Role of catalase in metabolism of hydrogen peroxide by the perfused rat heart

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Cardiac metabolism of H_2O_2 was studied by determining the concentration dependence for H_2O_2-stimulated release of GSSG, an indicator for flux through the glutathione peroxidase pathway, in perfused heart preparations. Treatment of rats with aminotriazole in vivo inhibited heart catalase by 83% and shifted the dose-response curve for GSSG release toward lower H_2O_2 concentrations. In aminotriazole-treated rats, 50 μM H_2O_2 elicited a maximal rate of GSSG release (about 5 nmol GSSG/min per g heart), whereas 200 μM H_2O_2 was necessary for obtaining a similar rate of GSSG release in control rat hearts. The results show that catalase, although present at low levels of activity in the heart compared to other organs, functions as a major route for detoxication of H_2O_2 in the myocardium.

Catalase *Hydrogen peroxide* *(Rat heart)* *Glutathione release* *Glutathione peroxidase* *Detoxication*

1. INTRODUCTION

Catalase and glutathione peroxidase are the major enzymes involved in detoxication of hydrogen peroxide in mammalian cells. Previous studies have shown that the catalase activity per g heart tissue is very low in comparison to other organs, being only about 2% that of liver in the rat [1,2]. On the other hand, glutathione peroxidase activity per g tissue is similar in both heart and liver [2,3]. Consequently, glutathione peroxidase has been suggested to function as the major route for H_2O_2 metabolism in the heart [3].

Organic hydroperoxides, such as *t*-BuOOH, are metabolized by glutathione peroxidase leading to an elevation of intracellular GSSG. High intracellular GSSG levels result in release of a fraction of GSSG to the extracellular environment via a specific transport system [3,4]. GSSG release has thus been employed as an indirect means of flux through the glutathione peroxidase pathway [3–7]. In liver, compounds which enhance endogenous H_2O_2 generation, such as glycolate [5,6], as well as the model redox cycling quinone menadione [7], also cause GSSG release.

To evaluate pathways for cardiac H_2O_2 metabolism, we have studied the dependence of GSSG release from the perfused heart on infused H_2O_2 concentration in control and aminotriazole-treated rats. Aminotriazole is an irreversible inhibitor of catalase [8], and was used as a way of selectively inactivating the catalase pathway.

Abbreviations: GSSG, oxidized glutathione; GSH, reduced glutathione; *t*-BuOOH, *tert*-butylhydroperoxide

2. MATERIALS AND METHODS

Male, Sprague-Dawley rats (300–350 g), fed standard chow diet, were used for all experiments. Rats were treated with 3-amino-1,2,4-triazole as described [5]. Hearts were perfused via the aorta in a non-recirculating mode at 6 ml/min with Krebs-Henseleit buffer containing 5.5 mM glucose and 2.5 mM $CaCl_2$ equilibrated with 95% O_2/5% CO_2 at 35–37°C. After 25 min, H_2O_2 was added to the

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medium. At 60 min hearts were frozen in liquid N_2 and stored at −70°C. Glutathione in the effluent perfusate and heart was measured spectrophotometrically by the catalytic assay [9]. GSSG and total glutathione (GSH + GSSG) were determined from samples with and without *N*-ethylmaleimide treatment; GSH levels were determined by difference [3,9]. Catalase activity of homogenates was assayed by following the decomposition of 12.5 mM H_2O_2 at 240 nm in a medium containing 50 mM KP_i, 1 mM EDTA and 0.1% cholate, at pH 7.0 [10].

3. RESULTS

Perfused rat hearts released glutathione in the form of GSH at basal rates corresponding to 0.1–0.3 nequiv. GSSG/min per g heart in different preparations. Exposure to H_2O_2 stimulated the release of glutathione to the perfusate (fig.1). At $\leq 25\ \mu M$ H_2O_2, little glutathione release above basal levels was detected in control rat hearts. At 50–200 μM H_2O_2, glutathione release increased markedly and was maintained at nearly steady rates. At 1 mM H_2O_2, glutathione release rapidly reached a maximum then subsequently declined. The decline in glutathione release at 1 mM H_2O_2 was accompanied by decreased and erratic heart rates, whereas at $\leq 200\ \mu M$ H_2O_2 heart rates were generally stable at 150–180 beats per min. In all cases, the peroxide-stimulated release of glutathione occurred mainly (88–100%) in the form of GSSG rather than GSH. The maximum rate of GSSG release observed under our conditions was about 5 nmol/min per g heart. A similar rate was also found in perfusions with *t*-BuOOH (100 μM).

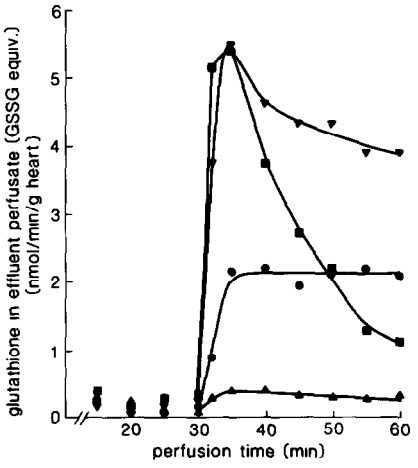

Fig.1. Representative experiments showing the time course for release of glutathione from control rat hearts perfused with medium containing H_2O_2 at 25 (▲), 50 (●), 200 (▼) and 1000 (■) μM as described in section 2. Initial contact of H_2O_2 with the heart occurred at approx. 30 min.

Hearts from rats treated with aminotriazole demonstrated GSSG release at lower H_2O_2 concentrations than control rats (fig.2). In aminotriazole-treated rats, 50 μM H_2O_2 produced a near maximal rate of GSSG release. Measurements in heart homogenates indicated that aminotriazole treatment had inhibited catalase by an average of 83%. Apparent first order rate constants were: control, 54.8 ± 8.7 $min^{-1} \cdot g^{-1}$, $n = 7$; aminotriazole-treated, 9.3 ± 2.5 $min^{-1} \cdot g^{-1}$, $n = 6$ ($p < 0.001$). Glutathione peroxidase activity was unaffected by aminotriazole treatment.

Total GSSG formed during exposure to peroxide was determined from measurements of residual intracellular GSSG and summation of GSSG released during the perfusion period. Plots of the

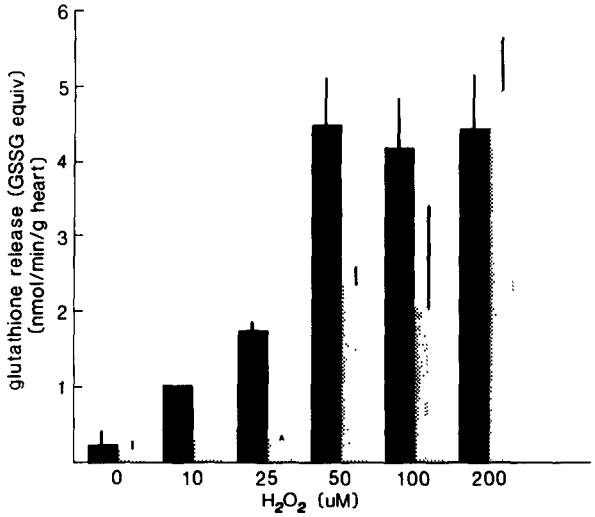

Fig.2. Dependence of the rate of glutathione release on infused H_2O_2 concentration for hearts from control (stippled) and aminotriazole-treated (solid) rats. Bars indicate mean and lines represent SD. Data are from 2–3 hearts at each H_2O_2 concentration, except for 10 μM which represent single experiments. Data for zero H_2O_2 indicate basal glutathione release before exposure to H_2O_2 ($n = 11$, control; 9, aminotriazole).

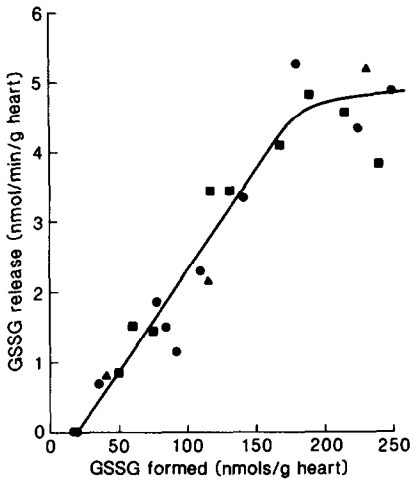

Fig.3. Relationship between the rate of GSSG release and total GSSG formed (released plus residual) during 30 min perfusion with various concentrations of H_2O_2 in control (●) and aminotriazole-treated (■) rats. Data for control hearts perfused with various concentrations of *t*-BuOOH (▲) are also included.

rate of GSSG release as a function of total GSSG formed during peroxide exposure showed an identical relationship for both aminotriazole-treated and control rat hearts perfused with H_2O_2 (fig.3). The same relationship was also found for perfusions with *t*-BuOOH. This result confirmed that the rate of GSSG release was a function of elevation of intracellular GSSG [3]. At all peroxide concentrations the increase of intracellular GSSG was accompanied by a compensating decrease in intracellular GSH. Mass balance determinations, measuring the sum of residual plus released GSSG and GSH, indicated that the total initial glutathione contents of the hearts were comparable at values of 1060 ± 82 (control, $n = 11$) and 1130 ± 138 nequiv. GSH/g heart (aminotriazole-treated, $n = 9$).

4. DISCUSSION

The shift of the dose-response curve for H_2O_2-stimulated GSSG release after catalase inhibition indicates that catalase does play a significant role in H_2O_2 metabolism in the heart. Inhibition of catalase allows relatively more H_2O_2 to be available to glutathione peroxidase, thus increasing flux through this enzyme. Although GSSG release does not provide an absolute measurement of the glutathione peroxidase reaction because part of the GSSG is reduced by glutathione reductase, the enhanced GSSG release observed after catalase inhibition must be at least proportional to the normal flux through catalase in the uninhibited state. Comparison of the concentration dependences of H_2O_2 and *t*-BuOOH for stimulating GSSG release likewise supports the involvement of catalase. Maximal rates of GSSG release are obtained with 20–50 μM *t*-BuOOH [3], whereas about 200 μM H_2O_2 was necessary to achieve the same maximal rate in control hearts. However, with isolated glutathione peroxidase similar catalytic rates occur with either *t*-BuOOH or H_2O_2 at a given substrate concentration [11]. Thus, the different concentration dependences for GSSG release cannot be attributed to an inherent difference at the level of glutathione peroxidase itself.

Studies of red blood cells [12] and liver [5], which contain high levels of catalase as well as glutathione peroxidase, have shown that both enzymes contribute to H_2O_2 metabolism. The relative contribution of each pathway is dependent on the concentration of H_2O_2, availability of GSH and hydrogen donors for the peroxidase mode of catalase [12], and is influenced by subcellular compartmentation [5]. While similar considerations apply to the heart, the present results demonstrate that catalase can provide a substantial contribution to cardiac detoxication of H_2O_2 in spite of its low tissue content relative to other organs. This may be important in regard to the actions of redox cycling compounds, such as the cardiac toxin adriamycin. The latter stimulates superoxide and H_2O_2 formation in vitro through redox cycling mechanisms involving flavoproteins [13], including cardiac mitochondrial NADH-dehydrogenase [1]. Many studies have attempted to evaluate adriamycin redox cycling in vivo by measurements of the glutathione status of the heart [14,15]. However, the present results show that enhanced H_2O_2 formation from redox cycling may not necessarily perturb the glutathione redox state of the myocardium.

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