as the CK flux rate, are also reduced [17–19]. The low PCr/ATP ratio in hearts with CHF occurred in the absence of myocardial hypoxia, suggesting that the HEP abnormalities were not the result of insufficient oxygen availability [17–19].

The aim of the present study was to investigate the bioenergetic response to K_{ATP} channel blockade and adenosine receptor blockade in animals with pacing induced heart failure using in vivo ^{31}P NMR spectroscopy.

METHODS

All experiments were performed in accordance with the animal use guidelines of the University of Minnesota, and the experimental protocol was approved by the University of Minnesota Research Animal Resources Committee. The investigation conformed to the *"Guide for the care and use of laboratory animals"* published by the National Institutes of Health (NIH publication No 85-23, revised 1985).

Surgical Instrumentation and Production of CHF

Studies were performed in 12 adult mongrel dogs of either sex weighing 20–27 kg. CHF was produced by rapid ventricular pacing in 12 dogs as previously described [13, 14]. Briefly, animals were sedated (acepromazine, 10 mg IM), anesthetized (sodium pentobarbital, 30 to 35 mg/kg IV), intubated, and ventilated with room air and supplemental oxygen to ensure that arterial blood gasses were maintained in the physiological range; then, a left thoracotomy was performed, and a heparin-filled polyvinyl chloride catheter (3.0 mm OD) was inserted into the internal thoracic artery and advanced until the tip was positioned in the ascending aorta. A fluid-filled catheter was placed in the left ventricle (LV) at the apex and secured in place. A unipolar epicardial pacing lead (Medtronic, Inc) was screwed into the right ventricle and the thoracotomy was closed in layers. A programmable pacing generator modified to allow rapid pacing (Medtronic 5385) was placed in a subcutaneous pocket in the lateral chest wall and connected to the pacing lead. One week after surgery the pacemaker was activated at 220 bpm. This rate was continued or increased to 250 bpm if evidence of CHF was not present within 3 weeks. Aortic and LV pressures were assessed weekly in normal sinus rhythm 1 hour after the pacemaker was deactivated. CHF was deemed to have developed when the LV end-diastolic pressure was >20 mmHg.

Experimental preparation for MRS study

Detailed surgical preparations for MRS study have been published previously [20–22]. Briefly, the dogs were anesthetized with sodium pentobarbital (30–35 mg/kg bolus followed by 4 mg/kg/hr, i.v.), intubated and ventilated with a respirator with supplemental oxygen to maintain arterial blood gases within the physiologic range. A heparin-filled polyvinyl

chloride catheter, 3.0 mm o.d., was introduced into the right femoral artery and advanced into the ascending aorta. A left thoracotomy was performed through the fourth intercostal space and the heart suspended in a pericardial cradle. A heparin-filled catheter (3.0 mm o.d.) was introduced into the LV through the apical dimple and secured with a purse string suture. A similar catheter was inserted into the left atrium through the atrial appendage. A homemade intra coronary catheter was placed into left anterior descending coronary artery (LAD) as previously described in details [10]. A 28 mm diameter MRS surface coil was sutured onto the epicardium of LV anterior wall. The pericardial cradle was then released and the heart allowed to assume its normal position in the chest. The surface coil leads were connected to a balanced tuned circuit and the animals were placed in the magnet.

MRS spectroscopy-general methods

MRS measurements were obtained with a 40-cm bore. 4.7-Tesla magnet interfaced with a Spectroscopy Imaging Systems Corporation (Fremont, CA) console. ^{31}P and ^{1}H-MRS frequencies were 81 MHz and 200.1 MHz, respectively. Data acquisition was gated to the cardiac cycle by using the LV pressure signal, and respiratory gating was achieved by triggering the ventilator to the cardiac cycle between data acquisitions [23, 24].

MR Spectroscopy detecting deoxy-myoglobin methods

Mb-δ levels were evaluated by using ^{1}H NMR[25, 26 and ^{1}H-MRS to measure the resonance of the proximal histidyl N-δ proton on Mb-δ [27]. Briefly, the N-δ proton of the proximal histidyl of Mb-δ was selectively excited with a single-pulse collection sequence and a Gaussian pulse (1 ms); the same pulse sequence also provided sufficient water suppression, because of the large difference (>14 kHz) between the chemical shifts of water and Mb-δ. The short T_1 value of Mb-δ enabled the use of a correspondingly short repetition time (TR=35 ms), and each spectrum was acquired within 6 min (10,000 FID); data acquisition did not need to be gated to the cardiac cycle, because the inherently broad line width of the Mb-δ peak masked any loss of signal due to the motion of the heart. The chemical shift of the Mb-δ resonance is temperature sensitive but remained virtually constant (at 71–72 ppm, relative to H_2O) throughout the study protocol, and no other resonances were detected within 5 ppm of the Mb-δ resonance. Furthermore, the results from phantom studies have shown that the sensitivity of Mb-δ detection is essentially flat across the thickness of the LV wall. Thus, the magnitude of the Mb-δ resonance corresponds to the average amount of Mb-δ at all depths of the LV wall and (unlike MRS assessments of high-energy phosphate [HEP] levels) does not need to be corrected for any loss of sensitivity in deeper myocardial layers.

^{31}P MR Spectroscopic technique

^{31}P MR spectra were acquired in late diastole with a pulse repetition time of 6–7 seconds. The repetition time was sufficient for full relaxation of the ATP and Pi resonances between acquisitions, but the PCr resonance remained slightly saturated (~10%), and measurements of PCr resonance intensities were corrected accordingly. RF transmission and signal detection were performed with a 28 mm diameter surface coil, and a capillary tube containing 15 μL of 3M phosphonoacetic acid was placed at the center of the coil to serve as a reference. The proton signal of the water resonance was used to homogenize the magnetic field and to adjust the position of the animal so that the coil was at or near the magnet and

gradient isocenter. Positioning was performed via a spin-echo experiment with a readout profile, and information gathered during same experiment was used to determine the spatial coordinates for spectroscopic localization. The PCr resonance was assigned a chemical shift of −2.55 ppm (85% phosphoric acid = 0 ppm), and all other chemical shifts were calculated from their positions relative to the PCr resonance.

Spatial localization within the LV wall was achieved via the RAPP-ISIS/FSW method; details of this method (e.g., voxel profiles, voxel volume) and its accuracy in phantom studies and in vivo have been published elsewhere [24, 28, 29]. Briefly, gradients and B_0 adiabatic inversion pulses were used to restrict the signal origin to an 18 mm × 18 mm column positioned coaxially to the surface coil and perpendicular to the left ventricular wall; then, the B_1 gradient was used to localize the signal within this volume to 5 voxels that spanned the left ventricular wall from the epicardium to endocardium. PCr, ATP, and Pi measurements were obtained for each voxel, the PCr and ATP levels were normalized to the values obtained in the basal state, and the PCr/ATP and Pi/PCr ratios were calculated. Each set of spatially localized transmural spectra consisted of a total of 96 scans accumulated in a 10-minute block, and resonance intensities were quantified by using the integration routines provided with the SISCO software.

The Mb-δ resonance detected with this method provides an unweighted average of the Mb-δ content across the entire LV wall; however, methods for obtaining a comparable whole-wall measurement of HEP levels have not been developed, because the sensitivity for detection of the phosphorus resonance decreases as a function of distance from the surface coil. Thus, the average whole-wall values for HEP were calculated from HEP levels measured in the subepicardial, midwall, and subendocardial voxels, which were acquired via the spatially localized spectroscopy technique described above [29].

Myocardial blood flow measurements

Myocardial blood flow was measured by injecting 2×10^6 radiolabeled (^{51}Cr, ^{85}Sr, ^{95}Nb and ^{46}Sc) microspheres (15 μm diameter) through the left atrial catheter while drawing a sample of arterial blood from the aortic catheter at a rate of 15 mL/minute. Radioactivity measured in the myocardial and blood reference specimens with a gamma spectrometer was used to determine tissue blood flow (Packard Instrument Company, Downers Grove, IL).

Study protocol

Hemodynamic and MRS assessments were initiated under baseline conditions, and microspheres were injected midway through the 20-minute data acquisition period for measurement of myocardial blood flow. Aortic and LV pressures were measured with fluid-filled pressure transducers positioned at mid-chest level. After completing the baseline measurements, adenosine receptor blockade was produced by administration of 8-Phenyltheophylline (8-PT; 5mg/kg iv). This dose has been previously shown to produce >90% inhibition of the coronary vasodilator response to adenosine [10]. Hemodynamic measurements and ^{31}P and ^{1}H MRS spectra were acquired after 8-PT infusion along with another microsphere injection. This was followed by intracoronary infusion glibenclamide at a rate of 20 μg/kg/min ic. After allowing 10 minutes to achieve steady state conditions,

pressure measurements were repeated and a microsphere injection was performed while ^{31}P and ^{1}H MRS spectra were again obtained.

Data analysis

Hemodynamic data were measured from the chart recordings. ^{31}P spectra were analyzed as described above. Transmural blood flow distribution was determined from the microsphere measurements. Data were analyzed with one-way analysis of variance for repeated measures. A value of $p<0.05$ was considered significant. When a significant result was found, individual comparisons were made using the method of Sheffé.

RESULTS

Hemodynamic Data

Rapid ventricular pacing resulted in heart failure with LV end-diastolic pressures greater than 20 mmHg during resting awake conditions. Hemodynamic measurements during each experimental condition in anaesthetized open chest dogs are shown in Table 1. At baseline, these heart failure dogs had a lower mean aortic pressure, lower LV systolic pressure and higher LV end-diastolic pressure as compared with normal dogs previously studied in our laboratory [10]. Infusion of 8-PT resulted in a significant increase in heart rate and consequently a higher rate pressure product. Infusion of glibenclamide caused no further change in heart rate, mean aortic pressure or LV systolic pressure. However, glibenclamide cause an increase of LV end-diastolic pressure from 19±2 to 29±2 mmHg ($p<0.05$), suggesting deterioration in LV function similar to that previously observed in normal dogs after K_{ATP} channel blockade [10].

Myocardial Blood Flow

Table 2 summarizes the myocardial blood flow (MBF) data under different experimental conditions. Resting mean MBF was 0.71±0.07 ml/min-g with a subendocardial-to-subepicardial ratio of 1.17±0.08. Adenosine receptor blockade caused a 21% increase in mean MBF (0.71±0.07 at BL *vs* 0.90±0.15 ml/min-g with 8-PT, $p<0.05$) with no change in the transmural gradient of perfusion. The subsequent addition of K_{ATP} channel blockade with glibenclamide resulted in a 32.7±0.1% decrease in MBF (0.90±0.15 ml/min-g with 8-PT *vs* 0.58±0.07 ml/min-g with 8-PT+GLIB, $p<0.05$). The reduction of blood flow produced by glibenclamide was more pronounced in the subepicardium than in the subendocardium, resulting in a significant increase in the subendocardial-to-subepicardial ratio from 1.13±0.14 to 1.42±0.15 ($p<0.05$). This is in contrast to previously studied dogs in which a decrease in MBF produced by a coronary stenosis was most marked in the subendocardium, resulting in a decrease in the subendocardium-to-subepicardial flow ratio [10].

Myocardial High Energy Phosphates and Oxygenation

^{31}P NMR spectra obtained during baseline conditions demonstrated prominent resonances corresponding to PCr and the three phosphates of ATP, whereas P_i was below the limit of detectability. The PCr/ATP ratio during baseline conditions was 1.90±0.14 in the subepicardial myocardium and 1.62±0.15 in subendocardial layer. These values are significantly lower than previously observed in this laboratory in normal dogs [10]. The

decreased PCr/ATP ratios in the heart failure animals during basal conditions were not the result of ischemia as no deoxymyoglobin peak (Mb-δ) was detectable.

Adenosine receptor blockade caused no significant change in myocardial PCr, ATP or the PCr/ATP ratio. In contrast to the lack of effects of adenosine receptor blockade on myocardial HEP, glibenclamide caused a significant decrease of PCr with no change of ATP, resulting in a significant decrease in the PCr/ATP ratio (Table 3, $p<0.05$). The decreased PCr/ATP ratio in response to glibenclamide was associated with a significant increase in $\Delta P_i/PCr$. These HEP changes were accompanied by the appearance of a Mb-δ signal indicating myocardial tissue hypoxia. [10]

Creatine Kinase Flux

Creatine kinase kinetic data are depicted in Table 4. The pseudo-first-order rate constant for ATP production via CK (k_f) did not change during either adenosine receptor or K_{ATP} channel blockade. However, the ATP production rate via CK was reduced by 35±0.08% during K_{ATP} channel blockade (normalized CK flux rate with 8-PT was 1.13±0.26 *vs* 0.73±0.08 with 8-PT+GLIB, $p<0.05$).

DISCUSSION

This is the first report examining the influence of adenosine receptors and K_{ATP} channel activity on myocardial energetics in the failing heart. We found that adenosine receptor blockade resulted in a modest increase in myocardial blood flow but no change in PCr or the PCr/ATP ratio. On the other hand, K_{ATP} channel blockade in the failing hearts caused significant reductions in PCr and the PCr/ATP ratio, as well as a decrease in the ATP production rate via CK. These changes produced by K_{ATP} channel blockade were associated with a decrease in myocardial blood flow and the development of tissue hypoxia. Thus, in failing hearts without coronary artery disease, the balance between myocardial ATP demands and oxygen delivery are critically dependent on functioning K_{ATP} channels.

Myocardial Energetics in Heart Failure

Our results confirm our previous findings that heart failure is associated with a decrease in myocardial PCr/ATP ratio during basal conditions that is not the result of ongoing myocardial ischemia [17–19]. It has been shown that this decrease in PCr/ATP ratio is associated with a decrease in CK flux [17–19] and a change in substrate utilization with increased glucose uptake and decreased fatty acid oxidation [30].

Adenosine Receptor Blockade in Heart Failure

We have previously shown that in the failing heart adenosine receptor blockade with 8-PT caused a modest but significant increase in myocardial blood flow that that was proportionate to an increase in myocardial oxygen consumption [14]. Adenosine receptor blockade did not alter the relationship between myocardial oxygen consumption and coronary venous pO_2, implying that adenosine receptors are not involved in the metabolic signaling between myocardial myocytes and the coronary resistance vessels [14]. The increase in myocardial blood flow produced by adenosine receptor blockade in the present study was

likely in part related to the increase in rate-pressure product (and therefore myocardial oxygen requirements) produced by 8-PT. Plasma and pericardial adenosine levels are increased in patients and animal models of heart failure and are strongly correlated with plasma norepinephrine. [3132] Adenosine A1 receptors in the sinoatrial node exert a negative chronotropic effect, so that blockade of these receptors in the failing heart where norepinephrine levels are increased could have resulted in the increase of heart rate observed in the present study. [33] Binding of adenosine to myocardial A1-receptors blunts adrenergic stimulation by activating the Gi protein, leading to attenuation of adenylyl cyclase activity and cAMP production. [34] Because myocardial energy utilization appears to be down regulated in CHF, whereas adenosine production is increased, it is possible that adenosine may moderate energy utilization in the failing heart through antiadrenergic effects on A1 receptors. Adenosine receptor blockade caused no significant change in myocardial PCr, ATP or the PCr/ATP ratio. This supports the concept that the increase in myocardial blood flow after 8-PT was a bioenergetically appropriate response to the increase in myocardial oxygen consumption.

8-PT was chosen as an adenosine receptor blocker because it has very low phosphodiesterase inhibitor activity. [35] Nevertheless, even weak phosphodiesterase blocking activity of 8-PT might have resulted in an increase of myocardial cAMP with a resultant increase in myocardial oxygen requirements. Importantly, however, the finding that 8-PT had no effect on myocardial ATP or PCr levels, and no effect on creatine kinase flux, supports the concept that adenosine receptors do not play a direct role in maintaining the metabolic balance between ATP production and utilization in the failing heart.

K^+ATP channel blockade in Heart failure

In the heart, K_{ATP} channels exist on the sarcolemma of the cardiac myocytes, the inner mitochondrial membrane, and the sarcolemma of the coronary smooth muscle cells. K_{ATP} channel activity results in tonic vasodilation of the coronary resistance vessels, so that in normal animals K_{ATP} channel blockade with glibenclamide resulted in a 12–20% decrease in myocardial blood flow [6] In the present study K_{ATP} channel blockade caused a 36% decrease in myocardial blood flow, in agreement with previous reports that K_{ATP} channels are of greater importance for maintaining coronary blood flow in the failing heart than in normal hearts [1436] Thus, Yammamoto et al [36] reported that K_{ATP} channel blockade with glibenclamide caused a greater decrease in coronary blood flow in open-chest dogs with pacing-induced CHF than in normal dogs and this was accompanied by lactate production, consistent with the production of ischemia. In unanesthetized swine with LV dysfunction 3 weeks after myocardial infarction, glibenclamide impaired the increase in coronary blood flow that occurred during exercise, indicating that dependence on vascular K_{ATP} channels was increased in the postinfarction hearts [37]

We previously reported that in normal animals K_{ATP} channels act to modulate myocardial oxygen demands when coronary blood flow is reduced. [10] Thus, the degree of myocardial myoglobin oxygen desaturation that occurred when coronary blood flow was decreased was markedly greater in animals treated with glibenclamide than in normal animals, indicating that K_{ATP} channels reduced oxygen demands when blood flow and oxygen delivery were

decreased. This implies that K_{ATP} channels play a pivotal role in maintaining the balance between ATP demand and delivery.

In the failing hearts in the present study we found that glibenclamide caused significant reductions of the PCr/ATP ratio and ATP production rate and that this was associated with tissue hypoxia. The degree to which these HEP changes were the result of hypoperfusion caused by glibenclamide vs. impairment of the ability of the myocardium to respond to the reduced oxygen availability is uncertain. It is of interest that the magnitude of the loss of PCr in response to glibenclamide in these failing hearts was very similar to that previously observed in normal hearts. This suggests that although the decrease in myocardial blood flow in response to glibenclamide was greater in the failing hearts than in normal hearts, the metabolic adjustment to this reduction of oxygen delivery was similar to normal hearts.

The coronary vasoconstriction and decreased coronary flow caused by K_{ATP} channel blockade appears to augment adenosine production in the heart. Samaha et al [9] reported that in anesthetized open chest normal dogs intracoronary glibenclamide caused an oscillating pattern of coronary blood flow with periods of decreased flow alternating with intervals of increased flow. Using 31P NMR spectroscopy to measure myocardial HEP, they found that PCr/ATP ratios fell during the flow troughs and increased during the flow peaks while Pi/ATP ratios changed in a reciprocal manner. Both the oscillations of blood flow and HEP changes were abolished by 8-phenyltheophylline, demonstrating that adenosine acted to modulate the effect of K_{ATP} channel blockade. For this reason, in insure stable HEP measurements in the present study adenosine receptor blockade was established prior to NMR spectroscopy to remove possible interfering effects of adenosine on the HEP measurements.

Conclusions

K_{ATP} channels but not adenosine receptors are important in maintaining the balance between oxygen delivery and demand in the failing heart.

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Table 1

Hemodynamic Data

	HR (Beats/min)	MAP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)	RPP (Beats.mmHg.min^{-1}×10^{-3})
Baseline	102±8	82±4	102±5	19±2	10.4±0.8
8-PT	120±10*	88±7	104±6	20±2	12.6±1.3*
8-PT + GLIB	121±11*	86±8	100±7	29±2*†	12.2±1.1*

Values are means ± SE. 8-PT, 8-Phenyltheophylline at 5mg/kg intravenous; GLIB, Glibenclamide at 20 μg/kg/min intracoronary; HR, Heart rate; MAP, Mean Aortic Pressure; LVSP, Left ventricular systolic pressure; LVEDP, Left ventricular end-diastolic pressure; RPP, rate pressure product;

*p<0.05 vs. baseline;

†p<0.05 vs. 8-PT.

Table 2

Myocardial Blood Flow

	EPI (ml/min/g)	MID (ml/min/g)	ENDO (ml/min/g)	Mean (ml/min/g)	Endo/Epi
Baseline	0.64±0.07	0.75±0.08	0.74±0.08	0.71±0.07	1.17±0.08
8-PT	0.83±0.12*	0.92±0.17*	0.94±0.18*	0.90±0.15*	1.13±0.14
8-PT+GLIB	0.47±0.05*†	0.63±0.11†	0.65±0.08†	0.58±0.07*†	1.42±0.15†

Values are means ± SE. 8-PT, 8-Phenyltheophylline at 5mg/kg intravenous; GLIB, Glibenclamide at 20 μg/kg/min intracoronary; EPI, subepicardium; MID, midmyocardium; ENDO, subendocardium;

*p<0.05 vs. baseline;

†p<0.05 vs. 8-PT.

Table 3

Myocardial high-energy phosphate levels

	PCr/ATP	ΔPi/PCr	Normalized PCr	Normalized ATP
Normal[#]	2.42±0.11			
Baseline	1.78±0.15	0	1	1
8-PT	1.65±0.17	0	0.92±0.06	0.99±0.08
8-PT+GLIB	1.11±0.15[*†]	0.63±0.26[*†]	0.65±0.09[*†]	1.02±0.09

Values are means ± SE. 8-PT, 8-Phenyltheophylline at 5mg/kg intravenous; GLIB, Glibenclamide at 20 μg/kg/min intracoronary; Pi, Inorganic phosphate; PCr, Phosphocreatine; ATP, Adenosine Triphosphate; EPI, subepicardium; ENDO, subendocardium;

[*] $p<0.05$ vs. baseline;

[†] $p<0.05$ vs. 8-PT.

[#] Cardiac myocardial HEP levels and PCr/ATP of normal canine hearts from a previous study[10], referenced here for the comparison.

Table 4

Creatine Kinase Kinetics

	ΔM/M	Tau (sec)	T1 (sec)	k_f (sec^{-1})	Normalized CK flux rate
Baseline	0.64±0.07	1.37±0.12	4.33±0.84	0.47±0.06	1
8-PT	0.62±0.07	1.17±0.14	3.28±0.37	0.58±0.14	1.13±0.26
8-PT+GLIB	0.66±0.06	1.47±0.07	4.55±0.82	0.48±0.04	0.73±0.08*

Values are means ± SE. 8-PT, 8-Phenyltheophylline at 5mg/kg intravenous; GLIB, Glibenclamide at 20 μg/kg/min intracoronary; ΔM/M, relative change of PCr resonance intensity; Tau, time constant that fits the integral of PCr magnetization decay as the time of saturation of ATPγ increased from 0 to infinite; T1, longitudinal relaxation time; k_f, forward rate constant of creatine kinase;

*p<0.05 vs. baseline.