J. DZIERŻKOWSKA, A. WITANOWSKA, E. WOJTECKA-ŁUKASIK,
T. DROŃ, J. A. MOUTIRIS, M. GUJSKI, P. POPPE, H. H. PREJBISZ, J. GAJEWSKA *,
M. GAJEWSKI **, S. MAŚLIŃSKI

ARE HISTAMINE H₁ RECEPTORS INVOLVED IN ISCHAEMIA/REPERFUSION INJURY IN RAT HEART?

Department of Pathophysiology, Medical Academy, Warsaw, Poland
* Department of Biochemistry, National Research Institute of Mother and Child, Warsaw, Poland
** Department of Biochemistry, Institute of Rheumatology, Warsaw, Poland

During ischaemia, there was no apparent change in malondialdehyde (MDA) content in rat myocardium. However, reoxygenation resulted in a significant increase in MDA content. The changes evoked by ischaemia and reoxygenation were significantly attenuated by addition of fenistil (histamine H_1 receptor antagonist). Enzymatic antioxidant systems were not significantly modified in the different periods of ischaemia and after 30 min of reoxygenation. It is suggested, that maintenance of an adequate endogenous antioxidant reserve during ischaemia may be important in recovery upon reoxygenation.

K e y w o r d s: *histamine H_1 receptors, ischaemia/reperfusion, rat heart*

INTRODUCTION

Readmission of oxygen during reperfusion of a previously ischaemic heart has been shown to exacerbate the injury. Although mechanisms responsible for reperfusion injury are poorly understood, the reactive oxygen intermediates (ROI) have been suggested to be involved (1, 2). These highly reactive ROI can cause lipid peroxidation (LPO) (3).

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione transferase (GST) have been reported to mitigate ROI induced injury and improve myocardial function during reperfusion (1, 3).

ROI produced by the occlusion and opening of the left anterior descending coronary artery induce a differential release of histamine in the perfusates with

a preferential liberation of histamine in the reperfusion phase, associated with an increase of ventricular arrhythmias (4). The release of histamine have been correlated with malondialdehyde (MDA, one of the many products of LPO production (5).

In the present study we have investigated the influence of histamine receptor H_1 blockade on the ischaemia/reperfusion process in rat heart.

MATERIALS AND METHODS

Male Wistar rats (body weight 250—270 g) were anesthetized with urethane (1 g/kg i.p.), placed on a heated table and respired artificially with air *via* a tracheal cannula. The electrocardiogram was recorded from limb leads. The thorax was opened and the left coronary artery was ligated about 1—2 mm from its origin. In different time intervals the coronary ligature was untied and reperfusion of coronary system was allowed to proceed for a further up to 60 min. Ventricular tissue specimens were taken at the end of each experiments for biochemical analyses. Some animals were pretreated with histamine H_1 receptor antagonist (6) — fenistil (1 mg/kg i.p. — 15 min before coronary artery ligation). Sham operated and saline treated animals served as the controls.

Determination of malondialdehyde (MDA)

To determine of lipid peroxidation MDA was assayed as in (7) by measuring the amount of thiobarbituric acid (TBA)-reactive materials. An aliquot of the tissue (0,3 ml) was mixing with 2 ml of ice-cold TBA-trichloroacetic acid (TCA)-HCL-butyl-hydroxytoluene (BHT) solution. The TBA-TCA-HCL solution was prepared by dissolving 41.6 mg TBA in 10 ml TCA (16,8% w/v in 0,25 N HCL). To 10 ml TBA-TCA-HCL, 1 ml BHT (1.5 mg/ml ethanol) was added. After heating (15 min, 80°C) and centrifugation (15 min; $4000 \times g$) the absorbance at 535 nm vs 600 nm was determined.

Antioxidant enzyme assays

The activity of glutathione peroxidase was measured by the coupled enzyme assay of Paglia and Valentine (8) with hydrogen peroxide as a substrate; glutathinne reductase by the method of Beutler et al. (9), glutathione S-transferase with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to Habig et al. (10) and catalase by the method of Beers and Sizer (11). Activity of superoxide dismutase was measured by the method of Misra and Fridovich (12).

Protein concentration was determined by the method of Lowry et al. (13).

Statistical analysis

Data from experimental groups and control were compared with a two-tailed Student's test. For two group comparisons, differences were considered significant when $P < 0,05$. Data were expressed as the mean $\pm$ SE.

RESULTS

In ischaemic animals premature contractions, atrial tachycardia and disturbances of conduction of heart developed. Reperfusion exaggerated the arrhythmia and in 50% of animals ventricular fibrillation was registered. Fenistil reduced the incidence of ventricular fibrillation (by 30%) and had no influence on other disturbances recorded. In some animals (ca 5%) fenistil increased the severity of observed arrhythmia.

Different antioxidant enzyme activities as well as malondialdehyde (MDA) content were examined in control, ischaemic and reoxygenated heart. There was no significant effect of ischaemia on the catalytic activity of superoxide dismutase (SOD) as compared to control hearts (*Table 1*). 5, 30 and 90 min of reperfusion lead to a non significant decrease in SOD activity, as compared to ischaemic non-reperfused hearts.

Glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities remained unchanged after 5, 30 and 90 min of ischaemia compared to control, whereas glutathione reductase (GR) activity was a non-significantly augmented during 90 min of ischaemia (*Table 1*). The minimal increase in CAT activity was seen after 90 min of occlusion. Thirty min of reperfusion do not lead to significant changes in the catalytic activity of CAT, compared to ischaemic hearts.

There was no significant change in the MDA content during ischaemia, as was shown in *Table 2*. However, reoxygenation of ischaemic hearts (15 min occlusion) resulted in about 200% increase in MDA content in comparison with hypoxic and over 200% increase when compared with normoxic hearts. Similarly, after 30 min of occlusion reoxygenation of ischaemic heart resulted in about 1000% increase in MDA content in comparison with ischaemic and normoxic tissue. Premedication of animals with fenistil significantly attenuated the reoxygnation induced increase in MDA content (*Table 2*).

DISCUSSION

A number of studies have shown the relation between reactive oxygen intermediates (ROI) and cardiac ischaemia/reoxygenation injury. However, little is known about oxidative stress in the human or rat hearts, which can be measured by malondialdehyde (MDA) formation.

The authors have reported MDA formation during coronary angioplasty procedures and pacing stress testing with ischaemic periods of a few minutes. On the other hand, Janssen et al. (14) showed that LPO which takes place in normal rat hearts, decreases after cold cardioplegia, but does not increase after ischaemia. Rat heart MDA content after reperfusion showed a tendency to

Table 1. Effects of ischaemia and reoxygenation on antioxidant enzyme activities in rat heart ($n = 5$ animals)

Glutathione peroxidase (U/mg protein)			
time (min)	control	occlusion	reperfusion
5	61.9 ± 8.5	60.9 ± 5.5	—
30	66.7 ± 5.2	58.7 ± 6.9	58.2 ± 3.7
90	70.2 ± 7.3	62.1 ± 5.2	—

Glutathione reductase (U/mg protein)			
time (min)	control	occlusion	reperfusion
5	20.4 ± 2.8	21.5 ± 2.5	—
30	19.7 ± 1.5	23.8 ± 3.7	18.1 ± 1.5
90	23.8 ± 3.2	27.5 ± 3.1	—

Glutathione S-transferase (U/mg protein)			
time (min)	control	occlusion	reperfusion
5	568 ± 45	534 ± 51	—
30	571 ± 52	552 ± 38	511 ± 50
90	558 ± 33	552 ± 40	—

Catalase (U/mg protein)			
time (min)	control	occlusion	reperfusion
5	1.22 ± 0.15	1.19 ± 0.21	—
30	1.19 ± 0.12	1.16 ± 0.17	1.19 ± 0.15
90	1.19 ± 0.22	1.42 ± 0.10	—

Superoxide dismutase (U/mg protein)			
time (min)	control	occlusion	reperfusion
05	8.92 ± 1.12	8.54 ± 1.24	8.41 ± 0.93
30	8.23 ± 0.80	8.15 ± 0.78	7.82 ± 0.87
90	8.11 ± 0.94	7.88 ± 1.00	6.45 ± 1.11

Gulatathione peroxidase, glutathione reductase activities are expressed as μmol NADP(H) transformed per min per mg protein. Glutathione S-transferase is expressed in μmol S-conjugate transformed per min per mg protein. One unit of catalase activity is the amount of enzyme which liberates half the peroxide oxygen from hydrogen peroxide solution of any concentration in 100 s at 25°.

Table 2. Effect of ischaemia and reoxygenation on lipid peroxidation (MDA content in nmol/g dry weight) in rat heart ($n = 5$—10 animals).

normoxia	ischaemia 15 min	ischaemia 15 min + FENISTIL	ischaemia 30 min
4.0 ± 0.2	5.0 ± 0.3	5.4 ± 0.5	5.2 ± 0.3
REOXYGENATION			
1 min	5.0 ± 0.2	5.0 ± 0.3	6.2 ± 0.3
5 min	7.0 ± 0.3	4.7 ± 0.8	8.0 ± 0.5
15 min	8.0 ± 0.4	4.4 ± 0.5	18.0 ± 1.1
30 min	11.8 ± 1.0	9.6 ± 0.6	50.0 ± 4.6

increase, in agreement with data of Ceconi (15) and experiments in our laboratory.

Endogenous antioxidants such as SOD, CAT, GPx, 5ST and GR have been shown to mitigate these oxidative changes and improve myocardial function upon reperfusion (16). These protective cellular antioxidants have been reported to change in response to both physiological and pathological conditions, such as age, exercise and hypertrophy (1). Depression in some of these antioxidants has also been reported during ischaemia and/or hypoxia (17).

In the present study enzymatic antioxidant status was assayed as a function of the duration of reperfusion in ischaemic rat heart.

We were unable to observe any significant increase in antioxidant activity.

GPx, CAT and SOD have key roles in the enzymatic defence system against ROI, they reduce superoxide anions, hydrogen peroxide and other lipid organic peroxides (1, 2). GPx can be inactivated and fragmented by ROI. In this way, a sudden increase in ROI production can destroy the main defence system of the cell. If the attack is not too strong, a quick removal of the modified enzyme will occur and the native enzyme will be synthesized. But if the destruction of the enzyme by ROI is substantial, the antioxidant enzymes will be destroyed, leading to a low defence potential and a possible destruction of the cell components by peroxidative reactions (3).

In our study, antioxidant system does not seem to be affected during 90 min of ischaemia and 30 min of reperfusion, as compared to control animals. This could mean that hearts were not overwhelmed by ROI production.

Early reperfusion remains one of the most effective means of reducing cardiac damage in acute myocardial damage (18). Current data are highly suggestive of the important role of progressive migration of neutrophils as primary mediators of reperfusion injury by damaging myocardial cells, causing capillary plugging and producing large guantities of ROI (19). The recent experiments have indicated ROI as mast cells histamine releasers (4, 5). The existence of at least three separate populations of histamine receptors has been demonstrated (20).

The results obtained by Masini et al. showed preferential liberation of histamine mainly in the reperfusion phase. The correlation between histamine release and MDA production in the ischaemic left ventriculum was observed (4, 5).

In the present study we have investigated indirectly the effects of the released histamine during reperfusion with the aim of disclosing whether histamine H_1 receptors mediate a significant component of reperfusion injury. In fact, it has been reported that the extent of myocardial damage shows a close linear relationship with the degree of mast cell degranulation (5).

Our results support the data on histamine involvement in reperfusion injury. The way by which histamine H_1 receptor blockade exerts its partial beneficial protective effect is to be established. The direct effect of histamine on infiltrating heart neutrophils (21) or influence on coronary arteries (22) have to be considered.

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Author's address: J. Dzierżkowska, Department of Pathophysiology, Medical Academy, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland.