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Administration of Exogenous ATP to Ischemic Skeletal Muscle Induces an Energy Sparing Effect: Role of Adenosine Receptors

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Abstract

Ischemia and reperfusion (IR) injury is a devastating complication that occurs in allotransplantation and replantation of limbs. Over the years, several preservation strategies have been employed to conserve critical levels of intracellular ATP during ischemia to sustain ion gradients across membranes and thus the viability of tissues. Administration of exogenous ATP to ischemic tissues is known to provide beneficial effects during reperfusion, but it is unclear whether it provides protection during ischemia. The purpose of this study was to determine the effect of ATP administration on high-energy phosphate levels in ischemic skeletal muscle and examine the role of purinergic and adenosine receptors in mediating the response to exogenous ATP.

Materials and Methods—Extensor Digitorum Longus (EDL) muscles of Fischer rats were subjected to ischemia and treated with different concentrations of ATP with or without purinergic and adenosine receptor blockers. ³¹P-NMR was used to measure the rate of decay of ATP, phosphocreatinine (PCr) and the formation of AMP and acidification. Phosphorylated compounds were analyzed using a simple model of energy metabolism and PCr half-life was used as an index of internal depletion of ATP to distinguish between intracellular and extracellular ATP.

Results—PCr decay was rapid in all muscle groups and was followed by a gradual ATP decay. The half-life of PCr was significantly longer in ATP-treated muscles compared to vehicle controls, and was maximally prolonged by treating with slow hydrolyzing ATPγS. Purinoceptor (P2X) blockade with ATP treatment significantly increased the half-life of PCr, whereas adenosine receptor blockers blunted the response. Administration of adenosine to ischemic muscles significantly increased the half-life of PCr compared to vehicle controls.

Conclusion—Exogenous ATP administration to ischemic skeletal muscles appears to spare intracellular energy by acting primarily through adenosine receptors.

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Keywords

Ischemia reperfusion injury; ATP; purinoceptors; adenosine receptors; skeletal muscle; Phosphocreatine; nuclear magnetic resonance

INTRODUCTION

Ischemia-reperfusion injury (IRI) is inevitable in complex limb reconstructions such as re-implantation of amputated limbs and allotransplantation. During these procedures, skeletal muscles are subjected to prolonged periods of ischemia. Ischemia is accompanied by the depletion of glycogen stores and glucose, decreased glycolysis, and oxidative phosphorylation, leading to depletion of adenosine triphosphate (ATP).

ATP is the main source of intracellular energy for maintaining membrane potentials, repair, anabolic metabolism and mechanical processes such as contraction.[1-3] Although a few other molecules, such as phosphocreatine (PCr), PEP and other nucleotide phosphates (e.g., GTP, CTP, and UTP), can serve as a source of energy in skeletal muscle cells, their production is ultimately dependent on the ATP supply.[4-6] These processes have different requirements for ATP or the phosphorylation potential.[7] In the absence of capacity to regenerate ATP from ADP as in ischemia, ATP is spared for maintenance rather than for macromolecular biosynthesis. Eventually the ATP level drops below a critical value, at which point cells become necrotic.[8;9] As a consequence of ATP breakdown, adenosine, inosine and hypoxanthine accumulate in the ischemic tissue.[10-12]

Reestablishing blood flow to limbs after prolonged ischemia can result in the systemic release of toxic metabolites from skeletal muscle, resulting in the so-called reperfusion syndrome, a potentially lethal condition causing secondary failure of organs remote from the ischemic area.[13] It is widely accepted that in skeletal muscle the duration of ischemia correlates directly with the severity of the injury. Significant skeletal muscle injury occurs after 3 hours of warm ischemia and gradually progresses to complete necrosis after 6 hours.[14;15] During ischemia, myocyte glycogen and PCr are preferentially depleted over ATP, but little muscle necrosis occurs until ATP is depleted.[16]

Several studies in various tissues/organs have reported beneficial effects of exogenous ATP administration including improvement of function and survival following shock and sepsis, [17-20] improved liver preservation,[21] reduced brain injury following IRI,[22] limb preservation,[23] and improved cardiovascular function in pathologic conditions.[24-26] Since ATP is a charged molecule that is not freely permeable through cell membranes its protective effects must therefore involve an alternative mechanism than directly providing energy to the cells. Interestingly, ATP is also an important molecule for extracellular signaling, and is rapidly inactivated to adenosine subsequent to its release by various extracellular enzyme families including ectonucleoside triphosphate diphosphohydrolases, ecto-nucleotide pyrophosphatase/phosphodiesterases, ecto-5'-nucleotidase, and alkaline phosphatases.[27] One potential mechanism may involve the activation of A1 and A3 adenosine receptors, which play a role in eliciting a cardioprotective effect against IRI in a phenomenon known as ischemic preconditioning.[28;29]

Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P -NMR) is a non-invasive tool that can be used to assess intracellular and extracellular bioenergetics. [30;31] As optimal function in skeletal muscle is directly related to energy metabolism, measurement of phosphorylated compounds such as ATP, ADP, PCr, and Pi provides valuable indicators of metabolic stress. Initially during ischemia, the ATP concentration is maintained by at least

three processes in the absence of resynthesis by oxidative phosphorylation, namely the reactions catalyzed by creatine kinase (Eq 1) and adenylate kinase (Eq 2) and glycogenolysis plus lactic fermentation[32;33] (Eq 3):

However, as ischemia progresses the hydrolysis of ATP continues to drive essential reactions, but the supply of PCr becomes exhausted leading to a decrease in intracellular ATP along with an accumulation of inorganic phosphate, Pi (Eq 4), formation of AMP (Eq 2) and accumulation of H⁺. Under such conditions, supplying exogenous ATP should maintain the energy-dependent processes in the cell, and additionally limit the conversion of PCr to ATP and the production of AMP. Therefore, measurement of PCr and AMP in vivo are good surrogates for assessing the bioenergetic status of ischemic or excised muscle. Furthermore, in these experiments ATP is present both intra and extracellularly, whereas PCr is exclusively intracellular; PCr therefore reports on the intracellular energy metabolism in the intact tissue.

The purpose of this study was to examine the potential mechanism by which administration of exogenous magnesium ATP (Mg-ATP) elicits a protective effect on ischemic skeletal muscle. We hypothesized that extracellular enzymes metabolized exogenous Mg-ATP to adenosine, which in turn activated adenosine receptors to reduce myocyte energy consumption. We used ³¹P-NMR to measure the depletion of PCr and other high-energy phosphates over time in ischemic skeletal muscle. We report that administration of (Mg-ATP) to ischemic skeletal muscle increased the half-life of PCr, and antagonism of adenosine receptors reduced the Mg-ATP-induced energy sparing effect.

MATERIALS AND METHODS

Animals

Male Fischer-344 rats, 8-12 weeks old, weighing between 250 – 300 g were used in this study (Harlan, IN). Animals were housed in separate cages in rooms regulated in temperature (24°C), light (12 h/ d), and airflow. They were fed standard rat chow and given water ad libitum. All handling of animals was performed in accordance with the guidelines of the animal care and use committee of the Louisville School of Medicine and the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, Publication No. NIH 86-23).

Experimental groups

The animals (n=10/group) were allocated into 8 groups: Group I, Heparinized Lactated Ringer's (HLR) only; Group II and III, Mg-ATP 50 μmol and 100 μmol respectively; Group IV ATP 50 μmol plus PPADS (a purinergic receptor blocker); Group V, adenosine 5'-O-(3-thio)triphosphate (ATPγS) 50 μmol (slowly hydrolyzing form); Group VI, ATP 50 μmol plus 8-SPT and MRS 1523 (adenosine receptor blockers); Group VII and VIII, adenosine 50 μmol and 100 μmol respectively.

Procedure

Under intraperitoneal pentobarbital anesthesia, a right inguinal incision was made and the femoral vessels were isolated. The femoral artery was cannulated (PE 20, ID 0.38 mm, BD) and the lower limb was flushed with heparinized lactated Ringer's (HLR) solution for 5 min using a peristaltic pump. The effluent was collected through an adjacent femoral venotomy. The limb was then perfused with 10 mL of different treatment solutions containing 5 mM or 10 mM test compounds according to the groups described above. At the end of perfusion, the extensor digitorum longus (EDL) muscle was quickly harvested and transported in a 5 mm NMR tube containing HLR for NMR spectroscopy. The mean ischemia time for muscle preparation prior to its placement in NMR was 45 min approximately. A capillary containing methylene diphosphonate in D₂O was used for locking and as an external chemical shift reference. [34;35]

NMR spectroscopy

1D ³¹P-NMR spectra of excised rat muscles at 25 °C were obtained at 18.8 T on a Varian Inova spectrometer (323.7 MHz for ³¹P), using a single pulse experiment with ¹H decoupling at 799.7 MHz during the acquisition (1.5 s). 128 transients with a repetition rate of 2.35 s were collected in each bin for 5 minutes for a total of 180 minutes. The raw data were zero-filled once, apodized using an unshifted Gaussian function, and 10 Hz line broadening exponential was added. The integral values (area under the curve) of PCr peak was used to obtain the half-life of the PCr by plotting the values as a function of time and fitting it to a single exponential (Fig. 1).

Reagents

Mg-ATP, ATPγS, Adenosine, Pyridoxal phosphate-6-azo (benzene-2,4-disulfonic acid) tetrasodium salt hydrate (PPADS), 8-(p-Sulfophenyl)theophylline hydrate (8 SPT), MRS 1523 were purchased from Sigma-Aldrich, USA.

Data analysis

The spectral data were analyzed using VNMR (version 6.1C). The tissue pH was determined from the chemical shift of the inorganic phosphate, which was calibrated against an external standard using a predetermined pH-titration curve as previously described.[35] The changes in the concentrations of ATP, PCr, inorganic phosphate and phosphate monoesters were monitored by peak intensity as a function of time. Since the absolute amount of ATP/unit of mass varies and also depends on the amount of tissue harvested, the amount of ATP at the start of data acquisition (t=0) was taken as the actual functional amount for that sample. The time at which ATPγ peak dropped to 10% of its initial value (ATPγ-t₁₀) was calculated to determine the potential tissue viability during ischemia. Time courses were analyzed as linear, single or double exponentials by regression analysis with the program, Kaleidagraph (Synergy Software).

Statistical analysis

Spectral data of PCr half-lives were analyzed by Kruskal-Wallis test followed by pairwise comparison using Mann-Whitney rank-sum test. Data are expressed as mean ± SEM. A value of p < 0.05 was considered significant.

RESULTS

Energy metabolism in excised skeletal muscle

1-D ³¹P-NMR spectra were recorded in excised ischemic skeletal muscles to measure the half-life of PCr as described in the Materials and Methods. Figure 1 shows typical ³¹P NMR

spectra at different times post excision. The peaks were assigned as described previously, [30]. The intensity of the peak is directly proportional to the concentration. There was a rapid decrease in the intensity of the PCr peak, and a slower loss of the ATP peaks (Fig. 1, 2). Furthermore, the Pi peak grows in intensity and shifts upfield due to acidification from the hydrolysis of ATP and lactic fermentation (Eq 1-4). In the first spectra, the tissue was at around pH 7.2, and decreased to < 6.5 by 2.5 h, by which time there is no remaining PCr, and little ATP. In addition, the phosphate monoester region (PME) showed progressive appearance of a new resonance at -13 ppm which corresponds mainly to AMP + IMP as shown by analysis of extracts of the tissue by ^1H NMR (not shown).

In these experiments, half-lives or t_{10} values (see methods) were used as an index of maintenance of internal ATP levels in order to distinguish between intracellular and exogenously administered ATP in the treatment solutions (Figs 3, 4). In control animals treated with HLR solution, intracellular PCr broke down rapidly and essentially as a single exponential, with a half-life of 13.65 ± 0.83 min (Figs 2, 3). In the control samples, with no exogenous ATP, the time required for intracellular ATP concentration to reach 10% of its initial value was 170.6 ± 7.3 min and the actual time including 45 min of ischemia duration to reach $\text{ATP}\gamma\text{-}t_{10}$ was ~ 215.6 min (Table 1). Up to this time, the tissue remains viable.

Exogenous ATP prolongs PCr half-life and $\text{ATP}\gamma\text{-}t_{10}$

Figure 4 shows the time courses of the PCr and inorganic phosphate levels; the PCr decays exponentially, whereas inorganic phosphate remained constant over the first 60 minutes, before increasing rapidly over the second half of the time course. The amount of functional ATP or $\text{ATP}\gamma\text{-}t_{10}$ (representing a decrease to 10% of its initial value) showed maximal depletion at 170.6 ± 7.3 min in the vehicle group and prolonged to above 3 h (range: $186.5 \pm 7.7 - 203.2 \pm 6.9$ min) with Mg-ATP, $\text{ATP}\gamma\text{S}$ and adenosine (Table 1), prolonging muscle viability by 20 – 33 min.

Two groups of animals also received exogenous Mg-ATP (50 μmol and 100 μmol amounts) intra-arterially at the start of ischemia period. In these muscles, the PCr half-life increased significantly compared to HLR controls (Fig. 3). As expected, exogenously delivered ATP maintained the intracellular level of PCr. Furthermore, the time required for HLR control muscles to reach the crucial $\text{ATP}\gamma\text{-}t_{10}$ level was 170.6 ± 7.35 min (Table 1), and administration of Mg-ATP prolonged the time by 13% (50 μmol) and 14% (100 μmol). We also administered 50 μmol of $\text{ATP}\gamma\text{S}$ (a slowly hydrolyzing form of ATP) as a control that would not contribute energy for cell metabolism. Interestingly, the PCr half-life was significantly prolonged by $\text{ATP}\gamma\text{S}$ (Fig. 3), and the time required for muscles to reach the crucial $\text{ATP}\gamma\text{-}t_{10}$ was prolonged by 16%, which was noticeably higher than what was achieved by either dose of Mg-ATP ($p < 0.05$) (Table 1). Since the preparation of EDL muscles for perfusion with the various treatments and preparation for NMR analysis required 45 min of ischemia, the actual total ischemia time (ATIT) for muscles to reach the $\text{ATP}\gamma\text{-}t_{10}$ was calculated and is presented in Table 1. These values represent the time when muscles are effectively running out of energy and becoming necrotic at room temperature.

Adenosine receptors play a role in energy sparing effect

Administration of PPADS (a purinoceptor P2X antagonist) along with Mg-ATP resulted in an increased half-life of PCr compared to HLR controls (Fig. 3). In contrast, antagonism of adenosine receptors with 8-SPT and MRS 1523 along with Mg-ATP administration showed a non-significant change in PCr half-life (Fig. 3). Administration of adenosine (50 μmol) showed a non-significant increase for PCr half-life but at the 100 μmol dose it increased significantly (Fig. 3).

DISCUSSION

The beneficial effects of administration of exogenous Mg-ATP to treat ischemia and reduce necrosis in various tissues,[36-39] and in skeletal muscle have been reported.[40] As ATP is a charged molecule and cells do not possess a known ATP membrane transport mechanism it is not clear how this protection is elicited. In the present study, ^{31}P -NMR was utilized to observe the time-dependent depletion of high-energy phosphates in ischemic skeletal muscle perfused with Mg-ATP. However, as this technique is unable to distinguish between intracellular ATP and that present outside the cells, we relied on the half-life of PCr (a molecule present only in the cytosol) as an index of intracellular high-energy phosphate decay. We found that administration of exogenous Mg-ATP prolonged the half-life of PCr. To assess whether Mg-ATP was indirectly contributing to the energy sparing effect observed, we administered ATP γ S, a form of ATP that cannot be used by the cells for energy, and found that it significantly increased the half-life of PCr ($p < 0.05$) (Fig 3). These results suggested that Mg-ATP or its metabolites were eliciting the energy sparing response, perhaps by activating purinoceptors. Furthermore, blockade of P2 receptors with PPADS increased the PCr half-life indicating that these receptors were not directly involved in sparing energy, but rather may be associated with activation of energy consuming pathways. However, blockade of adenosine receptors with 8-SPT and MRS1523 blunted the prolongation suggesting an active role of these receptors.

Purine and pyrimidine release in the extracellular environment from nerve endings in neuromuscular junctions and from active skeletal muscle fibers has been observed.[41-43] The combination of specific purinoceptors and the rapid extracellular breakdown of purines and pyrimidines by ectonucleotidases and adenosine deaminase enable these compounds to serve as well as regulate extracellular signaling molecules.[41;44] Purinoceptors are divided into the P2Y and P2X receptor families. P2Y are G protein-coupled metabotropic receptors with eight subtypes (P2Y_{1,2,4,6,11,12,13,14}) and P2X are ligand-gated ion channels (P2X₁₋₇). In skeletal muscle, the isoforms P2X_{1,3,4,5} and P2Y_{1,2} have been identified.[41;45;46] It is known that physiological elevation of extracellular potassium concentration ($[\text{K}^+]_o$) potentiates the effect of agonists to the P2Y receptor family which may serve to maintain the neuromuscular transmission during intense exercise where muscle excitability is presumed to decrease due to elevated $[\text{K}^+]_o$.[47;48] Recent evidence suggests that extracellular Mg-ATP can improve excitability and contractile function in muscles where the excitability is depressed because of depolarization (due to increased $[\text{K}^+]_o$), and can also increase stimulation of energy consuming sodium-potassium pumps.[49]

Although in our study we only used the purinoceptor blocker PPADS, which is considered to be a preferential P2X receptor blocker [50;51], PPADS has also been shown to block the Mg-ATP effect on P2Y receptors in HEK-293 cells, smooth muscle of pulmonary arteries, and motor function of the small intestine.[52-54] Thus, antagonism of both P2X and P2Y receptors by PPADS may explain the prolongation of PCr half-life observed in our study.

However, if ATP activates purinoceptors that lead to energy consuming activities in myocytes, it is not clear how the administration of Mg-ATP prolongs the PCr? It is well known that extracellular Mg-ATP is rapidly metabolized by ectonucleotidases generating extracellular adenosine which is further metabolized by adenosine deaminase to inosine and other metabolites.[10-12] In cardiac infarct studies, agonism of adenosine receptors (A₁ and A₃) is cardioprotective by eliciting an energy sparing phenomenon known as ischemic preconditioning (IPC).[28;29] The protective phenomenon of IPC is also induced in skeletal muscle by systemic administration of adenosine, [55-57] and based on these facts, we hypothesized that degradation of exogenous Mg-ATP by ectonucleotidases to adenosine activated adenosine receptors, which mediated the energy sparing effect. Indeed,

administration of the antagonist combination of 8-SPT and MRS1253 to preferentially block A1 and A3 adenosine receptors prior to administration of exogenous Mg-ATP, shortened the PCr half-life to similar levels as vehicle controls suggesting involvement of these receptors. Furthermore, we tested the direct administration of two amounts of adenosine concentrations (50 and 100 μ M) assuming that equal amounts and concentrations of Mg-ATP would yield equivalent amounts of adenosine after degradation. We found that both Mg-ATP concentrations elicited a significant prolongation of PCr half-life but only the higher concentration of adenosine induced a significant response compared to HLR controls. It is conceivable that A1 and A3 adenosine receptors respond differently to direct administration of adenosine than to the gradual degradation of Mg-ATP to adenosine. This may also explain the significant prolongation of PCr half-life produced by ATP γ S compared Mg-ATP when equal amounts were administered. The more slowly hydrolyzing ATP γ S is degraded to adenosine at a slower rate than Mg-ATP, producing a sustained release effect over time. An alternative explanation to the prolonged energy sparing effect produced by Mg-ATP administration may involve a synergistic effect produced by the combined activation of P2 purinoceptors with adenosine receptors when the two agonists are simultaneously present.

Measurements of tissue viability by staining techniques have shown that 3 h of ischemia produces a significant amount of necrotic muscle, and that by 5 h, 90% of the tissue is necrotic.[14;15] In an effort to translate our findings to potential clinical relevance of salvaging ischemic/amputated extremities or transporting hand allografts with administration of exogenous Mg-ATP, we performed a temporal analysis of 31 P-NMR spectra to determine the duration of functional ATP (ATP γ -t₁₀) in ischemic muscles (Table 1). Since our muscles required 45 min to treat, harvest and position in the magnet, we calculated the ATIT to reach ATP γ -t₁₀ by adding 45 min to the ATP γ -t₁₀. In HLR control muscles not receiving Mg-ATP the ATIT at room temperature was 3 h and 35 min, a time that correlates with published skeletal muscle ischemia studies. Adding Mg-ATP or ATP γ S to preservation solutions may extend the critical point of necrosis by 20 to 30 min, however, adding adenosine to solutions may not be as helpful since neither concentrations prolonged the time to reach ATP γ -t₁₀ significantly compared to controls despite prolonging PCr half-life at higher concentrations.

CONCLUSIONS

We conclude that administration of exogenous Mg-ATP and ATP γ S to ischemic skeletal muscle elicits a high-energy phosphate sparing effect in skeletal muscle as demonstrated by a prolongation of the PCr half-life. Administration of exogenous adenosine prolonged the PCr half-life, and blockade of A1 and A3 adenosine receptors blunted the response; together these findings suggest a role for these receptors. Administration of Mg-ATP with blockade of P2 purinoceptors significantly enhanced the PCr half-life, which suggests the presence of yet another unknown mechanism. Based on our findings, administration of the slow hydrolyzing ATP γ S appears to provide the longest protection to ischemic skeletal muscle. However, the energy sparing effect is mildly robust and may work best with hypothermia under controlled conditions as performed during the preservation of composite tissue allografts.

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LIST OF ABBREVIATIONS

IRI	Ischemia/reperfusion injury
ATP	Adenosine Triphosphate
AMP	Adenosine monophosphate
ADP	Adenosine diphosphate
Mg-ATP	Magnesium and ATP salt
PCr	Phosphocreatine
31P-NMR 31	Phosphate nuclear magnetic resonance
HLR	Heparinized Lactated Ringer's
SEM	Standard error of mean
EDL	Extensor digitorum longus
ATPγS	Slowly hydrolyzing form of ATP
8-SPT	8-(p-Sulfophenyl)theophylline hydrate
MRS 1523	Adenosine receptor blocker
PPADS	Pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt hydrate

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Figure 1. ^{31}P -NMR spectra of excised rat skeletal muscle

Spectra were recorded at 5 minute intervals at 18.8 T, 25 °C on a control rat skeletal muscle treated with HLR solution. PCr: Phosphocreatine; PME : Phosphomonoesters (G6P, AMP and IMP). PCr was reasonably well described by an exponential decay with a rate constant of 0.058 min^{-1} (half-life of 11.89 minutes). ATP- γ peak shows a much slower decay: ca. 0.01 min^{-1} (half-life of 69 min). All three ATP peaks disappear at the same rate without a concomitant appearance of ADP.

Figure 2. NMR time courses of PCr and inorganic phosphate in excised muscle

NMR spectra were recorded sequentially on excised muscle by ^{31}P NMR as described in the methods. PCr intensity (red dots) disappeared over 60 minutes, whereas the inorganic phosphate, Pi (blue dots) showed a plateau value for the first 90 minutes followed by a rapid increase until 180 min

Figure 3. Dependence of the half-life of phosphocreatine (PCr) in rat EDL muscles on treatment conditions

Muscle PCr decay was monitored by ^{31}P NMR for 3 hours as described in the Methods. Ischemic hind limbs were treated with various control and Mg-ATP (5 & 10 mM) solutions with purinergic (PPADS) and adenosine (8-SPT & MRS1523) blockers and adenosine 5 and 10 mM solutions. All the groups compared to lactated ringers' (HLR) controls were significantly different ($P < 0.05$). Pairwise comparison of muscles treated with P2Y blockade (Mg-ATP + PPADS) to adenosine receptor blockade (Mg-ATP + Ado blocker) differed significantly ($*P < 0.05$). Adenosine receptor blockade reduced PCr half-life in the presence of Mg-ATP compared to control (HLR) ($\dagger = \text{NS}$). Muscles treated with 100 μmol of Adenosine showed significant increase in the PCr half-life as compared to HLR group ($\S P < 0.05$). There was a significant difference between muscles treated with 50 μmol Mg-ATP and Mg-ATP + PPADS ($\ddagger P < 0.05$) but the other groups did not show any difference. Data are presented as mean $\pm$ SEM.

Figure 4. NMR Time course of energy metabolites in EDL muscle under different treatments

The hind limb was flushed with HLR and perfused with different treatment solutions based on the groups as described in materials and methods. Following treatment, EDL muscle was harvested and immersed in a glass tube containing HLR and D₂O and ³¹P-NMR spectroscopy was obtained. Muscles treated with a) HLR only showing rapid decline of PCr (●) and ATP (○); b) When treated with 100 μmol of Mg-ATP, PCr values were prolonged with a less raise in Pi (□); c) 50 μmol of Mg-ATP and purinoceptor blocker (50 μmol PPADS) shows increased levels of ATP; d) whereas 50 μmol of Mg-ATP with adenosine receptor blocker (8-SPT, MRS 1523) shows a significant and rapid reduction in both ATP and PCr; e) 100 μmol of adenosine prolonged ATP and PCr levels in the tissue. Blue circle with no filling represents the gamma peak of ATP.

■PME, □Pi, ●PCr, ○ATP-Gamma.

Table 1Functional ATP of skeletal muscles, ATP γ -t₁₀

	HLR	5 mM Mg-ATP	10 mM Mg-ATP	5 mM Mg-ATP γ S	5 mM Adenosine	10 mM Adenosine
ATP γ -t ₁₀ MT (min)	170.6 $\pm$ 7.4	195.5 $\pm$ 5.7	199.4 $\pm$ 9.2	203.2 $\pm$ 6.9 *	192.7 $\pm$ 7.8	190.2 $\pm$ 6
ATP γ -t ₁₀ ATIT (min)	215.6 $\pm$ 7.4	240.5 $\pm$ 5.7	244.4 $\pm$ 9.2	248.2 $\pm$ 6.9 *	237.7 $\pm$ 7.8	235.2 $\pm$ 6

MT= Measured Time; ATIT=Actual Total Ischemia Time; HLR= Heparinized Lactated Ringer's; ATP γ -t₁₀= Time at which ATP γ peak dropped to 10% of its initial value;

* p < 0.05 vs. HLR.