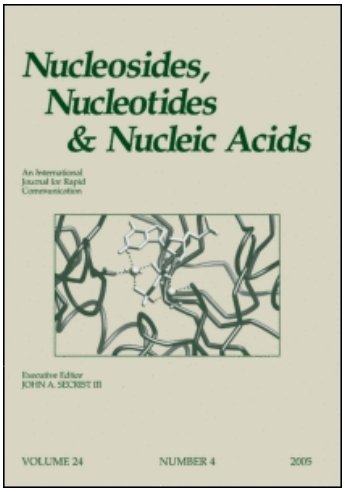

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Biological Efficiency of AMP Deaminase Inhibitor: 3-[2-(3-CARBOXY-4-BROMO-5,6,7,8-Tetrahydronaphthyl)Ethyl]-3,6,7,8-Tetrahydroimidazo[4,5]-[1,3]Diazepin-8-OL

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BIOLOGICAL EFFICIENCY OF AMP DEAMINASE INHIBITOR: 3-[2-(3-CARBOXY-4-BROMO-5,6,7,8-TETRAHYDRONAPHTHYL)ETHYL]-3,6,7,8-TETRAHYDROIMIDAZO[4,5]-[1,3]DIAZEPIN-8-OL

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□ *AMP deaminase could be a potential target for treatment of heart disease but experimental evaluation of this concept is difficult due to limited availability of inhibitors with proven efficiency in biological systems. This study evaluated the effect of 3-[2-(3-carboxy-4-bromo-5,6,7,8-tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo [4,5-d][1,3]diazepin-8-ol, an AMP deaminase inhibitor (AMPDI) on the pathways of nucleotide metabolism in perfused rat heart. We show that AMPDI at 0.3 mM concentration effectively inhibits AMP deaminase in this experimental model.*

Keywords AMP deaminase; hypoxia; heart; rat

INTRODUCTION

Inhibition of AMP deaminase with pharmacological agents is an attractive area of research interest in view of recent findings indicating that mutation in AMP deaminase (AMPD) resulting in lowered cardiac activity is protective in heart disease.[1,2] However, progress in this field is limited by lack of AMP deaminase inhibitors with proven biological efficiency. AMP deaminase inhibitors with potential cell permeability were established by Kasibhatla et al.,[3] and we have previously proven the effective inhibition in heart homogenates, with isolated enzyme and with isolated cardiomyocytes.[4] This study was designed to determine whether AMPDI added to

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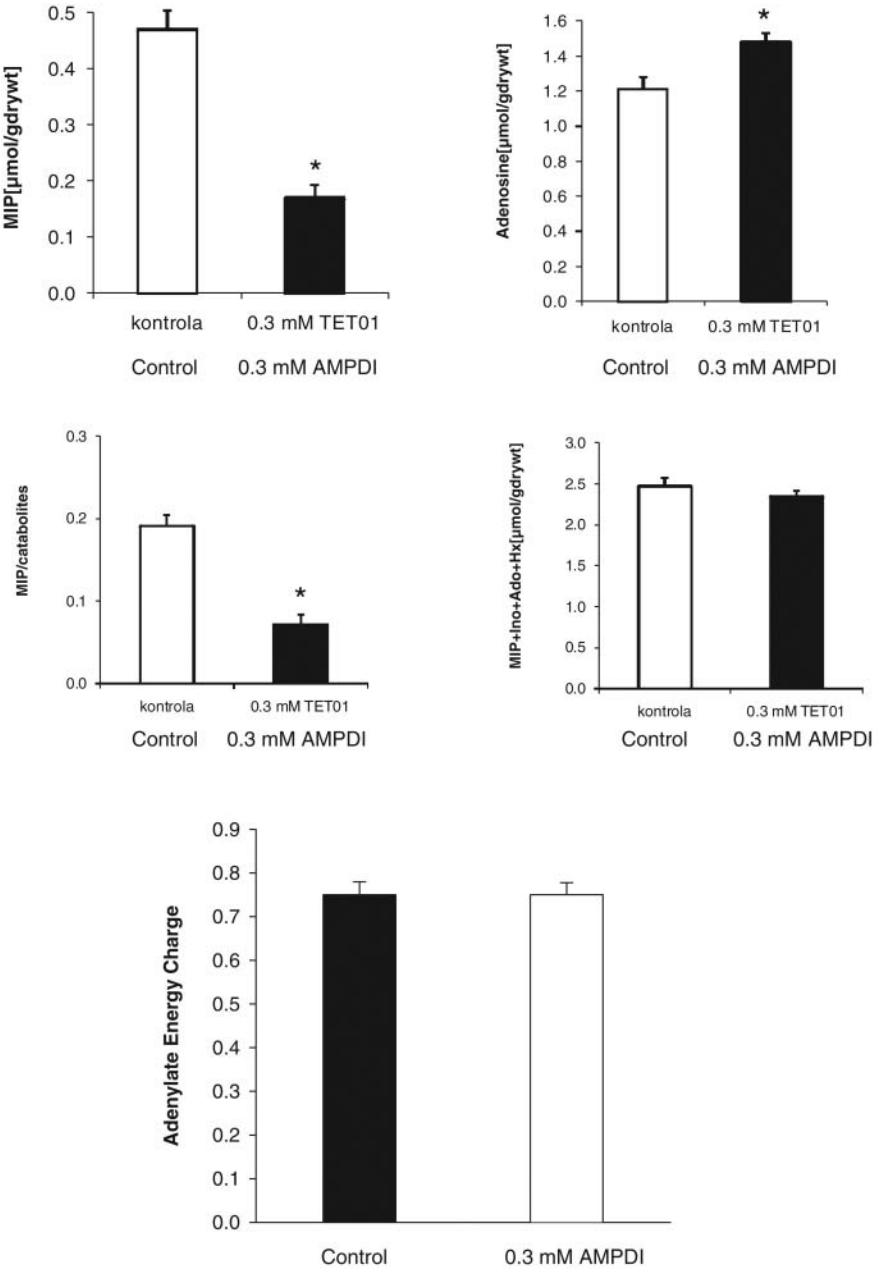

FIGURE 1 Effects of 10 minutes perfusion of rat heart with 0.3 mM AMPD inhibitor (AMPDI) on concentration of IMP, adenosine, IMP/catabolite ratio total catabolite concentration (IMP + hypoxanthine + adenosine + inosine) and adenylate energy charge following 5 minutes of global ischemia. Values are mean ± SEM, n = 3–4. *$p < 0.05$ versus control.

perfusion buffer at a concentration that effectively inhibits AMP deaminase in isolated cardiomyocytes can inhibit AMP deaminase in isolated perfused heart.

MATERIALS AND METHODS

An AMP deaminase inhibitor (AMPDI): 3-[2-(3-carboxy-4-bromo-5,6,7,8 tetrahydronaphthyl)ethyl]-3,6,7,8-tetrahydroimidazo [4,5-d][1,3]diazepin-8-ol was chemically synthesized according to the procedure established by Kasibhatla et al.[3] Young rats (50 g) were anesthetized with pentobarbital and hearts were rapidly excised and immediately attached to a Langendorff apparatus and perfused with Krebs buffer at a constant pressure as described previously.[5] After 15 minutes of initial stabilisation perfusion, AMPDI was added to the buffer and perfusion was continued in recirculation mode for an additional 10 minutes. In both AMPDI and control group, an adenosine deaminase inhibitor (EHNA, 5 μM) was included in the perfusate buffer to distinguish between the different pathways of nucleotide catabolism. After that, the perfusion line was closed and hearts were maintained in an ischemic state for 5 minutes at 37∞C. Hearts were then freeze-clamped and stored in liquid nitrogen for extraction and high performance liquid chromatographic (HPLC) analysis as previously described.[6]

RESULTS AND DISCUSSION

As illustrated in Figure 1, administration of AMPDI resulted in reduced IMP accumulation in ischemic hearts. This was associated with a higher adenosine concentration. Consequently, the ratio of IMP to catabolites (adenosine+inosine+hypoxanthine) profoundly decreased. This shift in profile was not associated with any change in the total concentration of purine catabolites. These results indicate that nucleotide catabolism shifted from deamination to dephosphorylation of AMP (by 5′nucleotidase). AMPDI had no effect on adenylate energy charge in the heart after 5 minutes of ischemia. These results indicate that AMPDI effectively inhibits AMP deaminase in perfused hearts, as we have previously noted in isolated cardiomyocytes.[4] This concentration range (about 0.3 mM) should be effective for *in vivo* targeting of AMP deaminase.

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