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Glyoxalase Activity in Sham- and Partially Hepatectomized Rats

Nicholas M. Alexander and James L. Boyer

Departments of Medicine [N. M. A., J. L. B.] and Molecular Biophysics and Biochemistry [N. M. A.], Yale University School of Medicine, New Haven, Connecticut 06510

SUMMARY

In male rats, hepatic glyoxalase activity and reduced glutathione, the cofactor for this enzyme system, approximately double 12 hr after partial (2/3) or sham hepatectomy, in which the liver lobes were handled during the sham operation. These changes precede by 10 to 12 hr the anticipated DNA synthesis after partial hepatectomy and by about 24 hr the smaller, but prolonged, DNA synthesis that occurs in peripheral hepatic cells after this type of sham hepatectomy. These findings indicate that the hepatic glyoxalase system is responsive to operative stress prior to increments in DNA synthesis and mitosis. Whether or not it regulates intracellular levels of methylglyoxal, a cell growth inhibitor, and thereby regulates cell division as postulated by Szent-Györgyi, remains to be established.

INTRODUCTION

Szent-Györgyi et al. (9-11, 15, 28-30) postulate that ketoaldehydes regulate cell growth since metabolites such as methylglyoxal inhibit cell division in bacterial (10) and mammalian (16) cell cultures and arrest tumor growth in mice (2, 3, 12). Methylglyoxal is rendered ineffective after conversion to D-lactate by an efficient, ubiquitous, dual enzyme system, glyoxalase I and II. GSH is required as a cofactor and the reaction proceeds as follows:

\[ \text{Methylglyoxal + GSH} \rightarrow \text{Lactoyl-SG} \rightarrow \text{D-lactate + GSH} \]

Thus, according to the theory of Szent-Györgyi et al. (29), methylglyoxal is “retine,” the cell growth retainer, and the glyoxalase enzyme system is “promine,” the cell growth promoter.

To test this theory in a rapidly dividing mammalian system, we measured hepatic glyoxalase and GSH levels in sham- and partially hepatectomized rats before, during, and after the expected maximum DNA synthesis and mitotic activity.

MATERIALS AND METHODS

Partial hepatectomies (17) were performed in 200- to 250-g male Sprague-Dawley rats under light ether anesthesia, and the excised lobes, equivalent to approximately 68% of the entire organ, were immediately homogenized with 9 volumes of 0.25 M sucrose in a Potter-Elvehjem Teflon-glass homogenizer. Livers from the hepatectomized or sham-operated rats were then removed at 6, 12, 18, 25, 42, 49, or 72 hr after operation and homogenized in the same manner. Sham hepatectomies consisted of exposing the median and left lateral hepatic lobes, severing the suspensory ligaments, and returning the lobes to the abdominal cavity. The cytosol of each liver homogenate (18) was collected and contained more than 99% of the glyoxalase activity in the whole homogenate.

Glyoxalase activity was measured by a spectrophotometric assay developed in this laboratory (1) which relies on the quantitative conversion of residual methylglyoxal to a disemicarbazone adduct that absorbs UV light with a peak at 286 nm and A = 32,000. The sensitivity of the assay, together with the high catalytic rate of the glyoxalase system, obviates interference from endogenous materials in the cytosol that might absorb light at this wavelength. Furthermore, there is negligible interference from hepatic α-ketoaldehyde dehydrogenase which converts methylglyoxal to pyruvate (21) at a rate only 1/200 as great as the glyoxalase reaction.

Both endogenous hepatic glyoxalase activity (dependent on endogenous GSH plus other undetermined factors) and absolute hepatic glyoxalase activities (Vmax of glyoxalase assayed with optimum concentrations of substrate and 2 mM GSH) were assessed. Protein concentration was determined by the method of Lowry et al. (19), with crystalline bovine serum albumin as the standard. Protein-free supernatant was obtained by mixing

1 Recipient of USPHS Grant AM-03788. Present address: University Hospital, University of California, 225 West Dickinson Street, San Diego, Calif. 92103.
2 Recipient of American Cancer Society Institutional Grant 31-J-4. To whom requests for reprints should be addressed at the Department of Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn. 06510.

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all of the nonprotein hepatic sulfhydryl component was GSH, was detected during the 2-min reaction with DTNB. Practically lactoyl-GSH thiol ester, and hence GSH was equal to the glyoxalase 1 (Sigma Chemical Company, St. Louis, Mo., Type reacted with methylglyoxal in the absence of glyoxalase I. and less than 5% of the thiols in this fraction spontaneously thiol ester was slowly hydrolyzed at pH 8.0, but no hydrolysis difference between the 2 thiol determinations. Lactoyl-GSH thiol ester after 5 min at room temperature by 25 ¿ig of supernatant, all of the GSH was converted to lactoyl-GSH 200-jul aliquots of protein-free supernatant by reacting the as follows. Total sulfhydryl content was measured in 100- and glyoxalase activity was expressed as a percentage of change from activity obtained in the excised lobes at the time of partial hepatectomy. The mean activity of the control liver cytosol in the absence of added GSH was 0.21 (± 0.03) unit (¿¿moles of methylglyoxal converted to D-lactate/min/mg of protein), and with added GSH the mean was 1.30 (± 0.14) units. The p value was <0.01 at 25 hr with added GSH and at 12 and 42 hr in the absence of added GSH when sham and regenerating livers were compared to the control by the paired t test.

9 volumes of cytosol with 1 volume of 50% trichloroacetic acid and centrifuging for 10 min at 10,000 X g. Because the large absorbance at 240 nm by trichloroacetic acid in the supernatant prevented assay of GSH with glyoxalase I alone (23), GSH was determined with DTNB3 (13), and glyoxalase I as follows. Total sulfhydryl content was measured in 100- and 200-µl aliquots of protein-free supernatant by reacting the supernatant with DTNB for 2 min. In other aliquots of supernatant, all of the GSH was converted to lactoyl-GSH thiol ester after 5 min at room temperature by 25 µg of glyoxalase I (Sigma Chemical Company, St. Louis, Mo., Type 1) after first mixing in 0.6 ml of 0.3 M phosphate buffer, pH 7.4, and 0.6 µmole of methylglyoxal. The sulfhydryl content was then measured with DTNB, which does not react with lactoyl-GSH thiol ester, and hence GSH was equal to the difference between the 2 thiol determinations. Lactoyl-GSH thiol ester was slowly hydrolyzed at pH 8.0, but no hydrolysis was detected during the 2-min reaction with DTNB. Practically all of the nonprotein hepatic sulfhydryl component was GSH, and less than 5% of the thiols in this fraction spontaneously reacted with methylglyoxal in the absence of glyoxalase I.

3The abbreviations used are: GSH, reduced glutathione; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid).

RESULTS

The bottom of Chart 1 depicts the changes in endogenous glyoxalase activity of livers from sham- and partially hepatectomized rats 6, 12, 18, 25, 42, 49, and 72 hr after surgery. The endogenous activity remained essentially unchanged except at 12 hr when a 2-fold increase in activity occurred in both groups and at 42 hr when regenerating livers increased by 20% while the livers from the sham hepatectomy diminished by 30%.

Since maximum DNA synthesis and mitosis in regenerating rat liver would have been expected to occur about 20 and 28 hr after surgery, respectively (4, 5), increased endogenous glyoxalase activity precedes these key events by several hr. The second but smaller increase in glyoxalase activity which occurs in regenerating liver at 42 hr (Chart 1, bottom) also precedes by 6 hr a second but smaller anticipated wave of mitosis that has been described after partial hepatectomy in weanling rats. Furthermore, the increase in hepatic glyoxalase activity at 12 hr in sham-operated rats also precedes by about 24 hr the moderately enhanced DNA synthesis in the inflammatory cells and peripheral and capsular hepatocytes that occurs after the liver lobes have been handled (34).

Absolute levels of hepatic glyoxalase activity increased 40 to 50% in both sham- and partially hepatectomized animals at 25 hr (Chart 1, top), coinciding with the expected maximum mitotic activity that is observed in regenerating rat liver. Because optimum amounts of GSH (1, 22) were added to the incubation mixtures in these assays, it is possible that the concentration of the hepatic glyoxalase enzyme system increases after both surgical procedures. However, the increased glyoxalase activity could rather reflect the alteration of a fixed amount of the glyoxalase system by some undetermined factors which are manifested during mitosis or after the liver has been handled during sham hepatectomy.

Inasmuch as the levels of endogenous hepatic GSH influence endogenous glyoxalase activity (22), the concentration of this tripeptide in liver after sham and partial hepatectomy was determined (Chart 2). The 1.5- to 2-fold increase in hepatic
GSH parallels the doubling in endogenous glyoxalase activity at 12 hr in regenerating and sham-operated livers. However, thereafter in both regenerating and sham-operated livers GSH levels did not parallel the endogenous activity of glyoxalase. Clearly, other unknown factors must affect glyoxalase activity in addition to hepatic GSH.

DISCUSSION

Because sham and partial hepatectomies led to nearly identical increments in hepatic glyoxalase activity and GSH concentration, these changes appear related to operative stress. Whether they are also important in the control of hepatocyte division remains speculative, although, presumably in both instances, hepatic levels of the postulated cell growth inhibitor, methylglyoxal, might be diminished (9-11, 15, 28-30). However, several other biochemical phenomena observed in hepatic regeneration may also be stimulated by stressful procedures. For example, silica injections in rats enhance hepatic RNA and protein synthesis in increments comparable to those seen in early liver regeneration (31). In addition, DNA synthesis is accelerated and intensified in regenerating liver if rats are prestressed with an injection of silica particles or by simple laparotomy 4 to 8 hr before partial hepatectomy (6, 25). The increased synthesis of protein and nucleic acid synthesis in regenerating liver are apparently independent of the adrenocortical hormones, whereas the increases seen in the stressed animals receiving silica injections alone are abolished by adrenalectomy. Nevertheless, the role of the adrenal glands in liver regeneration remains uncertain since adrenocortical hormones inhibit and adrenalectomy enhances regeneration (4, 6). It has been suggested that some common biochemical pathways respond to either stress or preparation for cell division and that the irreversible pathways leading to mitosis become active only after liver loss (6). These same irreversible pathways may also be manifested in peripheral hepatocytes during manipulation and handling of the liver lobes since a moderately increased thymidine incorporation into DNA persists for 1 to 4 days after the liver has been treated in this manner (34). The hepatic glutathione-glyoxalase enzyme system may be one of these common pathways that responds to either stress or liver loss. Whether this system is essential for cell division is still uncertain, however, and more experimentation is required to clarify this question.

Our results confirm and extend 2 previous reports (7, 14) that noted identical increases in hepatic GSH content 6 and 24 hr after partial hepatectomy in the rat. However, another group of workers (35) reported a 13% decrease in GSH at 24 hr after hepatectomy. The reason for this discrepancy is unknown, but since GSH increases prior to cell division in other species (24, 26) as well, GSH may play a prominent role in the control of cell growth, possibly through regulation of glyoxalase activity. However, in some tumor-bearing animals, absolute hepatic glyoxalase activity increases during the initial growth period (27), whereas other results have shown that hepatomas (8) and human leukemic leukocytes (20) have depressed glyoxalase activity. Furthermore, the activity in some rat carcinomas is normal (33).

These differences in glyoxalase activity may be related in part to the time when assays were obtained since both glyoxalase and GSH may increase only in the initial stage of the regenerative process. This undoubtedly accounted for the failure of others to observe changes in glyoxalase activity in regenerating rat liver 7 days after hepatectomy (8). Furthermore, glyoxalase activity is influenced by the GSH concentration (Chart 1 and Ref. 32), where the activity of the control liver cytosol incubated in the absence of added GSH is 1/6 as great as the activity obtained with added optimum amounts of GSH. All previous glyoxalase assays (8, 20, 27) have been performed with reaction mixtures that contained optimum concentrations of added GSH, and thus alterations in the endogenous activity may have been overlooked.

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