late 5-HT influx by permitting a faster return of the carrier to the outside of the membrane; K^+ may act by altering the conformation of the carrier and/or the outward directed K^+ efflux may provide part of the energy for the movement of the carrier. The failure to stimulate 5-HT influx in normal platelets following K^+ loading may indicate that in this case the K^+-induced return of the carrier is operating at maximum efficiency and, therefore, not influenced by increasing the internal K^+ concentration.

Our results suggest that a component of the functional impairment of 5-HT transport seen in D.S. platelets is due to disturbances in the Na^+ and K^+ gradients and that temporarily increasing the intracellular K^+ concentration restores functional integrity to the membrane carrier.

Acknowledgements—This work was supported by a grant from the Canadian Medical Research Council. J. M. Sneddon is in receipt of an Alberta Heritage Medical Research Visiting Scientist Award.

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Biochemical Pharmacology, Vol. 32, No. 11, pp. 1795–1798, 1983.
Printed in Great Britain.

0006–2952/83 $3.00 + .00

Effect of prolonged alcohol administration on activities of various enzymes scavenging activated oxygen radicals and lipid peroxide level in the liver of rats

(*Received* 4 *August* 1982; *accepted* 2 *December* 1982)

Many studies have demonstrated that biochemical changes as found in humans with alcoholic liver injury occur in the liver of animals treated with alcohol for a long period [1, 2]. It has been suggested that lipid peroxidation among the biochemical changes is involved in liver damage due to chronic alcohol ingestion [3, 4]. MacDonald [5] showed that the activities of hepatic glutathione peroxidase (GSH peroxidase) and glutathione reductase (GSSG reductase) were enhanced with increases in hepatic mitochondrial and microsomal lipid peroxide contents in rats following chronic alcohol administration, implying the involvement of activated oxygen radicals formed in alcohol metabolism in lipid peroxidation. Hence, the level of lipid peroxides in the liver of alcohol ingesting animals can be considered to vary with the balance between the potential to form activated oxygen radicals and the capacity to remove the activated oxygen radicals and lipid peroxides.

The present study was undertaken to clarify the relationship between the generation of activated oxygen radicals and the accumulation of lipid peroxides in the liver of rats given 20% alcohol as drinking water for 4 weeks. We determined the contents of hepatic lipid peroxide and reduced glutathione (GSH), which participates in decomposing hydrogen peroxide (H_2O_2) and lipid peroxides [6] and scavenging superoxide anions (O_2^-) [7], and the changes in activities of various enzymes to produce or to eliminate activated oxygen radicals closely related to the formation of lipid peroxides.

Materials and methods

Male Wistar rats weighing about 250 g were separated in two groups. The diets were closely controlled and consisted of Oriental M powder diet (Oriental Yeast Co. Ltd.) (3.7 kcal/g) which contained 55% carbohydrate, 24% protein and 5% lipids. The contents of vitamin E, vitamin C, choline, cystine and methionine in this diet were 0.006, 0.005, 0.1, 0.3 and 0.3%, respectively. Both groups of rats were maintained on this powder diet for one week before used and during the period of the experiment, four weeks. One group of rats received 20% ethanol as the only source of drinking water; the second one received an isocaloric amount of glucose in its drinking water, throughout the period in which the experiment was performed. The ingestion of ethanol amounted to 9.4 ± 1.1 g/kg body weight per day ($n = 10$). In this manner, the experimental animals consumed 30% of their total daily calories in the form of ethanol. Totally they gained 203.1 ± 43.9 kcal/kg per day ($n = 10$), whereas the control rats gained 220.3 ± 42.5 kcal/kg per day ($n = 5$). There was no difference in body weight gain or liver weight between control and alcohol-treated rats. Both groups of rats were starved for 15 hr prior to sacrifice. Liver were removed, washed and homogenized in 4 vol. of ice-cold 0.15 M KCl. The homogenates were centrifuged at 10,000 *g* for 20 min, and then the postmitochondrial supernatants were recentrifuged at 105,000 *g* for 60 min to prepare microsomes and soluble

fractions. The remaining microsomal pellets were washed once with 0.15 M KCl.

Lipid peroxide content in the liver homogenate or its microsomes was estimated by the method of Ohkawa *et al.* [8] using malondialdehyde (MDA) as a standard. The measurement of hepatic GSH content was performed with 5,5-dithiobis-(2-nitrobenzoic acid) as described by Beutler *et al.* [9]. Superoxide dismutase (SOD) [10], catalase [11], GSH peroxidase [12], GSSG reductase [13] and glucose-6-phosphate dehydrogenase (G-6-P dehydrogenase) [14] in the soluble fraction were assayed at 37°C using Beckman model 25 spectrophotometer. The soluble fraction was dialyzed against 50 mM Tris–HCl buffer, pH 7.5, overnight before used for SOD assay. Microsomal NADPH oxidase activity was followed by formation of H_2O_2 as described by Werrigloer [15]. H_2O_2 formed was determined by the ferrithiocyanate method [16]. NADPH-cytochrome *c* reductase was assayed by the method of Takesue and Omura [17]. Microsomal NADPH-dependent-and ascorbic acid-dependent lipid peroxidation reactions were followed by formation of MDA. MDA formed was measured as described by Beuge and Aust [19]. Protein was determined by the method of Lowry *et al.* [20]. All chemicals used were of reagent grade.

Results and discussion

The liver homogenates from rats given 20% alcohol as drinking water for 4 weeks contained 1.5 times more lipid peroxide than those from control rats (Table 1). This is in agreement with the previous studies in which prolonged alcohol treatment caused increased lipid peroxidation in liver tissues, including their microsomes and mitochondria [4, 5, 20–22]. The content of hepatic GSH in the alcohol-treated rats, also, was 1.5 times higher than that in the controls (Table 1). These findings indicate that an increase in lipid peroxide content does not necessarily lead to GSH depletion in the liver of rats chronically treated with alcohol.

Then, we determined the activities of various enzymes taking part in the elimination of activated oxygen radicals or lipid peroxides. The activity of hepatic cytosolic SOD, which dismutates superoxide anions to hydrogen peroxide and molecular oxygen to protect the tissue against oxygen toxicity [23], was decreased by 25% of control value, whereas the activity of hepatic cytosolic catalase, an enzyme to decompose H_2O_2, remained unchanged in this condition (Table 1). Table 1 also presents the activities of GSH peroxidase, GSSG reductase and G-6-P dehydrogenase in the liver cytosols of control and alcohol-treated animals. The chronic alcohol treatment had no effect on the activities of GSH peroxidase and GSSG reductase as a detoxifying system decomposing H_2O_2 and lipid peroxides to inactive metabolites [6, 24]. But, there was a 35% decrease in the activity of G-6-P dehydrogenase, which plays a physiological role in supplying NADPH for cytosolic GSSG reductase [25] and microsomal mixed-function oxidase system [26], in the alcohol-treated rats when compared with that in the controls.

Besides, we determined lipid peroxide content and the activities of NADPH-dependent lipid peroxidation and NADPH-cytochrome c reductase in the liver microsomes from both groups of rats. The results are shown in Table 2. The content of microsomal lipid peroxide in the alcohol-treated group was 1.4 times higher than that in the control group. This finding indicates that microsomal lipid peroxide formation contributes to an increase in hepatic lipid peroxide level due to prolonged alcohol ingestion. In contrast, there were no significant differences in the activities of NADPH-dependent lipid peroxidation, an enzymatic process [27], and NADPH-cytochrome *c* reductase, which is indicated to be associated with the enzymatic lipid peroxidation [28], between the control and alcohol-treated groups. Furthermore, the studies on hepatic microsomal ascorbic acid-dependent lipid peroxidation, a non-enzymatic process, failed to elicit any significant difference between the two groups of rats (Table 2). Then, the activity of hepatic microsomal NADPH oxidase, an enzyme known to generate O_2^- and H_2O_2 [15, 29], was measured by formation of H_2O_2. The chronic ingestion of alcohol caused a significant increase in this enzyme activity, and the alcohol-treated animals had about 1.5 times the activity of this enzyme as did the controls (Table 2). These findings

Table 1. Effect of prolonged alcohol administration on the levels of lipid peroxide and reduced glutathione and on the activities of various enzymes scavenging activated oxygen radicals or lipid peroxides

	Control (4)	Alcohol (8)	P
Lipid peroxide content (μmol MDA/g protein)	1.13 ± 0.07	1.67 ± 0.21	$P < 0.01$
Reduced glutathione content (μmol GSH/g protein)	13.61 ± 1.71	20.84 ± 1.45	$P < 0.001$
Superoxide dismutase (units/mg protein)	93.5 ± 7.5	70.1 ± 6.7	$P < 0.001$
Catalase (units/mg protein)	560 ± 60	586 ± 51	N.S.
Glutathione peroxidase (μmol NADPH/min/mg protein)	1.43 ± 0.23	1.28 ± 0.12	N.S.
Glutathione reductase (μmol NADPH/min/mg protein)	0.13 ± 0.01	0.12 ± 0.01	N.S.
Glucose-6-phosphate dehydrogenase (nmol NADPH/min/mg protein)	102 ± 19	66 ± 13	$P < 0.001$

Male Wistar rats were given drinking water containing either 20% alcohol or isocaloric amount of glucose as controls for 4 weeks. One unit of SOD activity is defined as the amount of enzyme which inhibits by 50% the spontaneous autoxidation of pyrogallol. One unit of catalase is the amount which catalyzes the decomposition of 1 μmol H_2O_2 per min. GSH peroxidase was measured using H_2O_2 as a substrate by coupling NADPH via added yeast GSSG reductase. GSH peroxidase, GSSG reductase and G-6-P dehydrogenase activities were determined by the amount of NADPH decreased or increased using a milimolar extinction coefficient of 6.27 $mM^{-1}\,cm^{-1}$ at 340 nm for calculation. Values represent means ± S.D. with the number of animals in parentheses. The significance was assessed by the Student's *t*-test. N.S., not significant.

Table 2. Effect of prolonged alcohol administration on lipid peroxide level and lipid peroxide formation in rat liver microsomes

	Control (4)	Alcohol (8)	P
Lipid peroxide content (μmol MDA/g protein)	3.22 ± 1.03	4.35 ± 0.56	$P < 0.05$
NADPH-dependent lipid peroxidation (nmol MDA/20 min/mg protein)	29.30 ± 4.95	25.48 ± 4.25	N.S.
Ascorbic acid-dependent lipid peroxidation (nmol MDA/20 min/mg protein)	56.80 ± 1.64	54.73 ± 3.40	N.S.
NADPH-cytochrome *c* reductase (nmol cytochrome *c* reduced/min/mg protein)	67.20 ± 9.96	63.55 ± 4.79	N.S.
NADPH oxidase (nmol H_2O_2 formed/15 min/mg protein)	39.53 ± 4.56	59.76 ± 8.61	$P < 0.01$

Male Wistar rats were given drinking water containing either 20% alcohol or isocaloric amount of glucose as controls for 4 weeks. For NADPH-dependent lipid peroxidation the reaction mixture contained 0.2 mg microsomal protein/ml, 1.0 mM KH_2PO_4 and NADPH generating system (2 mM glucose-6-phosphate, 1.2 mM $NADP^+$ and 0.2 unit of yeast G-6-P dehydrogenase/ml) in 0.1 M Tris–HCl buffer, pH 7.4. For ascorbic acid-dependent lipid peroxidation the reaction mixture contained 0.5 mM ascorbic acid in place of NADPH generating system in the above reaction medium. The amount of MDA formed in the thiobarbituric acid reaction was calculated by using a milimolar extinction coefficient of 156 mM^{-1} at 535 nm [18]. NADPH-cytochrome *c* reductase was assayed at 30° in a Beckman model 25 spectrophotometer. The other reactions were performed at 37° under an air atmosphere in a metabolic shaking water bath. Values represent means ± S.D. with the number of animals in parentheses. The significance was assessed by the Student's *t*-test. N.S., not significant.

suggest that the alcohol-induced microsomal lipid peroxide formation is mainly controlled by microsomal NADPH oxidase.

The decrease in the activity of liver cytosolic SOD found after chronic alcohol treatment (Table 1) would result in an increase in hepatic O_2^- level. Although no changes in the activities of catalase, GSH peroxidase and GSSG reductase were found *in vitro* in these conditions (Table 1), decomposition of H_2O_2 and lipid peroxides could be insufficient. The reason is that catalase is primarily concerned with the disposal of peroxisomal H_2O_2 [24] and the GSH peroxidase–GSSG reductase couple could become ineffective due to deficient supplies of NADPH depending on the decrease in G-6-P dehydrogenase activity (Table 1) and the increase in microsomal NADPH oxidase activity (Table 2). Accordingly, it is suggested that O_2^- generated reacts with H_2O_2 to give rise to the more reactive hydroxyl radicals ($OH^\cdot$), Haber–Weiss reaction [24], thereby leading to stimulation of lipid peroxide formation. The increase in hepatic GSH content observed following chronic alcohol consumption (Table 1) would play a role in protecting the liver against the toxic effects of activated oxygen radicals and/or lipid peroxides and would depend on an increase in the activity of glutathione synthesizing system as described by Guerri and Grisolia [30].

In conclusion, prolonged alcohol administration brings about significant enhancements of the contents of lipid peroxides and GSH in the liver of rats. These seem to depend not only on stimulation of microsomal lipid peroxide formation, but also on depression of the ability to metabolize lipid peroxides and to scavenge activated oxygen radicals such as O_2^- and H_2O_2 in the liver cytosol.

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Biochemical Pharmacology, Vol. 32, No. 11, pp. 1798–1800, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + .00

Inhibition of glutaminase activity of rat brain by lithium

(*Received* 24 *August* 1982; *accepted* 3 *December* 1982)

The efficacy of lithium salts in the treatment of manic depressive psychosis is well documented [1–3] although the mechanism of its action is not well understood. Information available about its *in vivo* effects on glutamate metabolism [4–6] is however very scanty and *in vitro* experiments [7–9] indicate that lithium salts may influence the binding, uptake and metabolism of γ-aminobutyric acid and glutamate. No investigation seems to have been carried out regarding the possible effects of lithium salts on the synthesis and breakdown of glutamine. The present communication describes the effects of lithium chloride on glutamine hydrolysis in rat liver and brain.

Male albino rats (150–175 g) were killed by decapitation and the brain tissues were removed and homogenized in chilled 0.25 M sucrose to give a 10% suspension (w/v) and the crude mitochondrial fraction was prepared according to Brody and Bain [10] and served as the source of phosphate activated glutaminase (Glutaminase I). The liver extract was obtained by centrifuging a 20% (w/v) homogenate in ice cold glass distilled water [11] (pH 7.5) at 1000 *g* for 5 min and the resulting supernatant was then centrifuged at 105,000 *g* for 60 min. The supernatant obtained was used as the source of α-keto acid mediated glutaminase (Glutaminase II). Glutaminase I (E.C. 3.5.1.2) was assayed according to Horowitz and Knox [12], and α-keto acid activated glutaminase (E.C. 2.6.1.15) activity (Glutaminase II) was assayed according to the method of Errera [11]. Both glutaminase I and glutaminase II activities were determined by estimating the ammonia formed employing the diffusion technique of Conway and Byrne [13].

The enzyme was preincubated with lithium chloride for 15 min at 37° prior to addition of the substrate. Preliminary experiments indicated that under the experimental conditions employed the different enzyme activities assayed were linear as a function of time and concentration of enzyme.

Protein was estimated by the method of Lowry *et al.* [14] using bovine serum albumin as the standard. L-Glutamine, α-oxoglutaric acid, pyruvic acid, tri-hydroxy-methyl-aminomethane and maleic acid were the commercial products of Sigma Chemical Co., St. Louis, U.S.A. Other reagents used were of analytical grade.

Table 1 depicts *in vitro* effect of lithium chloride on glutaminase I activity of the crude mitochondrial fraction of rat brain. The assays were carried out at two different pH values, the optimum pH of 8.5 and the near physio-

Table 1. Effects of lithium on glutaminase I activity of rat brain mitochondria

Phosphate concentrations (M)	pH of assay system	Glutaminase I activity (μmoles of ammonia formed/hr/mg protein (±S.D.))		Per cent inhibition of glutaminase I activity
		Without lithium chloride	With lithium chloride (0.05 M)	
0.005	7.4	2.5 ± 0.02	0.1 ± 0.05	96
0.01	7.4	4.2 ± 0.02	1.6 ± 0.02	96
0.02	7.4	6.2 ± 0.05	1.7 ± 0.04	72
0.04	7.4	9.9 ± 0.20	0.9 ± 0.05	64
0.005	8.5	3.2 ± 0.16	0.6 ± 0.05	81
0.01	8.5	3.8 ± 0.07	1.2 ± 0.03	67
0.02	8.5	6.8 ± 0.16	3.9 ± 0.09	41
0.04	8.5	7.8 ± 0.05	6.7 ± 0.03	14

The reaction mixture contained 0.06 M Tris–maleate buffer of pH 7.4 or 8.5, 0.004 M L-glutamine and crude mitochondria of brain equivalent to 4 mg protein in a final volume of 3 ml. Varied concentrations of phosphate of respective pH values were added as indicated in the table. The enzyme was preincubated with lithium chloride adjusted to pH 7.4 or 8.5 for 15 min at 37° prior to the addition of the substrate and the results are average of six determinations. Other details are given in the text.