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THIOL STATUS AND OXIDATIVE STRESS

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Introduction

Thiol groups play a complex and multifactorial role in biochemical systems. Thiol groups of membrane proteins and enzymes are in fact involved in metabolic, biosynthetic and transport processes while low molecular weight thiols might have coenzymatic, protective, reparative and regulatory role[1,2]. In particular the thiol-disulfide exchange might be the specific reaction which is relevant in this respect.

Depletion of thiols and in particular of glutathione is one of the most important causes of oxidative stress and the ischemia/reperfusion syndrome is a condition which causes depletion of thiols[3-9].

Loss of glutathione by perfused heart and its prevention by pyruvate

When isolated and perfused rat hearts are maintained in no-flow ischemic conditions for 1 hour and then are reperfused with a normoxic medium, a massive efflux of glutathione together with protein, lactate and lactate dehydrogenase occurs in the perfusate[3-10]. After ischemia there is no release of glutathione while after one hour of reperfusion only about 30% of the total glutathione can be found in the heart[4].

A release of glutathione is also observed in the presence of nitrogen

instead of oxygen[8,9] and, upon reperfusion, a larger amount of glutathione, as compared to ischemia, is still found in the heart.

After ischemia, rat heart mitochondrial glutathione decreases of about 55%, while no modification of total glutathione is observed (Table I). When a short reperfusion of 5 minutes follows the ischemic period there is a large decrease of cytosolic glutathione (about 40%) while mitochondrial glutathione only slightly decreases (Table I). On the other hand, if the reperfusion period is longer (60 min) there is a further decrease of both cytosolic and mitochondrial glutathione (Table I). All this means that mitochondrial glutathione, during ischemia, is released from the mitochondrion into the cytosol; cytosolic glutathione would, in turn, be released into the interstitial space and when reperfusion occurs there is a rapid washout of the glutathione localized in the extracellular environment.

The loss of glutathione from cytosol and mitochondria of rat heart is generally proportional to the duration of ischemia; however, for longer times of ischemia it tends to level off.

Since mitochondria obtained from ischemic heart show a decrease in their content of glutathione, some conditions facilitating this release might occur. One condition might be a decrease in membrane potential[11-13] while another could be the large increase of inorganic phosphate formed after phosphocreatine and ATP degradation[14]. A depletion of mitochondrial glutathione was observed upon treatment of rat heart mitochondria in the presence of 10 mM phosphate. The GSH escaped from mitochondria was found as such in the external medium and therefore not only GSSG but also GSH can diffuse through the mitochondrial membrane.

The use of substrates of both glycolysis and Krebs cycle during reperfusion appear to improve the recovery of the contractile force of the heart[7,15]. Substrates can be given before ischemia or added to the reperfusion medium. If pyruvate is present before ischemia, the subsequent reperfusion with pyruvate completely inhibits glutathione efflux, while the action of other substrates (lactate and acetate) mimicks that of glucose perfusion[7].

TABLE I

EFFECT OF PYRUVATE ON TOTAL AND MITOCHONDRIAL GLUTATHIONE CONTENT DURING ISCHEMIA AND REPERFUSION

	HOMOGENATE		MITOCHONDRIA	
	GLUCOSE	PYRUVATE	GLUCOSE	PYRUVATE
CONTROL	9.7 ± 0.9	11.0 ± 0.9	1.2 ± 0.2	1.62 ± 0.10
ISCHEMIA 60 min	9.3 ± 0.5	10.3± 0.7	0.66 ± 0.3	1.61 ± 0.08
ISCHEMIA (60 min) /REPERFUSION (5 min)	5.8 ± 0.1	8.6 ± 0.6	0.64 ± 0.08	1.3 ± 0.02
ISCHEMIA (60 min) /REPERFUSION (60 min)	4.4 ± 0.2	7.85 ± 0.7	0.5 ± 0.05	1.2 ± 0.03

Rat hearts were perfused according to Langendorff non-recirculating method with Krebs-Henseleit buffer (pH 7.4) containing 11 mM glucose or 5mM pyruvate and gassed with 95% O_2 and 5% CO_2. Total glutathione (nmol/ mg protein) was determined with the glutathione reductase recycling assay using 5,5'-dithiobis-(2-nitrobenzoic acid).

The protective effect of pyruvate against glutathione depletion was compared in cytosol and mitochondria. In the presence of glucose, a short reperfusion after ischemia causes a large decrease of both cytosolic and mitochondrial glutathione. On the contrary, the presence of pyruvate before ischemia almost completely prevents the loss of glutathione (Table I); pyruvate appears then to act as a glutathione replenishing agent.

At variance with ischemia, both low flow ischemia and hypoxia do not show any release of mitochondrial glutathione, while the loss of total glutathione is far less marked.

Perfusion in the presence of oxidizing agents such as diamide or hydrogen peroxide determines a large release of glutathione together with a decrease in protein sulfhydryl groups[4,5]. With diamide the rate of release of glutathione rapidly reaches a maximum and progressively decreases thereafter. It is interesting to note that at the end of the treatment with diamide the sum of glutathione found in the heart and glutathione released in the perfusate is lower than the amount of total glutathione indicating the formation of mixed disulfides between glutathione and protein thiols[4,5]. The perfusion with diamide or hydrogen peroxide also determines an extensive oxidation of total thiol groups. The decrease of glutathione is proportionally greater since about 80 % of it is lost in the presence of diamide and 60% in the presence of hydrogen peroxide. The fraction of glutathione remaining in the heart is about 50% in the oxidized form after perfusion with either hydrogen peroxide or diamide[4,5]. Hence mitochondria appear particularly protected from the challenge of the oxidizing agents and this might be referred to a protective action exerted by the mitochondrial substrates.

Thiol oxidation and lipid peroxidation

Oxidation and/or depletion of glutathione determines a perturbation of the protein oxidation-reduction state with a shift towards a more oxidized state. This condition can elicit an oxidative stress not only

because of the lack of GSH, but also because, at membrane level, a condition of potential lipid peroxidation is being created.

In isolated mitochondria diamide does not induce, *per se*, any peroxide formation; however, when mitochondria are pretreated with diamide, the rate and extent of lipid peroxide formation initiated by iron/ascorbate is strongly enhanced[16]. It can consequently be assumed that, in mitochondrial membranes, lipid peroxidation is partially prevented until membrane thiols are preserved; when a critical amount of thiols has been oxidized, peroxide formation is no longer prevented and proceeds autocatalytically. Peroxide formation takes place only when about 15-20% of thiol groups are oxidized[16]. It appears that a critical decrease of about 15% of membrane thiols constitutes a prerequisite for lipid peroxide formation, at least in mitochondria. Therefore it is conceivable that any condition such as ageing involving a decrease of thiol groups potentially promotes lipid peroxidation.

Conversely, peroxides arising from the lipid peroxidation process are able to decrease the content of membrane thiol groups. It is well known that lipid peroxidation causes the oxidation of protein thiol groups[17,18] even though the resulting products remain to be determined.

Since lipid peroxidation and thiol oxidation appear to be two strictly related phenomena, it is of interest to know the protective effect elicited by antioxidants not only towards lipid peroxidation, but also on membrane thiol groups. Butylated hydroxytoluene (BHT), Trolox C and a new benzofuran derivative (5-hydroxy-4,6,7-trimethyl-2,3-dihydrobenzofuran-2-acetic acid, BFA) are able to inhibit the oxidation of sulfhydryl groups consequent to lipid peroxidation, when the peroxidizing system is NADPH/Fe^{2+}/ADP[19]. In any case thiol groups appear to be more sensitive to oxidation than unsaturated lipids; in fact, when lipid peroxidation is almost completely inhibited still a large amount of thiols undergo oxidation. On the contrary, when lipid peroxidation is elicited by cumene hydroperoxide no protection on thiol groups is afforded by antioxidants that, on the other hand, strongly inhibit the peroxidative process[19].

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