Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 4, pp. 491-497, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00320-3

The Effect of In Utero Administration of Buthionine Sulfoximine on Rat Development

EDWARD REYES,[1] STEPHANIE OTT, BARBARA ROBINSON AND REGINA CONTRERAS

Department of Pharmacology, University of New Mexico School of Medicine, Albuquerque, NM 87131

Received 4 June 1993

REYES, E., S. OTT, B. ROBINSON AND R. CONTRERAS. *The effect of in utero administration of buthionine sulfoximine on rat development.* PHARMACOL BIOCHEM BEHAV **50**(4) 491-497, 1995.—Glutathione (GSH) is a tripeptide that is thought to be an essential cell component playing an important role as a cellular antioxidant and scavenger of free radicals. GSH depletion has been shown to render cells more sensitive to various insults. GSH has a protective effect. GSH levels can be decreased by inhibition of its synthesis with buthionine sulfoximine (BSO), which inhibits γ-glutamylcysteine synthetase. Several studies have shown that treatment with BSO enhances the toxicity of some drugs and radiation. A previous study indicated that the effects of BSO on the developing embryo were short lived and did not persist to birth. In the above-mentioned study, mothers were treated with BSO only on days 10 and 11 of gestation. The objective of the present study was to determine the effects of BSO administration on GSH depletion throughout pregnancy on the developing rat. Timed pregnant Sprague–Dawley rats were placed on a liquid BioServ diet containing BSO starting on day 1 of pregnancy. The mothers received a daily dose of BSO ranging from 2 to 6 mmol/kg/24 h. The mothers were maintained on the diet until gestation day 21 when they were anesthetized with sodium pentobarbital and the pups delivered by Cesarean section. GSH levels were measured in brain and liver, and various parameters relating to development were assessed. A dose–response curve showed that a maximum depletion (86%) of GSH in the mother's liver was produced by the 6 mmol/kg dose of BSO. However, no change was seen in brain GSH levels of the mothers. GSH levels in brain and liver of the offspring were decreased by 60% and 66%, respectively. No significant effect of treatment with BSO was observed on growth-related parameters, such as body or brain weight. A significant decrease in neuron-specific enolase (NSE) activity in cerebellum and an increase in liver γ-glutamyl transpeptidase activity were observed in pups born to mothers treated with BSO. A decrease in NSE activity consistent with delayed development was observed. Therefore, we conclude that although a decrease in GSH may not produce obvious or observable teratogenic effects, it may produce a delay in development and may have a permissive role in teratogenic effects produced by other drugs by virtue of GSH depletion.

Development Brain Buthionine sulfoximine γ-Glutamyltranspeptidase Neuron-specific enolase Glutathione Teratogenic

GLUTATHIONE (GSH) is a tripeptide abundant in the cells of most organisms. GSH is a major nonprotein thiol associated with a number of critical cellular regulatory functions. GSH appears to have a vital role in maintance of health. The role of GSH deficiency in diseases has recently been reviewed (47). GSH may play a variety of roles including conjugation with electrophilic metabolites, reaction with oxidants, and free radical scavenging reactions. The cytosolic pool of GSH functions in microtubule assembly and as a cysteine reservoir during protein synthesis (44). It is important in protecting living cells against damage produced by many toxic xenobiotics (5,17). Increased intracellular GSH protects the cell against the toxic effects of a variety of drugs, against radiation, as well as from hyperbaric oxygen toxicity (15,39). GSH protects against potassium bromate-induced oxidative DNA damage as well as cytotoxicity in kidney (38). Endogenous brain GSH is an important factor in the defense mechanism against lesion formation after ischemia (25). Cells depleted of GSH are more

[1] To whom requests for reprints should be addressed.

prone to membrane damage than normal cells due to oxidative stress (16).

GSH has been implicated as a possible common link in events leading to abnormal growth (12). GSH can play an important role in the regulation of chemically elicited neural-tube abnormalities in the developing conceptus (11).

Fasting as well as drugs have been shown to decrease GSH levels in liver (6,41). L-Buthionine (SR)-sulfoximine (BSO) effectively lowers hepatic and renal GSH by specifically inhibiting γ-glutamylcysteine synthetase (γ-GCSyn), the rate-limiting enzyme for GSH synthesis (4,7,9). BSO appears to have few, if any, effects other than the depletion of GSH (9,48). Skeletal muscle degeneration (23) and potentiation of drug toxicities (13,26,36) following BSO treatment are a result of decreased levels of GSH. BSO appears to decrease GSH levels in brain only in young animals, but does not appear to decrease GSH in brain of adults (3). BSO depressed cell growth in cultures of heart cells (18).

In vitro evidence suggests that GSH modulates the response of the embryo to a variety of insults. When given to pregnant rats, BSO decreases GSH levels of fetal and maternal tissues, including the fetal liver (24). BSO treatment of mothers for short periods of time did not produce malformations in mice; however, when given at the time corresponding to fetal eye development, it caused cataracts (24). The depletion of GSH with BSO in whole embryo culture systems potentiated the embryotoxicity of some teratogens (42). BSO also caused a significant increase in incidence of malformed embryos and growth retardation in 10.5-day rat embryos (43).

It has been reported (10) that the treatment of pregnant rats with BSO on days 10 and 11 of gestation produced a decrease in the embryonic GSH concentration and malformations in the embryos when observed at day 12 of gestation. However, the malformations observed in day 12 embryos apparently do not persist during later development. The malformations observed at day 12 were, according to the authors, reversed apparently due to a little understood repair mechanism in the embryo (10). The study implies that BSO produces intrauterine death, but no effects were seen in litter size when pregnancy outcome was assessed on day 20 of gestation.

It was the purpose of the present study to further investigate the in vivo effects of glutathione depletion with BSO on the developing rat when it was given throughout gestation. We hypothesize that BSO will produce teratogenic effects due to decreases in GSH levels, which will be manifested by decreased body, brain, and liver weights in the pups (31,33–35).

METHOD

Chocolate-flavored liquid diet was purchased from BioServ (Frenchtown, NJ) and prepared to contain varying amounts of L-buthionine sulfoximine. L-Buthionine sulfoximine, γ-glutamyl-p-nitroanilide, L-α-aminobutyrate, 2-phospho-D-glycerate (sodium salt), and other chemicals were purchased from Sigma Chemical Co. (St. Louis). Other chemicals were purchased from Fisher Scientific.

Experimental Paradigm

Sprague–Dawley rats used in these experiments as breeding stock for the experimental paradigm were purchased from Harlan Labs, Indianapolis, IN. The females were approximately 90 days of age. The rats were maintained on a 12 L : 12 D cycle with lights on between 0700–1700 h and given Purina Breeder Blocks and water ad lib. Breeding was performed by placing one male in an individual hanging cage with two mature female rats at 1600 h. The females were examined at 0900 h for evidence of copulation. When copulation was confirmed, the female was assumed pregnant and placed in a separate cage and started on the appropriate diet. The liquid diet was formulated to contain 1000 kcal per liter. The diets contained BSO in the appropriate concentrations such that the mothers would receive the following dose in a 24-h period: 0, 2, 3, 4, or 6 mmol per kg. The mothers were adapted to the BSO diet over a period of a week by gradually increasing the concentration of BSO to ensure that they consumed adequate amounts of nutrients in the early stages of pregnancy. The 0, 2, 3, and 4 mmol groups were yoked to the 6 mmol group via a simultaneous pair-feeding system (BioServ, Frenchtown, NJ). Fresh liquid diet was provided daily at 1400 h. Each mother consumed an average of 100 ml of liquid diet per day. Another group of mothers (LC) received Purina Breeder Block chow and water ad lib and served as a control for the liquid diet. Three mothers per group were used. The limited number of mothers was due to the high cost of BSO required to treat a pregnant mother throughout gestation.

On gestational day 21, mothers were anesthetized using sodium pentobarbital (60 mg/kg IP) at approximately 1000 h and the pups delivered by Cesarean section. An abdominal incision was made to expose the uterine horns. The uterine horns were opened and the fetuses delivered, weighed, and brain and liver samples were obtained for analysis.

Glutathione Assay

Tissue samples (whole brain or whole liver) were homogenized in 2 ml of ice-cold perchloric acid (0.2 N) and centrifuged at 1000 × *g* for 10 min to remove protein precipitant. Glutathione levels in brain and liver were determined by the spectrophotometric method described by Tietze (46). Absorption of the samples at 412 nm were compared against a standard curve and the concentration of GSH in the sample determined and reported as mmoles reduced glutathione/g of tissue (wet weight). The standard curve was linear between 0.0015 and 0.050 mg/ml with a coefficient of correlation (*r*) of 0.99.

γ-Glutamyl Transpeptidase Assay

γ-GTP activity was determined by reaction rate kinetics as described by Rosalki and Tarlow (37) and Reyes and Prather (32), using a Beckman model DU40 spectrophotometer to measure the increase in absorbance produced by the formation of p-nitroaniline at 410 nm at 37°C. Tissue samples (whole brain or liver) were homogenized in Tris buffer containing 10% deoxycholic acid (DOC), refrigerated overnight, and centrifuged at 1000 × *g* for 10 min to produce a clear supernatant fluid. The supernatant fluid was assayed for γ-GTP activity. A typical reaction mixture was as follows: protein source 0.1 ml (0.4 to 0.7 mg), 5.4 mM γ-glutamyl-p-nitroanilide; 110.5 mM glycylglycine; and 92 mM Tris-HCl (pH 8.5), in a total volume of 2.0 ml. The reaction was stopped with 10% trichloroacetic acid (TCA). Protein was determined in brain and liver samples by the method described by Lowry et al. (19), using bovine serum albumin as standard. Activity is reported as units per mg protein. One unit of γ-GTP activity was defined as the amount of enzyme that will form 1 mmol of p-nitroaniline per minute.

γ-Glutamylcysteine Synthetase Assay

γ-Glutamylcysteine synthetase (γ-GCSyn) activity was determined in reaction mixtures containing 10 mM sodium gluta-

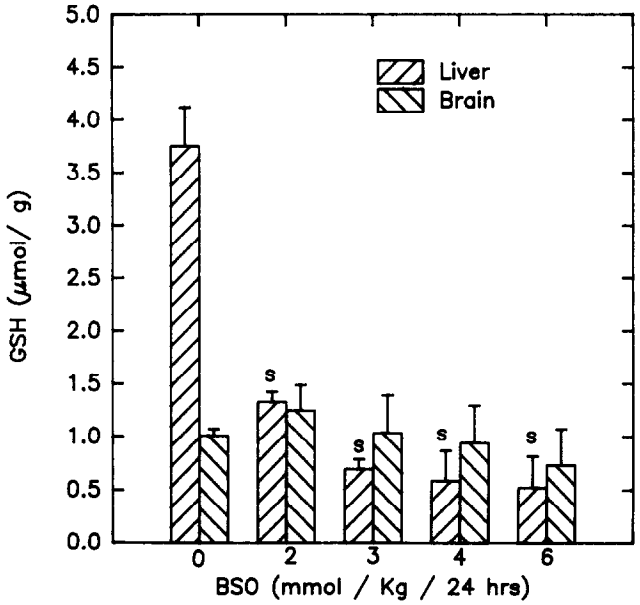

FIG. 1. Effects of BSO administration on GSH levels in liver and brain of pregnant rats. Values are presented as means with standard error (SE) bar. A significant difference from the control (0) at $p < 0.05$ is denoted by an s over the SE bar ($n = 6$ for the control group and 3 for each of the other treatment groups).

mate, 10 mM L-α-aminobutyrate, 20 mM $MgCl_2$, 5 mM Na_2ATP, 2 mM Na_2EDTA, 100 mM Tris-HCl buffer (pH 8.2), and 10 μg bovine serum albumin in a final volume of 1.0 ml, as described by Griffith et al. (8) and Sekura and Meister (40). The reaction was initiated by the addition of enzyme (0.5 to 0.9 mg). After incubation at 37°C for 20 min, the reaction was terminated by the addition of 0.5 ml of 10% TCA. The inorganic phosphate (Pi) formed was determined from absorbance at 720 nm, as described by Taussky and Shorr (45). Activity was determined from a standard curve and is reported in units/mg protein. One unit is that amount of enzyme that will form 1 mmol of Pi per minute.

Neuron-Specific Enolase Assay

Neuron-specific enolase (NSE) activity was determined by monitoring the formation of phosphenolpyruvic acid at 240 nm on a Beckman model DU40 spectrophotometer as described by Marangos et al. (20–22) and Baranowski and Wolna (2). Nonneuronal enolase activity was removed by heat treatment (28). The assay mixture contained 43.3 mM Tris buffer (pH 6.8), 0.5 mM KCl, 3.0 mM $MgSO_4$, 1.0 mM 2-phospho-D-glycerate (sodium salt), and enzyme (0.19 to 0.23 mg) in a total volume of 3.0 ml at 25°C. The enzyme reaction was initiated by the addition of 50 ml of a 20% brain homogenate to the reaction mixture and the initial velocity determined. The cerebellum was removed from the rest of the brain and each section was assayed for NSE activity. NSE activity is expressed as units per mg protein. One unit is that amount of enzyme that will form 1 mmol of product per minute.

Statistical Analysis

Data are presented as the mean ± SE, and analyzed by one-way analysis of variance (ANOVA) with BSO dose as the independent variable. A Newman-Keuls post hoc test was used to determine which groups differed from the control group. Two pups from each of three litters were used for determinations of GSH levels, γ-GTP, γ-GCSyn, and NSE activities. The use of an equal number of pups from each litter in the analysis prevents the inappropriate influence of an abnormal litter as suggested by Abel (1). In determinations of other values, such as pup body, brain, and liver weights, all the pups from a litter were used and the data was analyzed using a nested design.

RESULTS

The effects of BSO administration on GSH levels in liver and brain of pregnant mothers is shown in Fig. 1. GSH levels are reported as mmol per g of wet tissue. Pair-fed control levels in liver and brain were determined to be 3.75 and 1.01 mmol per gram, respectively. The levels of GSH in brain were not altered by the daily consumption of BSO in up to 6 mmol per kg. BSO produced a significant, $F(4, 13) = 20.0$, $p < 0.05$, dose-dependent decrease in liver GSH levels. Newman-Keuls post hoc tests indicated that all doses of BSO used were effective in lowering GSH levels in liver. An 86% decrease in GSH was produced by 6 mmol BSO.

γ-Glutamylcysteine synthetase was measured in the liver and brain of the mothers and found not to differ significantly between the various treatment groups. Control values in liver and brain were found to be 13.16 and 17.66 units per mg protein, respectively. No differences were found in γ-GTP activity in brain or liver of mothers in the various treatment groups.

The BSO treatment had no effect on the litter size, $F(4, 13) = 0.25$. Litter sizes of 11.3, 10.7, 13.3, 13.3, and 13.0 were

TABLE 1

CHARACTERISTICS OF OFFSPRING BORN TO RATS TREATED WITH L-BUTHIONINE SULFOXIMINE THROUGHOUT GESTATION

	L-Buthionine Sulfoximine, mmol/kg/24						
Characteristic	LC(6)	0(3)	2(3)	3(3)	4(3)	6(3)	*F*-Value
Body weight, g	3.59 ± 0.04	3.16 ± 0.05	3.10 ± 0.08	3.20 ± 0.05	3.25 ± 0.05	3.36 ± 0.06	(4,12) = 0.54*
Brain weight, mg	170.88 ± 1.88	170.77 ± 2.33	174.34 ± 2.91	174.10 ± 2.38	171.95 ± 3.29	172.87 ± 2.93	(4,12) = 0.24*
Liver weight, mg	244.67 ± 4.80	189.63 ± 5.48	179.22 ± 6.33	178.20 ± 4.14	181.87 ± 5.48	189.18 ± 5.65	(4,12) = 0.37*
Litter size	12.66 ± 0.95(6)	11.33 ± 2.53(6)	10.67 ± 4.71(3)	13.33 ± 1.47(3)	13.33 ± 1.47(3)	13.00 ± 1.23(3)	(4,13) = 0.25†

Values are presented as means of litter means ± SE. Values in parenthesis represent the number of litters used.
*Statistical analysis by nested analysis of variance design.
†Statistical analysis by one way ANOVA.

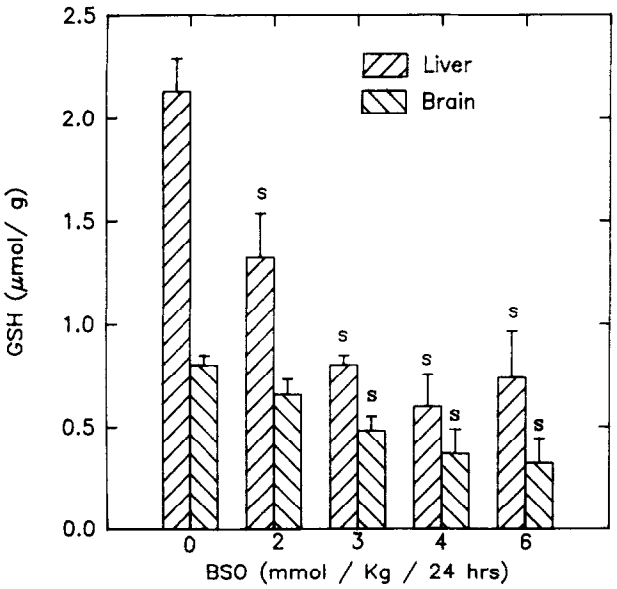

FIG. 2. Effects of in utero administration of BSO on GSH levels in liver and brain of g21-day-old rats. Values are presented as means with SE bar. A significant difference from the control (0) at $p < 0.05$ is denoted by an s over the SE bar ($n = 10$ for the control group and 6 for each of the other treatment groups).

found for the groups treated with 0, 2, 3, 4, and 6 mmol BSO, respectively.

The effects of the in utero administration of BSO on body, brain, and liver weights are presented in Table 1. The in utero administration of BSO did not produce a significant, $F(4, 12) = 0.54$, $p < 0.05$, effect on the body weight of the pups at gestational age 21 days. The pups of the mothers treated with 6 mmol BSO were heavier than those of the other treatment groups. However, there was no difference between the LC pups and the pups from the mothers treated with 6 mmol of BSO. There were also no effects on brain and liver weights observed.

The effects of in utero administration of BSO on liver and brain GSH of offspring is presented in Fig. 2. Values are reported in mmol of GSH per g of wet tissue weight. A statistically significant, $F(4, 28) = 14.84$, $p < 0.05$, effect of BSO dosage was produced. Newman-Keuls post hoc test showed that all four treatment groups had lower levels of GSH than did the control group. The greatest effect was produced by 4 mmol of BSO, which produced a 72% decrease.

Also shown in Fig. 2 are the effects of BSO on brain GSH levels. There was a statistically significant, $F(4, 28) = 6.62$, $p < 0.05$, decrease in GSH produced by the in utero administration of BSO. The 2 mmol dose of BSO did not differ from the control, although higher doses of BSO did differ statistically from the pair-fed control. A maximum decrease of 60% was produced by the 6 mmol dose.

Figure 3 shows the effects of in utero administration of BSO on γ-GTP in liver and brain of the offspring. Values are reported as munits of γ-GTP activity per mg protein. γ-GTP activity in liver was increased by the administration of BSO, $F(4, 27) = 3.65$, $p < 0.05$. The 2 and 3 mmol doses of BSO produced an increase in γ-GTP activity, although it was not statistically significant. The 6 mmol BSO dose produced a greater than 100% increase in activity over the control, from 22.4 munits to 55.1 munits.

Figure 3 also shows that brain γ-GTP activity was not affected by BSO. The 6 mmol dose of BSO produced only a

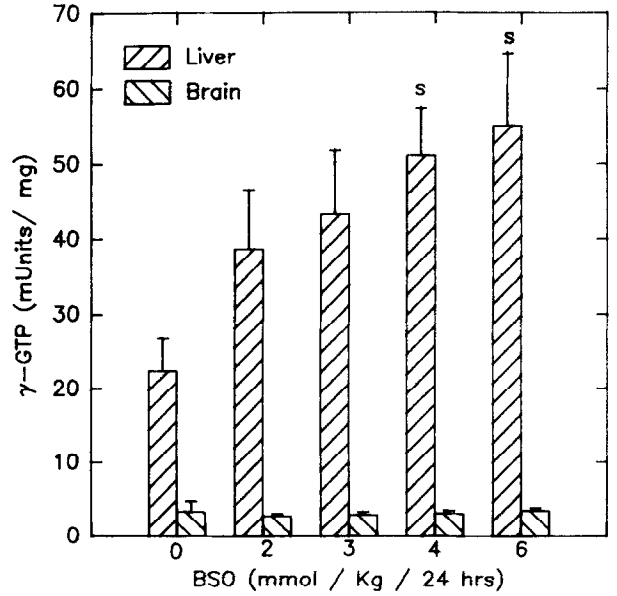

FIG. 3. Effects of in utero administration of BSO on γ-GTP in liver and brain of g21-day-old rats. One unit of γ-GTP activity is defined as the amount of enzyme that will form on μmol p-nitroaniline per minute. Values are presented as means with SE bar. A significant difference from the control (0) at $p < 0.05$ is denoted by an s over the SE bar ($n = 10$ for the control group and 6 for each of the other treatment groups).

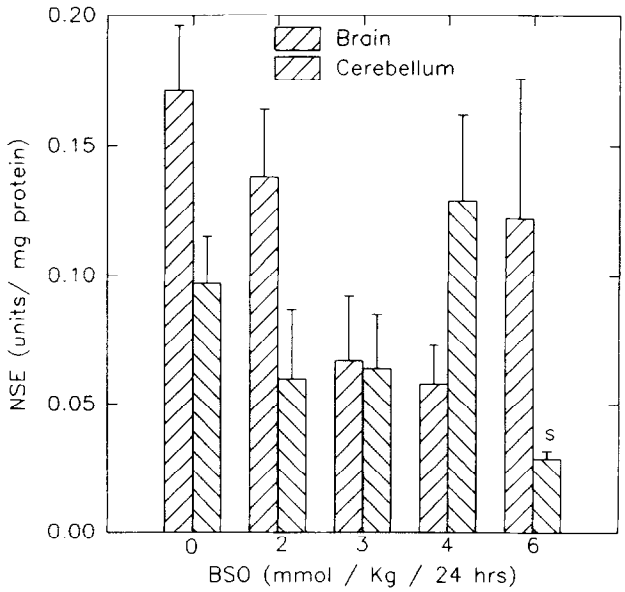

FIG. 4. Effects of in utero administration of BSO on NSE activity in cerebellum and brain of g21-day-old rats. Values are presented as means with SE bar. A significant difference from the control (0) at $p < 0.05$ is denoted by an s over the SE bar ($n = 10$ for the control group and 6 for each of the other treatment groups).

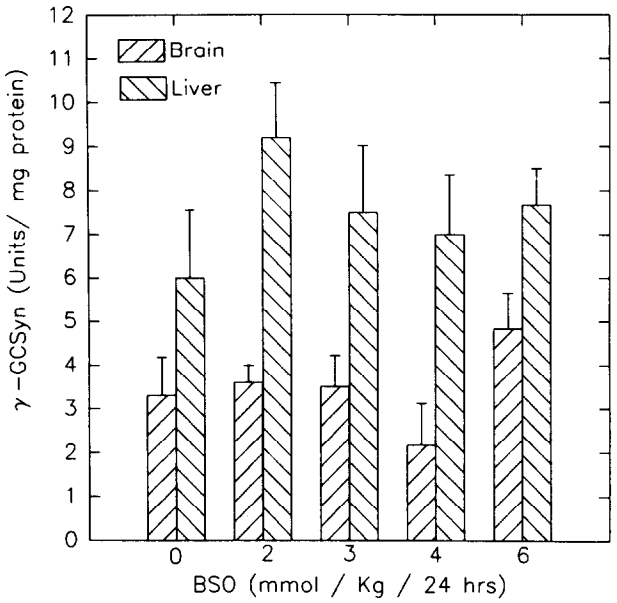

FIG. 5. Effects of in utero administration of BSO on γ-GCSyn in brain and liver of g21-day-old rats. One unit of γ-GCSyn activity is definded as the amount of enzyme that will form one μmol of Pi per minute per mg protein. Values are presented as means with SE bar. A significant difference from the control (0) at $p < .05$ is denoted by an s over the SE bar ($n = 10$ for the control group and 6 for each of the other treatment groups).

slight increase in γ-GTP activity from that seen in the pair-fed control.

The effects of in utero administration of BSO on NSE activity is seen in Fig. 4. Values are reported as units per mg protein. BSO produced a decrease in NSE activity in brain (with cerebellum removed) although not statistically significant. Lack of statistical significance may be due, in part, to an increase in NSE activity produced by the 6 mmol dose of BSO resulting in a U-shaped dose-response curve. In the cerebellum, a statistically significant decrease in NSE activity was produced, $F(4, 28) = 3.03$, $p < 0.05$. Post hoc analysis showed that the only significant difference was between the 4 and 6 mmol treatment groups. The 6 mmol dose of BSO produced a 30% decrease in NSE activity from control in cerebellum, $t(14) = 2.8$, $p < 0.05$.

Figure 5 shows the effects of the in utero administration of BSO on brain and liver γ-GCSyn activity. γ-GCSyn activity in brain ranged from 2.17 to 4.83 units. The pups from the group treated with 4 mmol of BSO had the lowest activities of γ-GCSyn (2.17 units), while the pups from the mothers treated with 6 mmol had the highest activity (4.83 units). γ-GCSyn activity in liver was higher than that in brain, and ranged from 6.00 to 9.20 units. BSO had no statistically significant effect on liver or brain γ-GCSyn activity.

DISCUSSION

The daily administration of BSO in doses from 2 to 6 mmol per kg produced a decrease in liver GSH levels in the mothers to approximately 14% of control. Even the lowest dose of BSO (2 mmol) produced a significant decrease in GSH levels. Brain levels of GSH were not affected. This is consistent with previous studies (7,9,14), and may be due to the fact that BSO does not cross the blood-brain barrier (3).

The in utero administration of BSO produced a dose-dependent depletion of GSH in brain and liver of the offspring. A 60% decrease in brain GSH was produced by the 6 mmol dose of BSO. The 4 mmol BSO dose produced a 72% decrease in liver GSH levels. The administration of BSO throughout gestation produced a greater decrease of liver GSH than did the one time treatment on day 12 of gestation (10), or a 4 day treatment at various times during gestation (24).

The administration of BSO to pregnant rats produced no effects on γ-GCSyn, the rate-limiting enzyme associated with synthesis and metabolism of GSH, in the mothers. Several reasons for this are possible. It is conceivable that the effects of BSO on γ-GCSyn were obliterated by the addition of buffer and deoxycholic acid to the liver samples to make them ready for in vitro assay. Interference from other ADP-forming systems on the enzyme assay may have contributed to a lack of observable effects of BSO on γ-GCSyn in the mothers (27). Certainly, the decreased GSH levels in liver indicate that BSO inhibited γ-GCSyn in the mothers. γ-GTP activity, a key enzyme in the metabolism of GSH, in the mothers was not affected by BSO. Aside from decreasing GSH levels in liver, BSO had no apparent affect on the mothers.

BSO treatment of the mothers produced a decrease in GSH levels in both liver and brain of gestational day 21 pups. Although BSO does not cross the blood-brain barrier in an adult, BSO is apparently able to enter the brain when administered in utero.

No effects of BSO treatment were seen on litter size, body weight, brain weight, or liver weight of the offspring. BSO did not appear to have produced teratogenic effects in these animals, as defined by alteration of the parameters measured and gross examination. This is similar to results obtained in mice, where the only observable abnormalities were lens epithelial cell damage (24). We did not examine the pups for lens epithelial cell damage.

Because NSE activity has been associated with the differentiation and functional maturation of neuronal cells in rat brain (22), the decrease in NSE activity in cerebellum may represent a delay in the development of the offspring (22). This finding agrees with the delay in the development of a number of the parameters that made up the morphological score seen in rats exposed to BSO on day 10 and 11 of gestation (10). Decreased NSE activity may be a more sensitive parameter to measure than brain weight, to ascertain effects on development. As suggested by Rajpert-De Meyts et al. (29), γ-GTP activity may be increased to respond to decreased levels of GSH and to provide protection against GSH depletion. The altered γ-GTP activity, at a time when the enzyme activity is changing rapidly, may also reflect an alteration of development (30).

BSO in and of itself produced no teratogenic effects. This finding, however, is important because it makes it possible to use BSO in studies that investigate the role of GSH in mechanisms of teratogenesis. If GSH is involved in the teratogenic mechanism of a particular drug, the depletion of GSH by BSO administration should result in a shift to the left of the drug's teratogenic dose response curve.

ACKNOWLEDGEMENTS

This study was supported in part by NIAAA AA08072 and MBRS Program Grant RR 08139.

REFERENCES

1. Abel, E. L. Behavioral teratology of alcohol. Psychol. Bull. 90: 564-581; 1981.
2. Baranowski, T.; Wolna, E. Enolase from human muscle. In: Anonymous, ed. Methods in enzymology. New York: Academic Press; 1975:335-338.
3. Bien, E.; Vick, K.; Skorka, G. Effects of exogenous factors on the cerebral glutathione in rodents. Arch. Toxicol. 66:279-285; 1992.
4. Campbell, E. B.; Hayward, M. L.; Griffith, O. W. Analytical and preparative separation of the diastereomers of L-buthionine (SR)-sulfoximine, a potent inhibitor of glutathione biosynthesis. Anal. Biochem. 194:268-277; 1991.
5. Chasseud, L. F. The role of glutathione and glutathione-s-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res. 29:175-274; 1979.
6. Gallagher, E. P.; Hasspieler, B. M.; Di Giulio, R. T. Effects of buthionine sulfoximine and diethyl maleate on glutathione turnover in the channel catfish. Biochem. Pharmacol. 43:2209-2215; 1992.
7. Griffith, O. W. Mechanism of action, metabolism and toxicity of buthioninesulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J. Biol. Chem. 257:13704-13712; 1982.
8. Griffith, O. W.; Larsson, A.; Meister, A. Inhibition of c-glutamylcysteine synthetase by cystamine; an approach to a therapy of 5-oxoprolinuria (pyroglutamic aciduria). B.B.R.C. 79: 919-925; 1977.
9. Griffith, O. W.; Meister, A. Glutathione: Interorgan translocation turnover and metabolism. Proc. Natl. Acad. Sci. USA 76: 5606-5610; 1979.
10. Hales, B. F.; Brown, H. The effect of in vivo glutathione depletion with buthionine sulfoximine on rat embryo development. Teratology 44:251-257; 1991.
11. Harris, C.; Stark, K. L.; Juchau, M. R. Glutathione status and the incidence of neural tube defects elicited by direct acting teratogens in vitro. Teratology 37:577-590; 1988.
12. Harris, J. E. Hepatic glutathione, metallothionein and zinic in the rat on gestational day 19 during chronic ethanol administration. J. Nutr. 120:1080-1086; 1990.
13. Ishikawa, M.; Sasaki, K.; Takayanagi, Y. Injurious effect of buthionine sulfoximine, an inhibitor of glutathione biosynthesis, on the lethality and urotoxicity of cyclophosphamide in mice. Jpn. J. Pharmacol. 51:146-149; 1989.
14. Kocak-Toker, N.; Mutlu-Turkoglu, U.; Alptekin, N.; Uysal, M. Effect of depletion of glutathione by buthionine sulfoximine on lipid peroxidation in rats acutely treated with ethanol. Int. J. Biochem. 25:1765-1768; 1993.
15. Komadina, K. H.; Duncan, C. A.; Bryan, C. L.; Jenkinson, S. G. Protection from hyperbaric oxidant stress by administration of buthionine sulfoximine. J. Appl. Physiol. 71:352-358; 1991.
16. Kosower, N. S.; Kosower, E. M. The glutathione status of cells. Int. Rev. Cytol. 54:109-160; 1978.
17. Larsson, P. ; Tjälve, H. Binding of aflatoxin B1 metabolites in extrahepatic tissues in fetal and infant mice and in adult mice with depleted glutathione levels. Cancer Res. 52:1267-1277; 1992.
18. Lewko, W. M. Glutathione levels in cultured heart cells influence of buthionine sulfoximine an inhibitor of glutathione synthesis. Biochem. Pharmacol. 36:219-223; 1987.
19. Lowry, D. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 192:265-275; 1951.
20. Marangos, P. J.; Schmechel, D. E. Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. Annu. Rev. Neurosci. 10:269-295; 1987.
21. Marangos, P. J.; Zomzely Neurath, C.; York, C. Determination and characterization of neuron specific protein (NSP) associated enolase activity. Biochem. Biophys. Res. Commun. 68:1309-1316; 1976.
22. Margangos, P. J.; Schmechel, A. M.; Goodwin, F. K. Developmental profile of neuron specific (NSE) and non-neuronal (NNE) enolase. Brain Res. 190:185-193; 1980.
23. Martensson, J.; Meister, A. Mitochondrial damage in muscle occurs after marked depletion of glutathione and is prevented by giving glutathione monoester. Proc. Natl. Acad. Sci. USA 86: 471-475; 1989.
24. Martensson, J.; Steinherz, R.; Jain, S.; Meister, A. Glutathione ester prevents buthionine sulfoximine-induced cataracts and lens epithelial cell damage. Proc. Natl. Acad. Sci. USA 86:8727-8731; 1989.
25. Mizui, T.; Kinouchi, H.; Chan, P. H. Depletion of brain glutathione by buthionine sulfoximine enhances cerebral ischemic injury in rats. Am. J. Physiol. 262:H313-H317; 1992.
26. Mizutani, T.; Satoh, K.; Nomura, H.; Nakanishi, K. Hepatotoxicity of eugenol in mice depleted of glutathione by treatment with DL-buthionine sulfoximine. Res. Commun. Chem. Pathol. Pharmacol. 71(2):219-230; 1991.
27. Nardi, G.; Cipollaro, M.; Loguercio, C. Assay of gamma-glutamylcysteine synthetase and glutathione synthetase in erythrocytes by high-performance liquid chromatography with fluorimetric detection. J. Chromatogr. Biomed. Appl. 530:122-128; 1990.
28. Neumann, M.; Wilisch, A.; Diddens, H.; Probst, H.; Gekeler, V. MDR hamster cells exhibiting multiple altered gene expression: Effects of dexniguldipine-HCl (B859-35), cyclosporin A and buthionine sulfoximine. Anticancer Res. 12:2297-2302; 1992.
29. Rajpert-De Meyts, E.; Shi, M.; Chang, M.; Robison, T. W.; Groffen, J.; Heisterkamp, N.; Forman, H. J. Transfection with gamma-glutamyl transpeptidase enhances recovery from glutathione depletion using extracellular glutathione. Toxicol. Appl. Pharmacol. 114:56-62; 1992.
30. Reyes, E. The effects of prenatal alcohol exposure on γ-glutamyl transpeptidase. In: Watson, R. R., ed. Drug and alcohol abuse review: Liver pathology and alcohol. Totowa: Humana Press; 1991:117-132.
31. Reyes, E.; Ott, S.; Robinson, B. Effects of in utero administration of alcohol on glutathione levels in brain and liver. Alcohol.: Clin. Exp. Res. 17:877-881; 1993.
32. Reyes, E.; Prather, E. E. Subcellular and regional localization of γ-glutamyl transpeptidase in sheep brain. Neurochem. Res. 1: 251-259; 1976.
33. Reyes, E.; Rivera, J. M.; Saland, L. C.; Murray, H. M. Effects of maternal administration of alcohol on fetal brain development. Neurobehav. Toxicol. Teratol. 5:263-267; 1983.
34. Reyes, E.; Wolfe, J.; Savage, D. D. The effects of prenatal alcohol exposure on radial arm maze performance in adult rats. Physiol. Behav. 46:45-48; 1989.
35. Robinson, S. R.; Smotherman, W. P. The emergence of behavioral regulation during fetal development. Ann. NY Acad. Sci. 662:53-83; 1992.
36. Ronis, M. J. J.; Huang, J.; Crouch, J.; Mercado, C.; Irby, D.; Valentine, C. R.; Lumpkin, C. K.; Ingelman-Sundberg, M.; Badger, T. M. Cytochrome P450 CYP 2E1 induction during chronic alcohol exposure occurs by a two-step mechanism associated with blood alcohol concentrations in rats. J. Pharmacol. Exp. Ther. 264:944-950; 1993.
37. Rosalki, S. B.; Tarlow, D. Optimized determination of γ-glutamyltransferase by reaction rate analysis. Clin. Chem. 20: 1121-1124; 1974.
38. Sai, K.; Umemura, T.; Takagi, A.; Hasegawa, R.; Kurokawa, Y. The protective role of glutathione, cysteine and vitamin C against oxidative DNA damage induced in rat kidney by potassium bromate. Jpn. J. Cancer Res. (Amsterdam) 83:45-51; 1992.
39. Sanders, A. P.; Currie, W. D.; Woodhall, B. Protection of brain metabolism with glutathione, glutamate, gamma-aminobutyrate and succinate. Proc. Soc. Exp. Biol. Med. 130:1021-1027; 1969.
40. Sekura, R.; Meister, A. γ-Glutamylcysteine synthetase. JBC 252: 2599-2604; 1977.
41. Shimizu, M.; Morita, S. Effects of fasting on cadmium toxicity, glutathione metabolism, and metallothionein synthesis in rats. Toxicol. Appl. Pharmacol. 103:28-39; 1990.

42. Slott, V. L.; Hales, B. F. Enhancement of the embryotoxicity of acrolein, but not phosphoramide mustard by glutathione depletion in rat embryos in vitro. Biochem. Pharmacol. 36:2019–2025; 1987.
43. Slott, V. L.; Hales, B. F. Effect of glutathione depletion by buthionine sulfoximine on rat embryonic development in vitro. Biochem. Pharmacol. 36:643–688; 1987.
44. TateishiI, N.; Higashi, T.; Naruse, A.; Shiozaki, H.; Sakamoto, Y. Rat liver glutathione: Possible role as a reservoir of cysteine. J. Nutr. 107:51–57; 1974.
45. Taussky, H. H.; Shorr, E. A Microcolorimetric method for the determiniation of inorganic phosphorus. JBC 202:675–685; 1953.
46. Tietze, F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Anal. Biochem. 27:502–522; 1969.
47. White, A. C.; Thannickal, V. J.; Fanburg, B. L. Glutathione deficiency in human disease. J. Nutr. Biochem. 5:218–226; 1994.
48. Wu, D.; Griffith, O. W.; Reidenberg, M. M. Lack of effect of glutathione depletion by L-buthionine-S,R-sulfoximine on gentamicin nephrotoxicity in rats. Pharmacology 40:250–257; 1990.