Changes in the cardiac glutathione status after ischemia and reperfusion

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Summary. In the isolated and perfused rabbit heart ischemia induced a rapid decline of contractility, associated with a reduction of the content of tissue GSH with no significant changes in GSSG. Reperfusion induced a small recovery of contractility, a substantial release of total glutathione and a further decrease in the content of tissue GSH with a significant increase of tissue GSSG. Glutathione reductase and glutathione peroxidase activities were not affected by ischemia and reperfusion. This study suggests a possible role for glutathione in the determination of functional damage induced by myocardial ischemia and reperfusion.

Key words. Glutathione heart; ischemia; reperfusion.

The tripeptide glutathione is present in most living cells as reduced glutathione (GSH), oxidized glutathione (GSSG) and as mixed glutathione disulfide (X-SSG). Glutathione metabolism has been widely studied, particularly in the liver where it plays an important role in defense mechanisms against the toxic effects of reactive metabolites of oxygen and of chemical intermediates including various drugs[3]. Relatively few studies have been undertaken to investigate the metabolism and the function of glutathione in heart muscle.

Wendel et al.[4] found in isolated and perfused hearts a GSH/GSSG ratio close to 50, as a consequence of the glutathione reductase activity. In addition, Harrish and Mahmound[5] and Harrop et al.[6] demonstrated that X-SSG concentration in the myocardium is more elevated than in the liver. This finding is important because the turnover of cardiac glutathione is slow[7] and GSH content can be rapidly reduced by different situations such as starvation[5] or chemotherapeutic treatment[8]. We have previously shown[9,10] that hypoxia also causes a depletion of myocardial GSH content, associated with its release into the coronary effluent.

The aim of the present study was to investigate the effect of myocardial ischemia, a condition different from hypoxia, and reperfusion on glutathione status, and to correlate these changes with myocardial function.

Materials and methods. Adult male rabbits were used. The hearts were excised and subjected to Langendorff non-recirculating perfusion with Krebs-Henseleit buffer at pH 7.4, containing 11 mM glucose and gassed with 95% O_2 5% CO_2 as previously described[11].

Each heart was paced at 180 beats/min. After 30 min of aerobic perfusion (coronary flow 22 ml/min), the hearts were made ischemic by reducing the flow to 1 ml/min. During the reperfusion the flow was restored to 22 ml/min. Left ventricular temperature was maintained at 37 °C, irrespective of coronary flow. Left ventricular pressures were monitored by means of a fluid-filled balloon inserted in the left ventricle[12].

Total glutathione was assayed using the catalytic assay with 5,5'-dithiobis (nitrobenzoate) and glutathione reductase[13]. GSSG was measured after preliminary reaction of GSH with 20 mM N-ethyl maleimide followed by complete removal of unreacted sulfhydryl reagent with diethylether. Glutathione reductase activity was measured following the oxidation of NADPH[14], and glutathione peroxidase activity as described by Grankvist et al.[15]. Proteins were estimated by the method of Bradford[16]. Coronary effluents were collected and assayed for CPK activity[12] and total glutathione[15].

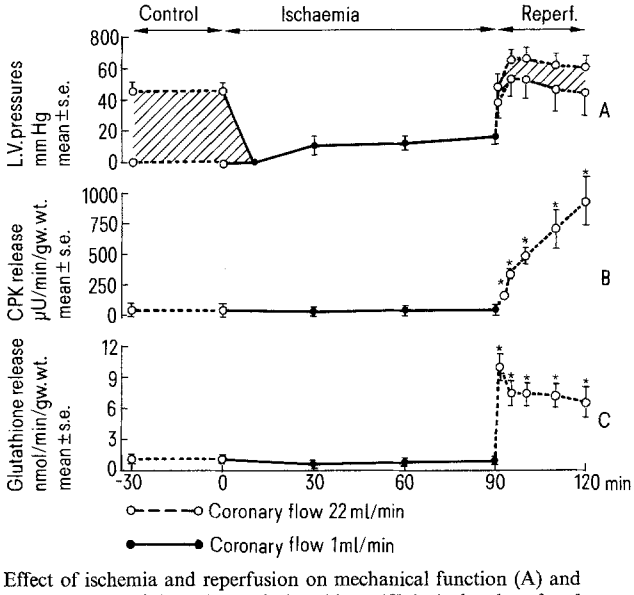
Results. The effects of ischemia and reperfusion on mechanical function are shown in the figure A. Reduction of the coronary flow induced a rapid decline of systolic pressure and a slight increase of diastolic pressure. The following reperfusion produced a further marked increase of diastolic pressure and a partial recovery of systolic function (32% of the preischemic value).

The ischemic perfusion did not cause a significant increment of CPK (fig., B) or glutathione release (fig., C). On the contrary, reperfusion induced a progressive leakage of CPK and a substantial release of glutathione, which reached a peak in about 3 min. The table shows that ischemia induced a significant diminution of tissue GSH together with a slight reduction of GSSG

Effect of ischemia and reperfusion on rabbit heart tissue glutathione content and on glutathione reductase and glutathione peroxidase activities

	Control	Ischemia	Ischemia + reperfusion
GSH	11.76 ± 0.67	6.60 ± 0.55*	4.50 ± 0.58*
GSSG	0.26 ± 0.08	0.17 ± 0.03 (NS)	0.55 ± 0.02*
	(45.2)	(38.8)	(8.2)
Glutathione reductase	45.6 ± 2.8	48.1 ± 1.8 (NS)	41.0 ± 3.7 (NS)
Glutathione peroxidase	20.9 ± 1.2	22.5 ± 1.9 (NS)	23.0 ± 1.6 (NS)

The hearts were perfused under ischemic conditions for 90 min, followed by 30 min of reperfusion. Glutathione content was expressed as nmoles · mg $prot^{-1}$. Numbers in parentheses indicate GSH/GSSG ratio. The enzymatic activities are expressed as nmole NADPH oxid · mg $prot^{-1} \cdot min^{-1}$. Each result is expressed as mean of six experiments ± SE. * Significantly different from control ($p < 0.01$); NS, not significantly.



Effect of ischemia and reperfusion on mechanical function (A) and on the release of CPK (B) and glutathione (C) in isolated perfused rabbit hearts. The results are mean values ± SE of six experiments. p relates to the significance of the difference between the results obtained during control and those obtained during ischemia and reperfusion.

level. Reperfusion resulted in a further depletion of tissue GSH content concomitant with a significant increase of GSSG ($p < 0.01$), resulting in a severe reduction of the GSH/GSSG ratio. The table also shows that ischemia and reperfusion did not alter the glutathione reductase and glutathione peroxidase activities measured in the heart homogenates.

Discussion. This study shows that in the isolated rabbit heart ischemia and reperfusion alter the glutathione status.

These alterations cannot be ascribed to a modification of glutathione reductase or glutathione peroxidase activities as both values remained relatively constant under our experimental conditions. They could be the result of the reperfusion-induced lesion of the cell membrane leading to a breakdown of the permeability barrier to molecules such as CPK and glutathione (fig., C).

However, there are three observations that support the idea that the reperfusion-induced glutathione release is not the only cause of the cellular changes which we have observed: 1) tissue GSH content was reduced after ischemia, when the release of glutathione was low, probably as result of the severe reduction of coronary flow; 2) the net amount of glutathione released during reperfusion does not quantitatively account for the cellular reduction of GSH; 3) the finding of a significantly increased GSSG level after reperfusion suggests an enhanced cellular oxidation of GSH into GSSG.

Therefore, it is likely that the cellular alterations of the glutathione status which we observed are the results not only of the glutathione leakage, but also of an enhanced metabolic utilization of GSH, mainly via glutathione peroxidase activity as demonstrated by the increased tissue GSSG[17].

A low value of the GSH/GSSG ratio is deleterious for cell function[18], and in muscle preparations it may increase lipid membrane peroxidation[19] and impair contractile activity[20]. Recently it has been proposed that intracellular thiols, and particularly GSH, may prevent alteration of Ca^{2+} homeostasis reducing lipid peroxidation of the membrane and protecting thiol groups critical for several enzymatic activities, as for example microsomal Ca^{2+}-ATPase[21].

In our experimental conditions, the severe tissue GSH depletion was coincident with an increase of diastolic pressure, an abnormality linked to an enhanced cytosolic Ca^{2+} concentration[12]. This suggests a possible role for glutathione in the determination of the functional damage occurring in post-ischemic reperfusion.

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Longitudinal continuity of the subrhabdomeric cisternae in the photoreceptors of the compound eye of the drone, *Apis mellifera*[1]

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Summary. It is shown that the subrhabdomeric cisternae of the honey bee drone photoreceptor cell constitute a single structure with a continuous lumen, that extends over at least 15 µm and perhaps the whole length of the cell. In this case, the structure of the cisternae might subserve the propagation of light adaptation along the cell.
Key words. Honey bee; *Apis mellifera;* drone; compound eye; photoreceptors; cisternae, subrhabdomeric.

The basic functional unit behind each facet of the drone compound eye is a cluster of six large and three small photoreceptor cells which constitute the retinula, a structure about 400 µm long and 20 µm across[3]. Each cell contributes microvilli to an orderly array, the rhabdom, about 2 µm by 6 µm, which runs down the center of the retinula. In vivo, light passes along the rhabdom, which acts as a light guide, and is absorbed by photopigment in the membranes of the microvilli. Light absorption causes an increase in Na^+ conductance of the cell membrane[4,5] which leads to the electrical response. In addition, there is evidence that light causes an increase in cytosolic free $[Ca^{2+}]$[6,7], in agreement with more extensive evidence from another microvillar photoreceptor cell, that of *Limulus* ventral eye[8,9]. One probable function of this increase in $[Ca^{2+}]$ is to adapt the sensitivity of the photoreceptor to different mean light intensities[6,10].