

Oxidative Damage & Repair

Chemical, Biological and Medical Aspects

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MITOCHONDRIAL GLUTATHIONE IN ISCHEMIA-REPERFUSION INJURY OF ISOLATED AND PERFUSED RAT HEART.

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Introduction

Glutathione has several important roles in cellular metabolism such as transport, catalysis and protection against toxic compounds of endogenous and exogenous origin. Much less defined is the role of mitochondrial glutathione and its major or unique function appears to be a protective effect in the oxidative stress.¹ In this paper we examine the role of cytosolic and mitochondrial glutathione of isolated and perfused rat heart undergoing different types of applied stress such as ischemia, ischemia/reperfusion and treatment with oxidizing agents.

Release of glutathione from isolated and perfused rat heart.

When perfused hearts are maintained in ischemic conditions and are then reperfused with a normoxic medium, a massive efflux of glutathione together with protein, lactate, lactate dehydrogenase and creatine kinase takes place.²⁻⁸ A similar efflux of glutathione can also be obtained with glutathione oxidizing agents such as diamide and hydrogen peroxide.^{3,4} Perfusion with 0.1mM diamide or 0.1mM hydrogen peroxide determines a relatively large release of glutathione that rapidly reaches a maximum and subsequently decreases progressively even when diamide or hydrogen peroxide are removed from the perfusion medium.^{3,4}

Total SH groups and glutathione content, do not undergo a significant alteration in their concentration after 60 min of ischemia, while they strongly decrease when one hour of reperfusion follows the ischemic period.^{3,4} In particular, total glutathione is decreased to about one third of that present in the control and about 20% of this residual amount is found in the oxidized form;^{3,4} the decrease of glutathione is very dramatic and the cell is abruptly left without one of the most relevant defence systems against oxidative stress.

Total SH groups are extensively oxidized during the perfusion with diamide (25% decrease) or hydrogen peroxide (30% decrease).^{3,4} The decrease in glutathione is proportionally larger since about 50% of it leaks out from the heart after 30 minutes of perfusion in the presence of hydrogen peroxide or diamide.

Glutathione is not completely lost even though the reperfusion is carried on

for relatively long times, indicating that some cell compartment might selectively retain glutathione. Cytosolic and mitochondrial glutathione concentrations of hearts undergoing different types of stress were then comparatively measured.

TABLE I

Cytosolic and mitochondrial total glutathione content of rat heart after ischemia/reperfusion or treatment with oxidizing agents.

	CYTOSOL %		MITOCHONDRIA %	
CONTROL	9.2 ± 1.2	100	1.20 ± 0.08	100
ISCHEMIA(60 min)	9.2 ± 0.5	100	0.66 ± 0.03	55
REPERFUSION(5 min)	5.8 ± 0.1	63	0.61 ± 0.06	51
REPERFUSION(60 min)	4.6 ± 0.2	50	0.30 ± 0.04	25
DIAMIDE(60 min)	1.2 ± 0.3	13	1.01 ± 0.04	84
H ₂ O ₂ (60 min)	4.2 ± 0.1	46	0.96 ± 0.10	80
CONTROL (PYRUVATE)	12.5 ± 0.7	100	1.50 ± 0.06	100
REPERFUSION(5 min) (PYRUVATE)	9.5 ± 0.8	76	1.30 ± 0.06	87

Rat hearts were perfused according to Langendorff non-recirculating method with Krebs-Henseleit buffer (pH 7.4) containing 11mM glucose or, where indicated, 5 mM pyruvate and gassed with 95% O₂ and 5% CO₂. Diamide and H₂O₂ concentrations in the perfusion medium were 0.1 mM. Total glutathione (expressed as nmol/mg protein) was determined with the glutathione reductase recycling assay using 5,5'-dithiobis (2 nitrobenzoic acid) (DTNB).

As reported in Table I, rat heart basal mitochondrial glutathione concentration is about 1.2 nmol/mg protein. After ischemia, mitochondrial glutathione decreases by about 55%, while no modifications of total glutathione are observed; this indicates that mitochondrial glutathione is released into the cytosol and, in turn, into the extracellular environment. When a short reperfusion (5 min) follows the ischemic period, there is a large decrease of cytosolic glutathione (about 50%) while mitochondrial glutathione decreases only slightly. Moreover, if the reperfusion period is longer (60 min) there is a further decrease of mitochondrial glutathione to about 25% of the control. The loss of glutathione observed during reperfusion is proportional to the duration of ischemia, even though for longer times of ischemia it tends to level off (not shown).

Unlike ischemia/reperfusion conditions, diamide and H₂O₂ determine a large decrease of cytosolic glutathione, while mitochondrial glutathione is almost totally preserved (Tab I). Hence mitochondria appear to be particularly protected

from the challenge of oxidizing agents and this might be referred to a protective action exerted by the mitochondrial substrates; in fact, it was previously shown⁹ that, at least rat liver mitochondria, were protected by succinate against diamide-induced glutathione depletion.

At variance with the treatment with oxidizing agents, mitochondria obtained from ischemic heart show a decrease of glutathione; consequently, they might be more or less damaged. In fact, it is reported that the membrane potential of mitochondria isolated from ischemic heart is lower¹⁰ and, in addition, the differences were markedly accentuated upon exposure to Ca^{2+} ions¹⁰. The decrease of the membrane potential could be one of the major causes responsible for mitochondrial glutathione release¹¹ together with a large increase of inorganic phosphate deriving from phosphocreatine and ATP hydrolysis during ischemia. Previously, a depletion of mitochondrial glutathione was observed¹² upon ageing of rat liver mitochondria in the presence of phosphate concentrations comparable to those formed in the heart during ischemia.

Prevention of glutathione loss by pyruvate.

Respiratory substrates prevent lipid peroxidation in mitochondria and, in general, the presence of substrates of either glycolysis or Krebs cycle during perfusion appears to improve the recovery of the contractile function of the heart. When the hearts, equilibrated in the presence of glucose, are reperfused with pyruvate, a complete recovery of the contractile force is obtained, while glucose itself, acetate and lactate are far less effective.¹³ If the perfusion before ischemia is performed in the presence of pyruvate, the recovery on reperfusion occurs either with glucose or with pyruvate itself.¹³ Pyruvate appears then to be a better substrate as compared with glucose and this is confirmed by the estimation of the concentration of the high energy phosphate compounds in various experimental conditions. Phosphocreatine is particularly preserved when pyruvate is present in the perfusion medium before the induction of ischemia.¹³ The action of pyruvate is far more striking if total cellular glutathione and mitochondrial glutathione are considered. If pyruvate is present before the onset of ischemia and during the subsequent reperfusion a complete protection of the glutathione efflux from both the mitochondrial and cytosolic compartment is apparent (Table I). Pyruvate appears then to act as a glutathione-preserving agent. Besides, pyruvate is an excellent oxidative substrate for the heart and is recognized as a potential cardioprotective agent,¹⁴ nevertheless the mechanisms of its beneficial action in ischemia/reperfusion are not yet completely clarified. From the reported results it appears that pyruvate, in addition to metabolic effects, is also able to counteract the oxidative stress by efficaciously preserving cellular glutathione.

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