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Very low doses of ethanol induce behavioral changes involving dopamine D2 receptors

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Abstract

In male rats, pretreatment for 20 days with very low (0.5, 1%, v/v) but not with high (5, 10%, v/v) oral doses of ethanol delayed the initiation and reduced the duration of narcosis induced by an acute high intraperitoneal (i.p.) dose of the drug (3 g/kg in 25% saline solution). Furthermore, the treatment improved the acquisition of shuttle-box active avoidance response but did not affect the emission of ultrasonic calls, an index of emotional state of animals. These effects were inhibited by peripheral administration of the dopamine D2 receptor antagonist, sulpiride (1 mg/kg). A higher dose of sulpiride (10 mg/kg) prolonged the duration of narcosis in rats pretreated with high-dose ethanol and reduced the number of conditioned avoidance responses in the shuttle-box paradigm. The pretreatment with the dopamine D2 receptor agonist, (±)-2-(*N*-phenethyl-*N*-propylamino)-5-hydroxytetralin (PPHT, 0.1 mg/kg), enhanced the effects of ethanol very low doses in delaying the initiation and reducing the duration of narcosis induced by an acute i.p. dose of the drug. A pharmacokinetic study in ethanol-pretreated animals revealed that administration of 0.5% or 1% ethanol for 20 days did not modify significantly the bioavailability of acute ethanol administered i.p. in a dose of 3 g/kg in 25% saline solution. Thus, repeated administration of ethanol very low doses may have affected the sensitivity of presynaptic dopamine D2 receptors. The influence on dopamine release through an action on presynaptic receptors may be involved in these effects of small doses of ethanol.

Keywords: Ethanol; Narcosis; Dopamine D2 receptor; Conditioned avoidance; Ultrasonic call; Pharmacokinetic

1. Introduction

The mechanism of action of ethanol at the neuronal level is still partly unknown, and the search for the neurochemical basis of its actions has been hampered by the lack of pharmacological specificity of the drug and the corresponding lack of pharmacological tools in studying the drug. In order to elucidate the biological mechanism of ethanol, research has been focused on detecting the biochemical and physiological alterations produced by this substance, most frequently using intoxicating doses.

Multiple receptor functions and signaling pathways are modulated by ethanol. Among these, the phospholipase C-mediated hydrolysis of phosphoinositides seems to be particularly sensitive to ethanol (Gonzales et al., 1986; Hoffman et al., 1986; Hoek et al., 1992). In high doses, ethanol is known to affect ion channel and ion channel–receptor complexes. In particular, the interactions with *n*-methyl-D-aspartate (NMDA) and γ-amino-butyric A ($GABA_A$) receptors are believed to be relevant for its intoxicating, amnesic and ataxic effects (Morrisett and Swartzwelder, 1993). However, the effect on ion fluxes seems to be of doubtful importance to ethanol reinforcement that is currently thought to be primarily related to the release of dopamine and serotonin (O'Brien et al., 1995). In particular, ethanol increases extracellular dopamine concentrations in the nucleus accumbens (Wozniak et al., 1991), while these concentrations are reduced during acute ethanol withdrawal (Rossetti et al., 1992). However, small-dose dopamine D2 receptor antagonists do not alter ethanol-induced conditioned place preference (Cunningham et al., 1992).

Indeed, little is known about the neurobehavioral consequences of chronic exposure to low doses of ethanol that may represent a research area of significant social interest. After 2-month treatment with a low dose of ethanol (3% v/v in drinking water) not inducing tolerance or dependence, rats perform better than controls in an active avoidance schedule (Govoni et al., 1994). In addition, ethanol-treated animals subjected to this task present a reduced ultrasonic

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vocalization, which is an indicator of their decreased emotional reactivity (Cuomo et al., 1992). Furthermore, a single oral dose of ethanol (1 g/kg) significantly decreased, whereas higher doses of ethanol (2 or 3 g/kg) significantly increased serum prolactin levels (Samochowiec et al., 1997). The dopamine D2 receptor agonist, (±)-2-(*N*-phenethyl-*N*-propylamino)-5-hydroxytetralin (PPHT), decreased the surge of prolactin in rats with ethanol withdrawal. The drug also reversed the abstinence syndrome of rats subjected to ethanol withdrawal (Samochowiec et al., 1995).

Although the neurochemical bases of the effects of low doses of ethanol are largely unknown, it may be possible that the effects of low doses of ethanol are opposite to those of large doses. Here we tested the hypothesis that repeated administration of very low doses of ethanol may influence the effect of a high acute dose of the substance. Furthermore, the hypothesis that very low doses of ethanol preferentially affect dopamine presynaptic receptors inducing their down-regulation has been verified.

2. Materials and methods

2.1. Animals

Four to five male rats of the Sprague–Dawley strain (Charles River, Italy) weighing 150–180 g were kept to a cage under standard environmental conditions (lights on between 0800 and 2000 h, at temperature of 21 ± 2 °C and humidity of 60%) and had free access to the standard laboratory diet and tap water. After a week of habituation in the facilities, they were subjected to the experimental procedures.

Animals were killed by decapitation at the end of behavioral procedures. Data were used only from those animals that showed no gross abnormalities on postmortem examination.

All experiments were performed blind to treatment between 1500 and 2000 h.

2.2. Drug treatment

Ethanol (Sigma, USA) was administered in 0.5%, 1%, 5% or 10% (v/v) water solution for 20 days as the only available beverage. The mean daily ethanol intake was function of the ethanol concentration, ranging from 0.6 g/kg (for the 0.5% v/v concentration) to 15.8 g/kg per day (for the 10% v/v concentration). Control rats received an equivalent diet in which ethanol was substituted by an equicaloric amount of sucrose, in order to avoid caloric imbalance between controls and chronically ethanol-treated rats. Twenty-four hours after the end of treatment, behavioral tests were performed. In ethanol-treated animals, at this time, plasma ethanol levels were undetectable (UV-method, Boehringer Mannheim Kit, detection limit = 2.2 mmol/l). A set of controls and ethanol-treated animals was sacrificed prior to behavioral testing.

The dopamine D2 receptor antagonist, sulpiride (supplied by Sigma) was freshly dissolved in saline and injected intraperitoneally (i.p.) in doses of 1 or 10 mg/kg/day for 20 days.

The selective dopamine D2 receptor agonist, PPHT hydrochloride (Seeman et al., 1985) was supplied by Sigma (P-105), suspended in appropriate vehicle and injected i.p. in a dose of 0.1 mg/kg/day for 20 days.

2.3. Ethanol-induced narcosis

Groups of animals were treated i.p. with the selective dopamine D2 receptor antagonist, sulpiride (Sigma) or saline. The drug was injected for 20 days, during the ethanol treatment period, in doses of 1 or 10 mg/kg/day. A different group of animals was treated i.p. with PPHT for 20 days.

Twenty-four hours after the last day of ethanol exposure, all animals were given an acute i.p. injection of ethanol (3 g/kg in 25% saline solution). The latency to drug-induced narcosis was measured as the time elapsing from the ethanol injection and the loss of the righting reflex; the duration of narcosis was established as the time elapsing from the loss and resumption of the reflex.

2.4. Behavioral tests

After a 20-day pretreatment with ethanol doses and/or sulpiride or PPHT, shuttle-box active avoidance acquisition was studied in a single session test as described elsewhere (Bohus and De Wied, 1981). Briefly, the rats were trained to avoid the unconditioned stimulus (US) of a scrambled electrical foot-shock (0.20 mA) delivered through the grid floor. The conditioned stimulus (CS) was a light presented for 10 s prior to the US. If no escape occurred within 20 s of CS/US presentation, the shock was terminated. A total of 60 conditioning trials was given with a variable inter-trial interval averaging 60 s. Index of learning capacity was the total number of con-ditioned avoidance responses.

Ultrasonic calls emitted during the 60-trial session were recorded by a QMC ultrasonic microphone placed at the center of the shuttle-box cover and connected to a receiver (QMC Bat Detector S200) transforming in real time the ultrasonic calls into audible sounds (Pascale et al., 1997). Microphone signals were relayed, via the high-frequency output socket, to Bruel and Kjaer (2033) High Resolution Signal Analyzer (HRSA) set on time-intensity mode to visualize the ultrasonic calls. The number of ultrasonic calls emitted by rats was counted manually by listening to the audible output of a headphone.

2.5. Ethanol pharmacokinetics

A pharmacokinetic study was performed in order to check whether chronic exposure to ethanol would have

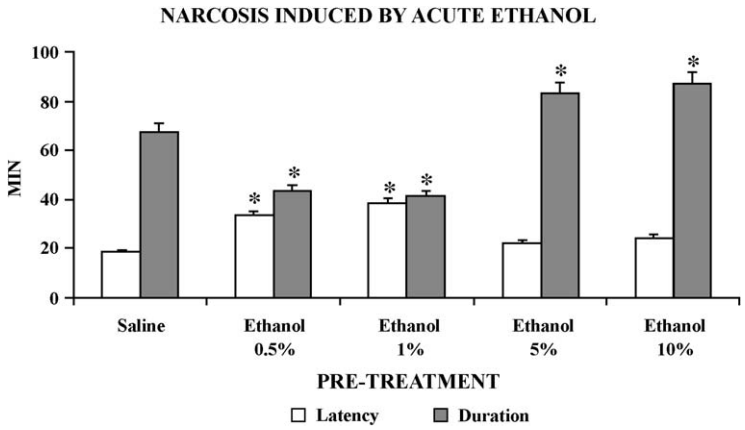

Fig. 1. Effects of oral pretreatment with very low (0.5% or 1% v/v in water) or high doses (5% or 10% v/v in water) of ethanol on the latency and duration of narcosis induced by an acute i.p. dose of ethanol (3 g/kg in 25% saline solution). Pretreatment was made for 20 days prior to injecting acute ethanol. Values are mean±S.E.M. expressed in min. The number of animals per each group was 7. The ANOVA revealed a significant drug effect ($F(4,34)=2.74$, $P<0.05$ for the latency; $F(4,34)=3.43$, $P<0.05$ for the duration). *Significantly different as compared to the latency or duration value of saline-pretreated controls ($P>0.05$, post-hoc Dunnett's test for multiple comparisons).

modified the level of ethanol injected acutely in a dose of 3 g/kg in 25% saline solution. Groups of ethanol-pretreated animals and control rats were sacrificed and their blood was collected 0, 15, 30 and 60 min after i.p. acute injection of ethanol. Plasma ethanol levels were measured with a UV-method (Boehringer Mannheim Kit, detection limit = 2.2 mmol/l).

2.6. Statistical analysis

Data were analyzed using the two-way analysis of variance (ANOVA) and the post-hoc Dunnett's test for multiple comparisons. A P level of 0.05 or less was considered as indicative of statistically significant differences.

3. Results

Fig. 1 shows that animals pretreated orally for 20 days with 0.5% or 1% v/v ethanol in water exhibited a belated initiation and a reduced duration of narcosis induced by acute ethanol (3 g/kg in 25% saline solution) as compared to those pretreated with saline. Opposite effects were found in animals pretreated with 5% or 10% v/v ethanol. These animals, in fact, showed an increased duration of narcosis induced by acute ethanol. Furthermore, pretreatment with very low doses of ethanol increased the number of conditioned avoidance responses of rats tested in a single session shuttle-box active avoidance test. In contrast, animals pretreated with 5% or 10% v/v ethanol showed a reduced

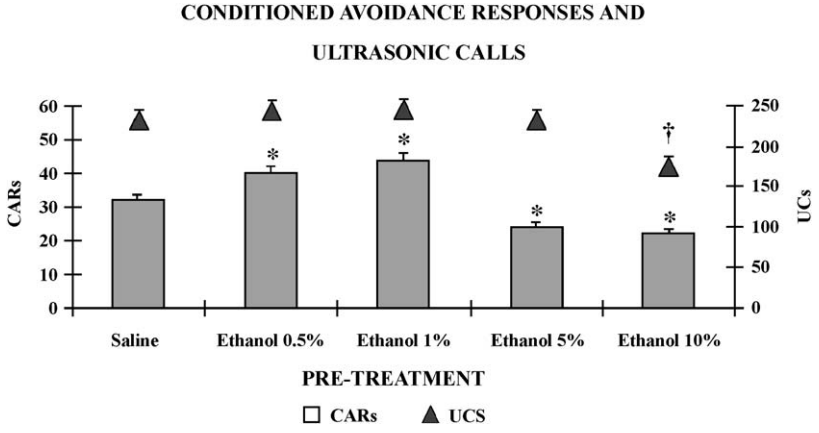

Fig. 2. Effects of oral pretreatment with very low (0.5% or 1% v/v in water) or high doses (5% or 10% v/v in water) of ethanol on the number of conditioned avoidance responses (CARs) and ultrasonic calls (UCs) emitted by rats trained in a shuttle-box active avoidance paradigm. Pretreatment was made for 20 days prior to performing the behavioral test. Values are mean±S.E.M. The number of animals per each group was 7. The ANOVA revealed a significant drug effect ($F(4,34)=3.84$, $P<0.05$ for the CARs; $F(4,34)=3.56$, $P<0.05$ for the UCs). *Significantly different as compared to CARs value of saline-pretreated controls ($P>0.05$, post-hoc Dunnett's test for multiple comparisons). †Significantly different as compared to UCs value of saline-pretreated controls ($P>0.05$, post-hoc Dunnett's test for multiple comparisons).

Table 1
Influence of sulpiride or PPHT on the effects of oral pretreatment with very low (0.5% or 1% v/v in water) or high doses (5% or 10% v/v in water) of ethanol for 20 days on the latency and duration of narcosis induced by an acute i.p. dose of ethanol (3 g/kg in 25% saline solution)

Groups	Latency (min)	Duration (min)
(1) Saline + saline (15)	18.7 ± 2.4	67.4 ± 3.9
(2) Saline + sulpiride 1 mg (7)	22.4 ± 2.0	65.7 ± 4.9
(3) Saline + sulpiride 10 mg (7)	23.4 ± 3.0	63.4 ± 5.5
(4) Saline + PPHT 0.1 mg (7)	21.4 ± 2.1	59.5 ± 5.4
(5) Ethanol 0.5% + saline (7)	33.4 ± 2.5[a]	44.5 ± 2.1[a]
(6) Ethanol 0.5% + sulpiride 1 mg (7)	21.5 ± 2.0	62.6 ± 4.8
(7) Ethanol 0.5% + sulpiride 10 mg (7)	32.4 ± 3.2[a]	43.5 ± 3.7[a]
(8) Ethanol 0.5% + PPHT (7)	51.5 ± 2.0[b]	29.0 ± 2.3[b]
(9) Ethanol 10% + saline (7)	24.2 ± 2.4	86.1 ± 5.2[a]
(10) Ethanol 10% + sulpiride 1 mg (7)	23.1 ± 2.9	89.3 ± 8.1[a]
(11) Ethanol 10% + sulpiride 10 mg (7)	24.2 ± 2.2	99.2 ± 7.0[c]
(12) Ethanol 10% + PPHT (7)	21.1 ± 1.8	87.4 ± 7.1[a]

Values are mean ± S.E.M. In parentheses is indicated the number of animals per each group. The ANOVA revealed a significant drug effect, $F(11,91) = 2.12$, $P < 0.05$.

[a] Significantly different as compared to group 1 ($P < 0.05$, Dunnett's test).

[b] Significantly different as compared to groups 1 and 5 ($P < 0.05$, Dunnett's test).

[c] Significantly different as compared to groups 1 and 9 ($P < 0.05$, Dunnett's test).

number of conditioned avoidance responses in this test as compared to control rats (Fig. 2). The number of ultrasonic calls recorded during the active avoidance session in animals pretreated with very low doses of ethanol was similar to that of control animals. In contrast, the pretreatment with 10% v/v ethanol reduced the emission of ultrasonic calls in rats (Fig. 2).

The repeated administration of 1 mg/kg sulpiride prior to the narcotic dose of ethanol prevented the inhibiting effect of pretreatment with very low doses, but did not affect the latency and duration of narcosis in rats pretreated with high doses of ethanol (Table 1). No such effect was observed when sulpiride was injected in a dose of 10 mg/kg, but this dose prolonged the duration of ethanol-induced narcosis in animals pretreated with 5% or 10% v/v ethanol. Furthermore, the pretreatment with the dopamine D2 receptor agonist, PPHT (0.1 mg/kg), enhanced the effects of ethanol very low doses in delaying the initiation and reducing the duration of narcosis (Table 1, data on animals treated with 1% and 5% v/v ethanol are omitted).

Table 2
Influence of sulpiride on the effects of oral pretreatment with very low (0.5% or 1% v/v in water) or high doses (5% or 10% v/v in water) of ethanol for 20 days on the number of conditioned avoidance responses (CARs) and ultrasonic calls (UCs) emitted by rats trained in a shuttle-box active avoidance paradigm

Groups	CARs	UCs
(1) Saline + saline (15)	31.7 ± 2.4	221.4 ± 23.9
(2) Saline + sulpiride 1 mg (7)	28.4 ± 2.5	237.3 ± 32.1
(3) Ethanol 0.5% + saline (7)	39.4 ± 2.5[a]	224.5 ± 22.1
(4) Ethanol 0.5% + sulpiride 1 mg (7)	31.5 ± 2.7	232.6 ± 27.8
(5) Ethanol 10% + saline (7)	21.4 ± 2.1[b]	156.2 ± 15.2[b]
(6) Ethanol 10% + sulpiride 1 mg (7)	23.4 ± 2.4[b]	169.0 ± 19.0[b]

Values are mean ± S.E.M. In parentheses is indicated the number of animals per each group. The ANOVA revealed a significant drug effect, $F(5,49) = 2.72$, $P < 0.05$.

[a] Significantly different as compared to group 1 ($P < 0.05$, Dunnett's test).

[b] Significantly different as compared to groups 1 and 3 ($P < 0.05$, Dunnett's test).

Table 3
Effects of oral pretreatment with very low (0.5% or 1% v/v in water) or high doses (5% or 10% v/v in water) of ethanol for 20 days on C_{max} and $t1/2$ values of plasma ethanol after an acute i.p. dose of the drug (3 g/kg in 25% saline solution)

Groups	C_{max} (μg/ml)	$t1/2$ (h)
(1) Saline (7)	425.6 ± 31.7	0.24 ± 0.07
(2) Ethanol 0.5% v/v (7)	410.1 ± 37.9	0.26 ± 0.08
(3) Ethanol 1% v/v (7)	441.1 ± 41.1	0.21 ± 0.08
(4) Ethanol 5% v/v (7)	423.1 ± 51.1	0.23 ± 0.09
(5) Ethanol 10% v/v (7)	432.3 ± 48.6	0.25 ± 0.08

Values are mean ± S.E.M. In parentheses is indicated the number of animals per each group. The ANOVA revealed no significant drug effect, $F(4,34) = 3.20$, $P < 0.05$.

The repeated administration of 1 mg/kg sulpiride prevented the effect of pretreatment with very low doses of ethanol on the number of conditioned avoidance responses and emission of ultrasonic calls of rats tested in a shuttle-box active avoidance paradigm (Table 2). The sulpiride treatment did not affect the behavioral response of animals given a high-dose ethanol (10% v/v).

Pretreatment with 0.5% or 1% ethanol for 20 days did not modify significantly the bioavailability of acute ethanol administered i.p. in a dose of 3 g/kg in 25% saline solution as compared to that of controls (Table 3). The pharmacokinetic parameters did not appear to be significantly changed also in animals pretreated with 5% or 10% v/v ethanol.

4. Discussion

The wide repertoire of pharmacological effects of ethanol includes various neurochemical and behavioral modifications that have been found to be dose-dependent. For instance, protein kinase C (PKC) has been reported to be affected by chronic ethanol intake at brain level (Battaini et al., 1989; Kruger et al., 1993). Two-month treatment with low doses ethanol (3% v/v), not inducing tolerance or dependence, caused a decrease in PKC activity both in the cortex and in the hippocampus (Pascale et al., 1997). Chronic ethanol, for a shorter time at higher concentrations (6% for 25 days), also decreased hippocampal and cortical PKC levels and Ca^{2+}-dependent PKC activity (Battaini et al., 1989), suggesting the existence of a dose–effect relationship.

However, in some cases, low and high doses of ethanol may exert opposite effects and an inverted U-shape curve may be found because of the bi-phasic action of the drug.

Low doses of ethanol (0.5 mg/kg) induce an increased release of dopamine from the nucleus accumbens but have no effect on release from the striatum. Larger doses (2.5–5 g/kg) also increase the release from the nucleus accumbens, but then a decrease is observed at higher doses while in the striatum, there is an increase (Di Chiara and Imperato, 1985). However, low-dose D2 receptor antagonists do not alter ethanol-induced conditioned place preference (Cunningham et al., 1992). A single low dose (1 g/kg) of ethanol decreases, whereas higher doses (2 or 3 g/kg) increase serum prolactin concentration (Samochowiec et al., 1997).

Here we show that very low doses (0.5% or 1% v/v) of ethanol may counteract the narcotic effect of a high dose of the drug. High doses (5% or 10% v/v) of the drug exert opposite effects. A pretreatment with very low, but not high doses of ethanol also improved the acquisition of active avoidance behavior without affecting the emission of ultrasonic calls in rats. The effects of very low doses of ethanol were prevented by a pretreatment with the dopamine D2 receptor antagonist, sulpiride and enhanced by a pretreatment with the dopamine D2 receptor agonist, PPHT. We do not provide data on the effects of PPHT treatment on the acquisition of active avoidance behavior of rats exposed to very low doses of ethanol. However, if these effects are parallel to those found in the ethanol-induced narcosis, we can predict that PPHT enhances the improving effect of a very low dose of ethanol on the acquisition of active avoidance responses. Furthermore, the present data are consistent with those of the previous studies showing that after 2-month treatment with low doses of ethanol (3% v/v), rats perform better than controls in an active avoidance schedule (Govoni et al., 1994). It is worth mentioning that 3% v/v ethanol treatment has been reported not to affect motor activity in rats (Lograno et al., 1993). Furthermore, low doses (3% v/v) of ethanol administered under the same experimental conditions as in the present experiments do not induce tolerance (as assessed by sleeping time measurement following acute ethanol) and dependence (as assessed by observation of the behavior following withdrawal of ethanol) (Govoni et al., 1994).

Ultrasonic vocalizations may be considered as a criterion for evaluating emotional and motivational state during various situations, such as learning (Cuomo et al., 1992). In the present experiments, a 20-day treatment with very low doses of ethanol did not influence the emission of ultrasonic calls of rats subjected to an active avoidance task. In contrast, high doses of ethanol induce a reduced emission of ultrasonic calls. Another study has shown that a 2-month treatment with 3% v/v ethanol reduced ultrasonic calls in rats subjected to a 75-trial session of shuttle-box active avoidance (Pascale et al., 1997), and this effect has been interpreted as an anxyolitic action of low doses of ethanol. Thus, it is possible that the duration of treatment as well as the dose administered have influenced the effect of ethanol on this parameter.

One of the functions of the mesolimbic dopamine system is to regulate the process of reinforcement, a process that is thought to influence ethanol self-administration. Hodge et al. (1997) have shown that injection of the dopamine D2 receptor antagonist, raclopride, into the nucleus accumbens reduced total responding of rats in an ethanol self-administration paradigm, while injection of the dopamine D2 receptor agonist, quinpirole, increased the response and response rate. The decrease in total responding produced by raclopride was enhanced by co-administration of the dopamine D1 receptor agonist, 1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol (SKF 38393) suggesting that D1 and D2 receptors in the nucleus accumbens interact in the regulation of ethanol self-administration. Furthermore, the dopamine D2 receptor agonist, *S*-5-hydroxy-2-dipropylaminotetralin (7-OH-DPAT) increased both ethanol intake and preference, but the dopamine D2 receptor antagonist, raclopride, did not affect ethanol consumption in a two-bottle, free-choice paradigm (Silvestre et al., 1996). Although in the present experiments, animals were not able to self-administer ethanol, it is possible that blockade of dopamine D2 receptors by sulpiride has induced a neurochemical change similar to the underlying reduced responsiveness of animals to repeated exposure to ethanol in the self-administration paradigm after injection of raclopride, i.e. a down-regulation of presynaptic dopamine D2 receptors. This hypothesis may be consistent with the lower reinforcing effect of ethanol in animals injected with raclopride into the nucleus accumbens, as shown by Hodge et al. (1997).

Sulpiride reversed the effect of very low-dose ethanol pretreatment only in a dose of 1 mg/kg/day, while the dose of 10 mg/kg/day was ineffective in this respect and prolonged the duration of narcosis induced by acute ethanol. Furthermore, a pretreatment with 1 mg/kg sulpiride prevented the effect of a very low dose of ethanol on the acquisition of shuttle-box active avoidance behavior, while it was uneffective in preventing the effect of a high dose of ethanol. Since the low dose of sulpiride used in the present experiments seems to primarily affect presynaptic dopamine D2 receptors (Seeman, 1981), these results suggest that these receptors may be involved in the inhibiting effect of very low-dose ethanol pretreatment on drug-induced narcosis. This is confirmed by the potentiation by the dopamine D2 receptor agonist, PPHT, of the effect of very low doses of ethanol on drug-induced narcosis. In particular, it is possible that repeated administration of very low-dose ethanol would have induced a down-regulation of presynaptic dopamine D2 receptors leading to increased release of dopamine. This may be sufficient to explain the inhibiting effect of repeated small-dose ethanol administration on drug-induced narcosis.

In general, evidence exists for a dopamine release in some brain areas after administration of high doses of ethanol, while effects are uncertain with low doses of the drug. An 8-week treatment with a low dose of ethanol (3% v/v) induced a significant increase in the number of dopamine D1 receptor

sites in the caudate-putamen. Conversely, no significant changes were observed in dopamine D2 receptor density or affinity (Lograno et al., 1993). However, a low dose of ethanol (1 g/kg) increases the release of dopamine in the rat brain (Samochowiec et al., 1995). Very high concentrations of ethanol in the perfusion fluid elicit a stimulation of extracellular dopamine release in the nucleus accumbens (Wozniak et al., 1991), while a decrease of dopamine content may be observed during acute ethanol withdrawal (Rossetti et al., 1992). Sustained doses of ethanol increase the release of dopamine from the nucleus accumbens, but have no effect on release from the striatum (Di Chiara and Imperato, 1985). The increased dopamine D2 receptor expression in the rat brain has been confirmed in rats after supplementation of a high dose of the drug, i.e. 10% for 5 weeks (Kim et al., 1998). Biochemical results were in agreement with these behavioral data, as amphetamine-induced locomotor hyperactivity was significantly higher in ethanol-treated rats in comparison to controls. Moreover, grooming behavior in response to SKF 38393, a selective agonist of D1 receptors, was potentiated in ethanol-treated rats, whereas locomotor hyperactivity induced by *trans*-(−)-4*aR*-4,4*a*,5,6,7,8,8*a*,9-octahydro-5-1*H*-pyrazolo[3,4-*g*] quinoline hydrochloride (quinpirole, a selective agonist of D2 receptors) was not affected by ethanol treatment. Some of these data seem to be in contrast with the present results, but it must be considered that two variables may be responsible for the difference, i.e. the dose level and the duration of treatment. In other words, doses lower than 3% v/v (0.5% or 1% v/v) and treatment shorter than 8 weeks (20 days) may have induced a down-regulation of dopamine D2 receptors that apparently was not present in other experimental conditions. Besides, no data exist in literature concerning treatments with ethanol in doses similar to those used in the present experiments and, hence, no comparison can be made with other experimental conditions. Consistent with the hypothesis put here is the finding that depression of GABA-mediated inhibitory post-synaptic potentials by amphetamine or cocaine seems to be mediated by the stimulation of dopamine D2 receptors in the striatum, as it is prevented by sulpiride and mimicked by quinpirole (Centonze et al., 2002). Thus, it is conceivable that repeated very low doses of ethanol under the present experimental conditions may have induced a release of small quantities of dopamine in some brain areas and these have caused a down-regulation of presynaptic dopamine D2 receptors. However, we are aware that no neurochemical data support this hypothesis, as the present experiments did not include a brain dopamine D2 receptor density or affinity analysis in animals treated with very low doses of ethanol.

It remains to be elucidated the fact that in other experiments, low-dose dopamine D2 receptor antagonists were found not to alter ethanol-induced place preference (Cunningham et al., 1992). However, this is a conditioned behavior that may depend on ethanol-dopamine interaction, while drug-induced narcosis is a non-conditioned phenomenon primarily due to the interaction of ethanol with central NMDA and $GABA_A$ receptors (Morrisett and Swartzwelder, 1993; O'Brien et al., 1995). The duration of treatment is also important in order to consider the induction of a presynaptic dopamine D2 receptor down-regulation. Again, the dose level of ethanol may also have influenced the behavioral responses in this case.

Interestingly, apomorphine acting as dopamine D2 receptor agonist in low doses (which do not provoke nausea or vomiting, but may induce down-regulation of presynaptic dopamine D2 receptors) has been reported to enhance sobriety maintenance (Jensen et al., 1977). Thus, the possible down-regulation of presynaptic dopamine D2 receptors by very low-dose ethanol may be relevant also for the reinforcement effect of the drug.

The pharmacokinetic study revealed that pretreatment with either very low or high doses of ethanol did not affect the C_{max} and $t1/2$ values of plasma ethanol after a single i.p. dose of the drug. This would exclude that the enhanced sensitivity of animals to the narcotic dose of ethanol may depend on a change in ethanol metabolism.

The results of studies on the effect of chronic ethanol administration on dopamine receptors in the brain seem to be contradictory, and other than on the dose, seem to depend on several interfering factors like the length of the treatment (Hamdi and Prasad, 1992), dietary changes related to ethanol consumption (Hietala et al., 1990), and the housing conditions of the experimental animals (Rilke et al., 1995). Thus, different variables should be taken into account when data on repeated administration of ethanol are evaluated.

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