

Acute aerobic swimming exercise increases nucleotidase activities in rat blood serum

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Objective: The purpose of this investigation was to examine the effect of an acute swimming exercise session on nucleotidase activities in rat blood serum. **Methods:** Male Wistar rats were divided in exercise and sedentary groups. In the exercise group, rats were submitted to one swimming session (60 minutes) with a constant load of 4% of body weight in the tail. Nucleotidase activities were gauged spectrophotometrically by measuring the inorganic phosphate from ATP, ADP or AMP hydrolysis or p-nitrophenol released from of p-Nph-5'-TMP hydrolysis. **Results:** The exercise group presented a significant increase in nucleoside triphosphate diphosphohydrolase (55.5% and 43.1%, for ATP and ADP, respectively), 5'-nucleotidase (57.4%), and nucleotide pyrophosphatase/phosphodiesterase (24.2%) activities. **Conclusion:** Our results have shown that nucleotidases are activated in rat blood serum after one session of aerobic swimming exercise, suggesting that these enzymes may promote an increase in adenosine levels, which might contribute to exercise-mediated vasodilatation.

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Key Words: Acute exercise; rat; serum; nucleotidase; ATP; adenosine

INTRODUCTION

Adenine nucleotides (ATP, ADP and AMP) and the derived nucleoside adenosine are important signaling molecules in the extracellular space, in which, they play important physiological roles. Evidence shows that nucleotides can be released from erythrocytes, endothelial cells and sympathetic nerve terminals upon muscle contraction (8, 20). The physiological stimuli for ATP release in vivo in the vasculature remains to be demonstrated. ATP regulates vascular tonus and cardiac function (24). Studies have shown that ATP, at milimolar concentrations, inhibits platelet aggregation via competitive and non-competitive mechanisms; however, at low concentrations, ATP may be stimulatory (31). It is well established that ADP induces changes in shape and platelet aggregation (18). Different studies have demonstrated the important role of these nucleotides in the processes of homeostasis and thrombi formation (9, 22). The

nucleoside adenosine, produced by nucleotide degradation, can act as a vasodilator and cardioprotective compound (12, 31).

The levels of extracellular nucleotides may be regulated by the action of ecto-nucleotidases and soluble nucleotidases, including enzymes of the NTPDase family (nucleoside triphosphate diphosphohydrolase; EC 3. 6. 1. 5), the 5'-nucleotidase (EC 3.1.3.5) and the NPP family (nucleotide pyrophosphatase phosphodiesterase; EC 3. 6. 1. 9) (37, 38). Studies have shown the presence of soluble NTPDases, NPPs, and 5'-nucleotidase in rat blood serum (21, 30). This enzyme cascade regulates the availability of ligands (ATP, ADP, AMP, and adenosine) for nucleotide and nucleoside receptors, and therefore the duration and increased receptor activation (7, 29). Roque et al. (2011) demonstrated that nucleotide hydrolysis is increased after moderate exercise training, which produces more adenosine, a potent vasodilator that may contribute to augmented

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blood flow. Nucleotide receptors are called P2 purinoceptors, and they are divided into two subclasses, P2X and P2Y. The P2X subclass consists of ionotropic receptors and mediates vasoconstriction. The P2Y subclass consists of metabotropic receptors and mediates vasodilation (25, 4, 5). Adenosine is a potent vasodilator that mediates its effects by means of a class of receptors called P1 receptors, which are divided into four subtypes: A₁, A_{2A}, A_{2B}, and A₃ (11, 13).

The precise blood flow and metabolism is especially important for skeletal muscle, where the energy supply demand can vary considerably for inactive or active periods (23). Neural vasoconstrictor activity and locally derived vasoactive substances regulate blood flow. Numerous local factors are released by skeletal muscle, endothelial cells and red blood cells; these factors also can include adenosine, ATP, potassium, hypoxia, hydrogen ion, nitric oxide, prostanoids and endothelium-derived hyperpolarizing factor (8).

Aerobic exercise induces cardiovascular responses such as an increase in heart rate, vasodilation and an increased blood flow, a mechanism necessary for a greater supply of oxygen and nutrients to the muscle during exercise. Considering these facts, this study hypothesized that the degradation pathway of an extracellular nucleotide is activated to produce adenosine, an important vasodilator. Therefore, the objective of this work was to evaluate the effect of acute aerobic swimming on the degradation of nucleotides by measuring NTPDase, NPP, and 5'-nucleotidase activities in rat blood serum.

MATERIALS AND METHODS

Chemicals

Nucleotides, p-nitrophenyl thymidine 5'-monophosphate, trizma base, malachite green, ammonium molybdate, polyvinyl alcohol, EDTA, EGTA, sodium citrate, Coomassie Blue G, bovine serum albumin, calcium and magnesium chloride were purchased from Sigma (USA). All other reagents used were of analytical grade.

Animals

Male Wistar rats (age—approximately 90 days, weight—260-320g) from our breeding stock were housed four to a cage, with food and water ad libitum. The animal house temperatures were kept between 22-23 °C with a 12-hour light/dark cycle (lights on at 7:00). Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology.

Acute exercise-test protocol

The rats were divided in two groups (n= 6): exercise (E) and sedentary (S). The E group was submitted to a 60-minute session of swimming exercise, supporting a constant load of 4% of body weight in the tail. This weight was chosen because it is already characterized in the literature as an aerobic exercise below the lactate threshold (15, 33). The swimming exercise was performed in cylinder tanks 45 cm deep and 32 cm in diameter. The sedentary group was placed into the same tank, but the water was added to a depth that the rat could stand on the bottom. The purpose of this procedure was to expose the sedentary group to water stress without promoting exercise stress. The water temperature was kept between 30-34 °C throughout the experiments (16).

Isolation of blood serum fraction

Blood samples were collected immediately after the exercise or water stress (34), and centrifuged in plastic tubes at 5000 g for 5 minutes at 20 °C. The serum samples were maintained on ice and used immediately in the experiments.

Determination of NTPDase and 5'-nucleotidase activities

ATP, ADP, and AMP hydrolysis were determined as described previously (21). The reaction mixture containing 3.0 mM ATP, ADP, or AMP as substrate, 112.5 mM Tris-HCl, and pH 8.0, was incubated with 0.7 mg to 1.0 mg of serum protein at 37 °C for 40 minutes in a final volume of 0.2 mL. The reaction was stopped by the addition of 0.2 mL of 10% TCA. The samples were chilled on ice for 10 minutes and centrifuged at 5000 x g for 5 minutes to eliminate the precipitate protein, and 100 µL of supernatant was used for the colorimetric assay. The amount of inorganic phosphate (Pi) released was measured as outlined previously (6). Incubation times and protein concentrations were chosen to ensure the linearity of the reaction. Controls with the serum fraction added after the trichloroacetic acid were used to correct non-enzymatic hydrolysis of the substrates. All samples were run in duplicate. Enzyme activities were expressed as nanomoles of Pi released per minute per milligram of protein.

Determination of nucleotide pyrophosphatase/phosphodiesterase activity (NPP)

The NPP activity was assessed using p-nitrophenyl

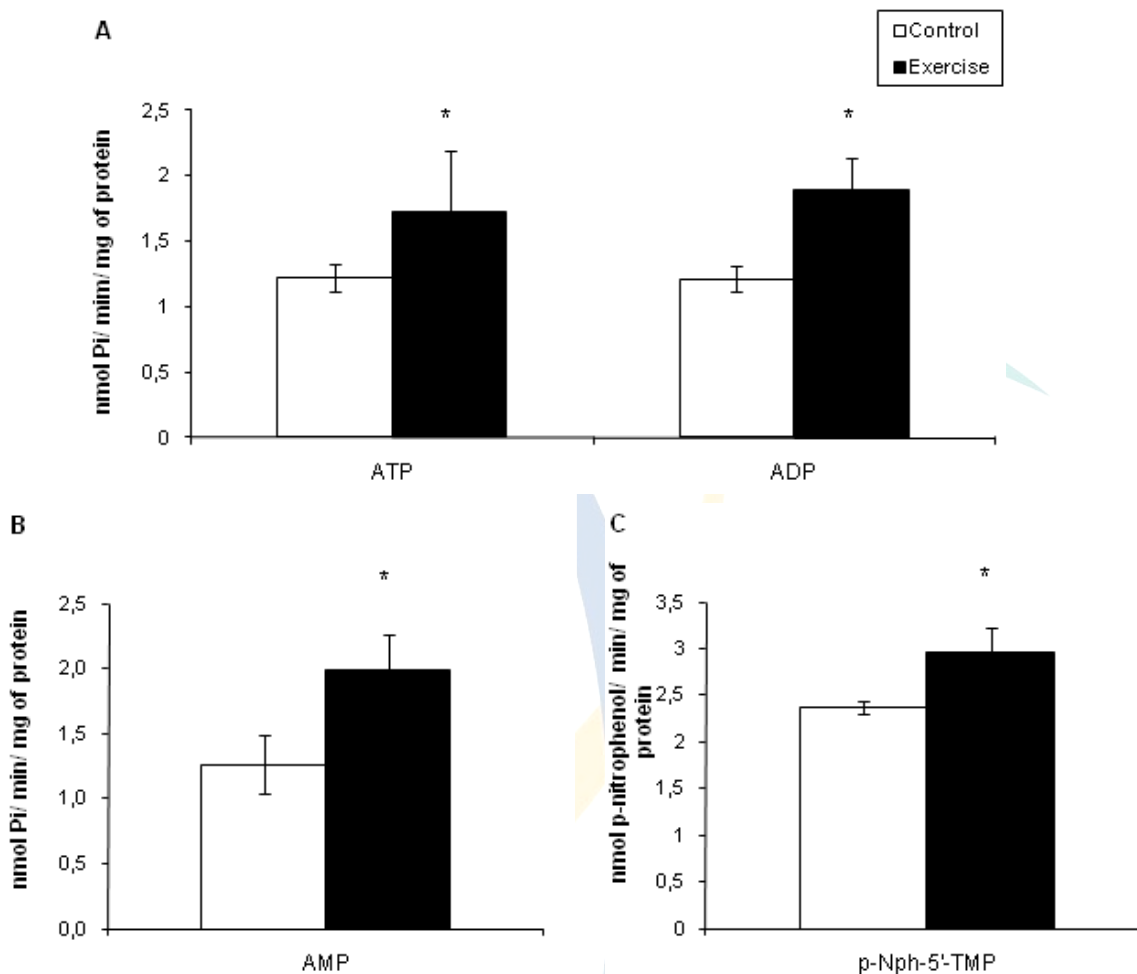


Figure 1. Effect of acute aerobic swimming exercise on serum nucleotide hydrolysis. (A) NTPDase (B) 5'-nucleotidase (C) NPP activity. Bars represent the mean \pm S.D. of six different experiments. * represents Student t-test considering $P \leq 0.05$ as significant).

thymidine 5'-monophosphate (p-Nph-5'-TMP), an artificial marker routinely used for the assay of this enzyme (30). The reaction mixture containing 0.5 mM of p-Nph-5'-TMP, as a substrate in 100mM Tris-HCl, pH 8.9, was incubated with approximately 1.0 mg of serum protein at 37 °C for 8 minutes in a final volume of 0.2 mL (21). The reaction was stopped by the addition of 0.2 mL of 0.2N NaOH. Incubation times and protein concentrations were chosen to ensure the linearity of the reaction. Controls with the serum fraction added after NaOH were used to correct non-enzymatic hydrolysis of the substrate. All samples were run in duplicate. Enzyme activity was expressed as nanomoles of p-nitrophenol released per minute per milligram of protein.

Protein determination

Protein was measured by the Coomassie Blue method (1), using bovine serum albumin as a standard.

Statistical Analysis

Data were expressed as means (n= 6) + S.D and analyzed by Student t-test. $P \leq 0.05$ was considered significant.

RESULTS

We evaluated the effect of acute swimming exercise on NTPDase, NPP, 5'-nucleotidase in rat blood serum. The data showed that acute swimming exercise resulted in a significant increase in all enzyme activities tested (Fig. 1). There was an increase in ATP (1.22 \pm 0.1 vs 1.89 \pm 0.4; 55.5%), ADP (1.21 \pm 0.1 vs 1.72 \pm 0.2; 43.1%) (Fig. 1A), and AMP (1.26 \pm 0.2 vs 1.99 \pm 0.2; 57.4%) (Fig. 1B) hydrolysis promoted by NTPDase and ecto-5'-nucleotidase activities present in blood serum of the exercise group, respectively, when compared to the control group. NPP activity, performed using the artificial substrate p-Nitrophenyl-

thymidine-5'-monophosphate (p-Nph-5'-TMP), also increased significantly (2.37 ± 0.1 vs 2.95 ± 0.2 ; 24.2%) in blood serum of rats subjected to exercise when compared to the control group (Fig. 1C).

DISCUSSION

In this study, we demonstrated that the enzymatic activities involved in extracellular nucleotide catabolism in blood serum are increased after an acute swimming exercise session. ATP and other nucleotides are released through lysis and/or cell death, as well as exocytosis. Besides ATP release, it is also important to observe that electrical stimulus also induces the release of soluble enzymes, which may act together with nucleotidases anchored in membranes in the nucleotide hydrolysis in blood serum (32, 35). Exercise can induce muscle damage and increase electrical stimulus; therefore it is easy to speculate that an increase in nucleotide and/or nucleotidases concentrations occurs in blood serum during exercise. Mortensen et al. (2011) demonstrated that ATP is released locally into the arterial and venous plasma of contracting muscle. Furthermore, nucleotide hydrolysis (ATP diphosphohydrolase and 5'-nucleotidase activity) is increased after chronic swimming training, suggesting that this enzymatic pathway is involved in functional adaptations of exercise (27). In physiological conditions, endothelial NTPDase maintain haemostasis through the rapid inactivation of released nucleotides (19). The endothelial metabolism generally prevails in microcirculation; however, in the larger veins, the ability to metabolize nucleotides also resides on the luminal surface of the vein wall and in circulating blood (9). Among the blood cellular elements, leukocytes contributed to the catabolism of nucleotides (17), but erythrocytes or platelets did not. The biological effect of nucleotides is mainly determined by its rate of release into the extracellular medium, the nucleotidase activities and their binding affinity to specific receptors (36). Nucleotidase-mediated modulation affects the nucleotide levels and acts on cell signaling mediated by these molecules in different cell types (2). The activation of the nucleotidase activities in rat blood serum after an acute swimming exercise session may decrease the effect of nucleotides (ATP, ADP, and AMP) via P2 receptors and increase the effect of adenosine via P1 receptors. Mortensen et al. (2011) used the intravascular microdialysis technique to show that ATP is rapidly degraded or taken up by cells because only 35-48% of the infused ATP could be detected in the artery ~20 cm downstream from the infusion. The short half-life in vivo of ATP can be caused by a rapid uptake in endothelial cells or degradation by soluble or membrane-bound enzymes. Our study demonstrated

an increase in the activity of soluble nucleotidases after exercise, reinforcing the role of this enzymatic pathway on acute physiological changes promoted by exercise.

ATP may have an antagonistic effect on the regulation of muscle blood flow, inducing constriction to maintain systemic blood pressure, and dilation to increase the supply of oxygen (28). It is widely assumed in the literature that the vascular effects of ATP are mediated by second messengers such as nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factors (10, 28). When ATP activates ionotropic P2X (found mainly in vascular smooth muscle cells), vasoconstriction occurs, while the activation of metabotropic P2Y receptors (mainly found in endothelial cells) produces vasodilatation (28). Studies have shown that ADP is potent in platelet recruitment via interaction with P2Y12 receptors (14). The end product of the degradation of nucleotides, adenosine, induces relaxation of vascular smooth muscle (26).

Our results have shown that nucleotidases are activated in rat blood serum after one session of aerobic swimming exercise, suggesting that these enzymes may promote an increase in adenosine levels, which might be one of the mechanisms responsible for exercise-mediated vasodilatation.

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