treatment selectively enhanced the rate of 5'-cyanonicotine formation shows quite clearly that there is great potential for numerous drugs including ethanol to modify nicotine metabolism by selectively altering the flux of nicotine through its various metabolic pathways.

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Department of Environmental Health Sciences
Case Western Reserve University
School of Medicine
Cleveland, OH 44106, U.S.A.

G. DAVID MCCOY*
GEORGE J. DEMARCO

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* Author to whom all correspondence should be addressed.

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Role of hepatic glutathione and glucocorticoids in the regulation of hepatic cholesterol 7α-hydroxylase

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Hepatic microsomal cholesterol 7α-hydroxylase (CH-7A, EC 1.14.13.7) is the rate-limiting enzyme of bile acid biosynthesis from cholesterol [1]. CH-7A has an important role in cholesterol homeostasis since the conversion of cholesterol to bile acids is quantitatively a very important pathway of removal of cholesterol from the body [2].

Recently, Hassan *et al.* [3] provided evidence, for the first time, for a role of hepatic reduced glutathione (GSH) content in the regulation of CH-7A activity. They showed that acute changes in hepatic GSH content *in vivo* significantly affect CH-7A activity assayed *in vitro* [3]. Depletion of hepatic GSH (using diethylmaleate [DEM]) significantly reduces CH-7A activity, whereas partial repletion of GSH (using L-cysteine), in previously GSH-depleted animals, partially restores CH-7A activity, although it is still significantly less than that in GSH-replete controls [3]. Based on these observations, Hassan *et al.* [3] suggested that physiological variations in hepatic GSH content may regulate CH-7A activity. Since both hepatic GSH content and CH-7A activity are known to be sensitive to the feeding status of the animal [4, 5], then it may be hypothesized that there is a temporal link between feeding and subsequent changes in hepatic GSH content and CH-7A activity.

The objective of this study was to show that there is a direct relation between the feeding-related change in hepatic GSH content and hepatic CH-7A activity. Additionally, since glucocorticoids are known to affect CH-7A activity [6], the effect of adrenalectomy (ADX) on CH-7A activity was studied to determine if there were any glucocorticoid/GSH interactions.

Methods

Animals and treatment. Male, Sprague–Dawley rats weighing between 40 and 45 g were obtained from the Holtzman Rat Co. (Madison, WI) and adapted to a 10-hr light cycle (8:00 a.m. to 8:00 p.m.). Food was only available for 2 hr between 8:00 and 10:00 a.m., whereas water was

available *ad lib*. Seven days after adaptation to the above conditions, the following studies were conducted.

Study 1. On the day of the study, the animals were randomly assigned to one of two groups and injected intraperitoneally with either buthionine sulfoximine (BSO, 1 mg/g body weight in 0.9% saline, pH 8.5) or an equivalent volume of vehicle only. Subsequently, the animals were allowed to eat for 2 hr. Four hours after the start of feeding, the animals were decapitated; the livers were rapidly removed, rinsed in homogenizing buffer, and processed as described below.

Study 2. Seven days *following adaptation* to the lighting and feeding conditions, the animals were bilaterally adrenalectomized (ADX) under ketamine/xyalzine anesthesia (100 mg/kg body weight and 13 mg/kg body weight respectively) [7]. Subsequently, the animals received a daily subcutaneous injection of deoxycorticosterone acetate (75 μg/50 μl propylene glycol) and were given 0.9% saline to drink *ad lib*. The feeding pattern established prior to the surgery was maintained, and 7 days *following ADX* the animals were randomly assigned to one of two groups and treated with BSO or vehicle as described above. Subsequent treatment of the animals was similar to that described above.

Hepatic GSH content. Unless otherwise specified, all subsequent procedures were carried out at 0–4°. The liver was homogenized (30%, w/v) in 0.1 M phosphate buffer, pH 7.4, containing 5 mM $MgCl_2$, 30 mM nicotinamide, 1 mM EDTA, and 30 mM NaF, using a Potter–Elvehjem homogenizer equipped with a Teflon pestle. An aliquot of the homogenate (0.5 ml) was added to an equal aliquot of chilled 4% sulfosalicylic acid, mixed with a vortex mixer, and centrifuged at 1500 *g* for 20 min. An aliquot of the protein-free supernatant fraction was used for GSH determination using the glutathione *S*-transferase method described by Asaoka and Takahashi [8].

Hepatic CH-7A activity. The activity of hepatic microsomal CH-7A was assayed as described previously [9] based on the methods of Mitropoulos and Balasubramaniam [10] and Shefer *et al.* [11]. Microsomal protein was measured by the Hartree [12] modification of the procedure of Lowry *et al.* [13], and CH-7A activity was expressed as picomoles of 7α-hydroxycholesterol formed/mg microsomal protein × min.

Statistical methods. The values between different groups were compared using Student's *t*-test for unpaired means. A P value of <0.05 (two-tailed *t*-test) was considered as statistically significant.

Results

All animals adapted well to the study paradigm and doubled their body weights during the study period. The animals consumed sufficient food during the 2 hr allowed so that the stomachs were still quite full when they were killed. The ADX animals were carefully examined for any evidence of remnant adrenal tissue, and none was found. While plasma corticosterone was not assayed, the fact that remnant adrenal tissue was not found combined with the requirement for deoxycorticosterone acetate is strong evidence that ADX was complete.

From Fig. 1, it can be seen that, regardless of whether the animal was intact or ADX, BSO treatment prevented the feeding-related increase in hepatic GSH content. There was no difference between the hepatic GSH contents of similarly treated intact and ADX animals.

Hepatic CH-7A activity in the various groups is also shown in Fig. 1. Regardless of whether the animal was intact or ADX, CH-7A activity in the saline-treated animals was significantly greater than that in the corresponding BSO-treated animals. Thus, changes in hepatic GSH levels due to feeding were associated with changes in hepatic CH-7A activity. While the same pattern held true for hepatic CH-7A activity in ADX animals the absolute levels were reduced significantly relative to corresponding values in intact animals. Correlation analyses carried out to determine if hepatic CH-7A activity was related to hepatic GSH content revealed that the two were highly correlated ($r = 0.81, P < 0.002$).

Discussion

This study has, for the first time, provided evidence for the physiological role of hepatic GSH in the regulation of hepatic CH-7A activity. In addition, it has also provided information on the possible interaction between glucocorticoids and GSH in the regulation of hepatic CH-7A activity.

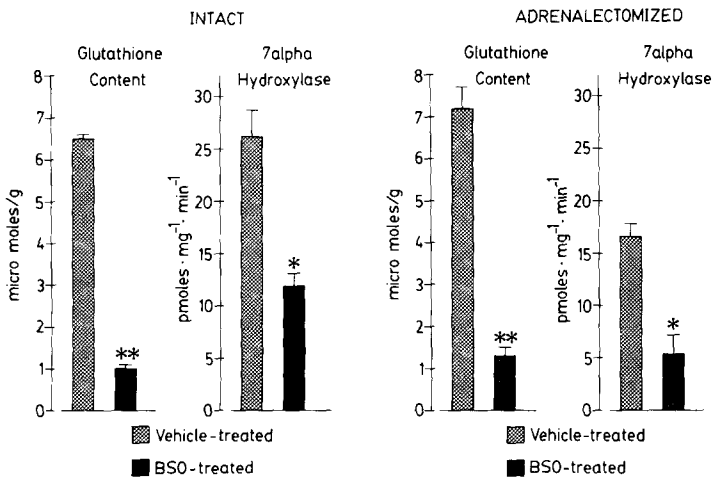

Fig. 1. Hepatic GSH content and CH-7A activity in vehicle or BSO-treated intact and ADX rats. The data are shown as the mean ± SEM of values from four to six animals in each group. Intact or ADX animals were adapted to being fed for 2 hr per day for 7 days prior to the study. On the day of the study, randomly selected animals were injected i.p. with either BSO (1 mg/g body wt) or vehicle, fed for 2 hr, and then killed 2 hr later for the assay of hepatic GSH content and CH-7A activity. Key: (*) significantly different from the value in corresponding vehicle-treated group, $P < 0.05$; and (**) significantly different from the value in corresponding vehicle-treated group, $P < 0.001$.

In the previous study by Hassan *et al.* [3], DEM was used to acutely *deplete* hepatic GSH content. Since there is concern that DEM may also result in other peroxidative damage [14] which might affect metabolic processes, BSO was used in the present study. BSO is a specific inhibitor of γ-glutamylcysteine synthetase [15] and does not have any effect on a number of other metabolic parameters [16]. From the results, it is evident that BSO effectively blocked the food-related increase in hepatic GSH content and that the effect of such an action on hepatic CH-7A activity was similar to that seen with DEM-mediated acute depletion of hepatic GSH. Since feeding-induced increase in hepatic GSH content is a physiological phenomenon [5], then the results of the present study suggest that hepatic GSH content has a physiological role in the regulation of hepatic CH-7A activity. It is now possible to account, at least in part, for the effects of feeding [4] and fasting [17] on hepatic CH-7A activity. Based on the results of the present study, it is reasonable to expect hepatic CH-7A activity to increase following feeding and to decrease upon fasting.

Although the hepatic content of glutathione disulfide (GSSG) was not estimated in the present study, the results are, nonetheless, in accord with the proposal of Gilbert [18] that "thiol/disulfide" status may act as an enzyme regulatory system *in vivo*. Since (a) changes in HMGCoA reductase (EC 1.1.1.34, the rate-limiting enzyme of cholesterol biosynthesis) activity are also linked to feeding [4] and (b) changes in CH-7A activity are often paralleled by changes in the activity of HMGCoA reductase, then the results of the present study support the recent suggestion that hepatic GSH/GSSG ratio may be involved in overall cholesterol homeostasis [19].

It is of interest to note that ADX did not alter the response of hepatic CH-7A to changes in hepatic GSH content. Thus, CH-7A activity was approximately 3-fold greater in the saline-treated ADX animals relative to that in BSO-treated ADX animals. This compares favorably with an approximate 2.2-fold difference in enzyme activity between saline-treated and BSO-treated intact animals. Since hepatic GSH content was similar in a given treatment group whether or not the animal was intact (Fig. 1), what is the basis for the difference in CH-7A activities between the intact and ADX animals? The simplest explanation for the difference in CH-7A activities between intact and ADX animals within a treatment group may lie in the fact that the true specific activity of CH-7A is not known since the content of CH-7A-specific CP-450 was not determined. It may be hypothesized that glucocorticoids regulate the *content* of CH-7A-specific CP-450 and that ADX reduces the content of this specific CP-450. Proof of this hypothesis will require isolation and purification of the specific CP-450 fraction, a direction currently being pursued in this laboratory.

In summary, this study has provided evidence that hepatic GSH levels probably have a physiological role in the more acute feeding-related regulation of the activity of CH-7A, whereas glucocorticoids may have a role in the regulation of the content of CH-7A.

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Department of Veterinary Biosciences
Division of Physiology
University of Illinois
Urbana, IL 61801, U.S.A.

ASLAM S. HASSAN*

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* Address all correspondence to: Aslam S. Hassan, Ph.D., Department of Veterinary Biosciences, University of Illinois, 2001 South Lincoln Ave., Urbana, IL 61801.