GLUTATHIONE TURNOVER IN HEPATOCYTES OF RAJA ERINACEA: WITH COMMENTS ON OXYGENATION DURING CELL ISOLATION

Thomas W. Simmons, Anh T. Truong, and Nazzareno Ballatori
Department of Biophysics, Environmental Health Sciences Center,
University of Rochester School of Medicine, Rochester, NY 14642

Glutathione plays a role in cellular defenses and maintenance of the thiol-redox status (Meister and Anderson, Ann. Rev. Biochem. 52:711-760, 1983), and in bile formation (Ballatori and Truong, Am. J. Physiol. 256:G22-G30, 1989). These functions are regulated in part by metabolism and turnover of glutathione, which is in turn dependent on transport of this tripeptide out of liver cells (Bannai and Tateishi, J. Membrane Biol. 89:1-8, 1986). Attempts to define the driving forces for glutathione transport across liver cell plasma membrane have yielded conflicting results. Inoue et al. (Eur. J. Biochem. 134:467-471, 1983) and Fernandez-Checa et al. (Am. J. Physiol. 255:G403-G408, 1988) argue for an electrogenic transport influenced by the transmembrane potential difference, whereas Wright et al. (Am. J. Physiol. 255:G547-G555, 1988) question this mechanism. In the present study, this problem was addressed from a comparative standpoint by investigating glutathione efflux from hepatocytes isolated from the little skate, Raja erinacea.

Glutathione was measured enzymatically according to Griffith (Anal. Biochem. 106:207-212, 1980), potassium was determined by flame photometry, and protein was quantified by the method of Lowry et al. (J. Biol. Chem. 193:265-275, 1951) using bovine serum albumin as the standard. Data are presented as mean ± standard deviation. Hepatocytes were prepared for initial experiments as described by Smith et al. (J. Exp. Zool. 241:291-296, 1987). During cell isolation, livers from male skates were perfused with oxygenated elasmobranch Ringer's solution, and isolated cells were preincubated under an atmosphere of 99% O_2/1% CO_2 for 30 min. Hepatocytes exposed to these conditions, displayed a GSH/GSSG ratio of 8.4±4.1 (n=3). In contrast, when oxygenation was omitted from the isolation procedure (i.e. air saturated solutions), the GSH/GSSG ratio of isolated hepatocytes was 25.9±4.9 (Fig. 1). In mammals, intracellular GSH/GSSG ratios are generally greater than ten and lower values are often indicative of oxidative stress (Halliwell and Gutteridge, "Free Radicals in Biology and Medicine," Oxford Univ. Press, NY, 1985). Consequently, perfusion solutions and isolated cells were not oxygenated in subsequent experiments, and a pH of 7.4 was maintained by addition of 10 mM Hepes/Tris buffer to the elasmobranch Ringer's solution.

Glutathione levels in skate livers (0.53±0.19 μmol GSH equivalents•g wet wt^{-1}, n=10) were approximately one-tenth of those found in mammals. Rate of glutathione release by hepatocytes at 15°C was exceedingly slow, 0.12±0.05 nmol GSH equivalents•mg $protein^{-1}$•hr^{-1} (n=3). This slow rate of efflux was unaffected by temperatures of 4°C (0.16±0.11) and 25°C (0.20±0.22); by supplementation with a mixture of amino acids containing 0.2 mM methionine, 0.5 mM alanine, 1 mM serine, 2 mM glycine and 5 mM glutamine (0.18±0.08); or by treatment with AT-125, an inhibitor of γ-glutamyltransferase (0.11±0.08). In addition, activity of this glutathione metabolizing enzyme was below detection in untreated liver homogenate. Therefore, the slow accumulation of extracellular glutathione observed was not due to metabolism of glutathione by this plasma membrane-bound ectoprotein. Intracellular potassium, GSH and GSSG levels were also unaffected by the above conditions, remaining at 0.59±0.09 μEquivalents•mg $protein^{-1}$, 6.87±2.23 and 0.28±0.09 nmol GSH equivalents•mg $protein^{-1}$, respectively. These data indicate that the slow rate of efflux was not limited by temperature, glutathione precursors or metabolism of extracellular glutathione. Furthermore, the lack of change in cell-

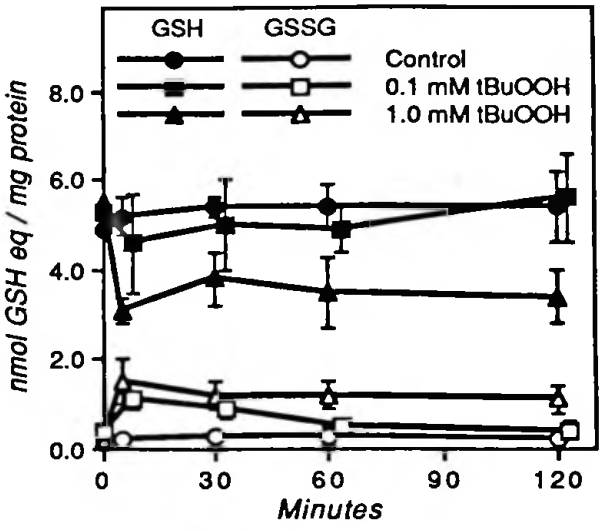

Figure 1. Effects of t-butyl hydroperoxide on reduced and oxidized glutathione levels in isolated hepatocytes.

Hepatocytes were resuspended at a density of 2 to 4 x10^6 cells•ml^{-1}. Cells were treated with 0, 0.1 or 1.0 mM t-BuOOH and incubated for 120 min at 15°C. Aliquots of 1.0 ml of cell suspension were removed at the appropriate time intervals for analyses. Data points represent mean ± S.D. of three experiments.

associated K^+ suggests that increases in extracellular glutathione did not simply reflect nonspecific leakage of intracellular solutes.

Enhanced GSSG transport out of mammalian liver cells after exposure to pro-oxidants is well documented (Sies et al., FEBS Lett. 27:171-175, 1972; Jones et al., Archiv. Biochem. Biophys. 210:505-516, 1981). Therefore, skate hepatocytes were exposed to t-butyl hydroperoxide (t-BuOOH) in an attempt to stimulate GSSG efflux. Treatment of hepatocytes with 0.1 and 1.0 mM t-BuOOH caused a rapid dose-dependent decrease in intracellular GSH/GSSG ratios from 25.9±4.9 to 5.4±2.2 and 3.2±1.0, followed by total and partial recovery, respectively (Fig. 1). Although elevated intracellular GSSG levels were sustained for at least two hours, rates of glutathione release by treated cells (0.14±0.06 and 0.063±0.085 nmol GSH eq•hr^{-1}•mg $protein^{-1}$ for 0.1 and 1.0 mM t-BuOOH, respectively) were as slow as untreated cells (0.15±0.06).

In conclusion, the results suggest that mechanisms for transport of glutathione across liver cell plasma membrane are not well-developed in R. erinacea. Since glutathione excretion appears to be a driving force for bile-acid independent bile flow (Ballatori and Truong, Am. J. Physiol. 256:G22-G30, 1989) and a determinant of xenobiotic elimination (Ballatori and Clarkson, Fundam. Appl. Toxicol. 5:816-831, 1985), an inefficient mechanism for glutathione export by skate hepatocytes may help to explain both the inordinately slow rate of bile formation and long biological half-times of certain xenobiotics in marine elasmobranchs (Ballatori and Boyer, Toxicol. Appl. Pharmacol. 85:407-415, 1986). In addition, the present findings indicate that the GSH/GSSG ratio is lower in cells exposed to a high pO_2, suggesting a deleterious change in the intracellular thiol redox status. Further studies are needed to define the physiologic significance of these alterations in the GSH/GSSG ratio, and the optimal pO_2 to support hepatocellular functions. (Supported by National Institutes of Health Grants ES03828, DK39165 and ES01247)