Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 263–266, 1983.

Liver Superoxide Dismutases and Catalase During Ethanol Inhalation and Withdrawal

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RIBIÈRE, C., J. SINACEUR, J. NORDMANN AND R. NORDMANN. *Liver superoxide dismutases and catalase during ethanol inhalation and withdrawal.* PHARMACOL BIOCHEM BEHAV **18**:Suppl 1, 263–266, 1983.—Manganese superoxide dismutase (Mn-SOD) studied during ethanol vapor inhalation shows no changes during the inhalation period (4 days) and a transient increase 12 hours after ethanol withdrawal. A significant decrease in cytosolic Cu-Zn-SOD is found at the end of the inhalation period and was sustained during 48 hours following ethanol withdrawal. It is suggested that this decrease in Cu-Zn-SOD activity might be related to an inactivation of the enzyme linked to the increase in hydroxyl radical production related to ethanol metabolism. Cytosolic catalase is reduced at the end of the ethanol inhalation period. This decrease could be related to an enhanced superoxide radical concentration linked to the reduced Cu-Zn-SOD activity.

Rats Ethanol inhalation Liver Superoxide dismutases Catalase

MANY results are consistent with a mechanism of microsomal ethanol oxidation involving the interaction of ethanol with hydroxyl radicals ($OH^{\cdot}$) that are generated through the microsomal electron transfer pathway [5, 6, 23]. Two H_2O_2-dependent pathways that are often evoked as sources of hydroxyl radicals in biological systems are the ferrous ion-catalyzed decomposition of H_2O_2 ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-$) [21] and the reaction between the superoxide radical ($O_2^{\cdot-}$) and H_2O_2 ($O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^-$) [9].

Moreover, acetaldehyde, the product of ethanol oxidation, could generate $O_2^{\cdot-}$ by its metabolism via oxidases such as aldehyde oxidase or xanthine oxidase [18]. The consumption of large amounts of alcohol may thus result in an enhanced production of $O_2^{\cdot-}$ leading itself to an increase in $OH^{\cdot}$ formation. Such $OH^{\cdot}$ radicals are known to contribute to cellular damage [4, 10, 11, 15]. Their formation is prevented by enzyme systems such as superoxide dismutase (SOD) (EC 1.15.1.1) and catalase (EC 1.11.1.6).

As a matter of fact, SOD catalyses the dismutation of $O_2^{\cdot-}$ ($O_2^{\cdot-} + O_2^{\cdot-} \xrightarrow{2H^+} O_2 + H_2O_2$) whereas catalase decomposes H_2O_2. Two forms of SOD occur in mammalian tissues, one containing copper and zinc (Cu-Zn-SOD) and localized essentially in the cytosolic compartment, the other containing manganese (Mn-SOD) and localized in the mitochondrial matrix. An *in vivo* cooperation between hepatic SOD and catalase has been recently described, SOD providing part of the H_2O_2 used as a substrate by catalase [8].

The purpose of the present study was to ascertain whether changes in SOD and catalase activities which could contribute to ethanol hepatotoxicity are induced by chronic ethanol administration.

METHOD

Animals

Male Sprague-Dawley rats (180±20 g body weight) were maintained on a standard laboratory diet (Iffa- Rat) containing 58% (in weight) carbohydrates, 3% lipids and 17% proteins. As it was observed that food consumption was about completely suppressed in most animals from the second inhalation day to the time of sacrifice, a control group consisting of rats fasted for 72 hr was run together with a control group consisting of animals having free access to the diet.

Procedures

Rats were placed in special chambers and exposed to increasing concentrations of ethanol vapor (15–25 $mg \cdot l^{-1}$) for a period of 4 days as described by Abu-Murad and Thurman [1]. Most animals showed physical dependence at the end of this inhalation period. Blood ethanol was determined after [22].

After sacrifice by decapitation, liver homogenates for SOD determinations were prepared in 0.33 M sucrose and mitochondria were prepared according to [3]. Superoxide dismutases were assayed by recording the inhibition, by the liver fractions tested, of ferricytochrome c reduction with xanthine and xanthine oxidase [16], EDTA being replaced by DETAPAC (1 mM). For cytosolic Cu-Zn-SOD assays the supernatant was used after chloroform-ethanol extraction according to [17]. For the assay of Mn-SOD activity in isolated mitochondria, 2 mM CN^- was included in the assay mixture in order to inhibit mitochondrial Cu-Zn-SOD and the

TABLE 1

EFFECTS OF ETHANOL VAPOR INHALATION DURING A 4 DAY PERIOD ON SOD ACTIVITIES

Treatment	Cystolic Cu-Zn-SOD	Mitochondrial Mn-SOD
Fed controls (20)	28.56 ± 2.61	12.11 ± 1.14
Fasted controls (11)	27.39 ± 0.69*	12.53 ± 1.41*
Ethanol inhalation (17)	22.17 ± 2.87†§	10.80 ± 1.53*‡

Values are expressed as $U \cdot mg^{-1}$ proteins in the cytosolic fraction for Cu-Zn-SOD and as $U \cdot mg^{-1}$ mitochondrial proteins for Mn-SOD. Reported values are means ± SEM with number of animals in parentheses.

Statistical comparison versus fed control: $*p>0.05$, $\dagger p<0.01$.

Statistical comparison versus fasted control: $\ddagger p>0.05$, $\S p<0.01$.

TABLE 2

EFFECTS OF ETHANOL VAPOR INHALATION DURING A 2 DAY PERIOD AND WITHDRAWAL ON LIVER SOD ACTIVITIES

Treatment	Cytosolic Cu-Zn-SOD	Mitochondrial Mn-SOD
Fed controls (12)	27.98 ± 2.35	10.64 ± 0.79
Ethanol inhalation (16)	25.73 ± 1.95*	10.50 ± 1.03*
Fed controls (6)	28.60 ± 1.75	11.05 ± 1.16
Ethanol withdrawal (12 hr) (6)	28.69 ± 3.60*	9.95 ± 0.81*

Values are expressed as $U \cdot mg^{-1}$ proteins in the cytosolic fraction for Cu-Zn-SOD and as $U \cdot mg^{-1}$ mitochondrial proteins for Mn-SOD. Reported values are means ± SEM with number of animals in parentheses.

Statistical comparison versus controls: $*p>0.05$

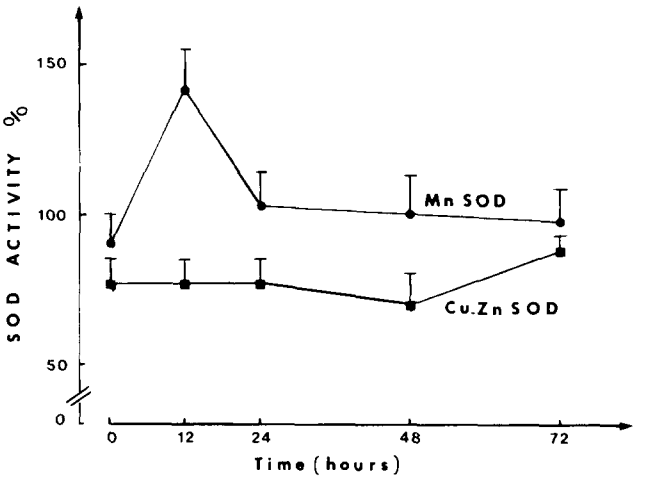

FIG. 1. Effects of ethanol withdrawal on rat liver superoxide dismutases following ethanol vapor inhalation during a 4 day period. The enzyme activity is expressed as percentage of the mean activity found at the same time in control rats. Each point is a mean of at least 10 determinations.

TABLE 3

EFFECTS OF ETHANOL VAPOR INHALATION DURING A 4 DAY PERIOD ON LIVER COPPER AND ZINC LEVELS

	Copper	Zinc
Fed controls (6)	29.36 ± 3.31	173 ± 8
Ethanol inhalation (6)	32.78 ± 7.49*	162 ± 19*

Values are expressed as $ng \cdot mg^{-1}$ total liver proteins. Reported values are means ± SEM with number of animals in parentheses.

Statistical comparison versus controls: $*p>0.05$.

reoxidation of reduced cytochrome c by cytochrome oxidase. One unit of SOD activity was defined as the amount of enzyme needed to obtain 50% inhibition of cytochrome c reduction. The unit activity was computed by plotting the reciprocal of the slope of cytochrome c reduction versus the reciprocal of the volume of sample used for the assay [20]. Liver copper and zinc were determined by atomic absorption spectrophotometry in the supernatant fraction (9,000 g).

For the determination of catalase activity, liver homogenates were prepared in ice-cold 0.33 M sucrose. The homogenates were centrifuged at 600 g for 10 min and the pellet was discarded. The supernatant was centrifuged at 12,000 g for 10 min. The new supernatant fraction (12,000 g) and the pellet (12,000 g) were used for catalase assays. Catalase activity was determined spectrophotometrically by measuring the decrease in absorbance of H_2O_2 at 240 nm [2]. One unit of activity is defined as the amount of enzyme that brings the k values to 1, k representing the decrease constant in extinction at 240 nm per second at 25°C. Protein determinations were done according to [14].

All results are given as mean values±SEM and Student's *t*-test was used for statistical interpretation.

RESULTS

Ethanol vapor inhalation during a 4 day period does not modify Mn-SOD activity (Table 1). A significant but transient increase in Mn-SOD activity occurs 12 hr after ethanol withdrawal (Fig. 1). Such an increase is not observed during the same withdrawal period following ethanol vapor inhalation during 2 days only (Table 2).

A significant decrease in cytosolic Cu-Zn-SOD activity was found at the end of the ethanol vapor inhalation period during 4 days (Table 1). At this time the blood alcohol level was 361 ± 44 mg · 100 ml^{-1}. The decrease in Cu-Zn-SOD is not likely related to a nutritional deficiency as fasting does not affect this activity (Table 1). Copper being involved in catalysis and zinc in the stability of the enzyme, a decreased Cu-Zn-SOD activity could be related to modifications in liver copper or zinc levels. The absence of significant differences in liver copper and zinc concentrations in alcohol intoxicated and control rats (Table 3) does not support such a hypothesis. The decrease in Cu-Zn-SOD was sustained during 48 hours following ethanol withdrawal and the activity returned to control values after 72 hours (Fig. 1). When the inhalation

TABLE 4

EFFECTS OF ETHANOL VAPOR INHALATION DURING A 4 DAY PERIOD ON CATALASE ACTIVITY

Treatment	Supernatant (12,000 g)	Pellet (12,000 g)
Fed controls (12)	0.334 ± 0.029	0.308 ± 0.040
Fasted controls (11)	0.302 ± 0.045*	0.211 ± 0.019*
Ethanol inhalation (13)	0.165 ± 0.024†‡	0.325 ± 0.034*‡

Values are expressed as $U \cdot mg^{-1}$ supernatant proteins and as $U \cdot mg^{-1}$ pellet proteins. Reported values are means ± SEM with number of animals in parentheses.

Statistical comparison versus fed control: $*p > 0.05$, $\dagger p < 0.01$.

Statistical comparison versus fasted control: $\ddagger p < 0.01$.

period was limited to 2 days, no changes in Cu-Zn-SOD were found during the intoxication period as well as following ethanol withdrawal (Table 2).

The findings concerning liver catalase (Table 4) show a decrease in the cytosolic enzyme activity which was highly significant when compared either to fed or to fasted controls. Contrarily to both forms of SOD and to cytosolic catalase, which are not markedly altered by a prolonged fast (Tables 1 and 4), fasting (in the absence of ethanol) reduced catalase activity significantly in the 12,000 g pellet as shown by the differences between normal fasted and normal fed rats (Table 4). The catalase activity of ethanol treated rats was unaltered in this 12,000 g pellet when compared to the fed controls but was enhanced significantly in comparison to the fasted controls (Table 4).

DISCUSSION

The present data show that the only significant change in mitochondrial Mn-SOD activity consists in a transient increase at the 12th hour following ethanol withdrawal in animals previously intoxicated by ethanol inhalation during a 4 day period. A correlation with physical dependence may exist as such an increase was not apparent during withdrawal following a shorter inhalation period (2 days).

Unlike Mn-SOD, cytosolic Cu-Zn-SOD was reduced significantly by ethanol inhalation. As our results show that this change does not appear to be related to nutritional factors such as copper or zinc deficiencies, the most likely mechanism responsible for the decreased Cu-Zn-SOD activity is an inactivation of the enzyme linked to the increase in $OH^{\cdot}$ production itself associated with ethanol oxidation. As a matter of fact, Sinet and Garber [19] have shown that human Cu-Zn-SOD is inactivated during exposure to $O_2^{\cdot-}$ and H_2O_2 and suggested that this inactivation is due to the action of $OH^{\cdot}$ generated from H_2O_2 at the active site of the enzyme. The lack of protective action of ethanol, which is a known $OH^{\cdot}$ scavenger, might be related to the fact that ethanol cannot gain access to the bound $OH^{\cdot}$ at this active enzyme site or that ethanol radicals produced by the reaction of ethanol with $OH^{\cdot}$ are themselves able to inactivate the enzyme [19]. It might thus be suggested that the overproduction of $OH^{\cdot}$ in such animals is at least partly responsible for the decrease in activity of the hepatic Cu-Zn-SOD. Our finding that Mn-SOD is not affected during ethanol inhalation is compatible with the results of [19] showing that $OH^{\cdot}$ radicals, while inactivating Cu-Zn-SOD, do not affect Mn-SOD.

The findings concerning liver catalase show a decrease in the cytosolic enzyme activity which is highly significant when compared either to the fed or to the fasted controls. De Master *et al.* [7] have recently reported that chronic alcohol feeding lowered the level of total catalase activity in the liver and suggested that this alteration may involve an inhibition of the biosynthesis of catalase without effecting the preformed enzyme. Kono and Fridovich [12], on the other hand, just reported that superoxide radicals inhibit catalase activity, a finding which seems to provide a basis for a synergism between SOD and catalase. The fact that the activity of both cytosolic SOD and catalase is reduced significantly after chronic ethanol vapor inhalation could reflect such a synergism. The enhancement in superoxide radical concentration due to the reduced Cu-Zn-SOD activity would be, partly at least, responsible for the decreased catalase activity in the same cellular compartment. Acetaldehyde resulting from ethanol oxidation could contribute to such an inactivation of cytosolic catalase [12].

The catalase activity of chronic ethanol treated rats was unaltered in the 12,000 g pellet when compared to the fed controls but enhanced significantly in comparison to the fasted controls; the mechanism involved in these changes needs further research.

As our determinations were made immediately after cessation of ethanol inhalation, it could be suggested that ethanol by itself would play a major role in the changes reported after a 4 day inhalation period. This does not hold true as the changes in liver SOD and catalase activities induced by a single ethanol administration (leading to a similar blood alcohol level) are quite different from those presently reported (results to be published).

The reduction in hepatic Cu-Zn-SOD activity may play a role in ethanol hepatotoxicity analogous to that previously suggested by Ledig *et al.* [13] concerning ethanol toxicity on the nervous system. This role would be favored by the simultaneous reduction in cytosolic catalase activity.

ACKNOWLEDGEMENTS

This work was supported by grants from the Université René Descartes (Faculté de Médecine Paris-Ouest) and from the Institut National de la Santé et de la Recherche Médicale. We would like to acknowledge the Service de Biochimie du Centre Hospitalier A. Mignot, Versailles (Pr. M. Pays) for their help in copper and zinc determinations.

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