Postischemic injury in isolated rat hearts is not aggravated by prior depletion of myocardial glutathione

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Abstract

The aim of this study was to test the hypothesis that a decreased myocardial concentration of reduced glutathione (GSH) during ischemia renders the myocardium more susceptible to injury by reactive oxygen species generated during early reperfusion. To this end, rats were pretreated with L-buthionine-S,R-sulfoximine (2 mmol/kg), which depleted myocardial GSH by 55%. Isolated buffer-perfused hearts were subjected to 30 min of either hypothermic or normothermic no-flow ischemia followed by reperfusion. Prior depletion of myocardial GSH did not lead to oxidative stress during reperfusion, as myocardial concentration of glutathione disulfide (GSSG) was not increased after 5 and 30 min of reperfusion. In addition, prior depletion of GSH did not exacerbate myocardial enzyme release, nor did it impair the recoveries of tissue ATP, coronary flow rate and left ventricular developed pressure during reperfusion after either hypothermic or normothermic ischemia. Even administration of the prooxidant cumene hydroperoxide (20 μM) to postischemic GSH-depleted hearts during the first 10 min of reperfusion did not aggravate postischemic injury, although this prooxidant load induced oxidative stress, as indicated by an increased myocardial concentration of GSSG. These results do not support the hypothesis that a reduced myocardial concentration of GSH during ischemia increases the susceptibility to injury mediated by reactive oxygen species generated during reperfusion. Apparently, myocardial tissue possesses a large excess of GSH compared to the quantity of reactive oxygen species generated upon reperfusion. (Mol Cell Biochem 156: 79-85, 1996)

Key words: ischemia, reperfusion, oxidative stress, glutathione, buthionine sulfoximine

Introduction

Reperfusion of ischemic myocardium is accompanied by an enhanced generation of reactive oxygen species [1-3]. If the quantity of reactive oxygen species generated upon reperfusion exceeds the capacity of the antioxidant defense mechanisms, these reactive species may cause injury additional to the injury caused by ischemia. This form of injury is termed reperfusion-induced injury. A major antioxidant defense mechanism in myocardial tissue is the glutathione redox cycle [4, 5]. The reaction of reduced glutathione (GSH) with reactive oxygen species leads to the formation of glutathione disulfide (GSSG). The majority of GSSG is reduced back to GSH by glutathione reductase, but part is actively transported out of the cell [6]. Under conditions of oxidative stress, GSSG accumulates intracellularly, which leads to an increased active outward transport of GSSG [4, 6, 7].

Several investigators have reported that during ischemia myocardial concentration of GSH declined by 30-55% [8-12]. A reduced concentration of GSH at the end of the ischemic period may render the myocardium more susceptible to injury by reactive oxygen species generated upon reperfusion [13]. This hypothesis has been tested by studying the effects of prior depletion of myocardial GSH on...
postischemic injury [11, 14–16]. The results have been controversial: three studies have claimed an aggravation of postischemic injury due to prior GSH depletion [11, 15, 16], but one study has not [14]. In the present study, we pretreated rats with L-buthionine-S,R-sulfoximine (BSO) to reduce the myocardial concentration of GSH. BSO is a highly selective inhibitor of glutathione synthesis [17]. In isolated perfused hearts, we subsequently determined whether postischemic reperfusion of GSH-depleted hearts was accompanied by oxidative stress, and whether these GSH-depleted hearts sustained more severe postischemic injury than non-depleted hearts. In addition, we tested whether the administration of an exogenous prooxidant (cumene hydroperoxide) to postischemic GSH-depleted hearts during the early phase of reperfusion aggravated postischemic injury.

**Materials and methods**

**Depletion of myocardial GSH**

Myocardial GSH was depleted by pretreating rats with L-buthionine-S,R-sulfoximine (BSO). BSO is a specific inhibitor of γ-glutamylcysteine synthetase, and therefore of GSH synthesis [17]. Preliminary studies showed that myocardial concentration of GSH was reduced by 15, 25, 45, and 48% at 4, 8, 24, and 48 h after one intraperitoneal injection of 1 mmol/kg BSO, respectively. A second injection of 1 mmol/kg BSO 24 h after the first injection caused a further 10% reduction 24 h later. In all subsequent experiments, myocardial GSH was depleted by two intraperitoneal injections of 1 mmol/kg BSO 48 and 24 h before the perfusion experiment. This dosage regimen resulted in a reduction of myocardial GSH by 55%.

**Isolated heart preparation**

Male Wistar rats weighing 280–340 g were anesthetized with diethyl ether. After intravenous injection of 300 IU of sodium heparin, the hearts were excised, placed in ice-cold heparinized perfusion buffer, and subsequently mounted on a non-recirculating Langendorff perfusion apparatus. The hearts were perfused retrogradely with Krebs-Henseleit bicarbonate buffer (37°C) at a constant perfusion pressure of 65 mmHg. The perfusion buffer contained (in mM): NaCl 118, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 25, CaCl$_2$ 2.5 and glucose 11, and was gassed continuously with 95% O$_2$ + 5% CO$_2$. The hearts were paced at 300–330 beats/min, which was started 15 min after mounting the heart and continued throughout the experiment. The whole perfusion apparatus was enclosed by a thermostated Perspex chamber. Myocardial temperature either decreased from 37°C to 30–31°C during no-flow ischemia (hypothermic ischemia) or was maintained at 37°C by immersing the heart in perfusion buffer during ischemia (normothermic ischemia). We selected both models to test for possible aggravation of postischemic injury in a model which sustains either relatively mild injury (hypothermic ischemia) or severe injury (normothermic ischemia).

Left ventricular pressure was measured via a 23 gauge needle inserted into the left ventricle through the apex. Coronary flow rate was measured electromagnetically (Skalar) and by timed collection of coronary effluent. Perfusion pressure, left ventricular pressure, and coronary flow rate were recorded on a chart recorder (Gould 2400). The experiments have been approved by the Institutional Committee on Animal Experiments.

**Experimental protocol**

First all hearts were allowed to stabilize for 30 min. Then groups of GSH-depleted and non-depleted hearts were subjected to 30 min of either hypothermic or normothermic no-flow ischemia, followed by 5 or 30 min of reperfusion. The 5 min time point was chosen not to miss the detection of a transient increase in myocardial GSSG due to reactive oxygen species generated during the initial minutes of reperfusion. Control groups of GSH-depleted and non-depleted hearts were subjected to 30 min of non-ischemic perfusion. To test whether GSH-depleted hearts could withstand an exogenous prooxidant during early reperfusion, we administered cumene hydroperoxide to separate groups of postischemic GSH-depleted hearts. Cumene hydroperoxide was administered to the perfusion buffer at the onset of reperfusion for 5 min, or during the first 10 min of the 30 min reperfusion period by means of an infusion pump. The average concentration of cumene hydroperoxide in the perfusion buffer during the first 10 min of reperfusion ranged from 17–28 μM among the hearts depending on the recovery of coronary flow rate. At the end of each experiment, the hearts were freeze-clamped for tissue analysis using Wollenberger tongs precooled in liquid nitrogen.

**Myocardial GSH and GSSG assay**

Heart tissue was processed as described by Akerboom and Sies [18]. Briefly, the freeze-clamped hearts were pulverized in an aluminum mortar at liquid nitrogen temperature. For determination of total glutathione (GSH+GSSG), frozen heart powder was homogenized in ice-cold 1 M perchloric acid containing 2 mM EDTA, whereas for selective determination of GSSG, 50 mM N-ethylmaleimide (NEM) was added to the perchloric acid/EDTA solution. NEM traps GSH and prevents oxidation of GSH to GSSG. GSH+GSSG and GSSG were