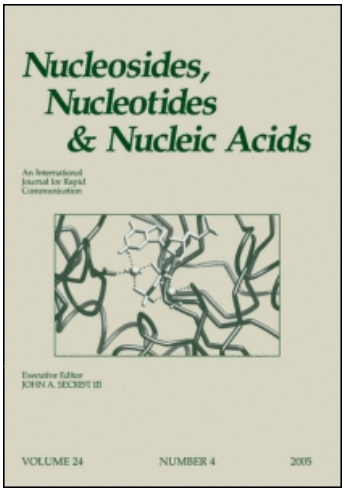

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Lidoflazine Combined with Nucleotide Precursors Increases ATP Content and Adenosine Production in Cardiomyocytes

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LIDOFLAZINE COMBINED WITH NUCLEOTIDE PRECURSORS INCREASES ATP CONTENT AND ADENOSINE PRODUCTION IN CARDIOMYOCYTES

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▫ *We have previously identified that the nucleoside transport blocker dipyridamole increases adenosine production but may cause depletion of the nucleotide pool in cardiomyocytes during extended exposure and that this effect was abolished by co-administration of adenine and ribose. The present study aimed to establish whether lidoflazine, a newer generation of nucleoside transport inhibitor with calcium antagonist properties, would cause a similar effect. We conclude that lidoflazine did not affect the nucleotide pool while the combined application of lidoflazine with precursors of nucleotide resynthesis increased ATP concentration and further enhanced adenosine production.*

Keywords Lidoflazine, Nucleoside Transport Inhibitors, ATP, Adenosine

INTRODUCTION

The property of lidoflazine (Clinium) as a long-acting coronary vasodilator was first identified in 1966 by Schaper et al.[1] In humans, lidoflazine was initially used clinically in the treatment of cardiac angina,[2] because it is a calcium antagonist and a nucleoside transport inhibitor. Application of nucleoside transport inhibitors (NTI) have been considered to enhance cardioprotection during heart transplantation, for the reduction of infarct size and for the improvement in the function of the failing heart.[3–5] Although biochemical mechanisms of these drugs have been studied extensively, few studies evaluated the effects on the nucleotide pool after prolonged exposure. We have previously shown that the nucleoside transport blocker dipyridamole causes depletion of the nucleotide pool in cardiomyocytes during long-term exposure.[6] The present study aimed to establish the effect of lidoflazine on the adenine nucleotide pool.

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MATERIALS AND METHODS

Adult rat cardiomyocytes were isolated using collagenase perfusion technique. Cells were then incubated at 37°C in HEPES buffered Krebs solution as described previously,[6] containing 2% albumin, for up to 8 h following isolation under the following conditions: 1) Control; 2) 10 μM lidoflazine (LIDO); 3) LIDO + 100 μM adenine (Ade); 4) LIDO + Ade + 5 mM inorganic phosphate (Pi); 5) LIDO + Ade + 2.5 mM ribose; and 6) LIDO + Ade + Ribose + Pi. The adenosine deaminase inhibitor erythro-9(2-hydroxy-3-nonyl) adenine (EHNA) was added at 5 μM concentration to all incubations. After 8 h, extracts of myocyte suspensions were analyzed by HPLC.[7] For comparison of ATP changes between the groups, one-way analysis of variance (ANOVA) was used followed by Student-Newman-Keuls test. For comparison of adenosine concentration changes we used nonparametric ANOVA.

RESULTS

ATP was unchanged after 8 h of incubation with lidoflazine alone, similar to control. In the presence of lidoflazine and the different combinations of nucleotide

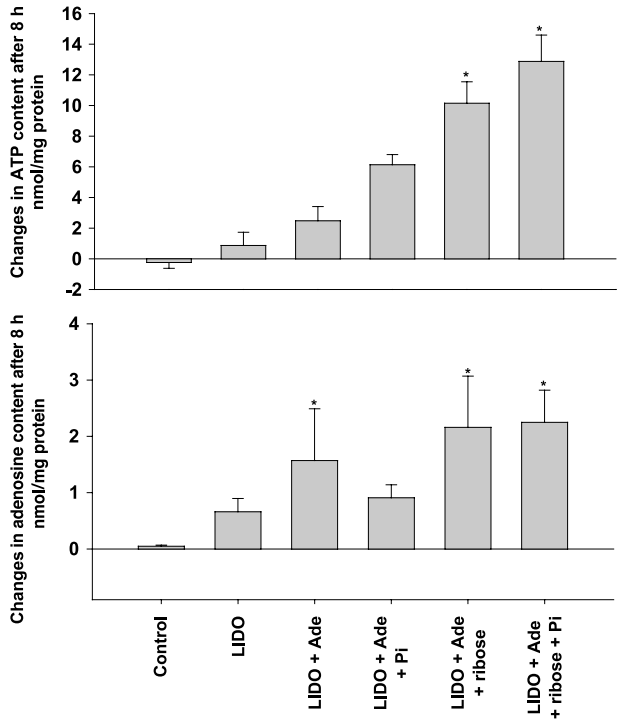

FIGURE 1 Changes in ATP and adenosine concentration after 8 h incubation of rat cardiomyocytes with lidoflazine and different combination of nucleotide precursors. Results are expressed as mean values ± SEM, (n = 4–6). *p < 0.05 vs. control.

precursors, ATP was maintained or significantly increased with adenine and ribose and inorganic phosphate. Presence of lidoflazine caused an increase in adenosine content that was further enhanced in combination with nucleotide precursors (Figure 1).

DISCUSSION

This study demonstrated that combined application of lidoflazine with precursors of nucleotide synthesis in cardiomyocytes increases ATP and extracellular adenosine concentration. Without nucleotide precursors, lidoflazine had no effect on ATP concentration. This indicates that the effect of NTIs on nucleotide metabolism could depend on the type of inhibitor. We have previously shown that dipyridamole caused a significant decrease in ATP concentration[6] while this was not shown here with lidoflazine under identical experimental conditions. Providing the precursors of nucleotides resynthesis adenine, ribose and inorganic phosphate resulted in increased adenosine concentration and restoration (dipyridamole) or increased (lidoflazine) ATP concentration. This difference could be caused by the nonspecific effects of NTIs not related to nucleoside transport inhibition. This includes dipyridamole-mediated inhibition of phosphodiesterases, increase in prostacyclin formation, interference with the transport of glucose, choline, and phosphate; these mechanisms could lead to ATP depletion.[8] In the case of lidoflazine, its calcium channel effects may reduce ATP demand and decrease the rate of adenosine cycling in cardiomyocytes. This in turn will reduce the amount of adenosine lost from cells due to inhibition of its re-uptake. An important conclusion from this study is that lidoflazine lacks deleterious metabolic effects previously identified with dipirydamole and therefore effects of these drugs may not only be related to transport inhibition.

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