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Effects of early ethanol input on the activities of ethanol-metabolizing enzymes in mice

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A system was developed in our laboratory which demonstrates the effect of prenatal and postnatal administration of ethanol (ETOH) in inducing long-term behavioral changes in mice [1]. The alcohol intake of the mice was mediated by their parents, who drank alcohol from weaning until after mating (males) or until 14 days post parturition (females). This early exposure to ethanol induced susceptibility to audiogenic seizure in the normally resistant C57BL/10/Bg strain and increased the incidence of seizure in the genetically susceptible DBA/1/Bg strain. Also, open-field activity was reduced in both strains [1]. These findings indicate that long lasting neurophysiological changes may be induced by ethanol during early development.

Ethanol is metabolized mainly in the liver by alcohol dehydrogenase (ADH), a cytoplasmic enzyme that has been shown by several investigators [2–4] to be inducible by chronic administration of ethanol. Recently, a microsomal ethanol-oxidizing system (MEOS) was described in liver [5, 6]. This enzyme, which is localized in the microsomal compartment in tissue, may be significant in the oxidative metabolism of ethanol. Moreover, as in the case of ADH, the microsomal enzyme also appears to be inducible by continuous administration of ethanol [6]. The present study used the system of parent-mediated ethanol administration in order to investigate the effects of ethanol during early development in the possible induction of prolonged changes in these ethanol-metabolizing enzymes.

Two strains of mice, C57BL/10/Bg and DBA/1/Bg, were used in this study. The parent mice were maintained under standard laboratory conditions until 28 days of age, when littermates were segregated by sex and housed four per cage in clear Plexiglas cages. Experimental animals were given food and 10% ethanol (v/v) in water available *ad lib.* as their only drinking supply. Control animals were given food and water available *ad lib.* The animals were maintained under their respective conditions until 60 days of age, at which time they were bred to animals of the same strain and treatment. Pregnant females were housed individually and remained on this regimen until 14 days post parturition, when the ethanol solution was replaced by tap water so that the offspring were never exposed directly to ethanol. The effects of this ethanol treatment on food intake by parent mice and on the physical development of offspring mice have been described elsewhere [7].

In order to assess the relative contribution of prenatal and postnatal exposure to ethanol, offspring were exchanged within the first 24 hr of birth between treated and control animals, from control to control and from treated to treated mothers as follows: (1) EH—offspring of parents fed 10% ETOH (E) were fostered by H_2O control (H) females; (2) HE—offspring of H_2O control parents were fostered by females which were fed with 10% ETOH; (3) EE—offspring of parents fed 10% ETOH were fostered by mothers other than their own who had also been fed 10% ETOH; and (4) HH—offspring of H_2O control mice were fostered by other control females. The offspring were weaned at 28 days of age and sacrificed between 44 and 46 days of age when their livers were removed for enzyme assays. An equal number of males and females was used in each group. There were no significant differences in body weight and liver weight between the groups in both strains at the time of sacrifice.

Whole liver was homogenized in 2 vol. of 1·5% potassium chloride. After freezing and thawing, the homogenate was centrifuged at 10,000 *g* for 15 min. The supernatant was used for the assay of the enzyme activities. Alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (AcDH) activities were measured by the procedures described by Ohno *et al.* [8], with some modification. For ADH, the incubation mixture (3·0 ml) consisted of 33 mM sodium pyrophosphate buffer, pH 8·8, 16 mM ethanol, 1·6 mM NAD, and 20 μl of liver preparation. For AcDH, the incubation mixture (3·0 ml) consisted of 3·3 mM sodium pyrophosphate buffer, pH 7·0, 16 mM acetaldehyde, 0·16 mM NAD, and 20 μl of liver preparation. In both cases, the initial rate of formation of NADH at 21° was followed fluorometrically for 3 min at 340 nm excitation and 460 nm emission. The microsomal ethanol-oxidizing system (MEOS) was measured by the method of Lieber and DeCarli [6]. The incubation mixture (3·0 ml) consisted of 80 mM sodium phosphate buffer, pH 7·4, 50 mM ethanol, 0·3 mM NADPH, 5 mM magnesium chloride, 20 mM nicotinamide, 8 mM sodium isocitrate, 6 mg isocitrate dehydrogenase (crude-type I; Sigma Chemical Co.), and 0·1 ml of liver preparation. After incubation at 37° for 15 min, the reaction was stopped by the addition of 0·5 ml of 70% trichloroacetic acid. The acetaldehyde produced was then allowed to react with 0·4 ml of 0·015 M semicarbazide in 0·17 M potassium phosphate buffer, pH 7·0, and the concentration of acetaldehyde bound to the semicarbazide was determined spectrophotometrically at 224 nm [9]. Protein was measured by the method of Lowry *et al.* [10].

The activities of alcohol dehydrogenase and microsomal ethanol-oxidizing system in liver are summarized in Table 1. There was an increase of 21 to 31 per cent in C57BL/10 and DBA/1 mice, respectively, in the ADH level among animals that were exposed to ethanol during early developmental stages (EE) compared to the control (HH) group. In the cross-fostered groups, the increase was found only among animals which were exposed to ethanol prenatally (EH). They exhibited the same levels as animals which were exposed to ethanol in both the pre- and postnatal periods (EE), while the level of ADH in the EE group was not higher than the control. The magnitude of increase was similar in both strains, although the initial level of ADH was significantly higher ($P < 0{\cdot}001$) in the C57BL/10 strain than in DBA/1 as previously reported by another laboratory [11, 12]. In the case of MEOS, the activity of MEOS was increased 23 and 30 per cent in C57BL/10/Bg and DBA/1/Bg animals, respectively, in the ethanol group (EE). The same increase was found whether the animals were exposed to ethanol only prenatally (EH), postnatally (HE), or for both periods (EE). The magnitude of increase was the same for both strains. It is interesting to note that, in contrast to ADH, the basal level of MEOS was significantly higher ($P < 0{\cdot}01$) in the DBA/1 strain than in C57BL/10. That the effects of ethanol treatment on ADH and MEOS were specific is shown by the activities of ace-

Table 1. Effect of early exposure to ethanol on liver ADH and MEOS activities in C57BL/10 and DBA/1 mice

Exposure period*	ADH activity† (nmoles/mg/min)		MEOS activity† (nmoles/mg/min)	
	C57	DBA	C57	DBA
HH	19·1 ± 0·5	15·3 ± 0·3	0·89 ± 0·04	1·04 ± 0·03
EH	23·0 ± 0·9‡	19·1 ± 0·4‡	1·06 ± 0·02§	1·29 ± 0·08‡
HE	18·8 ± 0·6	15·3 ± 0·5	1·20 ± 0·05‡	1·43 ± 0·04‡
EE	23·1 ± 0·8‡	20·0 ± 0·7‖	1·16 ± 0·08‖	1·28 ± 0·02‡

* See text for description of exposure period.
† Enzyme activities are expressed as nmoles/mg of protein/min. Each value is the mean ± S. E. M. from six homogenates of one to three livers.
‡ $P < 0.001$ as compared with control (HH).
§ $P < 0.05$ as compared with control (HH).
‖ $P < 0.01$ as compared with control (HH).

taldehyde dehydrogenase, measured in the same livers (Table 2). AcDH activities remained essentially unchanged after exposure to ethanol in all developmental periods tested.

These results indicate that early input of ethanol induced long-term increases in the activities of the ethanol-metabolizing enzymes (ADH and MEOS). The relation between the level of ethanol metabolism and drinking behavior in mice ("alcohol preference" [13]) was studied, and although it is suggested that the mice which are better able to metabolize ethanol may also be the ones that prefer it, the evidence is still equivocal [11, 12, 14–16]. If, however, there is a relationship between the activity of ethanol-metabolizing enzymes and ethanol-drinking behavior, offspring of "alcoholic" mice would be expected to exhibit greater preference for ethanol than normal mice.

The mechanisms underlying the developmental changes in the liver ADH and MEOS activities are presently unknown. In the developmental system used in this study, in which parents consumed ethanol continuously for more than 2 months, during which time the females were pregnant and lactating, possible nutritional disturbances might exist as contributing factors in the changes of the liver enzymes in the offspring. Indeed, some reduction in food intake in the mothers and in the gain of body weight of the offspring was observed as a result of this ethanol treatment [7]. However, the concurrent use of C57BL/10 and DBA/1 mice, two inbred strains that are known to exhibit different behavioral and physiological responses to ethanol [11, 12, 15, 16], permits the evaluation of possible nutritional involvement in these biochemical changes. While the offspring derived from the ethanol-treated DBA mice showed reduced body weight during postnatal period, those derived from ethanol-treated C57BL mice were normal [7]. Yet, in spite of these strain differences, the effect of early ethanol input in inducing increases of ADH and MEOS activities remained the same in both strains. Moreover, early input of ethanol induced only an increase in the activities of ADH and MEOS, but not that of AcDH. The fact that the activity of AcDH, another NAD-dependent dehydrogenase, remained unchanged indicates that there is a specificity in the effect of early ethanol input, and appears to argue against a case of generalized disturbance of protein metabolism.

Table 2. Effect of early exposure to ethanol on liver AcDH activity in C57BL/10 and DBA/1 mice

Exposure period*	AcDH activity† (nmoles/mg/min)	
	C57	DBA
HH	20·5 ± 1·1	19·3 ± 0·5
EH	18·8 ± 0·4	19·2 ± 0·8
HE	19·9 ± 1·1	21·0 ± 0·5
EE	18·6 ± 0·5	20·3 ± 0·3

* See text for description of exposure period.
† Enzyme activity is expressed as nmoles/mg of protein/min. Each value is the mean ± S. E. M. from six homogenates of one to three livers.

It should be emphasized that the offspring were never directly given ethanol. Prenatally, the fetus was exposed to ethanol to the same level as the drinking mother, since placenta barrier to ethanol does not exist [17]. After birth, the intake of ethanol was apparently through the milk supply. Although ethanol concentrations in the blood of these developing mice are not known, their daily intake can be estimated. Since milk and blood concentrations of ethanol are similar [18], ethanol concentrations in milk could reach about 40 mg/100 ml, the high values in blood during the night [7]. If daily milk intake was 0·2 to 0·3 ml/g body weight, daily ethanol intake would be approximately on the order of 0·1 mg/g body weight. Thus, continuous intake of ethanol during early development, even in such small daily doses, may result in prolonged metabolic alterations that extend into adulthood, as demonstrated in the present study.

In summary, mice derived from "drinking" mothers appear to have elevated levels of ADH and MEOS, as measured at adult age. The behavioral consequences of ethanol mediated via the mother, reported elsewhere [1, 7], include a possible induction of alcohol preference, which remains a subject of investigation; an increase in susceptibility to sound induced seizures for both strains; and a change in open field activity.

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Inhibition of phenylethylamine metabolism *in vivo*—Effect of antidepressants

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Despite the widespread use of tricyclic antidepressants, the mechanism of their antidepressant action remains unclear. Although it is widely acknowledged that the blockade of norepinephrine uptake may be a contributing factor [1], this mechanism is not exclusive of non-antidepressants nor is it inclusive of all tricyclic antidepressants [2]. In the search for an alternative mechanism, Fischer *et al.* [3] and Mosnaim *et al.* [4] have examined the effects of imipramine on brain phenylethylamine levels. They found that this putative ergotropic modulator was increased in rat brain after imipramine treatment and suggested that this elevation might be related to antidepressant efficacy. Although Fischer *et al.* did not determine the mechanism involved in the imipramine-induced elevation of phenylethylamine, recent experiments *in vitro* [5, 6] have indicated that tricyclic antidepressants may block the deamination of phenylethylamine by type B monoamine oxidase. The present experiments were designed to determine if antidepressants alter the metabolism of phenylethylamine *in vivo* at non-toxic doses.

Male mice (CF-1) or rats (Sprague–Dawley) were treated with several monoamine oxidase inhibitors and tricyclic antidepressants, either acutely or chronically, prior to intravenous administration of [^{14}C]β-phenylethylamine ([^{14}C]PEA, New England Nuclear, 9·86 mCi/m-mole). In the initial acute mouse study, compounds were injected intraperitoneally (i.p.) 1 hr before the labeled amine. In the chronic study, groups of four mice were placed on a diet containing 0·05% of one of several antidepressants for 1 week before receiving [^{14}C]PEA. Rats used in this study received one dose i.p. of either 50 mg/kg of pargyline or imipramine 24 hr prior to injection with the labeled amine. All mice received 0·1 μCi of [^{14}C]PEA and rats received 1 μCi. Ten min after the radioactive amine was injected, the animals were sacrificed by decapitation, and the brains were removed and placed on dry ice. The tissues were homogenized in 2 vol. of 0·1 N HCl, and 1 vol. of 30% $HClO_4$ was added [7]. After precipitated protein was removed by centrifugation, the pH of the supernatants was adjusted to above 11 with 10 N NaOH. One-ml aliquots were shaken for 10 min with 3 ml benzene and sufficient NaCl to saturate. Two and one-half ml of the benzene fraction was transferred and washed with 1 ml of 0·1 N NaOH and NaCl to saturate. Two ml of the washed benzene fraction was taken for radioactivity determination liquid scintillation spectrometry. The activity (cpm) was converted to dis./min using a correction factor obtained from counting a known amount of [^{14}C]PEA (counting efficiency = 86 per cent).

An acute single dose pretreatment of mice with standard monoamine oxidase inhibitors resulted in a large increase in [^{14}C]PEA brain levels (Table 1). However, similar treatment with the tricyclic antidepressants failed to alter the metabolism and disposition of [^{14}C]PEA.

Table 1. Effect of several monoamine oxidase inhibitors and antidepressants on metabolism *in vivo* of [^{14}C]phenylethylamine in mouse brain*

Drug	Dose (mg/kg)	[^{14}C]PEA (mean dis./min ± S. E. M.)
Control		69 ± 0
Pargyline	30	3605 ± 318
Tranylcypromine	30	7563 ± 389
Nialamide	30	1141 ± 78
Imipramine	30	69 ± 2
Iprindole	30	81 ± 7
Amitriptyline	30	71 ± 2

* Each figure is the result of four determinations. Drugs were administered intraperitoneally 1 hr before [^{14}C]PEA.