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Lead-induced catalase activity differentially modulates behaviors induced by short-chain alcohols

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Abstract

Acute lead administration produces a transient increase in brain catalase activity. This effect of lead has been used to assess the involvement of brain ethanol metabolism, and therefore centrally formed acetaldehyde, in the behavioral actions of ethanol. In mice, catalase is involved in ethanol and methanol metabolism, but not in the metabolism of other alcohols such as l-propanol or *tert*-butanol. In the present study, we assessed the specificity of the effects of lead acetate on catalase-mediated metabolism of alcohols, and the ability of lead to modulate the locomotion and loss of the righting reflex (LRR) induced by 4 different short-chain alcohols. Animals were pretreated IP with lead acetate (100 mg/kg) or saline, and 7 days later were injected IP with ethanol (2.5 or 4.5 g/kg), methanol (2.5 or 6.0 g/kg), 1-propanol (0.5 or 2.5 g/kg) or *tert*-butanol (0.5 or 2.0 g/kg) for locomotion and LRR, respectively. Locomotion induced by ethanol was significantly potentiated in lead-treated mice, while methanol-induced locomotion was reduced by lead treatment. The loss of righting reflex induced by ethanol was shorter in lead-treated mice, and lead produced the opposite effect in methanol-treated mice. There was no effect of lead on 1-propanol or *tert*-butanol-induced behaviors. Lead treatment was effective in inducing catalase activity and protein both in liver and brain. These results support the hypothesis that the effects of lead treatment on ethanol-induced behaviors are related to changes in catalase activity, rather than some nonspecific effect that generalizes to all alcohols.

Keywords: Lead acetate; Catalase; Methanol; Ethanol; 1-propanol; *tert*-butanol; Locomotor activity; Narcosis; Acetaldehyde

1. Introduction

Ethanol, through its actions in the central nervous system, is known to produce a large variety of behavioral effects in rodents. For example, acute IP administration of low doses of ethanol results in an enhancement of locomotion and open field behavior in mice (Draski and Deitrich, 1993; Pohorecky, 1977). Conversely, when ethanol is administered in medium to high doses, it induces locomotor suppression, motor incoordination and the loss of righting reflex (LRR) (Draski and Deitrich, 1993; Pohorecky, 1977).

It has been postulated that a number of these central effects of ethanol are mediated through its first metabolite, acetaldehyde (Aragon et al., 1985; Smith et al., 1997; Quertemont et al., 2005; Zimatkin and Deitrich, 1997). The presence of acetaldehyde in the brain under normal conditions is a topic of some controversy (Deitrich, 2004; Hunt, 1996; Quertemont et al., 2005) since ADH I is not present in the brain and acetaldehyde content in the blood is not detectable when ethanol intake is in a normal range. However, research in this area has led to the suggestion that acetaldehyde is formed in vivo from ethanol directly in the brain, in part via the enzyme catalase in conjunction with hydrogen peroxide (Aragon et al., 1991; Smith et al., 1997; Zimatkin and Deitrich, 1997). Supporting this idea, several studies have shown that rodents with different sensitivities to ethanol, or distinct patterns of ethanol-related behaviors, have different levels of catalase (Aragon and Amit, 1987; Correa et al., 2004a; Gill et al., 1996, 2000) thus suggesting that acetaldehyde is in part, responsible for the effects of ethanol. In this respect, it has been shown that mice genetically deficient in catalase have lower ethanol-induced locomotion than normal mice and also demonstrated

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significant differences in ethanol-induced LRR; acatalasemic mice have a longer duration of sleep time following ethanol administration than normal mice (Aragon and Amit, 1993; Aragon et al., 1992). Likewise, C57BL/6J mice display less locomotor activity and longer LRR time after an injection of ethanol, as well as lower brain catalase activity, compared to the DBA/2 strain or other recombinant or outbred strains of mice (Aragon and Amit, 1987; Correa et al., 2004a; Crabbe et al., 1982; Gill et al., 2000). Moreover, pharmacological studies also support the involvement of catalase in the effects of ethanol. Mice treated with catalase inhibitors have lower ethanol-induced locomotion than control animals (Aragon and Amit, 1993; Correa et al., 1999b, 2001b, 2004a; Sanchis-Segura et al., 1999a,b) and longer duration of LRR (Correa et al., 2001a). Conversely, manipulations that enhance the activity of the catalase–H_2O_2 system also enhance ethanol-induced locomotor activity (Correa et al., 1999a, 2000, 2001b, 2004a; Pastor et al., 2002; Sanchis Segura et al., 1999c) and decrease LRR (Correa et al., 2001a). All these findings support the proposed role of brain catalase, and thus acetaldehyde formed in the brain, in some of the behavioral effects induced by ethanol (Smith et al., 1997; Zimatkin and Deitrich, 1997).

Among all the different pharmacological tools used to manipulate catalase activity, acutely administered lead acetate is especially interesting because it produces, at several doses and times after its administration, a clear potentiation of brain catalase activity (Correa et al., 1999a, 2000, 2001b, 2004a,b; Valenzuela et al., 1989). Thus, it has been demonstrated that a single IP injection of this compound produced significant increases in both brain catalase activity and ethanol-induced locomotion a week after administration (Correa et al., 1999a, 2000, 2001b). Acute lead administration also has been shown to increase the latency and reduce the duration of LRR after an acute ethanol injection in rats and mice (Swartzwelder, 1984; Correa et al., 2001a). From these studies it has been suggested that lead acetate interacts with an ethanol-specific target in the central nervous system (i.e., catalase), since lead acetate specifically boosted the effects of ethanol without affecting spontaneous locomotion, amphetamine-induced locomotion or plasma ethanol levels.

However, it has been argued that lead acetate can be affecting other parameters of neural activity in addition to catalase (Deitrich, 2004; Quertemont et al., 2005; Sandhir et al., 1994). These actions could result in a nonspecific activational effect of lead, which could manifest itself as an interaction with the effects of ethyl alcohol. The present studies addressed this possibility by using acute administration of lead acetate as a pharmacological tool to increase catalase activity, and by comparing the effects of lead on ethanol-induced activity with its effects upon the locomotion and LRR induced by other alcohols (Dudek and Phillips, 1983; Miquel et al., 1999a,b; Palmer et al., 2002; Sanchis-Segura et al., 1999a). If the result of lead acetate administration is a general activational effect that increases locomotion and reduces LRR, then it would not be specific for ethanol, but instead should affect other alcohols that also induce these behaviors. Thus, in the present set of experiments, we studied the effects of lead acetate on the locomotion and LRR induced by two types of alcohols metabolized by catalase (i.e., ethanol and methanol, which also is a well known selective substrate for catalase in rodents; Bradford et al., 1993), and two other alcohols that seem not to be metabolized by catalase (i.e., 1-propanol and *tert*-butanol; Teschke et al., 1975; Reddy et al., 1995). We also assessed the effect of lead treatment on catalase synthesis and activity in liver and brain, and blood alcohol levels achieved after the administration of the different alcohols.

2. Materials and methods

2.1. Subjects

Male Swiss–Webster mice (32–42 g) purchased from Harlan-Interfauna Ibérica S.A. (Barcelona, Spain) were housed in groups of four per cage, with standard laboratory rodent chow and tap water available ad libitum. Subjects were maintained 7 days prior to experimentation at 22±1 °C with lights on from 8:00 to 20:00 h. Following this time, animals were randomly assigned to different groups; $n=8–10$ for the locomotion studies and $n=10–12$ for the LRR studies. Lead acetate (0 or 100 mg/kg) was injected (IP) 7 days before behavioral testing or biochemical assays were conducted. This time interval and dose of lead acetate were chosen following previous studies in which catalase showed maximal levels of induction (Correa et al., 1999a, 2000). Testing was conducted between 10:00 and 13:00 h. All experimental procedures complied with the European Community Council directive (86/609/ECC) for the use of laboratory animal subjects.

2.2. Drugs and selection of doses

All drugs were dissolved in saline solution and were administered intraperitoneally (IP). Lead acetate (Sigma-Aldrich S.A., Spain) was prepared at a concentration of 5 mg/ml and administered to the mice in a single injection (body weight/50). Doses used for the different alcohols were based on previous or pilot studies, and were selected based on the induction of locomotion and on the effect on the righting reflex that they produced. Methanol solutions (20% v/v) were prepared from 99.5% methanol (Panreac Química S.A., Spain). The locomotion-inducing dose of methanol was taken from Miquel et al. (1999b) and the LRR-inducing dose from pilot studies. Ethanol solutions (20% v/v) were prepared from 96% ethanol (Panreac Química S.A., Spain). The locomotion-inducing dose of ethanol was taken from Correa et al. (2000) and the LRR-inducing dose from Correa et al. (2001a). 1-Propanol solutions (13% v/v) were prepared from 99.5% 1-propanol (Panreac Química S.A., Spain). The locomotion-inducing dose of 1-propanol was taken from pilot studies and the LRR-inducing dose from Miquel et al. (1999a). *tert*-butanol solutions (10% v/v) were prepared from 99% *tert*-butanol (Sigma-Aldrich S.A., Spain). The locomotion-inducing dose of *tert*-butanol was taken from Sanchis-Segura et al. (1999a) and Palmer et al. (2002) and the LRR-inducing dose from pilot studies.

2.3. Locomotor activity test

After pretreatment, saline or one of the alcohols were injected to the animals (the control groups (0 mg/kg lead acetate-saline) were different for each experiment, and immediately afterwards the mice were individually placed in the open field chamber for 20 min. The open field apparatus consisted of a clear glass cylinder 25 cm in diameter and 30 cm high. Locomotor activity was registered by a computerized video tracking system (SMART, Letica S.A. Barcelona. Spain). Distance traveled (in cm) by each animal was recorded. Measures of locomotor activity are reported only for the last 10 min. This delay was chosen to decrease the effects of animal handling and the environmental novelty to the open field (Kelley, 1993). The behavioral test room was illuminated with a dim light, and external noise was attenuated.

2.4. Duration of loss of the righting reflex (LRR)

Another subset of independent groups of animals was used for these experiments. Seven days after lead acetate treatment (0 or 100 mg/kg), one of the alcohols was injected IP and immediately the animal was individually placed in a Plexiglas cage. The initiation of LRR was determined during a 10 min observation period at 2 min intervals after the IP injection. The LRR was defined as any case in which a mouse could not right itself twice in 1 min after being placed on its back. After mice lost the righting reflex, they were put on their back in a V-shape bed. The duration of LRR was defined as the time elapsed from the loss of the righting reflex to the time when the righting reflex was regained. Recovery was determined when mice could right themselves twice in 1 min after being placed on their backs (Miquel et al., 1999a). The behavioral room was illuminated with a dim light and external noise was attenuated.

2.5. Assay for blood alcohols

A naive group of animals was used for these experiments. Following lead acetate (0 or 100 mg/kg) treatment, one of the alcohols was injected to mice. Trunk blood was collected 15 or 60 min after the alcohol administration. Animals were sacrificed by decapitation under ether anesthesia. Each blood sample (1 ml approximately) was collected in heparinized microcentrifuge tubes and immediately placed in an Eppendorf centrifuge where the samples were spun down for 5 min at 5000 rpm. A micropipette was then used to extract 160 ml of plasma and to add it to 1.44 ml of TCA (20%). The mixture was spun down again (5 min at 5000 rpm) to obtain a clear, protein-free supernatant. This protein-free serum was then placed in Eppendorf tubes and frozen at −80 °C until chromatographic analysis. A CE Instruments GC 8000 gas chromatograph (Polyethylene glycol column: 122–7032 DB-WAX, 30 m × 0.25 mm in J&W Scientific) with an HS-850 headspace analyzer was used for methanol, ethanol, *tert*-butanol and 1-propanol analysis. Nitrogen was used as a carrier gas (flow rate 84 ml/min). The injector temperature was set to 90 °C, and the oven temperature was 60 °C (adapted from Yim and Gonzales, 2000). The retention times for the different alcohols were: methanol at 3.5 min, ethanol at 3.9 min, 1-propanol at 6.0 min, and *tert*-butanol at 3.4 min. The detection limits were: 0.5 μg/ml for methanol, and 0.5–1.0 μg/ml for ethanol, 1-propanol and *tert*-butanol. The concentrations of the alcohols were quantified by comparison to a standard curve.

2.6. Catalase activity determination

Brain and liver catalase activity was assessed in mice treated (IP) with lead acetate (100 mg/kg) or saline. A naive group of animals was used for these experiments. All mice were perfused with 50 ml of heparinized (1000 units/l) isotonic saline and sacrificed by decapitation 7 days after the treatment. The whole brain was removed and homogenized in a phosphate buffer (50 mmol/l; pH 7.0) with digitonin (0.01%). Brain homogenates were centrifuged at 10,000 rpm for 10 min in an Eppendorf microcentrifuge. Supernatant aliquots were used to determine brain catalase levels. Liver samples were homogenized in a sacarose buffer (0.25 M, pH 7.5) with EDTA (0.1 mM) and they were centrifuged at 14,000 rpm for 10 min in an Eppendorf microcentrifuge. Supernatant aliquots were diluted 2000 fold and were used to determine catalase levels. Catalase activity was assayed spectrophotometrically by measuring the decrease in absorbance of H_2O_2 at 240 nm ($\epsilon_{240}=0.00394$ $mmol^{-1} \times mm^{-1}$). Protein levels were determined from supernatants (Aebi, 1974).

2.7. Preparation of brain and liver lysates and Western Blot analysis

Brain and liver samples were homogenized in RIPA buffer (phosphate-buffered saline containing 1% Nonidet P-40, 0.5% sodium deoxicholate, 0.1% SDS, 1.25 mM PMSF, 40 μM leupeptin, 10 μg/ml aprotinin, and 1 mM sodium orthovanadate), and centrifuged at 10,000×*g*. After measuring the supernatant protein content with the Bio-Rad (Madrid, Spain) protein assay kit, the supernatants were mixed with equal volume of SDS buffer (0.125 M Tris–HCl, pH 6.8, 2% SDS, 0.5% (v/v) 2-mercaptoethanol, 1% bromophenol blue and 19% glycerol) and boiled for 5 min. Different amounts of proteins from brain and liver homogenates were separated with electrophoresis using 12% SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to a nitrocellulose membrane (Schleicher and Schuell, Barcelona, Spain), as previously described in Pascual et al. (2003). Then, membranes were blocked with 5% non-fat dried milk in TBS containing 0.05% Tween-20 (TBS-T) and incubated overnight with the anti-catalase polyclonal antibody (1:5,000) (Abcam Ltd., Cambridge, UK). Membranes were washed in TBS-T and then incubated 60 min with an alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG (1:1000). Proteins were visualized with alkaline phosphatase conjugate (Promega, Barcelona, Spain), nitrocellulose sheets were washed and photographed. The intensity of the bands was quantified with SigmaGel version 1.0 image analysis software (Jandel Scientific, Madrid, Spain). Under these experimental conditions, the optimal protein

concentration for the immunodetection of catalase in brain and liver homogenates was 100 μg and 4 μg, respectively. Therefore, we used these amounts of proteins to analyze the levels of catalase in liver and brain from control and lead acetate-treated animals.

2.8. Statistical analyses

Results are reported as mean ± SEM. Data were analyzed by means of *t*-tests (in the LRR, blood alcohol determinations and catalase activity studies) or analyses of variance ANOVA for independent measures (in the locomotion and immunoblot studies). Post hoc comparisons were undertaken if a significant main effect or interaction was found at $p<0.05$. These comparisons were made using Fisher's Least Significant Difference Tests (LSD). A computerized statistical program (STATISTICA 4.1) was used in this study.

3. Results

3.1. Effects of lead acetate on alcohol-induced locomotor activity

The results of the locomotor activity test are presented in Fig. 1. The effect of lead acetate pretreatment (0 or 100 mg/kg, 7 days before) on the locomotor activity induced by each alcohol was analyzed by means of a two-way factorial ANOVA. Fig. 1A represents locomotor activity induced by methanol (2.5 g/kg). The two-way factorial ANOVA revealed a significant effect for both main factors; lead acetate dose [$F(1,36)=7.10$, $p<0.01$] and methanol dose [$F(1,36)=37.33$, $p<0.01$], and for the interaction [$F(1,36)=10.20$, $p<0.01$]. The LSD post hoc test demonstrated that methanol-induced locomotion in the saline pretreated group ($p<0.01$), and also in the lead acetate-pretreated group ($p<0.05$), compared to their

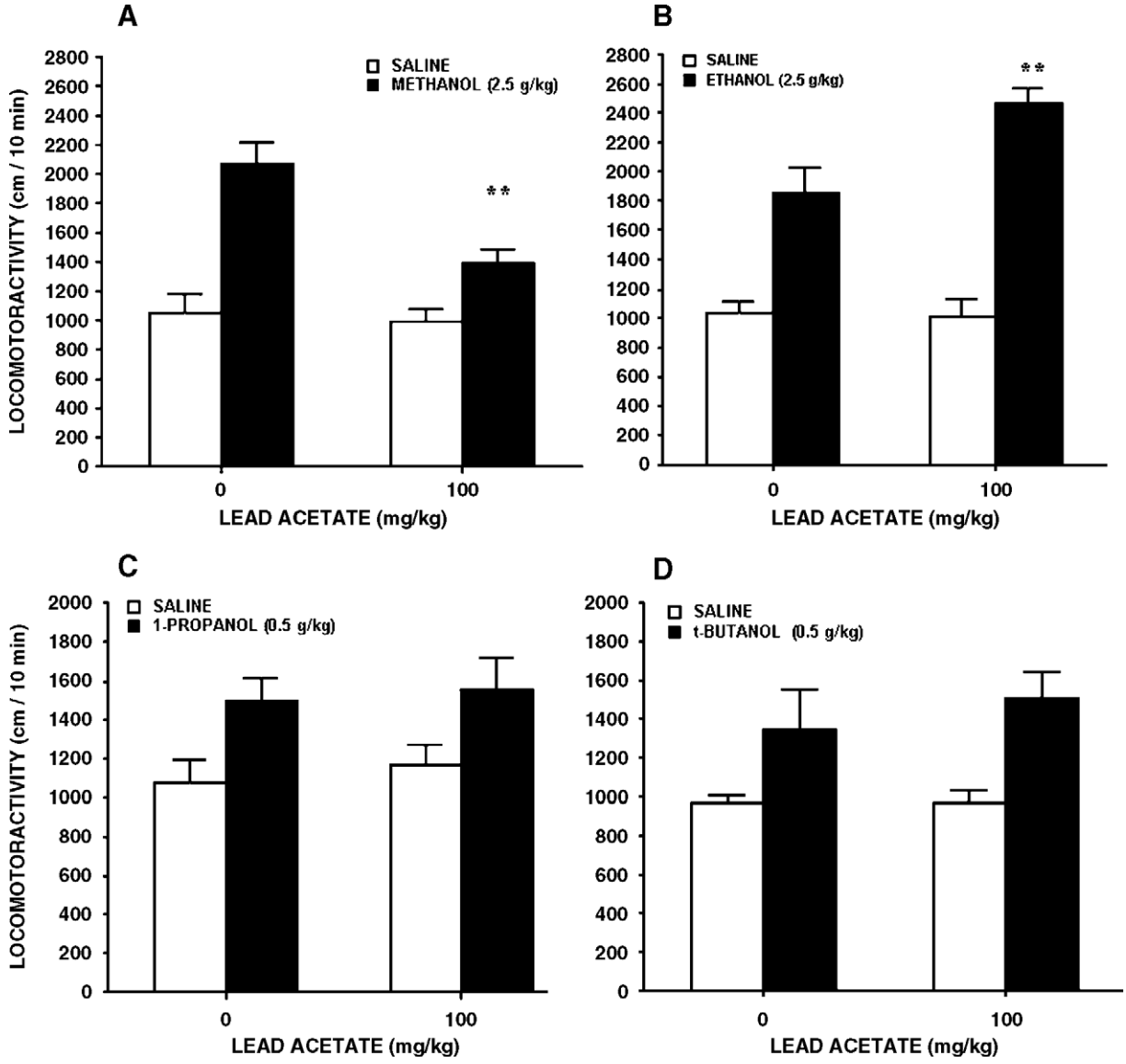

Fig. 1. Effect of an acute IP injection of lead acetate (0 or 100 mg/kg 7 days before test) on the locomotor activity induced by A) methanol (0.0 or 2.5 g/kg), B) ethanol (0.0 or 2.5 g/kg), C) 1-propanol (0.0 or 0.5 g/kg), and D) *tert*-butanol (0.0 or 0.5 g/kg). mean ± SEM centimeters in 10 min (**$p<0.01$, significantly different from 0 mg/kg lead acetate group in the same alcohol dose).

respective control (0 g/kg methanol groups). Lead acetate treatment significantly reduced locomotor activity in methanol-treated mice ($p<0.01$) compared to the saline–2.5 g/kg methanol-treated mice.

Ethanol (2.5 g/kg)-induced locomotor activity is shown in Fig. 1B. A two-way factorial ANOVA showed a statistically significant effect for the lead acetate dose factor [$F(1,32)=5.10$, $p<0.05$], the ethanol dose (0.0 or 2.5 g/kg) factor [$F(1,32)=78.70$, $p<0.01$], and the interaction [$F(1,32)=6.33$, $p<0.01$]). The post hoc LSD test demonstrated that the dose of ethanol produced a significant increase on locomotion in saline pretreated mice and in lead acetate-pretreated mice ($p<0.01$). It also showed that lead acetate modified locomotor activity only in the ethanol-treated group ($p<0.01$), this group displayed a higher level of locomotion that the ethanol– saline-treated group.

Fig. 1C and D depicts the effect of acutely administered lead acetate (0 or 100 mg/kg, 7 days before) on the locomotion induced by 1-propanol (0.0 or 0.5 g/kg) and *tert*-butanol (0.0 or 0.5 g/kg) respectively. A two-way factorial ANOVA demonstrated an effect of the main factor: *tert*-butanol dose [$F(1,32)=13.05$, $p<0.01$], but it showed neither effect of lead acetate dose [$F(1,32)=0.37$, $p=0.54$] nor a significant interaction [$F(1,32)=0.39$, $p=0.53$]. The same pattern of effects was observed for the 1-propanol experiment. The two-way ANOVA showed that 1-propanol dose was statistically significant [$F(1,26)=9.33$, $p<0.01$], but neither the factor lead acetate dose [$F(1,26)=0.28$, $p=0.60$], nor the interaction [$F(1,26)=0.00$, $p=0.92$] was significant. These results indicate that these alcohols significantly increase locomotion at this dose, and that this effect is independent of the lead acetate pretreatment.

3.2. Effects of lead acetate on alcohols-induced LRR

Fig. 2 shows the results for the LRR study. A series of *t*-tests were conducted in order to analyze the data concerning to the effect of the different alcohols in the LRR study. Lead acetate pretreatment (0 or 100 mg/kg, 7 days before) in ethanol (4.5 g/kg)-injected mice significantly reduced the duration of LRR ($t=3.13$, $df=26$, $p<0.01$) (see data in Fig. 2B). The opposite result was found in methanol (6.0 g/kg)-injected mice; lead acetate-pretreated mice had a significant increase in the duration of LRR induced by methanol ($t=3.14$, $df=22$,

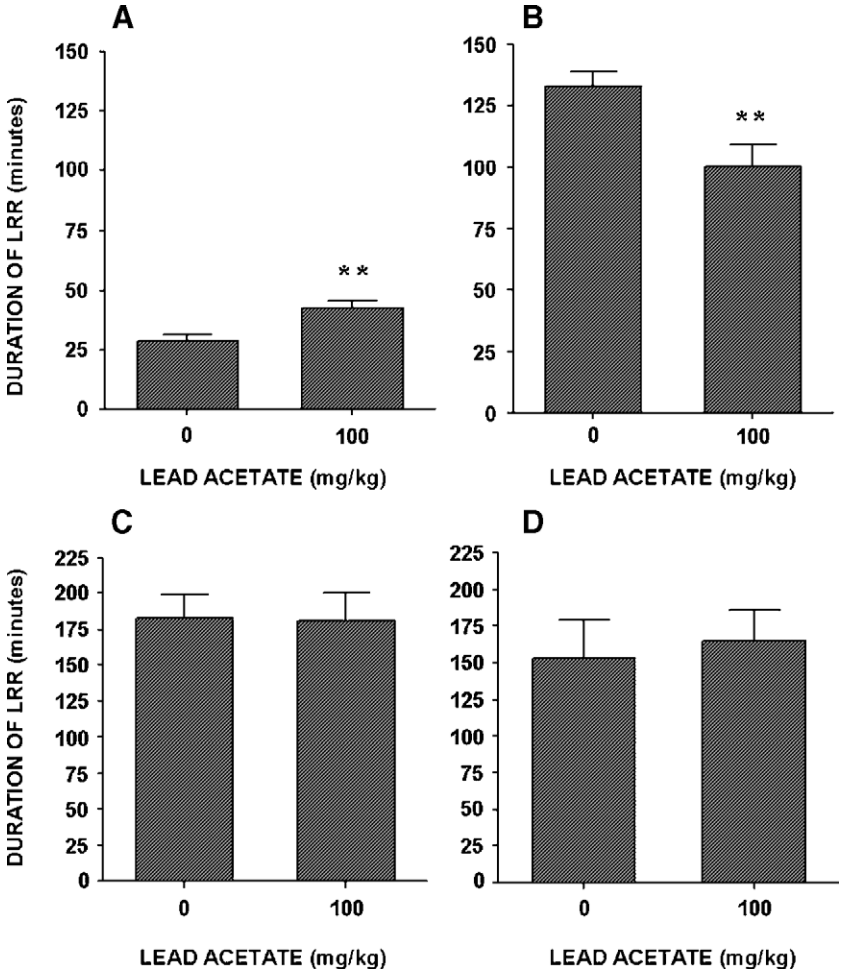

Fig. 2. Effect of an acute IP injection of lead acetate (0 or 100 mg/kg 7 days before test) on the duration of LRR induced by A) methanol (6.0 g/kg), B) ethanol (4.5 g/kg), C) 1-propanol (2.5 g/kg), and D) *tert*-butanol (2.0 g/kg). Mean±SEM min of LORR (**$p<0.01$, significantly different between groups in the same alcohol experiment).

Table 1A
Mean (±SEM) blood levels 15 min after an acute IP administration of one of the four alcohols (doses used in the locomotion study)

Blood levels (mg/dl)		
	Saline	Lead Acetate (100 mg/kg)
Methanol (2.5 g/kg)	415.3+21.6	322+18.3**
Ethanol (2.5 g/kg)	260.3+20.2	264.8+35.8
1-propanol (0.5 g/kg)	40.2+2.8	36.9+4.4
tert-butanol (0.5 g/kg)	58.1+3.1	55.6+1.0

Saline of lead acetate (100 mg/kg) were injected in mice 7 days before testing ($n=5-7$ per group).
** $p<0.01$, significantly different between groups in the same alcohol experiment.

$p<0.01$) (see data in Fig. 2A). However lead pretreatment did not significantly affect the duration of LRR in mice injected with either 1-propanol (2.5 g/kg) ($t=0.06$, $df=19$, n.s.) (Fig. 2C) or *tert*-butanol (2.0 g/kg) ($t=0.34$, $df=24$, n.s.) (Fig. 2D).

3.3. Effects of lead acetate on blood alcohols levels

The results shown in Tables 1A and 1B summarize the effects of lead acetate treatment (0 or 100 mg/kg, 7 days before) on methanol, ethanol, 1-propanol and *tert*-butanol blood levels. Table 1A shows the alcohols levels after 15 min of IP administration at the doses used to induce locomotor activity. Independent *t*-test for every alcohol shows that only methanol levels (2.5 g/kg) were different between saline and lead acetate-treated groups ($t=3.01$, $df=8$, $p<0.01$). None of the other alcohols had blood levels that were affected by the lead treatment compared to saline-treated mice: ethanol (2.5 g/kg) ($t=0.26$, $df=12$, n.s.), 1-propanol (0.5 g/kg) ($t=0.61$, $df=12$, n.s.) and *tert*-butanol (0.5 g/kg) ($t=0.61$, $df=8$, n.s.). Table 1B shows blood alcohols levels 60 min after the administration in the narcotic doses. The independent samples *t*-test showed that lead acetate only produced statistically significant different blood levels in mice injected with methanol (6.0 g/kg) ($t=2.39$, $df=8$, $p<0.05$). As with the lower doses, none of the other alcohols had blood levels that were affected by lead acetate treatment when compared to saline pretreated mice: ethanol (4.5 g/kg) ($t=0.74$, $df=7$, n.s.), 1-propanol (2.5 g/kg) ($t=0.66$, $df=10$, n.s.) and *tert*-butanol (2.0 g/kg) ($t=1.24$, $df=6$, n.s.). These results demonstrate that compared to their respective saline control, lead acetate only modified methanol levels but not the blood content of the other alcohols.

Table 1B
Mean (±SEM) blood levels 60 min after an acute IP administration of one of the four alcohols (doses used in the LRR study)

Blood levels (mg/dl)		
	Saline	Lead acetate (100 mg/kg)
Methanol (6.0 g/kg)	862.8+46.2	684.5+59.5*
Ethanol (4.5 g/kg)	351.0+25.0	364.2+22.9
1-propanol (2.5 g/kg)	233.8+21.7	217.4+13.8
tert-butanol (2.0 g/kg)	262.5+18.6	236.4+9.3

Saline of lead acetate (100 mg/kg) were injected in mice 7 days before testing ($n=5-7$ per group).
* $p<0.05$, significantly different between groups in the same alcohol experiment.

Table 2
Mean (±SEM) catalase activity after IP injection of saline or lead acetate (100 mg/kg) 7 days before ($n=6-7$ per group)

Catalase activity (mmol H_2O_2/min/mg protein)		
	Saline	Lead acetate (100 mg/kg)
Brain	1.02+0.06	1.47+0.10**
Liver	304.65+19.03	641.03+18.00**

** $p<0.01$, significantly different between groups in the same same organ.

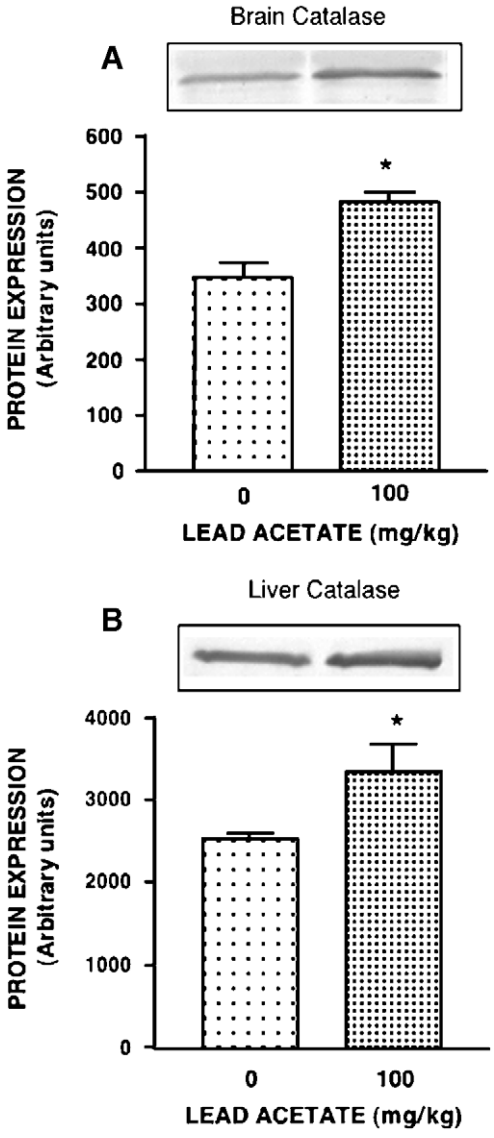

Fig. 3. Western blotting and densitometric analysis of catalase expression from control and lead acetate (100 mg/kg)-treated animals. A representative Western blot showed a unique band corresponding to catalase appeared with a molecular weight of ≈60 kDa. Each line contains A) 100 μg of total protein from brain homogenates or B) 4 μg of total protein from liver. Bars represent the mean±SEM of the densitometric quantification obtained from four different animals (*$p<0.05$ significantly different from 0 mg/kg lead acetate group).

3.4. Effects of lead acetate on catalase activity

Two separate *t*-test for independent samples were performed to analyze the effects of lead acetate (0 or 100 mg/kg, 7 days before) on catalase activity. Table 2 shows brain and liver catalase activity for both groups. The *t*-test demonstrated a significant effect of treatment in both organs (brain catalase, $t=3.57$, $df=10$, $p<0.01$; liver catalase, $t=5.98$, $df=11$, $p<0.01$). Thus, lead acetate potentiated brain (44%) and liver (110%) catalase activity.

3.5. Effects of lead acetate on catalase expression

Finally, we studied catalase protein expression by Western blot analysis in brain and liver homogenates of tissues obtained from 4 different animals treated 7 days before with lead acetate (0 or 100 mg/kg). Data are shown in Fig. 3A and B. A one way ANOVA for catalase expression demonstrated a significant effect of treatment in both organs; in the brain [$F(1,6)=8.66$, $p<0.05$], and in the liver [$F(1,6)=6.25$, $p<0.05$]. These results demonstrate that 100 mg/kg lead acetate significantly enhanced catalase protein expression in brain and liver tissues.

4. Discussion

The present studies demonstrated that the effects of lead treatment on behaviors induced by alcohols depend greatly upon whether or not a particular alcohol is metabolized by catalase. Although the effects of ethanol and methanol on locomotor activity and LRR were affected substantially by lead treatment, the effects of *tert*-butanol and 1-propanol were not. These results support the hypothesis that the facilitatory effect of lead treatment on ethanol-induced activity is related to changes in catalase activity, rather than some nonspecific activating effect that generalizes to all alcohols.

Lead-treated mice demonstrated a potentiation of ethanol-induced locomotor activity and a decrease in methanol-induced locomotor activity when compared to saline-treated mice. Mice that received 2.5 g/kg of ethanol and had been pretreated with lead acetate showed 32% more activity than control animals receiving the same dose of ethanol. For methanol the effect of lead acetate was the reverse; 2.5 g/kg of methanol induced locomotion in relation to saline-treated groups, but lead acetate pretreatment reduced methanol-induced locomotion by approximately 34%. The opposite pattern of results was found for the duration of the loss of the righting reflex produced by ethanol and methanol. Lead acetate reduced (33%) LRR in ethanol (4.5 g/kg)-treated mice, and conversely, the duration of LRR after methanol (6.0 g/kg) administration was potentiated (46%) by lead acetate pretreatment. The effects of these two short-chain alcohols in combination with lead acetate pretreatment were observed in the absence of other nonspecific behavioral effects. In the present paper, as well as in previous studies, we have observed that an acute lead acetate injection (100 mg/kg, 7 days before test) had no effect on spontaneous locomotor activity or in body and brain weight (Correa et al., 1999a, 2000, 2004b).

As expected, lead treatment was effective in increasing catalase activity as well as catalase protein expression in the liver and in the brain as measured spectophometrically and by immunoblot analysis. It has been suggested that this increase of catalase could constitute a defense mechanism against free radical generation (Montoliu et al., 1994; Tolosa et al., 1995), which in this case could have been induced by lead exposure. The increase in catalase content and activity may be related to the modulatory effects that lead seems to exert on methanol- and ethanol-induced behaviors. Methanol in rodents is mainly metabolized in the liver by catalase (Bradford et al., 1993). Previous work has demonstrated that the catalase inhibitor aminotriazole decreased ethanol and methanol metabolism by 75% in mutant mice lacking ADH (Bradford et al., 1993). Methanol has been used as a specific substrate to study the competition of this alcohol with ethanol for the Compound I (catalase–hydrogen peroxide) in vitro (Gill et al., 1992) and in vivo studies (Tampier et al., 1980). Since methanol is mainly metabolized by catalase in mice, and the liver is the primary organ for peripheral metabolism, in the present work, the lead-induced increase in liver catalase activity could account for the reduction in blood methanol content found at both doses (2.5 and 6.0 g/kg). Thus, if methanol reaches the brain in lower amounts and/or if it is more rapidly metabolized in the brain, that should modify its effects on behavior. This hypothesis is supported by the fact that in the rat, liver catalase is responsible for the oxidation of methanol to formaldehyde (Heck et al., 1990), and it has been demonstrated that this metabolite of methanol reduces locomotion in rats (Boja et al., 1985). The possibility that formaldehyde could also be responsible for the increase in the duration of LRR has not been studied yet.

The induction of catalase in the liver does not seem to have an effect on blood ethanol levels, and this is in accordance with the fact that ethanol metabolism in the liver is mainly mediated by ADH I. In contrast, catalase and cytochrome P450-2E1 seem to make only a minor contribution to liver ethanol metabolism under normal conditions (Hunt, 1996; Quertemont et al., 2005). In fact, catalase seems to contribute significantly to liver ethanol metabolism only in mice that lack ADH (Bradford et al., 1993). It has been suggested that since ADH I is not present in the brain, catalase could be the enzyme responsible of encephalic ethanol metabolism (Aragon et al., 1985; Cohen et al., 1980). Therefore, increasing catalase-mediated ethanol metabolism in the brain should increase cerebral acetaldehyde formation. The induction of brain catalase has been suggested to affect some of the behavioral effects of ethanol, including increases in locomotion and reductions in the duration of LRR in mice (Correa et al., 1999a, 2000, 2001a,b, 2004a; Pastor et al., 2002; Sanchis Segura et al., 1999c). These effects of centrally formed acetaldehyde are in accordance with papers demonstrating the stimulation of motor behaviors in rats after direct central administration of acetaldehyde (Arizzi et al., 2003; Arizzi-LaFrance et al., 2004; Correa et al., 2003). Although brain catalase activity (and by implication, centrally formed or administered acetaldehyde) can play a major role in the stimulant effects of ethanol (as the enhancement of

locomotor activity observed after low or moderate doses of ethanol), it only has a modulatory effect on the depressant effects of higher doses of ethanol (i.e., LRR). The inverse relationship between brain catalase activity and ethanol-produced LRR (Correa et al., 2001b), as well as the different size of the effect of catalase manipulations on different behavioral effects of ethanol (locomotion vs. LRR), could be understood as an opposite effect between catalase dependent and non-dependent effects in the brain after ethanol administration. In addition to the idea that central acetaldehyde plays a minor modulatory role in the LRR induced by ethanol, it also must be emphasized that the direct peripheral administration of acetaldehyde induces a very weak LRR relative to ethanol. This effect is achieved only when the dose of acetaldehyde is close to the lethal dose and only for a very brief period of time (6–10 min of LRR), in contrast to the persistent changes (more than 1 h of LRR) induced by ethanol doses that are well below the lethal dose (see Quertemont et al., 2005).

The results with the other two alcohols support the hypothesis that the behavioral interaction between methanol or ethanol and lead acetate (under the present conditions) is due to the induction of catalase. *tert*-butanol and 1-propanol are not metabolized by catalase (Poet et al., 1997; Teschke et al., 1975), and this is supported by the pharmacokinetic results in our study; lead acetate pretreatment did not affect *tert*-butanol or 1-propanol blood content in relation to saline pretreated mice. Thus, in our results, lead acetate did not affect the induction of locomotion, nor did it affect the duration of LRR induced by *tert*-butanol or 1-propanol. *tert*-butanol is a tertiary alcohol (branched chain alcohol) that is metabolized into acetone by a hydroxylation reaction (Poet et al., 1997; Baker et al., 1982). This alcohol lacks the carbonyl hydrogen required for ADH activity and it has been demonstrated that the administration of a catalase inhibitor such as aminotriazole is ineffective at modifying *tert*-butanol metabolism (Baker et al., 1982). Consistent with this observation, previous studies from our laboratory have demonstrated that *tert*-butanol-induced locomotion was not affected by sodium azide pretreatment under conditions that inhibited brain catalase activity (Sanchis-Segura et al., 1999a). Sodium azide is a selective inhibitor of catalase that has been demonstrated specifically to inhibit methanol and ethanol oxidation in rat hepatic microsomes without modifying propanol or butanol oxidation in this tissue preparation (Teschke et al., 1975). 1-Propanol is oxidized in the liver by ADH I (Ehrig et al., 1988) to propionaldehyde, which in turn is a substrate for ALDH (Pietruszko et al., 1984; Vasiliou and Marselos, 1989). These two alcohols have a relatively narrow range of doses that induce locomotion (Dudek and Phillips, 1983; Palmer et al., 2002). For example, in our pilot studies the 0.5 g/kg dose was the only dose that significantly stimulated locomotion in both cases. Nevertheless, lead acetate did not modify the induction of motor activity by this dose of 1-propanol or *tert*-butanol. Both alcohols were more effective and more potent in maintaining LRR than the shorter chain alcohols (methanol and ethanol). The observation about the potency of the different alcohols in inducing LRR (from least to most potent of methanol less than ethanol less than 1-propanol or *tert*-butanol) has been previously made about hypothermia and lipid solubility (Mohler and Gordon, 1991).

Thus, in the present paper, we have demonstrated that an acute administration of 100 mg/kg of lead acetate increases liver and brain catalase activity and content as measured 7 days later, and also affects the regulation of locomotion and LRR induced by the two alcohols that are metabolized by this enzyme. We also have found that, in contrast, lead treatment did not affect the behaviors induced by two other alcohols that have a different pathway of metabolism. The modulation of methanol-induced behaviors can be produced by the modulation of the peripheral metabolism of this alcohol.

Against the conclusion that lead acetate modulates ethanol-induced behaviors via its effects on brain catalase, it has been argued that lead has other neural effects (Deitrich, 2004; Quertemont et al., 2005). For example, lead acetate has been demonstrated to affect some neuronal parameters such as dopamine transmission (Cory-Slechta et al., 2004; Zuch et al., 1998). However, the route and pattern of administration (chronic administration in perinatal age) in those studies were very different from the one used in the present paper. Thus, although chronic perinatal administration of lead acetate interacted with spontaneous or amphetamine induced locomotion in young but not adult rats under stress conditions (Virgolini et al., 2004), our treatment (acute IP administration of lead acetate 100 mg/kg, to adult mice 7 days before test) did not produce a potentiation of either spontaneous or D-amphetamine-induced locomotion (Correa et al., 1999a), supporting the specificity of lead on ethanol-induced locomotor activity. In addition, it has been suggested that lead–ethanol interactions may be explained by changes in protein kinase C (Markovac and Goldstein, 1988). However, the actions of inorganic lead on protein kinase C activity are still a matter of debate (Murakami et al., 1993), and it is not clear that the in vitro effects of inorganic lead are highly related to the in vivo effects of lead acetate that underlie the present findings. Moreover, the hypothesis that lead-induced changes in the behavioral effects of ethanol are dependent upon changes in catalase activity is consistent with previous studies showing that, across a wide variety of treatments involving lead-related or other conditions, brain catalase activity is highly correlated with ethanol-induced increases in motor activity (Correa et al., 2001b; Sanchis Segura et al., 1999b,c; Pastor et al., 2002).

In summary, we cannot completely discard the possibility that some other unknown effects of lead are responsible for the modulation of ethanol-induced behaviors observed in the present and previous studies. Nevertheless, we propose that the most parsimonious explanation seems to be that the effects of lead acetate on ethanol-induced behaviors are mainly dependent upon the modulatory effects of lead on brain catalase activity. Therefore, the lead-induced modulation of the locomotor stimulation and LRR produced by ethanol may be a result of catalase-mediated increases in the local production of acetaldehyde in the brain.

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