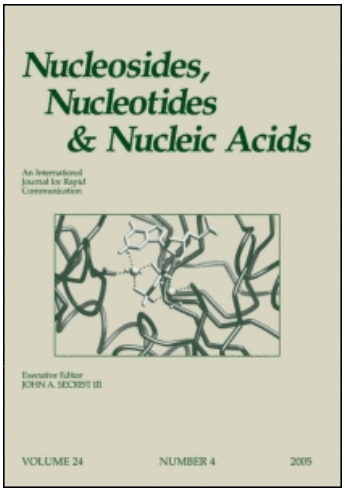

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Modulation of AMP Deaminase in Rat Hearts Subjected to Ischemia and Reperfusion by Purine Riboside

T. Borkowski[a]; M. Lipinski[b]; R. Kaminski[c]; E. Krzyminska-Stasiuk[c]; M. Langowska[a]; G. Raczak[c]; E. M. Slominska[a]; R. T. Smolenski[ad]
[a] Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland [b] Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdansk, Poland [c] Clinic of Cardiology and Electrotherapy, Medical University of Gdansk, Gdansk, Poland [d] Heart Science Centre, Imperial College London, Harefield, United Kingdom

To cite this Article Borkowski, T. , Lipinski, M. , Kaminski, R. , Krzyminska-Stasiuk, E. , Langowska, M. , Raczak, G. , Slominska, E. M. and Smolenski, R. T.(2008) 'Modulation of AMP Deaminase in Rat Hearts Subjected to Ischemia and Reperfusion by Purine Riboside', Nucleosides, Nucleotides and Nucleic Acids, 27: 6, 876 — 880
To link to this Article: DOI: 10.1080/15257770802146551
URL: http://dx.doi.org/10.1080/15257770802146551

Nucleosides, Nucleotides, and Nucleic Acids, 27:876–880, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802146551

Taylor & Francis
Taylor & Francis Group

MODULATION OF AMP DEAMINASE IN RAT HEARTS SUBJECTED TO ISCHEMIA AND REPERFUSION BY PURINE RIBOSIDE

T. Borkowski,[1] M. Lipinski,[2] R. Kaminski,[3] E. Krzyminska-Stasiuk,[3] M. Langowska,[1] G. Raczak,[3] E. M. Slominska,[1] and R. T. Smolenski[1,4]

[1]*Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland*
[2]*Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdansk, Poland*
[3]*Clinic of Cardiology and Electrotherapy, Medical University of Gdansk, Gdansk, Poland*
[4]*Heart Science Centre, Imperial College London, Harefield, United Kingdom*

□ *Changes in AMP deaminase (AMPD) activity influence heart function and progression of heart disease, but the underlying mechanism is unknown. We evaluated the effect of purine riboside (Purr) on the activity of AMPD in perfused rat hearts and in isolated rat cardiomyocytes. Brief perfusion of the pre-ischemic heart with 200 μM Purr resulted in activation of AMPD, more pronounced degradation of the adenine nucleotides, and reduced recovery of the adenine nucleotide pool during reperfusion. Brief incubation of rat cardiomyocytes with 200 μM Purr also activated AMPD, while prolonged exposure resulted in enzyme inhibition. We conclude that Purr activates AMPD, whereas metabolites of this compound may inhibit the enzyme.*

Keywords AMP deaminase; heart; cardiomyocytes; rat; purine riboside; ischemia-reperfusion

INTRODUCTION

The proposed cardioprotective effect of a C34T mutation in the AMP deaminase 1 gene,[1] which results in decreased AMP deaminase (AMPD) activity, has recently been challenged.[2] In the context of this clinical controversy it is important to develop pharmacological modulators of AMPD activity that can be used to test the role of this enzyme in different animal models such as isolated hearts, isolated cardiomyocytes, and experimental models of heart failure. In this study we examined the effect of 200 μM purine riboside (Purr), a known modulator of AMPD activity, on nucleotide metabolism in rat hearts subjected to ischemia using the

This study has been supported by the Ministry of Science of Poland (N401 101 31/2201, W-172, W-511).

Address correspondence to R. T. Smolenski, Heart Science Centre, Imperial College at Harefield Hospital, Harefield UB9 6JH, UK. E-mail: r.smolenski@imperial.ac.uk

Langendorff model. We also tested the effect of 200 μM Purr on AMPD activity and nucleotide metabolism in isolated cardiomyocytes incubated for 10, 30, and 60 minutes in normal and energy depleting conditions.

MATERIALS AND METHODS

Rats were anesthetized with pentobarbital and hearts were rapidly excised and immediately attached to a Langendorff apparatus and perfused with filtered Krebs buffer at a constant pressure as described previously.[3] Hearts treated with Purr and controls were studied to determine metabolic changes before ischemia (preisch C and preisch Purr groups), during ischemia (endisch C and endisch Purr groups) and after ischemia (reperf C and reperf Purr groups). Rat hearts connected to a Langendorff perfusion apparatus and initially perfused for 15 minutes, were perfused for another 5 minutes with 200 μM Purr or vehicle, then subjected to ischemia for 15 minutes at 37°C. Post-ischemic hearts were then reperfused for 30 minutes at 37°C. At the end of the initial perfusion (preisch C and Purr), ischemia (endisch C and Purr) and reperfusion (repef C and Purr) hearts were freeze-clamped and stored in liquid nitrogen for extraction and high performance liquid chromatographic (HPLC) analysis as described before.[4] Rat cardiac myocytes were obtained as described previously.[5] Viable myocytes were purified by centrifugation, then suspended and incubated in HEPES buffered Krebs solution containing 200 μM Purr for 10, 30, and 60 minutes followed by addition of 10 mM deoxyglucose and 1 mg/ml oligomycin for 45 minutes to induce breakdown of adenine nucleotides in the presence of selective adenosine kinase (5′-iodotubercidine [ITU]) and adenosine deaminase (erythro-9-(2-hydroxy-3-nonyl)adenine [EHNA]) inhibitors to discriminate between alternative AMP catabolic pathways. Cell suspensions were then extracted with perchloric acid and analysed with HPLC as described previously.[4]

RESULTS

Figure 1A presents ATP, NAD, and total adenine nucleotide (TAN) concentrations in rat hearts before ischemia, at the end of ischemia and after reperfusion. Concentration of ATP was reduced in both ischemic groups compared to preischemic conditions, but this reduction was more pronounced in the group treated with Purr. Recovery of ATP was observed in both reperfused groups, but this recovery was smaller in the Purr treated group. TAN and NAD followed changes noted for ATP. Concentrations of IMP, Ino and Ado (Figure 1B) in end-ischemic groups were greater in the group treated with Purr.

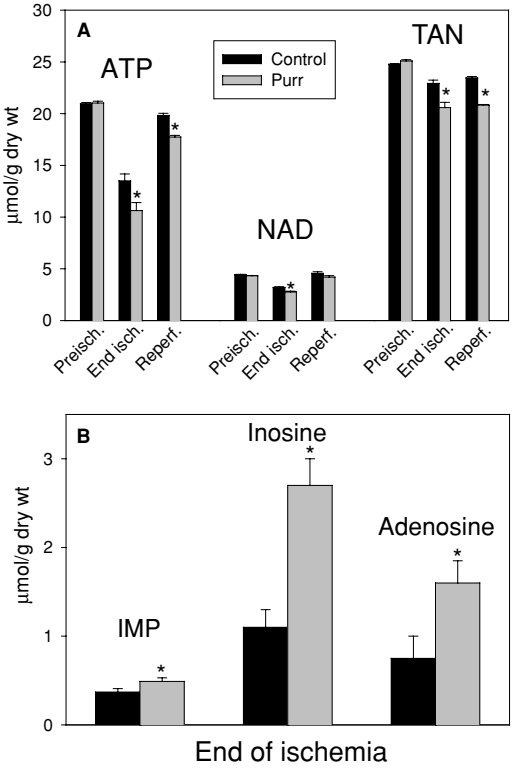

FIGURE 1 ATP, NAD, and TANconcentrations in rat hearts treated with 200 μM Purr and controls before ischemia, at the end of ischemia and after reperfusion (A) and IMP, inosine and adenosine concentrations at the end of ischemia (B). Values are mean ±SEM, n = 5, *p < 0.05 versus control.

Figure 2 demonstrates changes in IMP concentration (panel A) and in Ado/Ino+IMP ratio (panel B) in isolated rat cardiomyocytes preincubated with 200 μM Purr for 10, 30, and 60 minutes followed by 45 minutes stimulation of catabolism in the presence of 5 μM EHNA and 10 μM ITU. Ado/Ino+IMP ratio was decreased after 10 minutes preincubation, not changed after 30 min preincubation, and elevated after 60 minutes preincubation with Purr. IMP concentrations followed the same pattern.

DISCUSSION

Perfusion of the rat heart with 200 μM Purr before ischemia caused activation of AMPD that was indicated by increased concentrations of IMP in hearts subjected to ischemia. We observed more pronounced degradation of the adenine nucleotides. However, AMP dephosphorylation pathway has also been activates as indicated by increased adenosine production. This was not due to increased AMP concentration and seems to be a consequence of direct activation of catabolic enzymes. Experiments with rat cardiomyocytes

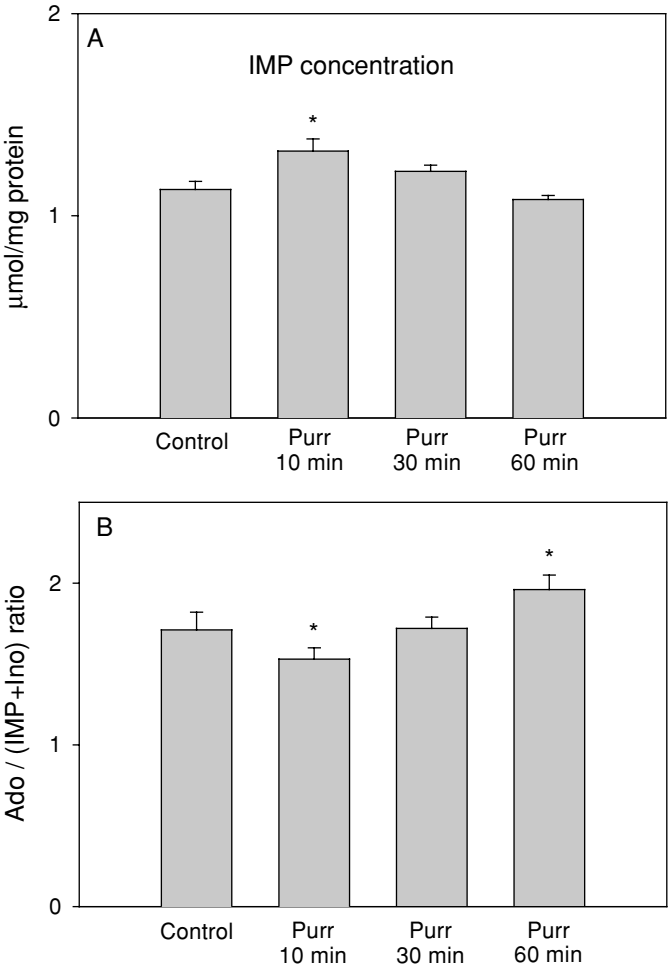

FIGURE 2 IMP concentration (A) and Ado/(Ino+IMP) ratio (B) in isolated cardiomyocytes treated with 200 μM Purr for 10, 30, and 60 minutes and then subjected to 45 minutes exposure to oligomycin and deoxyglucose in the presence of 5 μM EHNA and 10 μM ITU. Values are mean ±SEM, n = 5, $p < 0.05$ for control versus Purr 10 minutes and control versus Purr 60 minutes.

incubated with 200 μM Purr highlighted that effect of purine riboside on adenine nucleotide catabolism is more complex. Short preincubation with purine riboside caused activation of AMPD, as in perfused hearts but longer preincubation inhibited AMPD. We conclude that purine riboside causes diverse effects on AMP deaminase activity in the heart. The short-term effect is activation while prolonged exposure inhibits AMP deaminase. This diverse effect of purine riboside is most likely related to formation of intracellular phosphate metabolites of purine riboside that we previously found to be synthesized[6] but initially are absent, hence direct activation that turns to inhibition while those metabolites are formed.

REFERENCES

1. Loh, E.; Rebbeck, T.R.; Mahoney, P.D.; DeNofrio, D.; Swain, J.L.; Holmes, E.W. Common variant in AMPD1 gene predicts improved clinical outcome in patients with heart failure. *Circulation.* **1999**, 99, 1422–1425.
2. de Groote P.; Lamblin, N.; Helbecque, N.; Mouquet, F.; Hermant, X.; Amouyel, P.; Dallongeville, J.; Bauters, C. The impact of the AMPD1 gene polymorphism on exercise capacity, other prognostic parameters, and survival in patients with stable congestive heart failure: a study in 686 consecutive patients. *Am. Heart J.* **2006**, 152, 736–741.
3. Smolenski, R.T.; Amrani, M.; Jayakumar, J.; Jagodzinski, P.; Gray, C.C.; et al. Pyruvate/dichloroacetate supply during reperfusion accelerates recovery of cardiac energetics and improves mechanical function following cardioplegic arrest. *Eur. J Cardiothorac. Surg.,* **2001**, 19, 865–872.
4. Smolenski, R.T.; Lachno, D.R.; Ledingham, S.J.M.; Yacoub, M.H. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J. Chromatogr.* **1990**, 527, 414–420.
5. Smolenski, R.T. The elevation of adenylate pool in rat cardiomyocytes by S-adenosyl-L-methionine. *Acta Biochim. Pol.* **2000**, 47, 1171–1178.
6. Kozlowska, M.; Smolenski, R.T.; Makarewicz, W.; Hoffmann, C.; Jastorff, B.; Swierczynski, J. ATP depletion, purine riboside triphosphate accumulation and thymocyte death induced by purine riboside. *Toxicol. Lett.* **1999**, 104, 171–181.