Interaction between vitamin E and glutathione in rat brain—effect of acute alcohol administration

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Ethanol is known to produce membrane damage through increased lipid peroxidation leading to impairment of brain function. An investigation of the relative importance of vitamin E and glutathione (GSH) in rendering protection to the brain against ethanol-induced damage was undertaken. Normal, vitamin E-supplemented, and vitamin E-deficient rats were administered a single dose (1 mL 7 M/100 g body weight) of ethanol, and GSH levels and the activities of glutathione peroxidase (GP), glutathione reductase (GR), and glutathione-S-transferase (GST) were estimated in the cerebral cortex (CC), cerebellum (CB), and brain stem (BS) of rat brain. Vitamin E supplementation brought about an increase in GSH levels in CC and CB, elevation in GR and GST activities, and a decrease in GP activity in all three regions; while vitamin E deficiency brought about a decrease in the activities of GST (CC and CB) and GP (CB) only. Administration of ethanol to normal rats decreased GP activity in all three regions and GST activity in BS and increased GST activity in CC only. While no alterations either in GSH levels or activities of GP, GR, or GST in any of the regions were observed with vitamin E supplementation and ethanol treatment, vitamin E deficiency with ethanol administration led to depression in GP and GST activities in all three regions and GR activity in BS only. The results suggest that in the brain vitamin E acts both independently and in association with the GSH system to protect against ethanol induced damage.

Keywords: vitamin E; glutathione; ethanol toxicity; lipid peroxidation; antioxidants

Introduction

The ingestion of alcohol is known to increase lipid peroxidation and lead to membrane damage. In defense against this type of membrane damage several systems have evolved, including the glutathione (GSH) system and antioxidants like vitamin E. GSH is involved in three types of mechanisms: catabolism of the hydroperoxides formed, conjugation with reactive xenobiotics, and as a direct free radical scavenger.[1]

The metabolism of ethanol produces acetaldehyde, which conjugates with GSH either nonenzymatically[1] or by a reaction catalyzed by glutathione-S-transferase (GST). The increased lipid peroxidation due to ethanol ingestion[2] also leads to the formation of hydroperoxides that are removed by glutathione peroxidase (GP).[3,4] Both these reactions lead to a depletion of GSH levels.

While GSH is known to function as a free radical scavenger,[5] both vitamin E and GSH exhibit close interaction in their antioxidant function.[6] GSH has been shown to reduce the free radical form of α-tocopherol.[7] Aside from its antioxidant role in conjunction with GSH, vitamin E also functions as an important free-radical scavenger[8] *(Figure 1).*

Earlier studies by us showed an increase in malondialdehyde (MDA) levels (index of lipid peroxidation) in acute ethanol toxicity in the three brain regions (cerebral cortex (CC), cerebellum (CB), and brain stem (BS)) of rats.[9] Rats fed a vitamin E-deficient diet showed increased MDA levels in all three regions of the brain and further increase in CC and CB when the rats were subjected to acute ethanol toxicity. Vitamin E supplementation brought about a decrease in MDA levels, while there was no change in the levels due to acute ethanol toxicity. In this paper we present the GSH

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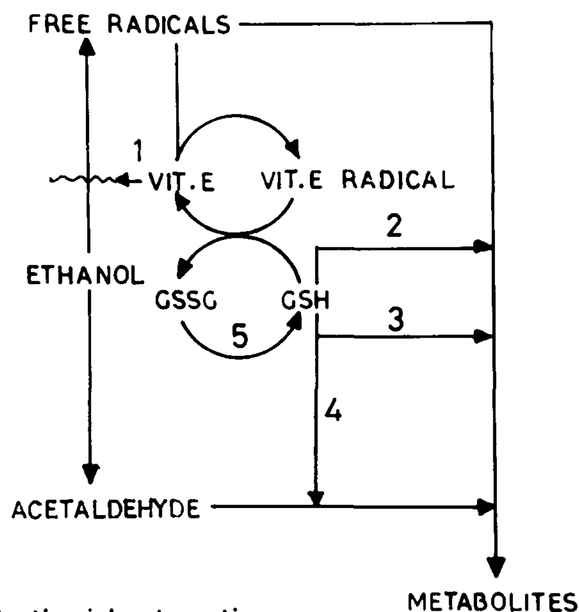

1. Antioxidant action.
2. Free radical scavenging action
3. Glutathione peroxidase.
4. Nonenzymatic or glutathione-S-transferase conjugation.
5. Glutathione reductase.

Figure 1 Vitamin E and GSH in ethanol toxicity.

levels along with the activities of GP, glutathione reductase (GR), and GST in the three brain regions (CC, CB, and BS) of normal, vitamin E-supplemented, and vitamin E-deficient rats subjected to acute ethanol toxicity.

Methods and materials

Animals and diet

Adult albino rats of either sex belonging to a local strain with body weight of 150–200 g were used. In each group six animals were used.

Vitamin E supplementation

Rats were fed orally dl-α-tocopherol at a daily dose of 10 mg/kg body weight in two divided doses for a period of 15 days as described by Marcus et al.[10]

Vitamin E deficiency

Vitamin E deficiency was induced by feeding a vitamin E-deficient diet ad libitum as suggested by Yang and Desai[11] with slight modifications for a period of 2 months as described by Marcus et al.[12] Coconut oil replaced vitamin E-stripped corn oil and the salt and vitamin mixtures were prepared as described by Raghuramulu et al.[13] No significant differences in food consumption were observed during the period of study. Control animals were fed standard laboratory chow diet. Random blood samples at the end of 2 months showed lowered serum vitamin E levels as assayed by the method of Kayden et al.[14]

Alcohol administration

Acute ethanol toxicity was induced by injecting 1 mL/100 g body weight of 7 M ethanol intraperitoneally as described by Marcus et al.[10] to a group of normal rats, a second group maintained on a vitamin E-supplemented diet, and a third group fed a vitamin E-deficient diet.

Preparation of homogenates

The animals were sacrificed 30 min after alcohol administration by decapitation. The brains were dissected out and the cerebral cortex (CC), cerebellum (CB), and brain stem (BS) were separated by the method of Sadasivudu and Lajtha.[15] Homogenates were prepared in appropriate media using a Potter-Elvehjem type homogenizer with a teflon pestle.

GSH assay

GSH levels were estimated in terms of nonprotein sulfhydryl groups according to the method of Sedlak and Lindsay.[16]

Enzyme assays

Glutathione peroxidase (GP) was assayed according to the method of Paglia and Valentine.[17] The assay mixture, consisting of 2.6 mL of 0.05 M phosphate buffer (pH 7) containing 1.125 M sodium azide, 0.5 units of glutathione reductase, 0.1 mL of 0.15 M GSH, 0.1 mL of 8.4 mmol/L NADPH, and 0.1 mL 2% homogenate of the tissue was allowed to equilibrate for 10 min at 37° C. The reaction was initiated by adding 0.1 mL of 2.2 M H_2O_2. The increase in absorbance was measured at 340 nm. The enzyme activity was calculated using a molar extinction coefficient of 6.1 $mmol/L^{-1}cm^{-1}$.

Glutathione reductase (GR) was determined by the method of Racker[18] as described by Carlberg and Mannervik.[19] The reaction mixture consisted of 2.5 mL of 0.1 M Tris buffer (pH 8), 0.1 mL NADPH (4 mmol/L), 0.1 mL EDTA (0.015 M), 0.1 mL GSSG (0.05 M), and 0.1 mL 10% homogenate. The decrease in absorbance at 340 nm was measured and enzyme activity was calculated using a molar extinction coefficient of 6.1 $mmol/L^{-1}cm^{-1}$.

GST activity was estimated by the method of Habig et al.[20] using 1-chloro 2,4-dinitrobenzene (CDNB) as substrate. The assay mixture consisted of 2 mL Tris buffer (0.15 M, pH 6.5), 0.3 mL GSH (0.03 M), 0.1 mL CDNB (0.03 M), 0.5 mL water, and 0.1 mL of the supernatant of the 10% homogenate of the brain tissue. The increase in absorbance was monitored at 340 nm after the addition of the homogenate. The enzyme activity was determined using a molar extinction coefficient of 9.6 $mmol/L^{-1}cm^{-1}$.

Statistical analysis

Statistical analysis was carried out using Student's *t* test. Comparisons made were between normal versus vitamin E-supplemented, normal versus vitamin E-deficient, ethanol-treated normal versus nonethanol-treated normal, vitamin E-supplemented plus ethanol versus vitamin E-supplemented minus ethanol, and vitamin E-deficient plus ethanol versus vitamin E-deficient minus ethanol.

Table 1 The levels of GSH and activities of GP, GR, and GST in different regions of normal, vitamin E-supplemented, and vitamin E-deficient rat brain with and without ethanol administration

	Diet	Cerebral cortex		Cerebellum		Brain stem	
		−EtOH	+EtOH	−EtOH	+EtOH	−EtOH	+EtOH
GSH[a]	N	1.24 ± 0.23	1.13 ± 0.31	1.00 ± 0.21	1.06 ± 0.33	0.90 ± 0.16	0.80 ± 0.19
	+E	1.70 ± 0.31*	1.20 ± 0.20	1.40 ± 0.20*	1.20 ± 0.16	1.10 ± 0.23	0.90 ± 0.20
	−E	1.20 ± 0.20	1.20 ± 0.13	1.10 ± 0.14	1.10 ± 0.13	0.85 ± 0.11	0.88 ± 0.13
GP[b]	N	52.50 ± 5.30	32.80 ± 1.47†††	57.70 ± 9.23	35.60 ± 3.25†††	51.20 ± 14.78	33.50 ± 4.29†††
	+E	24.50 ± 2.85***	28.50 ± 4.68	25.90 ± 3.25***	24.40 ± 3.04	26.60 ± 2.31***	28.03 ± 3.05
	−E	48.10 ± 3.78	30.70 ± 3.00†††	46.80 ± 3.03*	33.40 ± 3.06†††	48.30 ± 2.82	31.40 ± 3.32††
GR[b]	N	1.22 ± 0.29	1.19 ± 0.13	1.01 ± 0.09	0.98 ± 0.17	1.01 ± 0.14	1.07 ± 0.26
	+E	1.74 ± 0.42*	1.53 ± 0.14	1.54 ± 0.35**	1.28 ± 0.57	1.58 ± 0.28**	1.73 ± 0.24
	−E	1.01 ± 0.21	1.14 ± 0.21	1.40 ± 0.41	0.92 ± 0.24	1.04 ± 0.18	0.65 ± 0.13††
GST[b]	N	0.98 ± 0.20	1.41 ± 0.25††	1.21 ± 0.20	1.23 ± 0.08	0.98 ± 0.28	0.67 ± 0.11†
	+E	2.10 ± 0.76**	2.10 ± 0.32	1.62 ± 0.31*	1.36 ± 0.30	1.89 ± 0.49**	1.88 ± 0.54
	−E	0.74 ± 0.06*	0.46 ± 0.07††	0.90 ± 0.12*	0.52 ± 0.11†††	0.77 ± 0.13	0.41 ± 0.18†††

Mean ± SD. $n = 6$.
[a]Expressed as μmoles/g tissue.
[b]Expressed as μmoles product formed/g tissue/min.
Statistical analysis carried out using Student's *t* test.
Comparisons made: *(i) Normal vs. Vit E supplemented; *(ii) Normal vs. Vit E deficient; †(iii) EtOH treated normal versus non-EtOH treated normal; †(iv) vitamin E supplemented plus EtOH versus vitamin E supplemented minus EtOH; †(v) vitamin E deficient plus EtOH versus vitamin E deficient minus EtOH.

*** ††† $P < 0.001$.

** †† $P < 0.01$.

* † $P < 0.05$.

Abbreviations: GSH = Glutathione; GP = Glutathione peroxidase; GR = Glutathione reductase; GST = Glutathione-S-transferase; N = Fed with normal diet; +E = Fed with Vitamin E-supplemented diet; −E = Fed with Vitamin E-deficient diet; +EtOH = Ethanol treated; −EtOH = Without ethanol treatment.

Results

The GSH levels (μmol/g) and the activities of GP, GR, and GST (μmol product formed/g/min) are given with respect to the three brain regions, CC, CB, and BS of vitamin E-supplemented and vitamin E-deficient rats with and without ethanol administration in *Table 1.* In vitamin E-deficient rats the serum samples at random showed lowered levels of vitamin E (Normal 1.38 ± 0.29 mg/L, vitamin E deficient 0.52 ± 0.06 mg/L), and these values were similar to those reported by Tyopponen and Lindros.[21]

GSH levels

GSH levels were higher in CC and CB of vitamin E-supplemented rats compared with normals. Alcohol administration had no effect on GSH levels in any of the regions in any of the experimental conditions. However, in vitamin E-supplemented rats subjected to ethanol administration the elevated GSH levels in all brain regions showed a tendency to reach the levels seen in normal animals.

GP activity

Surprisingly, the GP activity was lowered in all three regions of vitamin E-supplemented, in CB of vitamin E-deficient, and in ethanol-treated rat brain regions when compared with the normal controls. Ethanol treatment brought about a decrease in GP activity in vitamin E-deficient animals but not in vitamin E-supplemented rats.

GR activity

In vitamin E-supplemented rats, GR activity was higher in all three brain regions, while in vitamin E-deficient animals the levels were similar to those in normal animals. While ethanol administration decreased the GR activity only in BS of vitamin E-deficient animals, it had no effect on GR activity in any of the regions either in normal or vitamin E-supplemented rats. However, in vitamin E-supplemented rats subjected to ethanol administration the GR activity was higher than in the normal animals.

GST activity

Vitamin E supplementation increased GST activity in all three regions, while vitamin E deficiency lowered GST in CC and CB. Ethanol administration brought about a significant decrease in GST activity in all regions of vitamin E-deficient rats and only in BS of normal rats but showed an increase in CC of normal rats. In vitamin E-supplemented rats the GST activity remained elevated even after ethanol administration.

Discussion

Vitamin E is an important lipid-soluble antioxidant found in living systems. The concentration of vitamin E in tissues has been inversely correlated to lipid peroxidation by Kornbrust and Mavis.[22] Vitamin E is also known to exhibit a close interaction with GSH in its antioxidant function.[6] The relative importance of the two systems in protection against peroxidative damages is an important area of study.

In this study, three groups of rats were used: one group fed a normal diet, a second group fed a vitamin E-supplemented diet, and a third group fed a vitamin E-deficient diet. The dosage (10 mg/kg weight/day) and period (15 days) of vitamin E supplementation brought about increased plasma vitamin E levels as observed by Nadiger.[23] The vitamin E deficiency induced in this study was relatively mild and did not produce clinically manifested signs of vitamin E deficiency. Similar animals with mild vitamin E deficiency were used by Tyopponen and Lindros,[21] who also observed that rats fed a low-vitamin E diet for 7 weeks showed vitamin E concentration in plasma and liver decreased by 60–70%. They also reported that the deficiency was not severe enough to produce clinical signs of deficiency in rats and would avoid the secondary nutritional effects.

Vitamin E supplementation

Vitamin E supplementation decreased MDA levels in CC and CB[2] and GP activity in all three brain regions; increased GSH levels in CC and CB and also the activities of GR and GST. Vitamin E has been shown to decrease lipid peroxidation[5] and its capacity to prevent lipid peroxidation is dependent on the vitamin E status of the animal. Yang and Desai[11] reported that an increase in dietary vitamin E significantly decreased the GP activity in liver and plasma of rats. While vitamin E prevents the formation of peroxides, GP is involved in the intracellular decomposition of lipid hydroperoxides.[24] Thus, vitamin E lowers the concentration of peroxides in tissues and spares GP activity.

The increased GR activity and GSH levels in vitamin E-supplemented rat brain reflects a state of preparedness to protect against increased peroxidative load. GSH can regenerate vitamin E and function as an independent radical scavenging system.[25] The increase in GST activity and decrease in GP activity would account for the increase observed in GSH levels in CC and CB.

Vitamin E deficiency

In a state of vitamin E deficiency, the increased lipid peroxidation[2] could be counteracted by GSH and GP activity. However, there is no alteration in GSH levels or GP activity in CC and BS, while in CB a significant decrease in both GP and GST activities was observed. There was no change in GR activity in any of the brain regions and the GST activity was depressed in CC and CB. The effects of vitamin E deficiency on GSH metabolising enzymes have been found to vary in different tissues. Chow et al.[4] found that the activity of GP was significantly increased in muscle and adipose tissues but not in lungs, liver, and kidney of male rats fed a vitamin E-deficient diet for 2 months. Yang and Desai[11] also reported that GP activity in the uterus of female rats fed a vitamin E-deficient diet was not lowered. Mehlert and Diplock[26] have shown that selenium and vitamin E deficiency caused a lowering of GP activity and consequently increased GST activity in rat liver. The observed changes in GP, GR, and GST activities could lead to an increase in GSH levels; however, such an increase is not seen suggesting that in the vitamin E-deficient state, GSH may be consumed while preventing lipid peroxidation to some extent. Thiriot et al.[8] have shown that vitamin E acts as a free radical scavenger and its antioxidant function occurs prior to that of GSH.

Acute ethanol toxicity

Acute ethanol administration to normal rats increased lipid peroxidation;[2] decreased GP activity in all the three regions; increased GST activity in CC; decreased GST activity in BS; and brought about no significant alteration in GSH levels, GR activity, and GST activity in CB. GR helps to maintain GSH in the reduced state, which would help to combat lipid peroxidation, promote GP and GST activities, and conjugate acetaldehyde formed from ethanol. Administration of ethanol increases the level of reduced coenzymes that help to maintain GR activity and thereby GSH levels. Chio and Tappel[27] have reported that lipid peroxides are capable of inactivating enzymes and sulfhydryl enzymes like GP are more susceptible. The decreased GP activity (probably due to inhibition by lipid peroxides) and GST activity in BS and no alteration in GST activity in CB would lead to elevated GSH levels. However, this elevation was not observed, suggesting that together with the available vitamin E GSH may be getting consumed in counteracting lipid peroxidation.

Vitamin E supplementation and acute ethanol toxicity

The administration of ethanol to vitamin E-supplemented rats did not alter MDA levels[2] and GSH levels. The higher basal levels of GR and GST activities observed in vitamin E-supplemented rat brains were maintained following ethanol administration. Some of the cytoplasmic GST isozymes have been shown to mimic GP activity,[28] and elevated GST activity would be advantageous to detoxify the lipid peroxides. McCay and King[5] observed that in the liver vitamin E supplementation provided sufficient scavenging capacity to protect the membrane from free radical damage, even at low GP levels. Acute ethanol administration did not further alter GP activity in the brain.

Vitamin E deficiency and acute ethanol toxicity

In vitamin E-deficient rat brains, administration of ethanol brought about a significant decrease in GST activity, GP activity, and GR activity only in BS. The decreased

GP activity in a state of vitamin E deficiency may cause the accumulation of H_2O_2, which interacts with free radicals causing increased peroxidation. Such an observation has been reported by us earlier.[2] The GSH levels remained unaltered despite a decrease in both GP and GST activities.

Thus, vitamin E both by itself and together with the GSH system *(Figure 1)* plays an important role in the brain to counteract the effect of acute alcohol administration by bringing about compensatory changes.

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