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The effect of cyanamide and 4-methylpyrazole on the ethanol-induced locomotor activity in mice

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Abstract

To assess the role of cyanamide and 4-methylpyrazole (4-MP) in mediating ethanol-induced locomotor activity in mice, they were pretreated with cyanamide (12.5, 25, or 50 g/kg) prior to one ethanol injection (2.4 g/kg) and showed significantly depressed locomotor activity compared with control groups. Cyanamide (25 mg/kg) also cancelled out the biphasic action of ethanol (0, 0.8, 1.6, 2.4, 3.2, or 4 g/kg) on locomotor activity. The action of cyanamide and 4-MP in combined administration was also tested. Our data show that pretreatment with 4-MP alone does not change the spontaneous or ethanol-induced locomotor activity. Conversely, when mice were pretreated with cyanamide and 4-MP, the depressive effect of cyanamide on the locomotor activity induced by ethanol disappeared, and the locomotor activity rose to levels similar to those of the control group, recovering the biphasic ethanol effect. These effects cannot be attributed to peripheral elevated blood acetaldehyde levels, as pretreatment with 4-MP prevents accumulation of acetaldehyde. These data might suggest some influence of brain catalase and aldehyde dehydrogenase (ALDH) on the effects of ethanol.

Keywords: Cyanamide–ethanol reaction (CER); Catalase; Aldehyde dehydrogenase; Cyanamide; 4-Methylpyrazole; Ethanol; Acetaldehyde; Mice

1. Introduction

The catalase and aldehyde dehydrogenase (ALDH) activity, using inhibitors of these enzymatic systems, have been researched for decades. After calcium cyanamide administration, voluntary ethanol drinking was suppressed in rats (Sinclair and Lindros, 1981); similar results were obtained in continual and alternate access to alcohol (Sinclair et al., 1980). In humans, this pretreatment reduces drinking by alcoholics (Brien et al., 1980; Peachey et al., 1981a), whereas in social drinkers or normal subjects, it does not produce enough aversion to curtail drinking totally (Peachey et al., 1981b,c, 1983). Varying data were obtained in this field of research (Sinclair and Gribble, 1985; Sinclair et al., 1980). Some authors (Sinclair and Lindros, 1981; Sinclair et al., 1980; Spivak et al., 1987a,b) state that the accumulation of acetaldehyde by cyanamide in the periphery after ethanol ingestion is not responsible for changes in several ethanol-induced behaviors.

These enzymatic systems, i.e., brain catalase and ALDH, could control the production and elimination of acetaldehyde in the brain. This hypothesis finds support in the results obtained from the manipulation of these systems. Changes in the activity of enzymatic systems produce modifications in ethanol-related behaviors, such as alcohol intake (Sinclair and Lindros, 1981), conditioned taste aversion (CTA) (Spivak et al., 1987a; Aragon et al., 1985a,b), or locomotor activity (Spivak et al., 1987b; Escarabajal et al., 2000). These results may indicate that brain catalase and ALDH could have an effect on ethanol metabolism by regulating the levels of acetaldehyde in the brain.

Some results obtained from correlational studies on the subject are in line with this hypothesis. Thus, levels of brain ALDH correlated better with ethanol consumption levels than liver aldehyde-oxidizing capacity (Socaransky et al., 1984). Similar data are available for catalase: catalase activity from rats naive to ethanol was significantly and positively correlated with later voluntary consumption of

Abbreviations: ALDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase; 4-MP, 4-methylpyrazole.

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ethanol (Aragon et al., 1985a,b; Amit and Aragon, 1988). Global brain ALDH activity also correlated with ethanol intake levels in rats (Aragon et al., 1985a,b; Socaransky et al., 1985).

According to this, if brain acetaldehyde levels determine ethanol effects, changes in central catalase and ALDH activity could show both the possibility of brain ethanol metabolism and the role of acetaldehyde on the psychopharmacological effects of ethanol.

The role of these enzymatic systems were analyzed here using a combined treatment with cyanamide, a catalase and ALDH inhibitor (Deitrich et al., 1976; Nagasawa et al., 1990), and 4-methylpyrazole (4-MP), an alcohol dehydrogenase (ADH) inhibitor (Theorell et al., 1969). These substances were selected for their effect on the enzymatic system that is involved in the metabolism of ethanol. The former, cyanamide, inhibits catalase and ALDH activity, which could show the role of these enzymes on ethanol-induced behaviors. The latter, 4-MP, inhibits the activity of ADH, which could reduce the acetaldehyde levels in the periphery after ethanol ingestion. This, in turn, could reduce the toxic effect of the peripheral acetaldehyde. In fact, 4-MP is used for the treatment of the toxic effect produced by high doses of alcohol (Lindros et al., 1981).

Combined treatment of cyanamide and 4-MP to prevent acetaldehyde accumulation in periphery also inhibits ALDH in the brain (Sinclair and Lindros, 1981). In our study, this manipulation was used to assess the role of peripheral and central action of acetaldehyde in the mediation of the locomotor activity. The hypothesis is that, if the levels of acetaldehyde in the brain are a physiological parameter that controls alcohol-induced locomotor activity, variations in brain catalase or ALDH activity in the brain may then cause differences in this behavior.

As shown above, most experiments on the cyanamide effect have been conducted with ethanol ingestion (Socaransky et al., 1985; Aragon et al., 1993) and few studies analyzed its effect on other ethanol behaviors (Spivak et al., 1987a,b). Our aim is to try to elucidate the role of ALDH and catalase enzymes through (a) a systematic study with a wide range of cyanamide doses, (b) the effect of cyanamide in a combined treatment with 4-MP preventing peripheral accumulation of acetaldehyde produced by cyanamide, and (c) assessment of the effect of the latter treatment on the locomotor activity in mice.

Specifically, we examined the effects of acetaldehyde on ethanol-induced locomotor activity by manipulating acetaldehyde-metabolizing enzymes.

2. Materials and methods

2.1. Subjects

The subjects were Swiss albino mice (Harlan Interfauna Ibèrica, Barcelona, Spain), 5 weeks old, and weighing 30–35 g at the start of the experiment. The animals were housed in groups of three or four per cage with laboratory chow (Panlab, Spain) and tap water available ad libitum. The animal colony room was illuminated on a 12-h day/night cycle, lights on at 8 a.m. and lights off at 8 p.m. Testing was always carried out during the light cycle. The mice were allowed 1 week of adaptation to the laboratory housing conditions prior to experimentation. All experimental procedures complied with the European Community Council Directive (86/609/ECC) for the use of laboratory animal subjects and in agreement with the UK Animals Scientific Procedures Act 1986.

2.2. Apparatus

The open-field chamber consisted of a clear glass cylinder 25 cm in diameter and 30 cm high. The floor of the cylinder was divided into four equal quadrants by two intersecting lines drawn on the floor. A locomotion score was assigned each time an animal crossed one of the lines with all four legs. The test room was illuminated with a soft white light and external noise was reduced.

2.3. Procedure

2.3.1. Experiment 1

The effect of doses of cyanamide (12.5, 25, and 50 mg/kg) on an acute ethanol injection was studied in the first experiment. Sixty-four Swiss male albino mice were treated with intraperitoneal injections of cyanamide or saline. Ninety minutes later, they received one saline injection, and 30 min after that they were injected with saline (control group) or ethanol (2.4 g/kg, experimental group). Following the latter treatment, mice were individually placed in the open-field chamber for 20 min and locomotor activity was recorded for the last 10 min. This delay was chosen to reduce the handling effect and the environmental novelty of the open field and to allow the drug to be absorbed (Kelley, 1993).

2.3.2. Experiment 2

In order to analyze the effect of cyanamide (25 mg/kg) on ethanol-induced locomotor activity, 96 animals were randomly assigned to two groups: control (cyanamide + saline + saline doses) and experimental (cyanamide + saline + ethanol doses). The time interval between injections and locomotor activity measure was similar to those described in Experiment 1.

2.3.3. Experiment 3

In Experiment 3, the effect of 4-MP on ethanol-induced locomotor activity was analyzed. Ninety-six mice ($n = 8$ per group) received intraperitoneal injections of saline. Ninety minutes after the injections, the animals were assigned to two pretreatment conditions: saline or 4-MP (10 mg/kg). This 4-MP dose was selected because previous studies

(Sinclair et al., 1980; Spivak et al., 1987b) proved that it does not produce a significant effect on locomotor behavior.

Half an hour later, the mice were injected with ethanol (0, 0.8, 1.6, 2.4, 3.2, or 4 g/kg) and were immediately placed in open-field chambers for 20 min and the locomotor activity was recorded for the last 10 min.

2.3.4. Experiment 4

The fourth study tested the effect of concurrent treatment of cyanamide and 4-MP on the locomotor activity induced by different doses of ethanol. Ninety-six mice were pretreated with cyanamide (25 mg/kg), as in Experiment 2 above. They were randomly assigned either to a control or to an experimental group. In the former case, animals received saline and several doses of ethanol injections; in the latter, they were treated with 4-MP and, after half an hour, with different doses of ethanol (0, 0.8, 1.6, 2.4, 3.2, or 4 g/kg).

2.3.5. Experiment 5

The effect of different doses of cyanamide when interacting with 4-MP was explored in another experiment. Sixty-four mice were pretreated with cyanamide (12.5, 25, or 50 mg/kg) half an hour before the injection of 4-MP (10 mg/kg). The animals were treated with ethanol (0, 0.8, 1.6, 2.4, 3.2, or 4 g/kg) or saline half an hour later and placed in the open-field chambers.

In all experiments, the animals were placed in their cages between injections.

2.4. Drugs

Cyanamide and 4-MP (Sigma-Aldrich Química, Madrid, Spain) were dissolved in saline (50 mg/20 ml and 20 mg/20 ml, respectively). Ethanol solution (Panreac Química, Madrid, Spain) was diluted in saline at 20% v/v from 96% solutions. All injections were administered intraperitoneally.

2.5. Statistical analysis

All data were analyzed by two-way analysis of variance (ANOVA). Pairwise comparisons were made using Fisher's Least Significant Difference Tests (LSD). In this study, SYSTAT 5.2 (SPSS, Chicago, IL) was used.

3. Results

Fig. 1 shows the dose–response effects on locomotion of cyanamide pretreatment (12.5, 25, or 50 mg/kg) 2 h before an ethanol challenge (2.4 g/kg). A two-way ANOVA revealed a significant cyanamide pretreatment effect [$F(3,56)=9.61$, $P<.000$] and an interaction effect [$F(3,56)=10.604$, $P<.000$], but it does not show a significant ethanol effect [$F(1,56)=1.713$, $P>.05$]. Pairwise comparisons using Fisher's test revealed that cyanamide did not change the spontaneous locomotion compared with the control group. Conversely, in the experimental group, the analyses showed that cyanamide pretreatment significantly reduced ethanol-inducing effect at all cyanamide doses evaluated: 12.5, 25, and 50 mg/kg ($P<.000$).

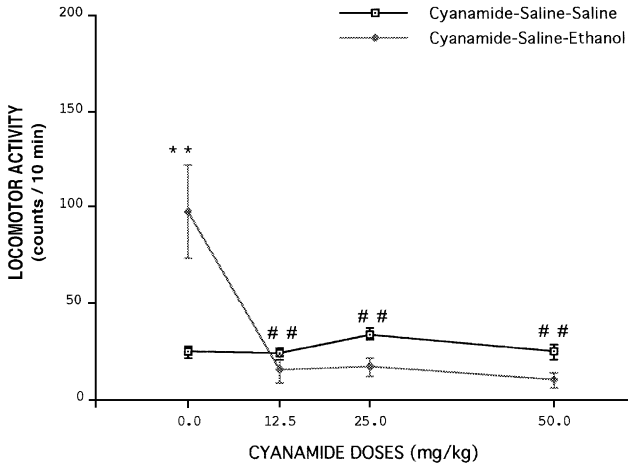

Fig. 1. Effect of different doses of cyanamide on locomotion induced by ethanol. Mean (±SE) locomotor activity (counts in 10 min) for all treatment groups ($n=8$). Mice were pretreated intraperitoneally with cyanamide (12.5, 25, or 50 mg/kg). Two hours after this treatment, ethanol (2.4 g/kg) was administered to the mice. $^{**}P<.01$, for differences between cyanamide–saline groups and cyanamide–ethanol groups. $^{\#\#}P<.01$, for differences between saline–ethanol and cyanamide–ethanol group.

Fig. 2 shows the locomotor activity of saline- and cyanamide-(25 mg/kg) pretreated mice 2 h before an acute injection of several doses of ethanol (0, 0.8, 1.6, 2.4, 3.2, and 4 g/kg). A two-way ANOVA yielded a cyanamide effect [$F(1,84)=23.058$, $P<.000$], an ethanol treatment effect [$F(5,84)=8.481$, $P<.000$], and a significant interaction between both effects [$F(5,84)=6.535$, $P<.000$]. LSD comparisons showed that cyanamide pretreatment significantly reduces ethanol-induced locomotor activity at the 1.6 [$t(14)=-2.791$; $P<.01$], 2.4 [$t(14)=-3.328$; $P<.00$], and 3.2 [$t(14)=-2.495$; $P<.03$] doses. Besides, cyanamide did not affect the spontaneous locomotor activity compared to the control group.

In order to decide whether 4-MP can be used as a pharmacological tool in later experiments, we examined its effect on locomotor activity induced by a wide range of ethanol doses (0, 0.8, 1.6, 2.4, 3.2, and 4 g/kg). The results are shown in Fig. 3. A two-way ANOVA (Group × Doses) revealed a significant ethanol pretreatment [$F(1,84)=12.535$, $P<.000$], but neither the 4-MP pretreatment [$F(5,84)=2.445$, $P>.05$] nor the interaction between two factors [$F(5,84)=1.449$, $P>.05$] were significant. Pairwise comparisons using Fisher's test showed that ethanol has a biphasic effect on locomotor activity increasing crossings significantly at 1.6 and 2.4 g/kg. It was also shown that 4-MP administration does not have any effect on spontaneous locomotor activity.

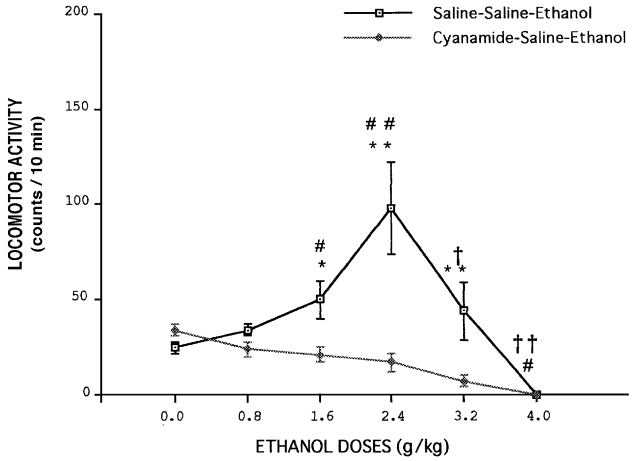

Fig. 2. Effect of cyanamide or saline on ethanol-induced locomotor activity. Mean (±SE) locomotor activity (counts in 10 min) for all treatment groups ($n=8$). Mice were pretreated intraperitoneally with saline or cyanamide (25 mg/kg) 2 h prior to ethanol administration (0.0, 0.8, 1.6, 2.4, 3.2, or 4.0 g/kg). $^{*}P<.05$, $^{**}P<.01$, for differences between saline–ethanol groups and cyanamide–ethanol group. $^{\#}P<.05$, $^{\#\#}P<.01$, for differences between saline–saline groups and saline–ethanol groups. $^{\dagger}P<.05$, $^{\dagger\dagger}P<.01$, for differences between cyanamide–saline and cyanamide–ethanol groups.

The results of the fourth experiment are given in Fig. 4. This experiment tests the effect of concurrent administration of cyanamide and 4-MP on ethanol-induced locomotor activity. A two-way ANOVA showed a significant ethanol effect [$F(5,84)=12.442$, $P<.000$], but neither the factor group [$F(1,84)=0.22$, $P>.05$] nor the interaction between both factors [$F(5,84)=1.017$, $P>.05$] were significant. Post hoc Fisher's LSD test revealed that ethanol has a similar biphasic effect on locomotor activity. Crosses are significantly increased at 1.6–2.4 and 2.4 g/kg for the control and experimental groups, respectively.

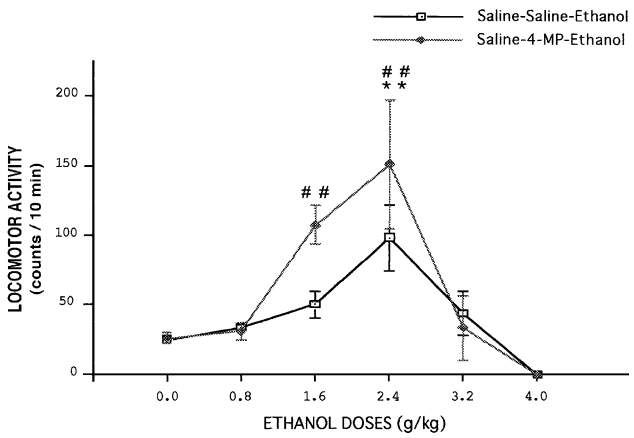

Fig. 3. Effect of 4-MP on ethanol-induced locomotor activity. Mean (±SE) locomotor activity (counts in 10 min) for all treatment groups ($n=8$). Mice were pretreated intraperitoneally with saline or 4-MP (10 mg/kg) half an hour prior to ethanol administration (0.0, 0.8, 1.6, 2.4, 3.2, or 4.0 g/kg). $^{**}P<.01$, for differences between saline–ethanol groups and saline–saline groups. $^{\#\#}P<.01$, for differences between 4-MP–etanol and 4-MP–saline groups.

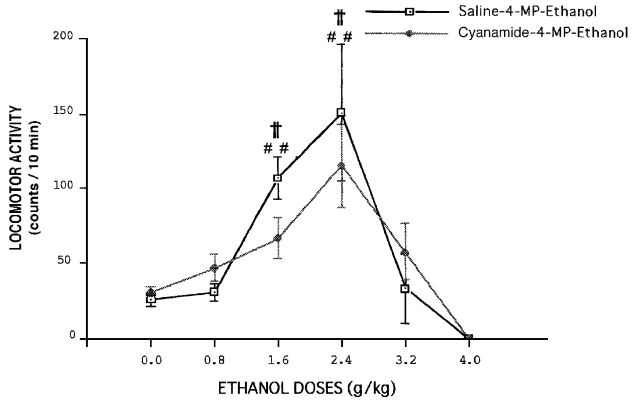

Fig. 4. Effect of concurrent administration of cyanamide and 4-MP or saline on ethanol-induced locomotor activity. Mean (±SE) locomotor activity (counts in 10 min) for all treatment groups ($n=8$). Mice were pretreated intraperitoneally with cyanamide (25 mg/kg) or saline (0.0, 0.8, 1.6, 2.4, 3.2, or 4.0 g/kg) 2 h prior to ethanol administration. $^{\#\#}P<.01$, for differences between saline–saline group and saline–ethanol groups. $^{\dagger\dagger}P<.01$, for differences between cyanamide–4-MP–saline group and cyanamide–4-MP–ethanol groups.

The fifth experiment studied the effect of the combined administration of 4-MP and different doses of cyanamide (12.5, 25, and 50 mg/kg) on locomotor and ethanol-induced locomotor activity (Fig. 5). A two-way ANOVA showed a significant effect only for the factor group [$F(1,56)=26.409$, $P<.000$], but no statistical effect for the factor dose [$F(3,56)=0.446$, $P>.05$] or the interaction [$F(3,56)=0.377$, $P>.05$]. LSD comparisons proved that none of the groups treated with the combined treatment show any effect on the spontaneous locomotor activity or between doses of cyanamide.

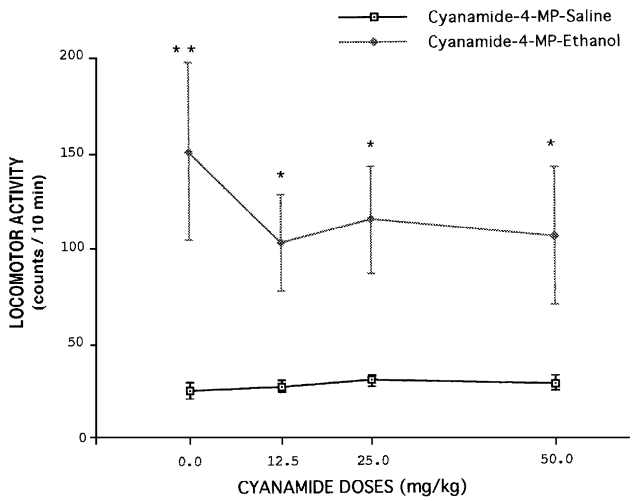

Fig. 5. Effect of concurrent administration of different doses of cyanamide and 4-MP on locomotion induced by ethanol. Mean (±SE) locomotor activity (counts in 10 min) for all treatment groups ($n=8$). Mice were pretreated intraperitoneally with cyanamide (12.5, 25, or 50 mg/kg). Two hours after this treatment, ethanol (2.4 g/kg) was administered to mice. $^{*}P<.05$, $^{**}P<.01$, for differences between cyanamide– 4-MP–ethanol groups and cyanamide– 4-MP–saline group.

4. Discussion

This paper gives evidence that cyanamide injections depress the stimulating effect that ethanol causes on the locomotor activity in a dose-dependent manner (Figs. 1 and 2). Cyanamide doses do not modify the spontaneous locomotor activity compared with control groups when administered alone.

Besides, when mice were pretreated with 4-MP injections (Fig. 3), we obtained an effect similar to that of saline-pretreated animals. Both groups showed the biphasic effect of ethanol on the locomotor activity in mice. The spontaneous locomotor activity did not change. These results may allow the use of this inhibitor as a pharmacological tool in the study of possible implications of catalase and ALDH on ethanol-induced behaviours.

The results of the fourth experiment showed that the combined treatment of cyanamide and 4-MP enhances the ethanol-induced locomotor activity similar to saline- and 4-MP-pretreated mice. As in other studies, cyanamide appears to cause a depression in ethanol-induced locomotor activity (Figs. 1 and 2). However, we also showed that the increase in the locomotor activity following treatment with the combination of cyanamide and 4-MP is not just an additive response, because administration of 4-MP alone (Experiment 3) gives similar results to those administered with saline only.

These results are not just the prevention of cyanamide-induced depression by 4-MP either: if we compare this result with those obtained when an inhibitor of ALDH, but not of catalase, is administered, a different pattern of behavior can be seen (Escarabajal, 2000). In this case (inhibitor + 4-MP), the locomotor activity differs significantly as compared with the control group (saline + 4-MP).

The difference between the results obtained from cyanamide and from disulfiram or its metabolites lies in that the former inhibits the catalase activity whereas the latter does not. Several studies have also shown that the treatment with cyanamide reduces the brain catalase activity in a dose-dependent manner (Shirota et al., 1982; DeMaster et al., 1986, 1988; Sanchis-Segura et al., 1999). According to these data, the results obtained here could be a reflection of the brain catalase in the ethanol-induced effects on behavior.

On the other hand, although treatment with 4-MP reduces the peripheral accumulation of acetaldehyde, the interaction between cyanamide and 4-MP does not enhance the locomotor activity over the levels of saline. This may be because cyanamide inhibits the activity of brain catalase and this may result in lower levels of acetaldehyde. This may find support in the fact that ALDH inhibition alone enhances the locomotor activity over the control group (Escarabajal, 2000) and in the data showing that, when only catalase is inhibited (Sanchis-Segura et al., 1999; Escarabajal et al., 2000), the cyanamide-induced depressing activity increases in animals treated with ethanol.

In this sense, several studies have analyzed the role of catalase (Gill et al., 1996; Sanchis-Segura et al., 1999; Escarabajal et al., 2000) and ALDH enzymes (Spivak et al., 1987a; Gill et al., 1996; Escarabajal, 2000) in the metabolism of ethanol. Consequently, acetaldehyde has been held responsible for some of the psychopharmacological effects of ethanol, for example, CTA (Aragon et al., 1985a,b; Spivak et al., 1987a), ethanol consumption (Sinclair et al., 1980; Socaransky et al., 1984, 1985), or locomotor activity (Spivak et al., 1987b).

This hypothesis is not without problems. First is the difficulty in detecting acetaldehyde in brain following exposure to normal amounts of ethanol (Eriksson, 1980); this is because the hepatic metabolism of acetaldehyde by ALDH is very effective and does not let acetaldehyde into the brain. Secondly, the metabolic barrier constituted by ALDH (Tabakoff et al., 1976; Zimatkin, 1991) prevents acetaldehyde from penetrating from blood to brain.

Identification of brain ethanol metabolizing enzymes, e.g., catalase (Zimatkin and Lindros, 1996; Hamby-Mason et al., 1997) and ALDH enzymes (Zimatkin and Lindros, 1989; Zimatkin et al., 1992) may indicate occurrence of acetaldehyde in the brain. This has been interpreted as an evidence of ethanol metabolism in the brain (Cohen et al., 1980; Zimatkin and Deitrich, 1997).

The pretreatment with ALDH inhibitors such as disulfiram (Deitrich and Erwin, 1971; Yourick and Faiman, 1989) or cyanamide (Brien et al., 1985) before ethanol challenge causes toxicity due to an increase in the peripheral acetaldehyde levels (Kitson, 1991; Kupari et al., 1983). This toxic effect may conceal the behavioral expression of ethanol induction.

Therefore, pretreatment with 4-MP could reduce the acetaldehyde levels after ethanol ingestion (Sinclair and Lindros, 1981; Sinclair et al., 1980), but this reduction would be only in the periphery because brain ADH plays a minor role in the metabolism of ethanol (Raskin and Sokoloff, 1972; Giri et al., 1989). Hence, there could be a larger accumulation of brain acetaldehyde, and this in turn could result in an increase in ethanol-induced behaviors (Experiment 4).

Our results show an antagonistic interaction between cyanamide and ethanol-induced effects. This is in accordance with previous studies (Sinclair and Gribble, 1985; Sinclair and Lindros, 1981; Aragon et al., 1993; Spivak et al., 1987a,b). Thus, different doses of cyanamide in mice treated with an acute dose of ethanol show a significant decrease in locomotor activity compared to the control group. Moreover, cyanamide (25 mg/kg) removes the biphasic effect of ethanol on locomotor activity, showing even larger levels of narcosis than in saline-pretreated animals.

The reduction of ethanol-induced behaviors may thus be caused by peripheral accumulation of acetaldehyde. In fact, this is the rationale of the pharmacological therapies that use cyanamide as a deterrent, as an increase in peripheral levels of acetaldehyde is toxic. In our study, this toxicity

could reduce the locomotor activity induced by ethanol, since the higher the dose of ethanol, the lower the locomotor activity. This is because the peripheral acetaldehyde increases too, sometimes reaching levels higher than those of the control group.

On the other hand, Experiments 4 and 5 showed (a) the suppression of the effect of cyanamide on ethanol-induced locomotor activity and (b) an increase in ethanol-induced locomotor activity after pretreatment with cyanamide and 4-MP, thus leading to the biphasic ethanol effect on locomotor activity. Contrary to the results obtained with drugs that only inhibit ALDH activity (Escarabajal, 2000), our results do not show any significative increase compared to the control group. This could be explained by the double cyanamide action that inhibits catalase (Shirota et al., 1982; DeMaster et al., 1988) and ALDH (Deitrich et al., 1976; Loomis and Brien, 1983) activity.

Overall, these results could suggest a role for the catalase in the mediation of locomotor activity induced by ethanol. They might also support the idea that some psychopharmacological actions of ethanol could be mediated by acetaldehyde.

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References

Amit Z, Aragon CMG. Catalase activity measured in rats naive to ethanol correlates with later voluntary ethanol consumption: possible evidence for a biological marker system of ethanol intake. Psychopharmacology 1988;95:512–5.

Aragon CMG, Spivak K, Amit Z. Blockade of ethanol-induced conditioned taste aversion by 3-amino-1,2,4-triazole: evidence for catalase mediated synthesis of acetaldehyde in rat brain. Life Sci 1985a;37:2077–84.

Aragon CMG, Sternklar G, Amit Z. A correlation between voluntary ethanol consumption and brain catalase activity in the rat. Alcohol 1985b;2:353–6.

Aragon CMG, Spivak K, Smith BR, Amit Z. Cyanamide on ethanol intake. How does it really work? Alcohol Alcohol 1993;28:413–21.

Brien JF, Peachey JE, Loomis CW. Calcium carbimide–ethanol interaction. Clin Pharmacol Ther 1980;27:426–33.

Brien JF, Tam GS, Cameron RJ, Steenaart NAE, Loomis CW. A comparative study of the inhibition of hepatic aldehyde dehydrogenases in the rat by methyltetrazolethiol, calcium carbimide and disulfiram. Can J Physiol Pharmacol 1985;63:438–43.

Cohen G, Sinet PM, Heikkila RE. Ethanol oxidation by rat brain in vivo. Alcohol: Clin Exp Res 1980;4:366–70.

Deitrich RA, Erwin VG. Mechanism of the inhibition of aldehyde dehydrogenase in vivo by disulfiram and diethyldithiocarbamate. Mol Pharmacol 1971;7:301–7.

Deitrich RA, Troxell PA, Worth WS, Erwin VG. Inhibition of aldehyde dehydrogenase in brain and liver by cyanamide. Biochem Pharmacol 1976;25:2733–7.

DeMaster EG, Redfern B, Shirota FN, Herbert T. Differential inhibition of rat tissue catalase by cyanamide. Biochem Pharmacol 1986;35:2081–5.

DeMaster EG, Stevens JM, Redfern B. Ethanol oxidation by cumene hydroperoxide- and hydrogen peroxide-supported peroxidatic activities of catalase. Biochem Arch 1988;4:319–27.

Eriksson CJP. Problems and pitfalls in acetaldehyde determinations. Alcohol: Clin Exp Res 1980;4:22–9.

Escarabajal MD. The mechanisms involved in alcohol-induced behaviors: the role of brain enzymes responsible for the metabolism of acetaldehyde. PhD dissertation, University Jaume I, Castellón, Spain, publications of the University of Jaén, 2000.

Escarabajal MD, Miquel M, Aragon CMG. A psychopharmacological study of the relationship between brain catalase activity and ethanol-induced locomotion on mice. J Stud Alcohol 2000;61:493–8.

Gill K, Amit Z, Smith BR. The regulation of alcohol consumption in rats: the role of alcohol-metabolizing enzymes—catalase and aldehyde dehydrogenase. Alcohol 1996;13:347–53.

Giri PR, Linnoila M, O'Neill JB, Goldman D. Distribution and possible metabolic role of class III alcohol dehydrogenase in the human brain. Brain Res 1989;481:131–41.

Hamby-Mason R, Chen JJ, Schenker S, Perez A, Henderson GI. Catalase mediates acetaldehyde formation from ethanol in fetal and neonatal rat brain. Alcohol: Clin Exp Res 1997;21:1063–72.

Kelley AE. Locomotor activity and exploration. In: Sahgal A, editor. Behavioral neuroscience: a practical approach, vol. 2. New York: Oxford Univ. Press, 1993. pp. 1–21.

Kitson TM. Effect of some thiocarbamate compounds on aldehyde dehydrogenase and implications for the disulfiram ethanol reaction. Biochem J 1991;278:189–92.

Kupari M, Lindros KO, Hillbom M, Heikkilä J, Ylikahri R. Cardiovascular effects of acetaldehyde accumulation after ethanol ingestion: their modification by β-adrenergic blockade and alcohol dehydrogenase inhibition. Alcohol: Clin Exp Res 1983;7:283–8.

Lindros KO, Stowell A, Pikkarainen P, Salaspuro M. The disulfiram (Antabuse)–alcohol reaction in male alcoholics: its efficient management by 4-methylpyrazole. Alcohol: Clin Exp Res 1981;5:528–30.

Loomis CW, Brien JF. Inhibition of hepatic aldehyde dehydrogenases in the rat by calcium carbimide (calcium cyanamide). Can J Physiol Pharmacol 1983;61:1025–34.

Nagasawa HT, DeMaster EG, Redfern B, Shirota FN, Goon DJW. Evidence of nitroxyl in the catalase-mediated bioactivation of the alcohol deterrent agent cyanamide. J Med Chem 1990;33:3120–2.

Peachey JE, Brien JF, Zilm DH, Loomis CW, Hemy MF, Maglana SM. The calcium cyanamide–ethanol interaction in man. Effects of repeated ethanol administration. J Stud Alcohol 1981a;42:208–16.

Peachey JE, Zilm DH, Cappell H, Robertson G. Comparative study of the disulfiram–ethanol reaction and the carbimide–ethanol reaction in nonalcoholic men: I. Effects of initial alcohol exposure. Clin Phamacol Ther 1981b;29:271.

Peachey JE, Zilm DH, Cappell H. Comparative study of the disulfiram–ethanol reaction and the carbimide–ethanol reaction in nonalcoholic men: II. Effects of repeated drinks. Clin Phamacol Ther 1981c;29:271.

Peachey JE, Zilm DH, Robinson GM, Jacob M, Cappell H. A placebo-controlled double-blind comparative clinical study of the disulfiram- and calcium carbimide–acetaldehyde mediated ethanol reactions in social drinkers. Alcohol: Clin Exp Res 1983;7:180–7.

Raskin NH, Sokoloff L. Enzymes catalyzing ethanol metabolism in neural and somatic tissue of the rat. J Neurochem 1972;19:273–82.

Sanchis-Segura C, Miquel M, Correa M, Aragon CMG. Cyanamide reduces brain catalase and ethanol-induced locomotor activity: is there a functional link? Psychopharmacology 1999;144:83–9.

Shirota FN, DeMaster EG, Nagasawa HT. Studies on the cyanamide–ethanol interaction. Dimethylcyanamide as an inhibitor of aldehyde dehydrogenase in vivo. Biochem Pharmacol 1982;31:1999–2004.

Sinclair JD, Gribble PA. Cyanamide injections during ethanol deprivation increase alcohol drinking. Alcohol 1985;2:627–30.

Sinclair JD, Lindros KO. Suppression of alcohol drinking with brain alde-

hyde dehydrogenase inhibition. Pharmacol, Biochem Behav 1981;14: 377–83.

Sinclair JD, Lindros KO, Tehro K. Aldehyde dehydrogenase inhibitors and voluntary ethanol drinking by rats. In: Thurman RG, editor. Alcohol and aldehyde metabolizing systems, vol. 4. New York: Plenum, 1980. pp. 481–7.

Socaransky SM, Aragon CMG, Amit Z, Blander A. Higher correlation of ethanol consumption with brain than liver ALDH in three strains of rats. Psychopharmacology 1984;84:250–3.

Socaransky SM, Aragon CMG, Amit Z. Brain ALDH as a possible modulator of voluntary ethanol intake. Alcohol 1985;2:361–5.

Spivak K, Aragon CMG, Amit Z. Alterations in brain aldehyde dehydrogenase activity modify ethanol-induced conditioned taste aversion. Alcohol: Clin Exp Res 1987a;11:513–7.

Spivak K, Aragon CMG, Amit Z. Alterations in brain aldehyde dehydrogenase activity modify the locomotor effects produced by ethanol in rats. Alcohol Drug Res 1987b;7:481–91.

Tabakoff B, Anderson RA, Ritzman RF. Brain acetaldehyde after ethanol administration. Biochem Pharmacol 1976;25:1305–9.

Theorell H, Yonetani T, Sjöberg B. On the effect of some heterocyclic compounds on the enzymic activity of alcohol dehydrogenase. Acta Chem Scand 1969;23:255–60.

Yourick JJ, Faiman MD. Comparative aspects of disulfiram and its metabolites in the disulfiram–ethanol reaction in the rat. Biochem Pharmacol 1989;38:413–21.

Zimatkin SM. Histochemical study of aldehyde dehydrogenase in the rat CNS. J Neurochem 1991;56:1–11.

Zimatkin SM, Deitrich RA. Ethanol metabolism in the brain. Addict Biol 1997;2:387–99.

Zimatkin SM, Lindros KO. A histochemical study of the distribution of aldehyde dehydrogenase activity in brain structures of rats with genetically different alcohol-related behavior. Alcohol 1989;6:321–5.

Zimatkin SM, Lindros KO. Distribution of catalase in rat brain: aminergic neurons as possible targets for ethanol effects. Alcohol Alcohol 1996;3: 167–74.

Zimatkin SM, Rout UK, Koivusalo M, Bühler R, Lindros KO. Regional distribution of low-Km mitochondrial aldehyde dehydrogenase in the rat central nervous system. Alcohol: Clin Exp Res 1992;16:1162–7.