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MYOCARDIAL GLUTATHIONE ALTERATIONS IN ACUTE CORONARY OCCLUSION IN THE DOG

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Glutathione (GSH) decreases in dog mycocardium upon acute coronary occlusion when compared with sham-operated dogs. Total glutathione content (GSHeq = GSH + 2GSSG) remains unchanged throughout the experiment (6 h after surgery) in both sham- and acute coronary occlusion-operated dogs. GSSG and GSH/GSSG ratio increases and decreases respectively in all animals but tends to reach the normal value after 6 h in sham-operated dogs. Both parameters (GSSG and GSH/GSSG ratio) remain altered in acute coronary occlusion-operated ones. This alteration of glutathione status in ischemic myocardium is discussed.

KEY WORDS: Glutathione, ischemia, myocardium.

INTRODUCTION

Glutathione alterations may play a role in the development of the changes in the mechanical function of heart muscle that occur during the early phases of the hypoxic response. Galvin and Lefer[1] reported a decrease in non-protein sulfhydryl compounds in mycocardial tissue during cardiogenic shock. It has also been suggested that changes during hypoxia might be due to a failure of the maintenance of calcium homeostasis.[2] This hypothesis fits with the reported involvement of cellular thiols, e.g. glutathione, in calcium movements across membranes and thus in cell viability.[3] Furthermore, oxygen free radicals have been implicated in the mechanisms of cell injury under ischemic conditions.[4]

The aim of this work was to study the content and status of glutathione in myocardial tissue when acute coronary occlusion is performed.

MATERIALS AND METHODS

Mongrel dogs (12–22 kg) fed on a stock diet (Cid, Vara de Quart, Valencia, Spain) were starved overnight and experiments were started between 9–10 a.m. to avoid changes in glutathione levels due to circadian rhythm.[5] Dogs were anesthetized with sodium thiopental (25 mg/kg, i.v.). Polyethylene catheters were inserted in the left

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external jugular vein and the right common carotid artery. Left ventricular pressure, mean arterial blood pressure, dP/dt and EKG were recorded with an ELEMA SHONANDER MINGOGRAF polygraf recorder. After establishment of artificial ventilation with a Harvard type respirator, the heart was exposed through a left thoracotomy and suspended in a pericardial cradle. The left anterior descending coronary artery was dissected out from surrounding tissue at a point just distal to the first major diagonal branch and a ligature placed under the vessel. Only in sham-operated dogs, the coronary artery was not occluded.

Biopsies were always taken from the left ventricular myocardium near the apex because this area is the most affected by coronary occlusion.[6] Tissue samples were placed in liquid nitrogen and homogenized in 6% perchloric acid containing 1 mM EDTA. Venous blood samples for GSHeq determination were drawn from the local vein draining the affected area through the polyethylene catheter properly placed. GSH was determined with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, and GSH S-transferase according to[7], and total glutathione content (GSHeq = GSH + 2GSSG) according to the method of Tietze.[8] Student's t test was applied to each pair of values for the same experimental time (one sham- and one acute coronary occlusion-operated).

RESULTS AND DISCUSSION

Figure 1A shows the time course of GSH concentration in myocardial tissue of sham- and acute coronary occlusion-operated dogs. GSH content in sham-operated dogs remains constant while a steep decrease to values less than 50% of controls can be observed 6 h after surgery in acute coronary occlusion-operated dogs, where irreversible myocardial damage is already established.[9] Interestingly, no significant difference was observed in total glutathione content (GSHeq) in either sham- or acute coronary occlusion-operated animals (Figure 1B). GSH/GSSG is affected in both types of experiments, though in sham hearts this ratio tends to return to its normal value after the initial decrease. A possible explanation for this alteration in GSH/GSSG could be the effect of surgical stress (Figure 1C).

Three different and related events can be observed in acute coronary occlusion conditions: (a) a steady decrease in GSH concentration, (b) the maintenance of total glutathione contents and (c) the accumulation of GSSG intracellularly, whereas no significant changes can be detected in serum of venous samples of the vein draining the affected area throughout the experiments (data not shown).

It is known that under normal conditions mitochondria can generate O_2^- and H_2O_2,[10] and indirect evidence has been put forward that ischemic injury might generate free radicals[4] that could be involved in damage to plasma and mitochondrial membrane. The latter has been reported to be more susceptible to free-radical damage than the former.[11] Moreover, once mitochondria are initially damaged, they themselves may contribute to an increased leaking of O_2^-. The intracellular distribution of glutathione in heart tissue shows only 7% of the total content in mitochondrial fractions (Romero & Sies, unpublished observations). An intramitochondrial GSH depletion promoted by the so-called O_2^- "univalent leak"[10] under ischemic conditions is likely to occur as the initial event and might trigger the free radical chain reaction. As an explanatory mechanism for the changes in the mechanical function, this mitochondrial damage would impair ATP production.[12]

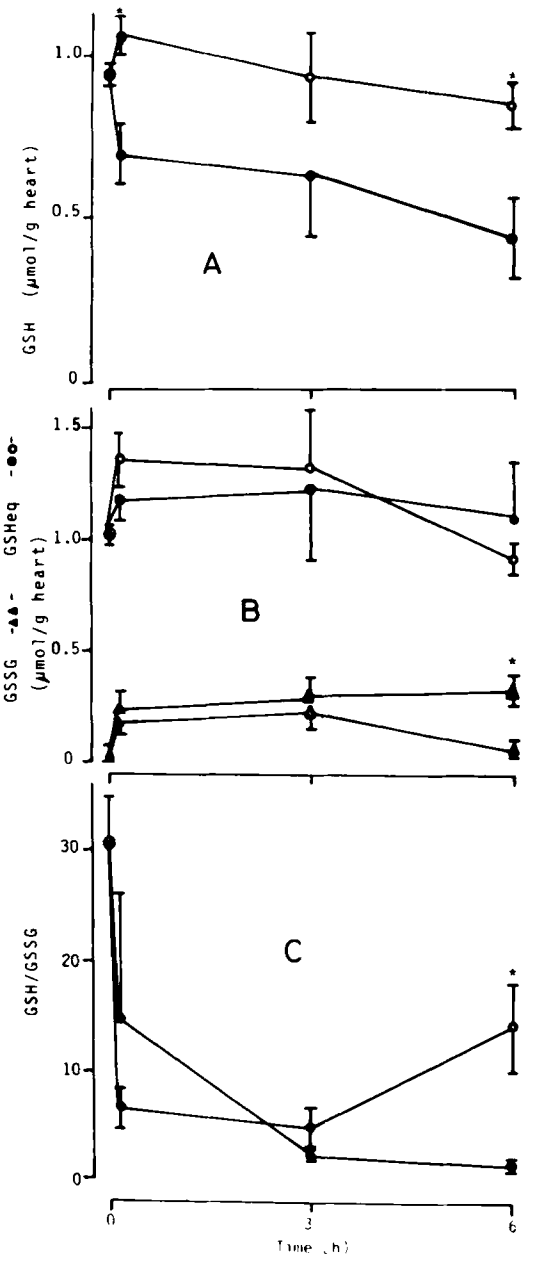

FIGURE 1 Time course of GSH (A), total glutathione (GSHeq) and GSSG contents (B) and GSH/GSSG ratio (C) in myocardial tissue of sham- (open symbols) and acute coronary occlusion-operated dogs (closed symbols). Results are mean ± S.E.M. of at least 4 different dogs. *$p < 0.05$

It has been reported a substantially lower capacity of heart for GSSG transport and reduction under oxidative stress promoted by organic hydroperoxides.[13] This being in agreement with the inability of the mycocardial tissue of the affected area in our conditions to reduce the GSSG formed and/or to translocate this GSSG out of the cell, if the latter is an energy-dependent process.[14]

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