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Administration of exogenous adenosine triphosphate to ischemic skeletal muscle induces an energy-sparing effect: Role of adenosine receptors

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ABSTRACT

Background: Ischemia–reperfusion injury is a devastating complication that occurs in allotransplantation and replantation of limbs. Over the years, several preservation strategies have been used to conserve the critical levels of intracellular adenosine triphosphate (ATP) during ischemia to sustain the ion gradients across the membranes and thus the tissue viability. The 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 the present study was to determine the effect of ATP administration on high-energy phosphate levels in ischemic skeletal muscle and to examine the role of purinergic and adenosine receptors in mediating the response to exogenous ATP.

Methods: The extensor digitorum longus muscles of Fischer rats were subjected to ischemia and treated with different concentrations of ATP with or without purinergic and adenosine receptor blockers. Phosphorus-31 nuclear magnetic resonance spectroscopy was used to measure the rate of decay of ATP, phosphocreatine (PCr), and the formation of adenosine monophosphate and acidification. Phosphorylated compounds were analyzed using a simple model of energy metabolism, and the 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 gradual ATP decay. The half-life of PCr was significantly longer in the ATP-treated muscles than in the vehicle controls and was maximally prolonged by treating with slow hydrolyzing adenosine 5′-O-(3-thio)triphosphate. Purinoceptor (P2X) blockade with ATP treatment significantly increased the half-life of PCr, and adenosine receptor blockers blunted the response. Administration of adenosine to ischemic muscles significantly increased the half-life of PCr compared with that in the vehicle controls.

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

1. Introduction

Ischemia–reperfusion injury is inevitable in complex limb reconstructions such as reimplantation of amputated limbs and allotransplantation. During these procedures, the 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), phosphoenolpyruvate (PEP), and other nucleotide phosphates (e.g., guanosine triphosphate, cytidine triphosphate, and uracil triphosphate), 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 the capacity to regenerate ATP from adenosine diphosphate (ADP), such as occurs during ischemia, ATP is spared for maintenance, rather than for macromolecular biosynthesis. Eventually, the ATP level decreases to less than a critical value, at which point the cells become necrotic [8,9]. Because of the ATP breakdown, adenosine, inosine, and hypoxanthine accumulate in the ischemic tissue [10–12].

Reestablishing the blood flow to limbs after prolonged ischemia can result in the systemic release of toxic metabolites from the 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 h of warm ischemia and gradually progresses to complete necrosis after 6 h [14,15]. During ischemia, myocyte glycogen and PCr are preferentially depleted instead of ATP, and little muscle necrosis occurs until ATP has been depleted [16].

Several studies of various tissues/organs have reported the beneficial effects of exogenous ATP administration, including improvement of function and survival after shock and sepsis [17–20], improved liver preservation [21], reduced brain injury after ischemia–reperfusion injury [22], limb preservation [23], and improved cardiovascular function in pathologic conditions [24–26]. Because 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. 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, ectonucleotide pyrophosphatase/phosphodiesterases, ecto-5′-nucleotidase, and alkaline phosphatases [27]. One potential mechanism could involve the activation of A1 and A3 adenosine receptors, which play a role in eliciting a cardioprotective effect against ischemia–reperfusion injury in a phenomenon known as ischemic preconditioning [28,29].

Phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) spectroscopy is a noninvasive tool that can be used to assess intracellular and extracellular bioenergetics [30,31]. Because the optimal function of skeletal muscle is directly related to energy metabolism, the measurement of phosphorylated compounds such as ATP, ADP, PCr, and inorganic phosphate (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):

$$\mathrm{PCr} + \mathrm{ADP} \Leftrightarrow \mathrm{ATP} + \mathrm{Cr} \quad (1)$$

$$2\mathrm{ADP} \Leftrightarrow \mathrm{AMP} + \mathrm{ATP} \quad (2)$$

$$\mathrm{Glyc_n} + 3\mathrm{Pi} + 3\mathrm{ADP} \rightarrow \mathrm{Glyn_{n-1}} + 3\mathrm{ATP} + 2\ \mathrm{lactate} \quad (3)$$

$$\mathrm{ATP} \Leftrightarrow \mathrm{ADP} + \mathrm{Pi} \quad (4)$$

However, as ischemia progresses, the hydrolysis of ATP continues to drive essential reactions. However, the supply of PCr becomes exhausted, leading to a decrease in intracellular ATP, along with an accumulation of Pi (Eq. 4), the 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, and PCr is exclusively intracellular. PCr, therefore, reports on the intracellular energy metabolism in the intact tissue.

The purpose of the present 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 would metabolize exogenous Mg-ATP to adenosine, which, in turn, would activate adenosine receptors to reduce myocyte energy consumption. We used ^{31}P-NMR spectroscopy to measure the depletion of PCr and other high-energy phosphates over time in ischemic skeletal muscle. We found that the administration of Mg-ATP to ischemic skeletal muscle increased the half-life of PCr and that antagonism of adenosine receptors reduced the Mg-ATP–induced energy-sparing effect.