Vol. 89, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 27, 1979 Pages 678-684

GLUTATHIONE PEROXIDASE ACTIVITY AND RELEASE OF GLUTATHIONE FROM OXYGEN-DEFICIENT PERFUSED RAT HEART

C. Guarnieri, F. Flamigni and C. Rossoni-Caldarera

Institute of Biochemistry, School of Medicine,
University of Bologna, 40126 Bologna, Italy

Received June 4,1979

Summary

The effect of cellular hypoxia on glutathione levels in rat hearts was determined. Hearts perfused with 95% N_2-5% CO_2 demonstrated a significant decrease in tissue reduced glutathione content when compared to control hearts perfused with 95% O_2-5% CO_2. The hypoxic perfusate contained reduced glutathione and its release was time dependent over a period of 60 minutes. The cellular depletion of oxidized glutathione and its release into coronary effluent were less evident with respect to reduced glutathione. Moreover during hypoxic perfusion we have observed a decrease of cytosol glutathione peroxidase activity. These results suggest that severe oxygen-deprivation causes in myocardial cells a significant perturbation of glutathione metabolism.

Introduction

The perfusion of isolated rat hearts under conditions of oxygen deprivation causes cell injury that can be measured in terms of release of intracellular compounds into coronary effluents (1, 2). Increased permeability and/or loss of membrane integrity are considered as early events associated with the appearance of an efflux of ions (3) and enzymes (4) from hypoxic hearts. In the first phase of hypoxia, the release of some compounds, such as nucleosides (1), promoting vasodilation, may represent a cellular defensive response to cellular damage. On the contrary, the loss of essential cellular components induced by prolonged oxygen deprivation, may contribute to the irreversibility of hypoxic cell damage (4).

0006-291X/79/140678-07$01.00/0

The effect of severe hypoxia on glutathione peroxidase activity and on cellular glutathione metabolism in perfused rat hearts were examined in this study. It is known that the perturbation of $GSH^*/GSSG^*$ ratio in the cells induced by several factors or agents, affects numerous biochemical and physiological processes, such as ion transport (5), neurotransmitter release (6) and muscle contraction (7). In fact GSH protecting the thiol groups of enzymes and membranes (8) plays an important role in many biochemical processes (9).

Moreover, reduced glutathione functioning as a hydrogen donor in reactions catalyzed by glutathione peroxidase, may protect sensitive membrane lipids from oxidation (10).

Our data indicate that the cardiac impairment caused by oxygen-deprived perfusion may be due to an alteration in glutathione metabolism.

Materials and Methods

Male Sprague-Dowley rats, weighing from 300 to 350 g and fed on standard pellet diet and water ad libitum, were used in this study.

The hearts were quickly removed from the animals anaesthetized with diethyleter and mounted on the perfusion apparatus and perfused with a constant perfusion pressure of 60 mm Hg through the coronary vessels as described by Langendorff (11). The temperature of the perfusate was maintained at 37°C. A Krebs-Henseleit bicarbonate buffer (12), pH 7.4, containing 11 mM glucose and equilibrated with 95% O_2 - 5% CO_2 (pO_2 > 600 mmHg) was used during aerobic perfusion. In hypoxic studies the fluid was equilibrated with 95% N_2 - 5% CO_2 (pO_2 < 6 mm Hg) and the glucose was replaced with 11 mM mannitol. The hearts during all the time of perfusion were paced at 180 beats/min. At the end of each experiment the heart was removed, excess fluid was blotted and the tissue weighed. The contents of GSH and GSSG in the tissues and coronary effluents were determined as described by Tietze (13). The incubation mixture for total glutathione determination contained: 0.1 mM DTNB*; 0.15 mM NADPH; 1 unit/ml of glutathione reductase, 50 mM phosphate buffer, pH 7.4, 1 mM EDTA and an appropriate volume of the neutralized

*Abbreviations used: GSH, reduced glutathione; GSSG, oxidized glutathione; DTNB, 5,5' dithiobis-(2-nitro benzoic acid); NEM, N-ethyl maleimide.

sample (final volume 1.0 ml). The GSSG was measured as described after preliminary reaction of GSH with 20 mM NEM* followed by complete removal of unreacted sulphydryl reagent with ether. The hearts were placed in an ice-cold solution containing 250 mM sucrose and 50 mM phosphate buffer, pH 7.2, and homogenized with Ultra-Turrax. Then they were utilized for glutathione peroxidase activities (E.C. 1.11.1.9). Homogenates were centrifuged at 750 g for 20 min, and the supernatants were centrifuged at 105,000 g for 60 min. The glutathione peroxidase was assayed on the cytosol by the coupled assay procedure of Paglia and Valentine (14), using 0.2 mM cumene hydroperoxide as substrate. Proteins were estimated as described by Bradford (15).

Results

The effects of oxygen deficiency on GSH and GSSG content are shown in fig. 1.

The hypoxic perfusion induced a progressive cellular fall in GSH content, whereas in the well oxygenated heart the GSH

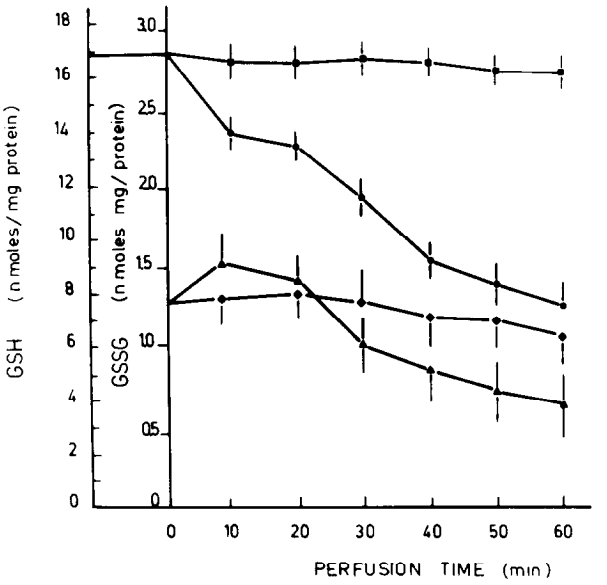

Fig. 1. GSH and GSSG content in rat heart after perfusion with or without oxygen. Hearts were perfused with Krebs-Henseleit bicarbonate buffer containing 11 mM glucose for 15 min (initial) or for an additional 60 min. ■——■, GSH 95% O_2-5% CO_2; ●——●, GSH 95% N_2-5% CO_2; ◆——◆, GSSG 95% O_2-5% CO_2; ▲——▲, GSSG 95% N_2-5% CO_2. Vertical bars indicate the mean ± SE of six separate experiments.

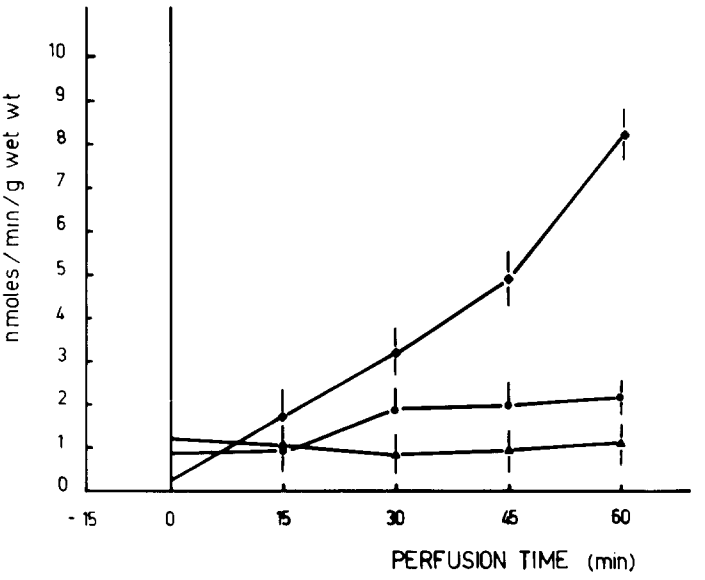

Fig. 2. Release of tissue GSH and GSSG of the isolated rat heart. Experimental conditions are the same as in fig. 1. ▲——▲, Total glutathione (GSH + GSSG) 95% O_2-5% CO_2; ◆——◆, GSH 95% N_2-5% CO_2; ●——●, GSSG 95% N_2-5% CO_2. Each value represents the mean ± SE of six separate experiments.

level is not affected. In the same experimental conditions, the content of cellular GSSG was depleted more slowly and a significant reduction was measured in the last 10 min of hypoxic perfusion.

Samples of perfusates collected at various time intervals were analyzed for GSH-GSSG content. The results are shown in fig. 2.

In well-oxygenated control hearts, a small quantity of total GSH-GSSG was detected in the coronary effluents. By contrast, perfusion with substrate free hypoxic Krebs-Henseleit solution resulted in a marked and prolonged release of GSH. A slow release of GSSG was induced by hypoxia perfusion that began after 30 min and gradually increased as the hypoxic perfusion continued.

The results presented in table 1 indicate that the cellular glutathione peroxidase activity changes are in response to hy-

Table 1. Effect of hypoxia on glutathione peroxidase activity in the cytosol of rat hearts (μmol NADPH oxidize/min/mg protein).

Time (min)	Aerobic perfusion	Hypoxic perfusion
10	0.214 ± 0.032	0.195 ± 0.032
20	0.218 ± 0.028	0.191 ± 0.030
30	0.200 ± 0.030	0.188 ± 0.026
40	0.195 ± 0.032	0.173 ± 0.022
50	0.203 ± 0.034	0.159 ± 0.022
60	0.191 ± 0.036	0.136 ± 0.020

All activities were measured in 105,000 g supernatant from fractions 20% (w/v) homogenates and were expressed as the mean ± SE of six separate experiments.

poxic perfusion. In regard to the activities found in well-oxygenated rat hearts that remained unchanged, the perfusions under hypoxic conditions resulted in a decrease in glutathione peroxidase activities. This effect was more evident about 30 min after the beginning of oxygen deprived perfusions.

Discussion

The experiments described here reveal that the release of GSH, induced in rat hearts by hypoxic perfusions, reduces significantly the intracellular content of this compound. This depletion, that increases with the time of exposure to oxygen deficiency, was accompanied with a parallel decrease of glutathione peroxidase activity. Probably this reduction results from cellular GSH depletion, since no glutathione peroxidase activity was found in the hypoxic coronary effluent. It is known that GSH can be released across the cell membrane after it has been oxidized by glutathione oxidases (6); nevertheless in our experimental hypoxic conditions it was present in reduced form in the coronary effluent. Evidence obtained from perfused rat hearts also suggests that increased cell membrane permeability is an early event following hypoxic injury (17). Under these

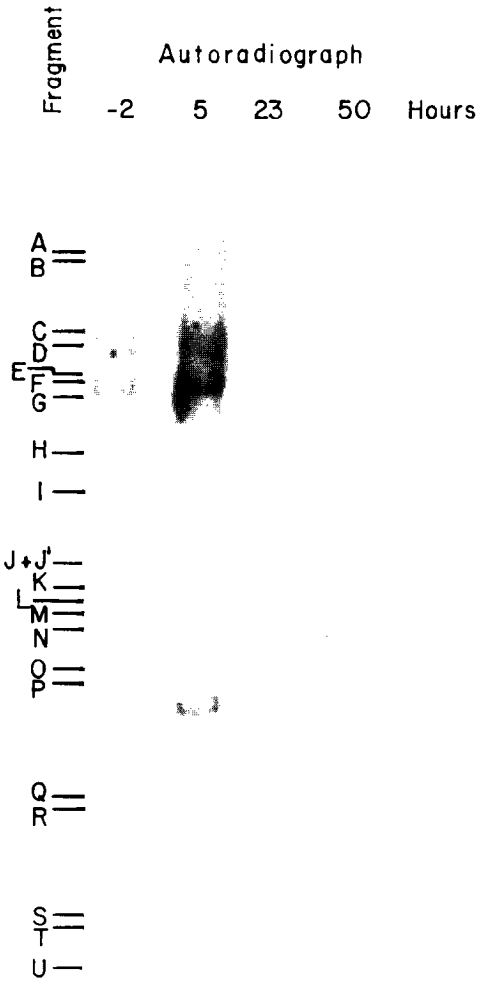

Figure 3. Autoradiographs of hybrids formed between EcoRI chloroplast DNA fragments and [^{32}P]-pulse labeled RNA. EcoRI chloroplast DNA fragments (.3 μg) were separated by electrophoresis in 0.8% (w/v) agarose gels. The DNA fragments were denatured and transferred to Millipore filters. [^{32}P]-labeled RNA from cells pulse labeled 2 hrs prior to transfer of all the flasks to the light (-2 hr) and from cells which had been exposed to light for 5, 23 and 50 hr was hybridized for 72 hr to the Southern imprints. The hybrids were detected by autoradiography.

Four different classes of EcoRI chloroplast DNA fragments exist; those which are: 1. continually transcribed in the dark and in the light, 2. transcribed only in the light and very little, or not at all in the dark, 3. transcribed in the dark and less in the light, 4. not transcribed (i.e. no detectable transcription). Some of the smaller EcoRI chloroplast DNA

H.D., eds., p. 206-215, Georg Thieme Publishers Stuttgart, Tübingen.
9. Meister, A. (1975) in "Metabolism of Sulfur Compounds", Greenberg D.M., ed., vol. VII, p. 102-169, Academic Press, New York.
10. Mills, G.C. (1959) J. Biol. Chem. 229, 189-197.
11. Langendorff, O. (1895) Pflugers Arch. Gesamte Physiol. 61, 291-332.
12. Krebs, H.A., and Hemseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33-66.
13. Tietze, F. (1969) Anal. Biochem. 27, 502-522.
14. Paglia, D.E., and Valentine, W.N. (1967) J. Lab. Clin. Med. 70, 158-169.
15. Bradford, M.M. (1976) Anal. Biochem. 72, 246-254.
16. Srivastava, S.K., and Beutler, E. (1969) Biochem. J. 114, 833-837.
17. Ganote, C.E., Worstell, J., and Kaltenbach, J.P. (1976) Am. J. Pathol. 84, 327-343.
18. Galvin, M.J., and Lefer, A.M. (1978) Am. J. Physiol. 235, H657-H663.