

METABOLIC RECOVERY FOLLOWING TEMPORARY REGIONAL MYOCARDIAL ISCHEMIA IN THE RAT

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The restoration of the cardiac ATP content after an ischemic insult takes a long period of time. Ribose, via stimulation of adenine nucleotide biosynthesis, accelerated the replenishment of the adenine nucleotide pool in the heart, in the kidney, however, it had no effect. In the myocardium, the ribose-mediated restoration of the adenine nucleotide content was dependent on the duration of the previous ischemic period and was not influenced by the β -receptor blocker atenolol.

INTRODUCTION

It is well known that the myocardium can restore its adenine nucleotide content only very slowly once it has been depleted during a brief ischemic period (2,10,19). The most likely explanation for this post-ischemic metabolic insufficiency is that the adenine nucleotide degradation products such as adenosine, inosine and hypoxanthine (6) permeate the cell membrane and are washed out during the reperfusion period so that they are not available for reutilization and resynthesis of ATP via the "salvage pathways". In this situation, the heart is mainly dependent on the de novo formation (= biosynthesis) of purine nucleotides. This synthetic process, however, is very slow in the heart. Even when it is stimulated during postischemic recovery, the complete restoration of the adenine nucleotide pool requires a long time. It is therefore a logical metabolic intervention to enhance adenine nucleotide biosynthesis. Ribose has been found to be an appropriate substrate to achieve this. The approach is based on the fact that the capacity of the oxidative pentose phosphate pathway, as evaluated by the activity of glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme (3), is very small in heart and skeletal muscle. Ribose bypasses this critical step and leads to an expansion of the 5-phosphoribosyl-1-pyrophosphate pool and to an acceleration of adenine nucleotide biosynthesis in the myocardium (16). In many pathological situations, the stimulation of cardiac adenine nucleotide synthesis was further enhanced by ribose. During the recovery period from a 15 min regional ischemia, the resulting enhancement was of such an extent that the restoration of the adenine nucleotide pool was achieved almost

ready after 12 hours, whereas about 72 hours were needed for ATP normalization without any intervention (19).

In view of the cardioprotective effect of ribose during the postischemic recovery period it was the aim of this study to answer the following questions: 1. After which period of regional ischemia can ribose compensate the decline of adenine nucleotides during a 24-hour recovery period? 2. Are catecholamines involved in the metabolic effect of ribose? 3. Does ribose accelerate the restitution of adenine nucleotides during postischemic recovery of the kidney?

MATERIAL AND METHODS

Female Sprague-Dawley rats (220-250 g) fed a diet of Altromin[®] with free access to tap water were used throughout the study. Temporary regional ischemia of the heart was induced after thoracotomy by ligation of the descending branch of the left coronary artery (1) using the special knot of Fabiani (4). The occlusion could be released at any time without another thoracotomy. The rats received continuous i.v. infusion of 0.9% NaCl or ribose through the jugular vein (18). Ischemia of the left kidney was also induced in ether anesthetized rats. The renal artery and vein were repeatedly ligated for 5 min each time with 1 min of reperfusion in between until a total ischemic period of 30 min was obtained.

Rates of myocardial adenine nucleotide biosynthesis were determined by relating the total radioactivity of adenine nucleotides due to the incorporation of ¹⁴C-thymine (specific activity 58 μ Ci/mole, Radiochemical Centre, Amersham, England) to the mean specific activity of the tissue glycine precursor pool (15). The contents of ATP, ADP and AMP were measured using the method of Gerlach et al. (5).

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Table 1. Myocardial adenine nucleotide biosynthesis in various experimental conditions.

Experimental condition	n	Without ribose (nmoles/p/h)	n	With ribose (nmoles/p/h)
Control	25	6.0 ± 0.7	7	26.7 ± 5.0
Recovery from asphyxia	6	12.6 ± 0.5	5	20.5 ± 2.2
Recovery from ischemia	4	14.4 ± 3.2	4	60.5 ± 11.2
Nonischemic myocardium	8	32.2 ± 5.5	6	48.2 ± 8.0

Mean values ± SEM, n = number of experiments.

Asphyxia was induced in ether-anesthetized rats by switching off artificial respiration four times for 1 min, followed by a last period of 30 seconds. A recovery time of 3 min was allowed between the individual asphyxic periods. Iostasphyxic recovery: 60 min; dose of ribose: 500 mg/kg/h. Ischemia was created by ligation of the descending branch of the left coronary artery, recovery time: 5 hours; dose of ribose: 200 mg/kg/h. The data obtained in the nonischemic myocardium were measured 48 hours after coronary artery ligation; dose of ribose: 200 mg/kg/h.

The β -receptor blocker atenolol was obtained from Rhein-Pharma, Planckstadt. D (-)-Ribose was purchased from Sigma Chemie, München. All other chemicals were of analytical grade.

RESULTS

In Table 1 rates of adenine nucleotide biosynthesis are compiled that were measured in rat hearts under control conditions and in experimental situations in which different forms of oxygen deficiency were applied. In addition, the effect of continuous i.v. infusion of ribose for various periods of time is shown. There was an enhancement within the first hour of recovery from 4.5 min of intermittent asphyxic periods (13). This was of the same order of magnitude as that measured during the last 5 hours of recovery after a regional ischemic period of 15 min duration (19). Interestingly, also in the nonischemic area of the heart after permanent coronary artery ligation, adenine nucleotide synthesis proved to be increased (17). Ribose stimulated adenine nucleotide de novo synthesis in the normal heart and induced a further increase in all pathological conditions.

Fig. 1 shows the content of myocardial adenine nucleotides in rat hearts under control conditions and after 24 hours of recovery from a 10, 15 and 20 min period of regional ischemia. There was a progressive decline in the adenine nucleotide content depending on the duration of the previous ischemic periods. Ribose induced an elevation of the adenine nucleotide pool in all these conditions. It was capable to replenish completely the cardiac adenine nucleotide pool within 24 hours of recovery from the 10 and 15 min periods of

To examine whether the marked effect of ribose on the replenishment of the post-ischemic cardiac ATP pool may be dependent on or facilitated by catecholamines, the β -receptor blocker atenolol was administered. Atenolol did not influence the ATP content after 24 hours of reperfusion when the animals had received only 0.9% NaCl nor did it interfere with the ribose-mediated complete ATP normalization (Fig. 2).

For comparison, multiple periods of ischemia were applied in rat kidney to make up a total of 30 min. Ribose was then administered as continuous i.v. infusion during the reperfusion period for up to 4 hours. The entire period of 30 min of ischemia induced a marked decline in the ATP as well as in the total adenine nucleotide pool (Fig. 3). There was already an appreciable spontaneous restitution of these high energy phosphates within the first hour of recovery. Ribose did not have any additional effect during the reperfusion period observed in this study. At the end of the fourth hour of recovery, both the ATP and total adenine nucleotide contents had achieved the normal levels irrespective of whether or not ribose had been administered. Thus, in contrast to the myocardium, the recovery time for adenine nucleotides is much faster and ribose does not accelerate the restitution of ATP and total adenine nucleotides in the kidney.

Metabolic Recovery Following Myocardial Ischemia

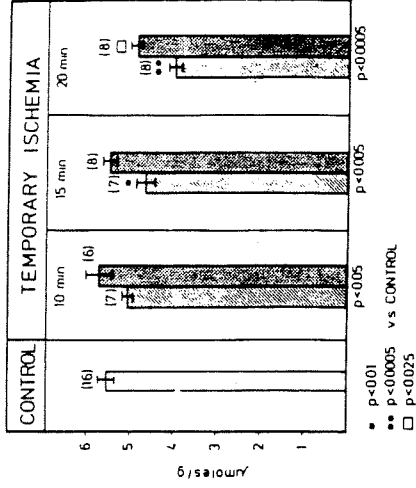


Fig. 1. Total adenine nucleotide content (ATP, ADP, AMP) in the myocardium of controls and 24 hours after release of temporary regional ischemia of 10, 15 and 20 min duration in rats that had received continuous i.v. infusion of 0.9% NaCl (hatched bars) or ribose (200 mg/kg/h, stippled bars). Mean values ± SEM, number of experiments in parentheses.

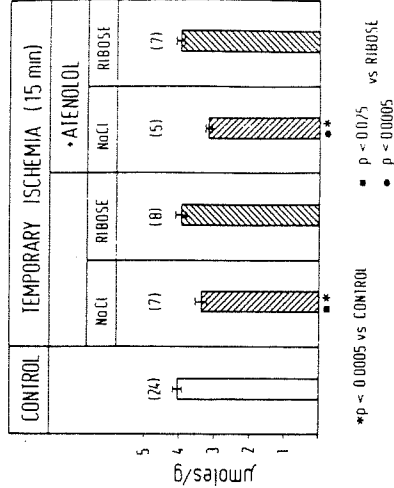


Fig. 2. Effect of atenolol (10 mg/kg, s.c., 1 hour prior to coronary artery ligation, 6 and 12 hours after reperfusion) on the ATP level after 24 hours of recovery from 15 min of regional myocardial ischemia. The animals had been infused with 0.9% NaCl or ribose (200 mg/kg/h). Mean values ± SEM, number of experiments in parentheses.

DISCUSSION

Ribose has been shown to lead to further stimulation of cardiac adenine nucleotide biosynthesis during the recovery from intermittent asphyxic periods (13), from temporary regional ischemia (19) and in the nonischemic myocardium (17). From a clinical point of view, the recovery period subsequent to temporary regional ischemia is interesting, since it has become possible during the last years to recanalize coronary arteries using systemic

or intracoronary thrombolysis (11) with or without accompanying percutaneous transluminal coronary angioplasty (8). In experimental studies, even a brief ischemic episode has been shown to cause a marked decline in the ATP level which can be compensated only after days (2, 10, 19). Thus, in this situation, the myocardium needs urgently a metabolic support to replenish its ATP content. This can be done by supplying the putative degradation products adenosine, inosine or hypoxanthine. Adenosine, however, has a marked

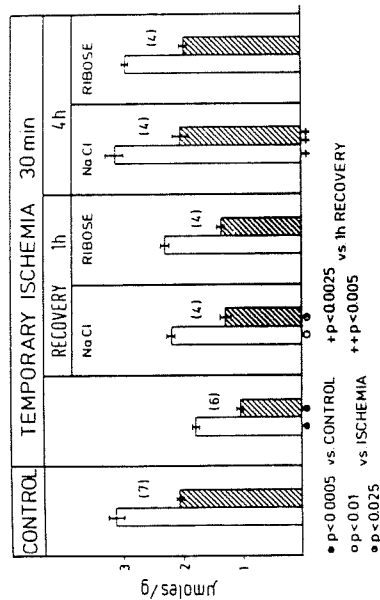


Fig. 3. Contents of ATP (hatched bars) and of total adenine nucleotides (ATP, ADP, AMP, open bars) in kidney of control rats, after 30 min of intermittent ischemic periods as well as 1 and 4 hours of postischemic recovery in rats with continuous i.v. infusion of 0.9% NaCl or ribose (450 mg/kg/h). Mean values \pm SEM, number of experiments in parentheses.

hypotensive effect (9), and inosine turned out to be also negative inotropic in closed-chest rats (21). It is therefore reasonable to administer ribose, a substance, which stimulates adenine nucleotide biosynthesis appreciably (Table 1) and which has no hemodynamic effects at all. That ribose may also be effective in the human heart is suggested by the fact that the activity of glucose-6-phosphate dehydrogenase, the first and regulating enzyme of the oxidative pentose phosphate pathway, is in the same order of magnitude in the human heart as in rat and guinea pig hearts in which the cardioprotective effects of ribose have been demonstrated (20). It can therefore be expected that ribose would exert its cardioprotective influence also in humans.

The ribose-induced restoration of the cardiac adenine nucleotide pool is clearly dependent on the duration of the previous ischemic period. However, the ATP decline induced by a 20 min period of ischemia could not be counteracted by ribose within 24 hours of reperfusion (Fig. 1). This can be interpreted to indicate that the transition from reversible to irreversible myocardial injury occurs between 15 and 20 min of ischemia in the rat heart. Alternatively, one can assume that it takes a longer period of time for ribose to restore the more depressed adenine nucleotide level in this experimental condition.

A potential explanation for the pronounced postischemic stimulation of cardiac adenine nucleotide synthesis by ri-

bose could be that catecholamines are involved. It has been demonstrated that noradrenaline and adrenaline are released from the isolated perfused rat heart during reperfusion after 15 min of total ischemia (12) and that catecholamines stimulate cardiac adenine nucleotide biosynthesis (15). To exclude this potential contribution, the β -receptor blocker atenolol was administered. In the presence of an effective β -receptor blockade the ribose-induced postischemic acceleration of ATP restoration was not impaired (Fig. 2). Thus, catecholamines seem not to participate in the ribose effect.

It is a particular feature that ribose exerts its stimulating metabolic effect only in muscular organs in which the activity of glucose-6-phosphate dehydrogenase and also the rate of adenine nucleotide de novo synthesis is very low. In contrast, in the kidney the activity of glucose-6-phosphate dehydrogenase, the available pool of 5-phosphoribosyl-1-pyrophosphate and adenine nucleotide biosynthesis are all much higher than in the myocardium (14). During postischemic recovery, adenine nucleotide biosynthesis in the kidney is increased about 9-fold (7) and cannot be stimulated any further by ribose. The metabolic restoration is complete within 4 hours of reperfusion (Fig. 3). Thus, ribose is a substrate that is effective only in muscular organs.

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