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Modification of behavioral effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin following chronic ethanol consumption in the rat: evidence for the involvement of 5-HT_{1A} receptors in ethanol dependence

Mark Kleven [a,*], Caroline Ybema [a], Elisabeth Carilla [a], Michel Hamon [b], Wouter Koek [a]

[a] *Centre de Recherche Pierre Fabre, 17, avenue Jean Moulin, 81106 Castres Cedex, France*

[b] *INSERM U. 288, Neurobiologie Cellulaire et Fonctionnelle, Faculté de Médecine Pitié-Salpêtrière, 91, boulevard de l'Hôpital, 75634 Paris Cedex 13, France*

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Abstract

Behavioral effects induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; i.e., lower lip retraction, flat body posture, and forepaw treading) were examined in rats during ethanol withdrawal following a 2-week period of access to a liquid diet containing 9% (v/v) ethanol. After an 18 h withdrawal period, tolerance to 8-OH-DPAT-induced flat body posture and, conversely, sensitization to the effects of 8-OH-DPAT on lower lip retraction were observed in the 9% ethanol group as compared to control rats fed an isocaloric diet. In contrast, 8-OH-DPAT-induced forepaw treading in the 9% ethanol group was not significantly different in comparison to control rats. Plasma corticosterone levels were significantly higher in the ethanol-exposed group than in control animals, an effect which was not additive with the increase in corticosterone levels normally observed after the administration of low doses of 8-OH-DPAT. Altered flat body posture and lower lip retraction responses to a submaximal dose of 8-OH-DPAT (2.5 mg/kg i.p.) were still observed as late as 3 days after withdrawal of the 9% ethanol liquid diet, but were no longer apparent at 7 days. Interestingly, prominent ethanol withdrawal signs such as tremor and rigidity, while occurring on the first day, were completely absent on the third day. Taken together, these results indicate that chronic ethanol exposure differentially alters sensitivity to several pharmacological effects of the 5-HT_{1A} receptor ligand 8-OH-DPAT. They further support the involvement of 5-HT (5-hydroxytryptamine, serotonin) systems in alcohol abuse and therapeutic interventions using 5-HT_{1A} ligands.

Keywords: 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); Ethanol tolerance; Ethanol withdrawal; 5-HT (5-hydroxytryptamine, serotonin) syndrome; Corticosterone

1. Introduction

There is increasing clinical and preclinical evidence that serotonergic mechanisms may play an important role in alcoholism and/or withdrawal from chronic ethanol exposure (for reviews, see LeMarquand et al., 1994a,b; Sellers et al., 1992). In animal studies, it has been demonstrated, for example, that 5-HT_{1A} receptor agonists reduce ethanol intake (Schreiber et al., 1993; Svensson et al., 1993) and attenuate apparent anxiogenic effects associated with ethanol withdrawal (Lal et al., 1991). Furthermore, clinical studies also suggest that 5-HT_{1A} receptor agonists may be beneficial in the treatment of alcoholism (Bruno, 1989; Tollefson et al., 1991). The present study is part of an effort to investigate further the basis for the clinical efficacy of 5-HT_{1A} receptor agonists in alcoholism by examining the effects of chronic alcohol on 5-HT_{1A} function.

A number of recent papers have reported effects of chronic ethanol treatment on central 5-HT (5-hydroxytryptamine, serotonin) receptors. In particular, Ulrichsen (1991) has demonstrated a significant decrease in [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) binding to 5-HT_{1A} receptors in the hippocampus both during chronic ethanol intoxication and during

* Corresponding author. Tel. (33) 63.71.42.68, fax (33) 63.35.66.29.

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the withdrawal phase. In contrast, Pandey et al. (1992) reported that hippocampal 5-HT_{2C} receptors remained unaltered after chronic ethanol consumption. With regard to ethanol preferring strains of rats, Wong et al. (1993) have found an increased labeling of brain 5-HT_1 receptors by [^{3}H]-5-HT as well as an increased B_{max} of 5-HT_{1A} receptors in these animals in comparison with alcohol non-preferring rats. These studies add to growing evidence of changes in serotonergic systems following chronic ethanol intake in animals (LeMarquand et al., 1994b).

In the present study, the influence of chronic exposure to ethanol-containing liquid diet on pharmacological effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, was examined at different time-points following an 18 h withdrawal period. Behavioral effects induced by 8-OH-DPAT (e.g., forepaw treading, flat body posture, and lower lip retraction) were used to characterize possible changes in sensitivity of 5-HT_{1A} receptors. Additionally, since both 5-HT_{1A} receptor agonist treatment (Bagdy et al., 1989; Przegalinski et al., 1989; Welch et al., 1993) and ethanol withdrawal (Gadek et al., 1987; Pohorecky et al., 1978; Sipp et al., 1993) activate the hypothalamus-pituitary-adrenal axis and consequently increase the release of corticosterone from the adrenal gland, the effects of 8-OH-DPAT on plasma corticosterone levels at various times after alcoholization were examined. The aim of these investigations was to tentatively explain the efficacy of 5-HT_{1A} receptor agonists to prevent ethanol consumption and withdrawal-induced alteration of behavior on the basis of the particular functional states of 5-HT_{1A} receptors in alcoholized animals.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (ICO: OFASD [IOPS], Iffa Credo, France), weighing 160–180 g upon arrival and 180–200 g at the beginning of the studies were used. They were housed individually in plastic hanging cages (28 cm × 21 cm × 18 cm) with metal grid floors (RC Iffa Credo). Water, filtered at 0.22 μm, was freely available from an automatic dispenser. The storage room and manipulation rooms were air-conditioned (temperature = 22 ± 1°C; hygrometric degree 55 ± 5%), with lighting on from 7:00 to 19:00 h. All rats were cared for in accordance with the principles of laboratory animal care (Guide for the Care and Use of Laboratory Animals, U.S. Department of Agriculture. Public Health Service. National Institutes of Health publication No. 85-23, Revised 1985), and the protocol was carried out in accordance with local ethical committee guidelines for animal research. They were held in quarantine for 4–8 days, with free access to standard laboratory food before being used for the experiments.

2.2. Chronic ethanol treatment

For a period of 14 consecutive days, rats were given access to a liquid diet (BioServ; Frenchtown, NJ, USA) containing 5–9% ethanol (v/v) or a control diet, in which ethanol was substituted isocalorically with dextrose, as their only source of nutrition. Liquid diet was provided in 100 ml calibrated drinking bottles (BioServ Liquid Diet, Frenchtown, NJ, USA) which were replaced every day (between 16:00 and 17:00 h) with fresh solution and the amount consumed was recorded. Rats were weighed each morning and the average daily ethanol intake (g/kg body weight) was calculated. Animals were assigned to ethanol or pair-fed control group using a stratified procedure balancing body weight.

Liquid diet containing concentrations of ethanol higher than 5% was introduced over a period of several days, i.e., on the first day, animals assigned to the ethanol group(s) received a liquid diet containing 5% ethanol, on day 2 they received 6.7% ethanol diet and on day 3, animals in the 9% group received a 9% ethanol diet. In order to maintain a similar degree of food-restriction, animals in the control group were presented the average amount of food consumed on the previous day by animals in the ethanol group(s). During the afternoon of the last treatment day, rats in the ethanol group received 5 g/kg of ethanol in liquid diet by oral gavage (40 ml/kg) whereas animals in the control groups each received an identical volume of control diet (40 ml/kg p.o.). Thereafter, a bottle containing 50 ml of control diet was presented to all of the animals. Bottles were removed the next morning (at 8:00 h on day 15).

2.3. Ethanol tolerance

In order to establish that tolerance to its acute effects was present following chronic consumption of ethanol, rectal temperature was measured at 0, 15, 30, 60, 120, and 180 min following the acute oral administration of ethanol on the 14th day of treatment using a Digi-Sense thermometer with a type K probe (Cole Parmer, Chicago, IL, USA). For the purpose of only this comparison, an additional group of animals ($n = 6$) was presented control diet for a period of 14 days, administered acute ethanol and no longer used. During several days prior to acute ethanol injection, rats were habituated to the insertion of the lubricated temperature probe.

2.4. Ethanol withdrawal

Behavior was rated for severity of withdrawal 18 h after exposure to ethanol (i.e., after the oral adminis-

tration of 5 g/kg of ethanol) following procedures which have been described previously (Lal et al., 1988). Ethanol dependence was characterized by three different signs which have been associated with moderate to severe dependence (i.e., rigidity, caudal tremor, and general tremor. Each sign was rated from 0 (less than normal) to 3 (maximal response), with 1 representing normal. The total score obtained could therefore vary from 0 to 9 (see Lal et al., 1988).

2.5. Behavioral effects of 8-OH-DPAT

Eighteen hours after the last ethanol exposure, animals received an injection of either 8-OH-DPAT (0.01–10.0 mg/kg i.p.) or saline (10 ml/kg). Behavioral observations were made at one time point, centered at 15 min after the injection and lasting for a total of 10 min. Four animals were observed individually during the 10 min period; the four rats were observed in turn, every 15 s with a period of 10 s of observation per animal. During each of these observation periods, the presence (1), or absence (0) of forepaw treading and lower lip retraction was recorded. The studied behavior was considered present if the animal showed uninterrupted signs for at least 3 s. This cycle was repeated 10 times during a 10 min period; thus, the incidence of a particular behavior could vary from 0 to 10 for any observation period. Flat body posture was scored as present (1) if it occurred for the entire observation period, otherwise, the corresponding score was 0. On each day, only two animals in each group (i.e., ethanol-treated, pair-fed) received the same dose of 8-OH-DPAT.

2.6. Blood ethanol and corticosterone determinations

On the morning of the last day of ethanol availability (day 14), blood samples were taken from the tail vein, frozen and subsequently analyzed for levels of ethanol by a gas-chromatographic method described previously (Lal et al., 1988). Immediately after the observation of behavioral effects of 8-OH-DPAT, rats were decapitated and trunk blood was collected in chilled tubes containing 50 μl 10% EDTA disodium (disodium ethylenediaminetetraacetate) solution and frozen until being analyzed for both ethanol (Experiments I and II) and corticosterone levels (Experiments II and III). Blood samples were centrifuged (4000 rpm, 20 min) and plasma was aliquoted and stored at -30°C and analyzed within 2 weeks after being frozen. Corticosterone levels were determined by radio-competitive binding assay of transcortin (Murphy, 1967), as reported elsewhere (Millan et al., 1992; Rivet et al., 1992) in which bound corticosterone was separated from free by using dextran-coated charcoal. The limit of detection of this assay was 50 pg/tube.

The purpose of Experiment I was to establish conditions of ethanol diet exposure that reliably produce tolerance and physical dependence. The effects of 2 weeks of intake of liquid diet containing varying ethanol concentrations on the development of tolerance (indicated by changes in acute, ethanol-induced hypothermia (Ritzmann and Tabakoff, 1971)) and physical dependence (indicated by the presence of behavioral signs of withdrawal) were examined. In the tolerance study, core body temperature was assessed immediately before and at 15, 30, 60, 120, and 180 min after ethanol administration. Withdrawal signs were scored 18 h after the acute ethanol administration.

The purpose of Experiment II was to examine the effects of chronic alcohol intake on behaviors induced by 8-OH-DPAT and blood corticosterone levels. On the basis of the results of Experiment I, a 2-week feeding period with 9% (v/v) ethanol liquid diet was used to examine alterations in the effects of 8-OH-DPAT i.p. on flat body posture, forepaw treading, and lower-lip retraction. Animals were fed either a liquid diet containing 9% ethanol (v/v) or the calorically equivalent, control diet for a 2-week period.

The purpose of Experiment III was to examine the duration of changes in behavioral effects of 8-OH-DPAT following chronic intake of a liquid diet containing 9% ethanol (v/v) or the calorically equivalent, control diet for a 2-week period. The effects of saline or 2.5 mg/kg 8-OH-DPAT i.p. were examined in separate groups of rats 1, 3, or 7 days after availability of the liquid diet was terminated. During the withdrawal period, animals were permitted free access to normal diet.

2.7. Data analysis

Data were analyzed using analysis of variance (Winer, 1971). Where appropriate, a posteriori individual comparisons were made using Duncan's or Dunnett's test with $P < 0.05$ as the lower limit of statistical significance. Areas under the dose-temperature curves were calculated for individual animals using the trapezoidal rule.

2.8. Drugs

The drugs used were 95% ethanol (Alcool Pharmaceutique et Industrie, St. Georges, D'Orques, France) and 8-OH-DPAT hydrobromide (Research Biochemicals International, Natuck, MA, USA). 8-OH-DPAT was dissolved in water and injected i.p. in a volume of 10 ml/kg.

3. Results

3.1. Experiment I

Effects of chronic ethanol

The effects of chronic intake of liquid diet containing varying concentrations of ethanol on intake, blood ethanol levels and body weight are shown in Table 1. In general, daily ethanol intake over the 2-week period was similar in the three ethanol groups (approximately 12 g/kg/day). In all cases, average daily ethanol intake initially increased during the first week of availability and then stabilized over the second week. Intake of ethanol among the groups during the second week differed significantly ($F(2,90) = 23.17$, $P < 0.001$), and post-hoc tests showed that intake was significantly higher in the 6.7% group than in the 5% group (Table 1). These differences in daily ethanol intake among the groups were also to some extent reflected in the significant differences in blood levels of ethanol measured on the final day of availability of ethanol liquid diet ($F(3,16) = 6.55$, $P < 0.001$). Thus, blood ethanol levels were significantly higher in animals given the 6.7% or 9% liquid diet as compared to the 5% diet (Table 1).

Chronic intake of ethanol significantly decreased body weight (Table 1; $F(3,20) = 16.5$, $P < 0.001$), measured on the last treatment day, with post-hoc tests showing differences between the 6.7% and 9% ethanol groups when compared to either the control, pair-fed group or the 5% ethanol group (Table 1). Body weights in the 5% group were higher than, but not significantly different from, those in the pair-fed group (Table 1). However, since the intake of liquid diet in control animals was limited to corresponding intake of matched animals in each of the three ethanol groups, direct comparisons with the overall control group are not informative. Nonetheless, it is clear that animals in the 9% ethanol group show a relatively large reduction in body weight at the end of the 2-week period.

Tolerance to acute ethanol-induced hypothermia

Fig. 1 shows the effects of acute oral administration of ethanol (5 g/kg) on core body temperature over a 180 min period in animals exposed to liquid diet containing varying amounts of ethanol or the pair-fed controls. Overall, ethanol produced significant hypothermia ($F(5,80) = 5.16$, $P < 0.001$) and a significant interaction with the liquid diet group ($F(15,80) = 1.92$, $P < 0.05$). Acute administration of ethanol (5 g/kg) produced hypothermia in pair-fed control animals which, although maximal at 60–120 min (change in temperature = -1.95 ± 0.49°C), persisted for the total duration of the observation period (Fig. 1, left panel). Acute ethanol produced a smaller temperature change in animals fed chronically with ethanol-containing liquid diet: this effect was most apparent in the 9% ethanol group and, to a lesser extent, in the 6.7% ethanol group, whereas the effect of acute ethanol in the 5% ethanol group was not significantly different than in controls. Fig. 1 also shows that basal temperature, measured prior to the administration of acute ethanol, was decreased in the ethanol groups ($F(3,16) = 3.94$, $P < 0.05$). Post-hoc tests revealed that basal temperature was significantly lower in the 9% group than in controls; in contrast, the (smaller) difference in basal temperature in the 6.7% ethanol group relative to control animals (−0.78°C) failed to achieve statistical significance. As a consequence of the lower basal temperatures, the area under the curve for the change in temperature due to acute ethanol was significantly lower for both the 6.7% and the 9% ethanol groups (Fig. 2, inset panel; $F(3,16) = 3.32$, $P < 0.05$), reflecting the relatively reduced effect of the treatment in the latter two groups.

Table 1
Effects of consumption of ethanol liquid diet on body weight, blood ethanol levels, and ethanol intake

Group	n	Weight (g)	Ethanol intake (g/kg)		[ethanol] (mg/ml)
			Last week	Last day	
Experiment I					
Control	6	231.8 ± 16.5	–	–	0.00 ± 0.00
5%	5	260.3 ± 9.4	11.6 ± 0.3	9.7 ± 1.5	0.45 ± 0.19
6.7%	4	195.3 ± 5.0 [a,b]	12.0 ± 0.4	13.1 ± 0.8 [b]	1.39 ± 0.51 [a,b]
9%	5	167.5 ± 4.0 [a,b]	14.2 ± 0.5	12.5 ± 0.8	1.19 ± 0.26 [a]
Experiment II					
Control	61	189.3 ± 1.9	–	–	0.01 ± 0.00
9%	57	165.6 ± 2.5 [a]	14.1 ± 0.2	13.6 ± 0.6	2.55 ± 0.13 [a]
Experiment III					
Control	42	215.7 ± 6.0	–	–	ND
9%	48	181.3 ± 6.5 [a]	13.6 ± 0.5	14.1 ± 0.3	ND

[a] $P < 0.05$ vs. control; [b] $P < 0.05$ vs. 5% ethanol; ND = not determined.

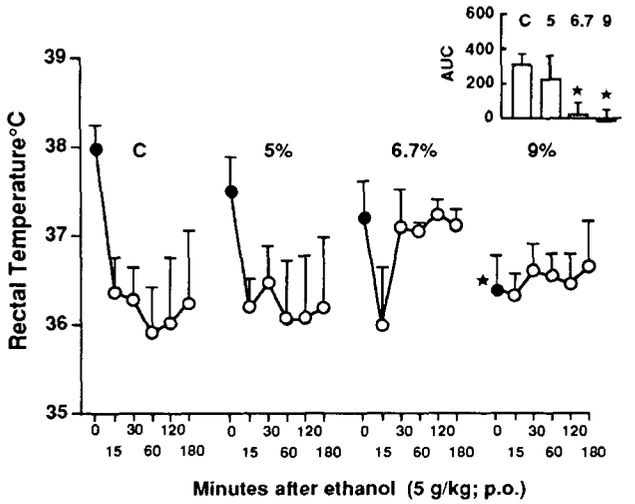

Fig. 1. Tolerance to acute ethanol-induced hypothermia in animals consuming ethanol. An acute dose ethanol (5 g/kg p.o.) was administered to rats that were given access to control diet or a diet containing 5%, 6.7%, or 9% of ethanol for 14 days. Values are means ± S.E.M. Closed symbols: basal temperature (i.e., the temperatures measured immediately before acute ethanol administration). Open symbols: temperature measured following acute ethanol administration. Data are also expressed as the area under the curve (AUC) of the change in temperature at the different time points after acute ethanol administration (inset panel). * Significantly different from control ($P < 0.05$).

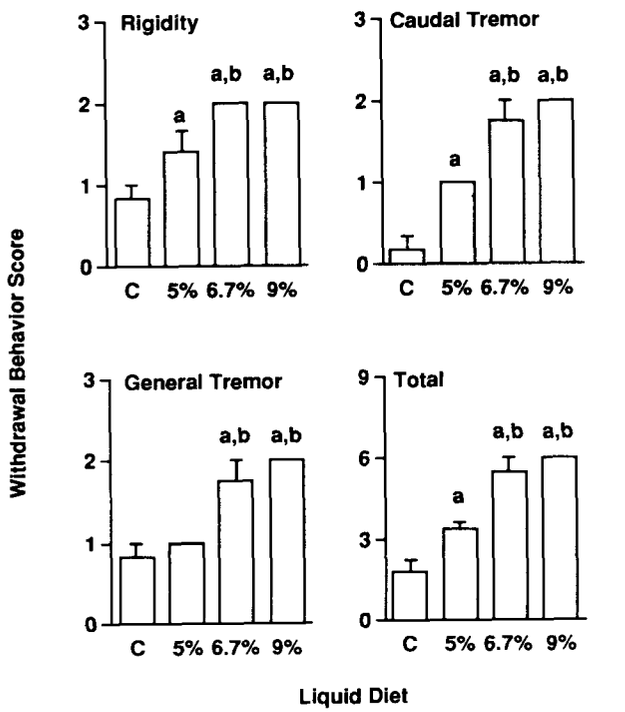

Fig. 2. Effect of ethanol concentration in liquid diet on the occurrence of withdrawal signs. Withdrawal signs were scored 18 h after the final exposure to ethanol (5 g/kg p.o.) in animals that were given access to control diet, or a diet containing varying concentrations of ethanol (i.e., 5%, 6.7%, 9%; see Materials and methods). Values are means ± S.E.M. [a]Significantly different from control ($P < 0.05$); [b]significantly different from 5% ethanol liquid diet ($P < 0.05$).

Withdrawal signs

Chronic exposure to ethanol containing liquid diet produced significant increases in behavioral signs of withdrawal upon its discontinuation (Fig. 2), with the magnitude of total withdrawal scores related to the concentration of ethanol in the diet. Withdrawal of the ethanol containing diet produced significant increases in rigidity ($F(3,16) = 13.0$, $P < 0.001$), caudal tremor ($F(3,16) = 36.9$, $P < 0.001$) and general tremor ($F(3,16) = 16.7$, $P < 0.001$) in each of the groups. Post-hoc tests showed that withdrawal scores of the higher ethanol groups differed significantly from those observed in the 5% group in each of the behavioral signs measured, as well as the total score. For all of the groups, total scores significantly increased in a concentration-related manner, indicating that the severity of ethanol withdrawal was related to its concentration in the liquid diet consumed.

3.2. Experiment II

Effects of chronic ethanol

The effects of ethanol availability for a 2-week period on body weight, ethanol intake, and ethanol blood levels are shown in Table 1. Animals in the 9% ethanol group consumed an average of 14.1 ± 0.2 g ethanol/kg during the second week of liquid diet availability. Average daily intake on the last day, 13.6 ± 0.60 g/kg, was comparable to that observed in the same group in Experiment I and resulted in blood ethanol levels of 2.55 ± 0.13 mg/ml. No ethanol could be detected in the blood taken 18 h after the last exposure to ethanol (data not shown). Animals in the 9% ethanol group showed a significant mean weight loss of approximately 25 g in comparison to pair-fed control animals (Table 1; $F(1,119) = 58.1$, $P < 0.001$). It is likely, in this case, that this weight loss represented an effect of ethanol other than caloric restriction since food intake of the pair-fed control animals was matched closely to that of the 9% ethanol group (unlike in Experiment I). As in the previous experiment, comparable, significant increases in rigidity ($F(1,107) = 286.4$, $P < 0.001$), caudal tremor ($F(1,107) = 353.3$, $P < 0.001$) and general tremor ($F(1,107) = 380.0$, $P < 0.001$) scores were observed in animals withdrawn from the 9% ethanol liquid diet, which were reflected by total withdrawal scores of 7.55 ± 0.23 vs. 3.07 ± 0.04, ethanol vs. control diet, respectively.

Effects of 8-OH-DPAT

Administration of 8-OH-DPAT produced a dose-related increase in flat body posture in both control and ethanol-treated animals (Fig. 3A, $F(6,107) = 51.5$, $P < 0.001$). In control animals, flat body posture was observed following doses between 0.16 and 0.63 mg/kg and the maximal score was obtained (1.0 ± 0.0) for

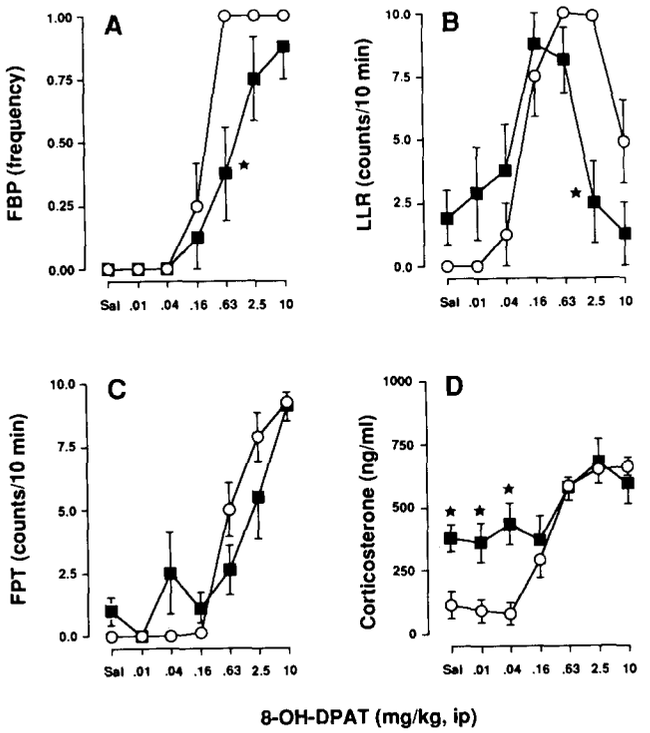

Fig. 3. Effects of chronic ethanol consumption on 8-OH-DPAT-induced behavioral effects and elevation of corticosterone. Values are means ± S.E.M. obtained in animals that were given access to control diet (open symbols) or a diet containing 9% ethanol (solid symbols). A: Effect of chronic ethanol exposure on 8-OH-DPAT-induced flat body posture (FBP). The presence (1) or absence (0) of flat body posture was noted if it occurred at any time during a 10 min period starting 10 min after the administration of 8-OH-DPAT (i.p.). B: Effect of chronic ethanol consumption on 8-OH-DPAT-induced lower-lip retraction (LLR). The number of 10 s periods in which the behavior was present was noted, yielding a maximum score of 10. C: Effect of chronic ethanol exposure on 8-OH-DPAT-induced forepaw treading (FPT). D: Effect of chronic ethanol consumption on 8-OH-DPAT-induced elevation in serum corticosterone levels. Corticosterone was assayed in trunk blood collected 1 h after administration of 8-OH-DPAT. Values are means ± S.E.M. $^{*}P < 0.05$ vs. control.

higher doses up to 10 mg/kg (Fig. 3A). In animals withdrawn from the 9% ethanol diet there was a significant difference in the main effects of 8-OH-DPAT ($F(1,107) = 12.2$, $P < 0.001$) as well as a significant interaction with the liquid diet group ($F(6,107) = 3.41$, $P < 0.01$). Post-hoc tests showed that the effects of 8-OH-DPAT were significantly lower in the ethanol group as compared to the pair-fed group following administration of the 0.63 mg/kg dose (1.0 ± 0.0 vs. 0.38 ± 0.18, pair-fed vs. ethanol, respectively).

Administration of 8-OH-DPAT produced lower lip retraction in both control and ethanol-exposed animals (Fig. 3B, $F(6,107) = 15.5$, $P < 0.001$) which followed an inverted U-shaped dose-response function. In control animals, lower lip retraction increased progressively following doses between 0.04 and 2.50 mg/kg and then decreased following the highest dose, 10 mg/kg. There was no significant main effect of group ($F(1,107) = 12.2$, $P < 0.001$); however, there was a significant interaction ($F(6,107) = 4.93$, $P < 0.001$), with an apparent shift to the left of the entire dose-response curve in animals withdrawn from the 9% ethanol diet. Post-hoc tests showed that the effects of 8-OH-DPAT were significantly reduced in the ethanol group as compared to the pair-fed control group following administration of the 2.5 mg/kg dose (9.88 ± 0.13 vs. 2.63 ± 1.64, pair-fed vs. ethanol, respectively).

Administration of 8-OH-DPAT produced a significant (Fig. 3C, $F(6,107) = 39.6$, $P < 0.001$) dose-related increase in forepaw treading in both control and ethanol-exposed animals. Although there was no significant difference between the pair-fed control and ethanol groups, a significant interaction ($F(6,107) = 2.50$, $P < 0.05$) was found between diet group and dose of 8-OH-DPAT. Since none of the post-hoc comparisons reached significance, the interaction may perhaps be explained by the finding that a small degree of forepaw treading occurred following injection of saline or the 0.04 mg/kg dose of 8-OH-DPAT in the ethanol group, whereas higher doses produced relatively lower levels of forepaw treading in this group (Fig. 3C).

Administration of 8-OH-DPAT produced a significant ($F(6,107) = 17.8$, $P < 0.001$), dose-related increase in blood corticosterone levels in both control and ethanol-exposed animals (Fig. 3D). In control animals, 8-OH-DPAT produced a dose-related elevation of plasma corticosterone which reached a plateau following administration of the 2.5 or 10 mg/kg i.p. doses (Fig. 3D). Although there was a significant main effect of group ($F(1,107) = 14.1$, $P < 0.001$), there was also a significant interaction ($F(6,107) = 3.1$, $P < 0.01$) which is explained by the fact that basal plasma corticosterone levels were significantly elevated in animals withdrawn from the 9% ethanol diet and administered saline or low doses of 8-OH-DPAT (0.01–0.16 mg/kg i.p.). Further evidence of an interaction is that the higher doses of 8-OH-DPAT, which produce either intermediate or maximal elevations of plasma corticosterone levels in control animals failed to produce an additive increase in the 9% ethanol group. Thus, relative to basal levels, 8-OH-DPAT produced a much smaller increase in plasma corticosterone levels in the ethanol group than in the control group.

3.3. Experiment III

Effects of chronic ethanol

The effects of ethanol diet on average daily ethanol intake, body weight, and withdrawal signs were comparable to those noted in previous experiments involving identical treatments (Table 1 and Fig. 4). Body weights were slightly higher than in previous experiments since different groups were started at later time-points after

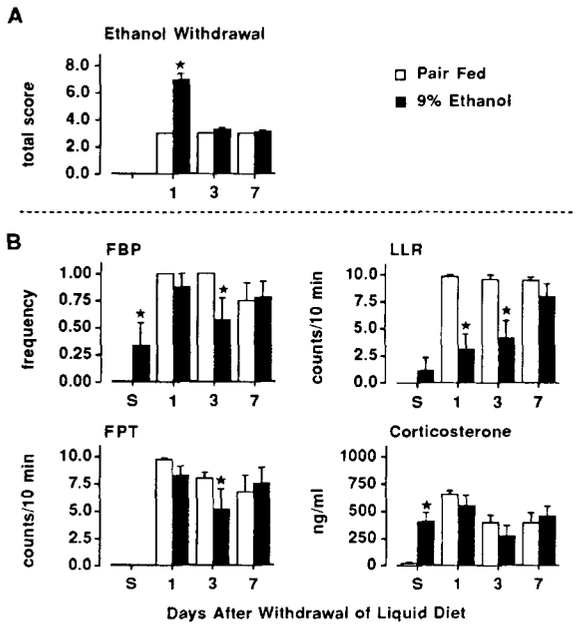

Fig. 4. Effects of chronic ethanol on 8-OH-DPAT-induced behavioral effects and elevation of plasma corticosterone levels measured in different groups of animals on the first, third, and seventh day following withdrawal of the ethanol-containing diet or calorically matched diet. Values are means ± S.E.M. For comparison purposes, the total withdrawal scores measured prior to the administration of 8-OH-DPAT are shown in panel A (see Materials and methods for details). Panel B: Effects of 8-OH-DPAT (2.5 mg/kg, days 1, 3, 7) or saline (S, day 1 only) on flat body posture (FBP), lower lip retraction (LLR), forepaw treading (FPT) and corticosterone in ethanol withdrawn or control animals. With the exception of corticosterone levels, saline produced scores of zero in all control animals. See legend to Fig. 3 and Materials and methods for details. $^{*}P < 0.05$ vs. control.

arrival. In this experiment, withdrawal signs as reflected by the total withdrawal score, were significantly elevated on the first day of withdrawal of ethanol containing liquid diet (Fig. 4), but were not different from control on subsequent days of withdrawal.

Effects of 8-OH-DPAT

As in the previous experiment, 8-OH-DPAT, at a dose of 2.5 mg/kg, produced a significant increase in flat body posture ($F(1,73) = 130.4$, $P < 0.001$) and a significant interaction between diet group and 8-OH-DPAT ($F(1,73) = 4.47$, $P < 0.05$; Fig. 4). Although the effect of withdrawal day approached significance ($P < 0.07$) no interaction between day and 8-OH-DPAT or diet group was found. Post-hoc tests showed that the effect of 8-OH-DPAT on flat body posture was significantly reduced on the third day following withdrawal of the ethanol diet (Fig. 4), but was not different on the first or seventh day. There was a low, but significant, incidence of flat body posture in animals withdrawn from the ethanol diet and treated acutely with saline. Since flat body posture was not observed either in comparable groups or in animals treated with low doses of 8-OH-DPAT in the previous experiment, the reliability of this result is not clear.

Again as in the previous experiment, 8-OH-DPAT produced significant increases in lower lip retraction ($F(1,73) = 213.1$, $P < 0.001$) as well as an interaction between diet group and 8-OH-DPAT ($F(1,73) = 25.3$, $P < 0.001$). In this case, there was a significant interaction between diet group, 8-OH-DPAT, and withdrawal day ($F(2,73) = 3.58$, $P < 0.05$), and post-hoc tests indicated a significant reduction in lower lip retraction following administration of 8-OH-DPAT on the first day (i.e., 18 h) and on the third day following withdrawal of the ethanol diet (Fig. 4). Interestingly, lower lip retraction was observed in several animals following saline treatment in the ethanol group on the first withdrawal day (0.33 ± 0.21, mean ± S.E.), but not on subsequent days (data not shown).

The pattern of effects of 8-OH-DPAT on forepaw treading in pair-fed and ethanol diet animals was similar to that seen on flat body posture (Fig. 4). However, in this case, the analysis of variance revealed only a significant main effect of 8-OH-DPAT ($F(1,73) = 178.0$, $P < 0.001$) and the absence of interactions. Post-hoc tests showed that the effect of 8-OH-DPAT was significantly reduced on the third day following withdrawal of the ethanol diet (Fig. 4), but was not different on the first or seventh days.

Like other effects of 8-OH-DPAT, there was a significant main effect of 8-OH-DPAT ($F(1,73) = 68.9$, $P < 0.001$) on plasma corticosterone levels. However, in this case, there was also a significant effect of withdrawal day ($F(2,73) = 9.9$, $P < 0.001$) and an interaction between diet group, 8-OH-DPAT, and withdrawal day ($F(2,73) = 3.58$, $P < 0.05$). Since post-hoc tests failed to show a difference in the effects of 8-OH-DPAT between diet groups at any of the three withdrawal days, the significant interaction can be explained by the marked elevation of mean basal corticosterone levels on the first day of withdrawal of the ethanol diet (415 ± 74 vs. 22.4 ± 12 ng/ml, ethanol vs. pair-fed group, respectively) which returned toward control levels by the third and seventh days (100.8 ± 62 vs. 94.5 ± 57.6, day 3 vs. day 7, respectively).

4. Discussion

The most important finding of this study is that chronic ethanol exposure causes alterations in the behavioral effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, which were observed both in the presence and absence of overt ethanol withdrawal signs. It is evident that sensitivity to several of the effects of 8-OH-DPAT, most notably flat body posture and lower lip retraction,

is altered as a result of chronic ethanol exposure. Interestingly, these effects are in opposite directions, with tolerance to effects on flat body posture and sensitization to the effects of 8-OH-DPAT on lower lip retraction. Nonetheless, the results clearly suggest that chronic ethanol exposure changes several well characterized pharmacological effects of 8-OH-DPAT (Berendsen et al., 1989; Hjorth et al., 1982; Moore et al., 1993).

The results of the first experiment showed that exposure to 9% and 6.7% ethanol liquid diet produces both tolerance, evident on the last day of exposure, and physical dependence upon withdrawal of liquid diet, consistent with a number of previous studies using this procedure (Miller et al., 1980). Interestingly, ethanol intake was very similar for both the 5% and 6.7% groups, whereas only the latter showed significant signs of both tolerance and physical dependence. This suggests that overall levels of ethanol intake, when expressed on a g/kg basis, do not necessarily predict the degree of tolerance or physical dependence. Continuous availability of the 9% diet resulted in daily intake of ethanol (10–15 g/kg/day) comparable to that in previous studies using similar concentrations of ethanol in different liquid diet preparations (File et al., 1989; Lieber and DeCarli, 1989; Miller et al., 1980). Not surprisingly, this level of ethanol intake produced what are described as mild signs of ethanol withdrawal upon discontinuation of the diet (Lal et al., 1988). These signs consisted primarily of general tremor, caudal tremor, and rigidity, as opposed to convulsions and death which occur after prolonged intake or administration of higher doses of ethanol (Majchrowicz, 1975; Majchrowicz and Hunt, 1976).

In the present study, 8-OH-DPAT produced, in a dose-related manner, flat body posture and forepaw treading, elements which are part of the '5-HT syndrome' produced by direct and indirect 5-HT receptor agonists (Tricklebank et al., 1984). While not originally included as a component of the '5-HT syndrome', 8-OH-DPAT also reliably produces lower lip retraction, an element presumably mediated by 5-HT_{1A} receptors (Berendsen et al., 1989), which was observed to follow an inverted U-shaped dose-response function. A low incidence of lower lip retraction was observed in the absence of 8-OH-DPAT treatment in animals withdrawn from the ethanol diet, suggesting that there is a sensitization of 5-HT_{1A} receptors as a result of chronic ethanol. Furthermore, the fact that the entire dose-response curve is apparently shifted to the left (see Fig. 3B), including the effects at the higher doses of 8-OH-DPAT, suggests that there is an additive level of lower lip retraction induced by withdrawal from ethanol. The latter effect is, by itself, interesting since the mechanism by which higher doses of 8-OH-DPAT reduce the incidence of lower lip retraction is unclear. The present data suggest that this reduction is mediated by 5-HT_{1A} receptors since there is an apparent shift to the left of both the ascending and descending limbs of the dose-response function.

The finding that rats show elevations in plasma corticosterone levels following withdrawal of ethanol is in agreement with previous studies (Gadek et al., 1987; Pohorecky et al., 1978; Sipp et al., 1993). However, it is interesting that lower than maximal doses of 8-OH-DPAT which increase corticosterone levels in control rats (see Fuller and Snoddy, 1990) failed to show additive effects in animals withdrawn from ethanol. If these results are expressed in relation to the basal levels, there is clear tolerance to the effects of 8-OH-DPAT following ethanol consumption. Although it is possible that a ceiling is reached in terms of the maximal stimulation of corticosterone secretion by 8-OH-DPAT, the results with low and intermediate doses clearly fail to show an additive effect in ethanol-exposed animals. Reduced sensitivity to 8-OH-DPAT was not observed on subsequent days following withdrawal of the ethanol diet due to a concomitant decrease in sensitivity to 8-OH-DPAT in control animals. It is not clear whether this is due to variability or is a consequence of the return to normal diet. The former is suggested by increased variability in other effects of 8-OH-DPAT such as forepaw treading and flat body posture measured at the same time.

The finding that chronic ethanol exposure results in tolerance to the effects of 8-OH-DPAT on flat body posture while producing sensitization to its effects on lower lip retraction supports the idea that different receptor populations mediate these behavioral effects. Recently, it was demonstrated that 8-OH-DPAT was more potent in producing lower lip retraction when injected into the median raphe in comparison to dorsal raphe injection (Berendsen et al., 1994). Pharmacological evidence also supports differences in pre-versus post-synaptic mediation of lower lip retraction and of flat body posture. That is, lower lip retraction is believed to be mediated by activation of pre-synaptic (i.e., somatodendritic) 5-HT_{1A} receptors (Berendsen et al., 1989), while flat body posture is mediated postsynaptically (Tricklebank et al., 1984). Thus, both pharmacological and anatomical evidence are consistent with the dissociation between sensitization to lower lip retraction and tolerance to flat body posture observed in the present study. This correspondence may also extend to 8-OH-DPAT-induced forepaw treading and corticosterone secretion, which are also reportedly mediated by stimulation of postsynaptic 5-HT_{1A} receptors (Welch et al., 1993). However, in the present study, the evidence for ethanol-induced tolerance to 8-OH-DPAT's effects on forepaw treading or corticosterone secretion is much weaker than that for lower lip retraction or flat body posture. It is therefore likely that chronic ethanol

exposure causes a differential decrease in selected postsynaptic 5-HT_{1A} receptor populations, or alters the balance between pre-and post-synaptic receptors mediating different behavioral and/or neuroendocrine effects.

In parallel studies, Nevo et al. (1995) reported that labeling of somato-dendritic 5-HT_{1A} receptors by [^{3}H]8-OH-DPAT and [^{3}H]WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) was increased in the dorsal raphe nucleus, whereas labeling by the same receptor ligands of postsynaptic 5-HT_{1A} sites in the hippocampus and cerebral cortex was decreased following chronic ethanol consumption in the rat. Therefore, the variation in the responses to 8-OH-DPAT noted above might reflect an upregulation of somatodendritic 5-HT_{1A} receptors (which mediate the lower lip retraction response) and a downregulation of post-synaptic 5-HT_{1A} receptors (which mediate the flat body posture response) in rats exposed to chronic alcoholization (see Nevo et al., 1995). It is suggested that the biochemical basis for these receptor alterations involves ethanol-induced increases in corticosterone release which were consistently observed following withdrawal of ethanol (Nevo et al., 1995).

While a definitive explanation of the altered sensitivity to acute effects of 8-OH-DPAT as a consequence of chronic ethanol consumption awaits further investigation, a recent study suggests that tolerance to 8-OH-DPAT is due to similar adaptive processes which are responsible for ethanol withdrawal. It has been recently demonstrated that pretreatment of rats with the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine (MK-801), blocks the development of tolerance to 8-OH-DPAT-induced corticosterone secretion (Ross et al., 1992) as well as 5-HT-mediated tolerance to ethanol (Khanna et al., 1992,1993). It has already been established that chronic ethanol consumption causes hypersensitivity to NMDA receptor agonists as a consequence of chronic inhibition of the NMDA receptor (Davidson et al., 1993), a finding which can explain the development of withdrawal seizures (Grant et al., 1990; Gulya et al., 1991). It is therefore conceivable that chronic exposure to ethanol augments the development of tolerance to 8-OH-DPAT via alteration of NMDA receptors.

In conclusion, the present results demonstrate that consumption of an ethanol-containing diet for a period of 2 weeks results in a relatively enduring change in sensitivity to the behavioral effects of 8-OH-DPAT. These results are consistent with the involvement of central serotonergic neurons in acute effects as well as adaptation during chronic ethanol exposure. It remains to be demonstrated whether a reduced magnitude of tolerance or physical dependence also invokes similar changes in response to 5-HT_{1A} receptor ligands.

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