Pharmacology Biochemistry and Behavior, Vol. 64, No. 3, pp. 563–566, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00104-5

Conditioned Taste Aversion Induced by Ethanol in Alcohol-Preferring Rats: Influence of the Method of Ethanol Administration

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Received 11 November 1998; Accepted 23 April 1999

CICCOCIOPPO, R., S. ANGELETTI, M. CHHADA, M. PERFUMI, R. FROLDI AND M. MASSI. *Conditioned taste aversion induced by ethanol in alcohol-preferring rats: Influence of the method of ethanol administration.* PHARMACOL BIOCHEM BEHAV **64**(3) 563–566, 1999.—A recent study of our group has shown that ethanol evokes conditioned place preference (CPP) in Marchigian Sardinian alcohol-preferring (msP) rats following intragastric (IG) administration by means of an indwelling IG catheter, but not following administration by gavage or by intraperitoneal (IP) injection. The present study evaluated in ethanol-naive msP rats the influence of the method of administration (IG injection by indwelling catheter vs. IP injection) on ethanol-induced conditioned taste aversion (CTA). The dose of 0.35 g/kg of ethanol did not evoke aversion either by IG or by IP administration. Following IG injection, 0.7 g/kg of ethanol, the amount that msP rats voluntarily ingest in a short (2–5 min) drinking episode, did not evoke CTA, and 1.5 g/kg induced a modest CTA. On the other hand, IP injection of 0.7 g/kg of ethanol evoked CTA, and 1.5 g/kg induced a very pronounced CTA. These findings show that the aversive properties of ethanol in msP rats are influenced by the method of administration, and suggest that the IG injection by catheter may reveal more faithfully than the IP injection the motivational properties of amounts of ethanol that alcohol-preferring rats voluntarily ingest. © 1999 Elsevier Science Inc.

Conditioned taste aversion Marchigian Sardinian alcohol-preferring rats Intragastric ethanol administration Intraperitoneal ethanol administration

A recent study of our group (5) has shown that conditioned place preference (CPP) can be evoked in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats, following intragastric (IG) administration of ethanol by means of an indwelling IG catheter (14). In ethanol-experienced msP rats CPP was observed in response to 0.7 and 1.5 g/kg of ethanol; in ethanol-naive msP rats CPP was evoked only by 0.7 g/kg. On the other hand, the same doses of ethanol failed to evoke CPP after administration by gavage or by intraperitoneal (IP) injection. These results indicate that the method of ethanol administration can be crucial to reveal the positive motivational properties of ethanol in alcohol-preferring rats.

The present study evaluated in msP rats the influence of the method of ethanol administration in the conditioned taste aversion (CTA) paradigm (2,11,12,21). In this test the consumption of a novel and detectable taste solution is paired with the administration of ethanol. Aversion to ethanol is indicated by reduced intake of the taste solution in subsequent exposures. In the present study, ethanol was administered either by IG injection, by means of an indwelling catheter, or by IP injection. The administration by gavage was not used in the present study, in relation to the problems raised by the gavage administration to rats that have ingested a large volume of fluids shortly before, as it occurs in the CTA paradigm.

Our interest in the present study was strengthened by the fact that CTA studies in alcohol-preferring rats have usually employed just the IP route of administration. In ethanol-naive alcohol-preferring (P) rats (13), IP injection of 0.5 g/kg of ethanol evoked neither CTA nor conditioned taste preference; CTA was evoked by IP injection of 1 g/kg, and a very pro-

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nounced CTA was evoked by 1.5 g/kg (9,21). These amounts of ethanol can be voluntarily ingested by P rats in a single drinking episode (15). In the study of Froehlich et al. (9), mild conditioned taste preference was observed in ethanol-naive P rats by IP injection of 0.25 g/kg. A reduction in ethanol-induced CTA has been reported following prior exposure to ethanol in both genetically selected P rats (21) and in unselected rats (4,18); in this regard, the present study employed only ethanol-naive msP rats.

METHOD

Animals

Male genetically selected alcohol-preferring rats were employed; they were bred for 26 generations in the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy), starting from Sardinian alcohol-preferring (sP) rats of the 13th generation (1,7,10). They are referred to as Marchigian Sardinian alcohol-preferring (msP) rats. The rats' body weight ranged among 400 and 450 g at the moment in which the experiments were carried out. Rats were kept in individual cages in a room with a reverse 12L:12D cycle (lights off at 1000h), temperature of 20–22°C and humidity of 45–55%. They were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). All animal testing was carried out according to the Italian ethical rules on animal care.

In Experiment 1, ethanol-experienced rats were employed. They were selected for ethanol preference by offering them 24-h access to 10% ethanol for a week, at the age of 2 months. Afterwards, to evaluate their ethanol drinking behavior in a short time interval, they were offered ethanol in their home cage 2h/day for 10 days before the experiment, while they had food and tap water freely available.

Three-month-old msP rats without previous ethanol experience (ethanol-naive) were tested in the CTA paradigm. Ten days after the end of the taste conditioning experiments, rats were offered 24-h access to both water and 10% v/v ethanol. All the animals employed in the study showed over 90% ethanol preference [ml of ethanol solution/ml of total fluids (water + 10% ethanol) ingested in 24 h × 100] and a daily ethanol intake ranging among 6 and 7 g/kg body weight.

Tap water, 10% ethanol, as well as the sweet solution used in the CTA paradigm were provided in the rats' home cage in 50-ml graduated drinking burettes, equipped with a metallic drinking spout, and their consumption was measured to the nearest 0.1 ml.

Intragastric Surgery

Rats were anesthetized by IP injection of 100–150 μl/100 g body weight of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml). A polyethylene catheter (PE-50, Clay Adams) was permanently implanted into the stomach, according to the method of Lukas and Moreton (14). The PE tubing was run subcutaneously to reach the skin between the scapulae, where it was exteriorized. Rats were allowed a week to recover from surgery before testing began.

Experimental Procedure

Experiment 1: Ethanol intake of msP rats in a 2-h period of access to ethanol. The rationale for this experiment was to evaluate, in the generation of msP rats employed in the present study, the amounts of ethanol that they voluntarily ingest in a short time interval. The same amounts have been given in the following experiments by IG or IP administration. Freely feeding and drinking msP rats ($n = 9$) were offered access to 10% ethanol 2 h/day (from 1000 to 1200 h) for 10 days before the experiment. On day 11, 10% ethanol was offered again and ethanol intake (g/kg body weight) was recorded after 2.5, 5, 15, 30, 60, and 120 min.

Experiment 2. Effect of IP or IG injections of ethanol in the CTA paradigm. Taste conditioning was carried out offering rats a sweet solution (containing 0.125% saccharin + 3% glucose). This sweet solution was chosen, because it is highly palatable, it is consumed in huge amounts by the sated rat, and it has a low caloric content (20,23). Once a day, in the 2 days before the experiment, a few drops of sweet solution were sprinkled over the rat mouth, so that it could taste it. This expedient was adopted to familiarize the rat with the new tastant.

Eight groups of seven to eight msP rats, with free access to food and water, were employed. For 7 consecutive days (at 1000h) all of them were offered the sweet solution for 20 min, while food and water were removed from the cage. The amount of sweet solution ingested was recorded and expressed as ml/kg body weight. Immediately after the 20 min of access, four groups of seven to eight animals received IP injection of 0.35, 0.7, or 1.5 g/kg of ethanol or isotonic saline (vehicle). The other four groups of seven rats received an IG injection of 0.35, 0.7, or 1.5 g/kg of ethanol or isotonic saline (vehicle). These doses of ethanol were chosen on the basis of the results in Experiment 1. Each dose of ethanol was administered in a constant volume of 10 ml/kg; thus, the concentration of the ethanol solutions ranged between 5 and 20%.

Experiment 3: Blood alcohol levels (BAL) following IG and IP injection of ethanol. Six groups of ethanol-naive msP rats were used. At 1000 h, as in Experiment 2, three groups of five to six rats received IG ethanol administration, while other three groups of five to six rats received IP ethanol injection. The ethanol doses employed (0.35, 0.7, or 1.5 g/kg) were dissolved in isotonic saline and administered in a constant volume of 10 ml/kg, as in Experiment 2. Blood samples (50–100 μl) were taken from the tail vein 15, 30, 60, and 120 min after ethanol administration. BAL were measured by gas chromatography (6).

Statistical Analysis

Data are presented as means ± SEM. Data were analyzed by means of split-plot analysis of variance (ANOVA), with between-groups comparisons for method of administration and within-groups comparisons for time. Pairwise comparisons were carried out by means of the Dunnett's test. Statistical significance was set at $p < 0.05$. The slope of the decreasing portion (30–120 min) of the BAL curve was calculated for the three doses, following each method of administration; the values obtained were compared according to Tallarida and Murray (22).

RESULTS

Experiment 1: Ethanol intake of msP rats in a 2-h period of access to ethanol

In Fig. 1, the pattern of ethanol intake in freely feeding and drinking msP rats during a 2-h period of access to 10% ethanol is reported. In the first 2.5 min rats ingested from 0.35 to 0.7 g/kg of ethanol, usually in a single, continuous drinking episode. The cumulative 5-min ethanol intake averaged about 0.7 g/kg. The 2-h cumulative ethanol intake was slightly lower than 1.5 g/kg. These ethanol doses were chosen for the taste conditioning experiments.

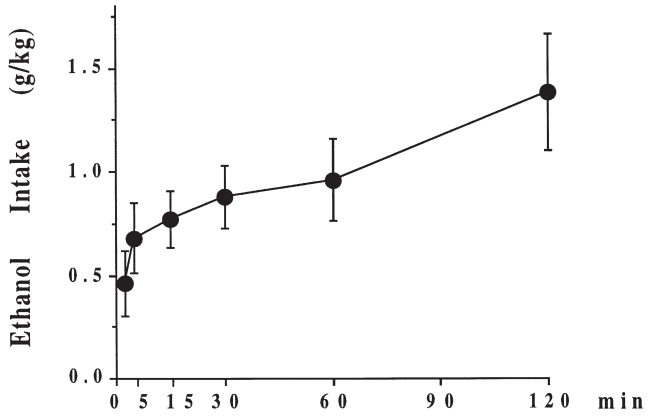

FIG. 1. Cumulative ethanol drinking in msP rats offered 10% ethanol 2-h/day. Values are means ± SEM of nine rats.

Experiment 2: Effect of IP or IG injections of ethanol in the CTA paradigm

As shown in Fig. 2, IP ethanol injections evoked pronounced CTA. The overall ANOVA revealed a statistically significant treatment effect, $F(3, 25) = 75.3$, $p < 0.0001$, time effect, $F(5, 125) = 3.7$, $p < 0.01$, and treatment–time interaction, $F(15, 125) = 2.5$, $p < 0.01$. A significant CTA was evoked by 0.7 or 1.5, but not 0.35 g/kg of ethanol, after the first ethanol pairing. After 3 days of pairing with 1.5 g/kg of ethanol, the intake of the sweet solution was almost completely abolished.

For the IG ethanol administration, the ANOVA revealed a statistically significant treatment effect, $F(3, 24) = 4.4$, $p < 0.05$, but nonsignificant time effect, $F(5, 120) = 1.8$, $p > 0.05$, and treatment–time interaction, $F(15, 120) = 1.6$, $p > 0.05$ (Fig. 3). Neither 0.35 nor 0.7 g/kg of ethanol significantly reduced the intake of the sweet solution in the 7-day treatment. A significant CTA was evoked only by 1.5 g/kg; however, this dose only slightly reduced the intake of the sweet solution by IG injection, while it completely abolished the intake following IP administration.

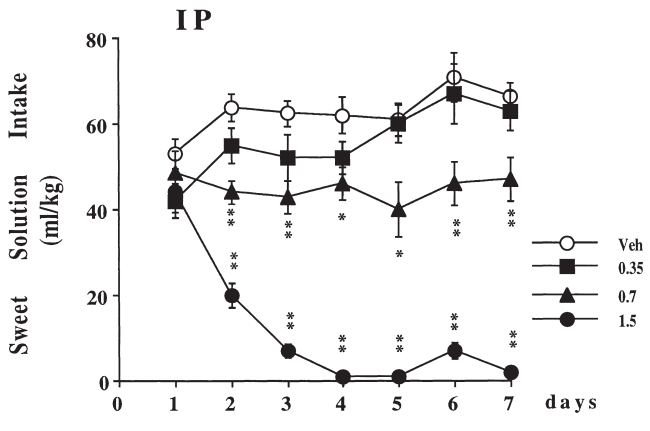

FIG. 2. Effect of IP administration of ethanol (0.35, 0.7, or 1.5 g/kg) or vehicle (Veh) on the intake of 0.125% saccharin + 3% glucose solution in the CTA paradigm. Values are means ± SEM of eight subjects for 0.7 g/kg and of seven subjects for the other treatments. Statistical difference from controls: * $p < 0.05$, ** $p < 0.01$; where not indicated, difference from controls was not statistically significant.

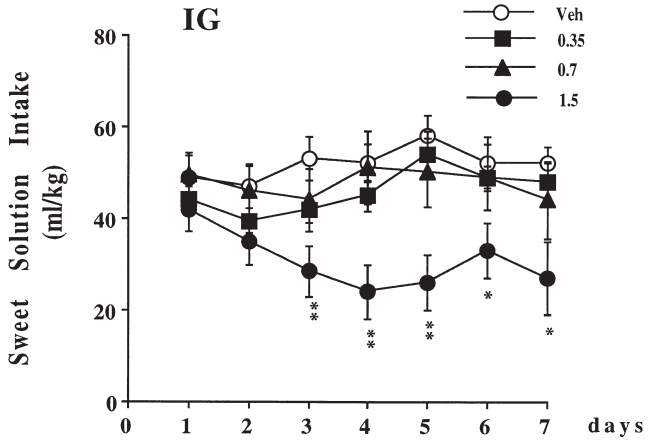

FIG. 3. Effect of IG administration of ethanol (0.35, 0.7, or 1.5 g/kg) or vehicle (Veh) on the intake of 0.125% saccharin + 3% glucose solution in the CTA paradigm. Values are means ± SEM of seven subjects. Statistical difference from controls: * $p < 0.05$, ** $p < 0.01$; where not indicated, difference from controls was not statistically significant.

Experiment 3. BAL following IP or IG injection of ethanol

BAL after either IG or IP ethanol injection are reported in Fig. 4. Detectable BAL were measured at the lowest dose, 0.35 g/kg, following both methods of administration, and progressively increased with the dose. Following IP injection of the three doses of ethanol, BAL were slightly higher than those following IG administration; however, the difference did not reach statistical significance either in response to 0.35, $F(1,10) = 2.5$, $p > 0.05$, to 0.7, $F(1, 8) = 0.45$, $p > 0.05$, or to 1.5 g/kg, $F(1, 8) = 0.45$, $p > 0.05$).

The slopes for the decreasing portion of the BAL curves, following each method of administration, were not significantly different ($p > 0.05$) at the three doses tested.

DISCUSSION

A previous study of our group (5) has shown that the method of ethanol administration markedly affects the re-

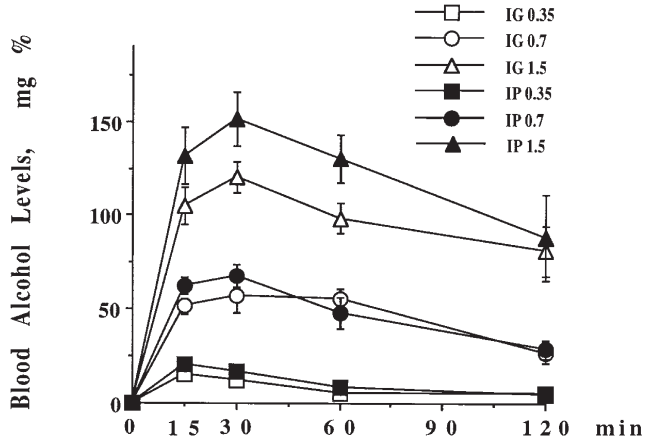

FIG. 4. Blood alcohol levels following IP or IG ethanol (0.35, 0.7, or 1.5 g/kg) administration. Values are means ± SEM of six subjects for 0.35 g/kg and of five subjects for the other treatments. Statistical difference between route of administration for each ethanol dose was never statistically significant.

warding properties of ethanol in msP rats. In that study, amounts of ethanol that msP rats voluntarily ingest induced CPP following administration by an IG catheter, but not by gavage or by IP injection. The results of the present study show that the aversive properties of ethanol in msP rats are also strongly influenced by the method of administration.

Usually msP rats voluntarily ingest 0.7 g/kg of ethanol in a short (2–5 min) drinking episode. The IG administration of this dose by catheter did not produce aversion in the CTA paradigm, and elicited CPP in our previous study (5). The IG dose of 1.5 g/kg produced a slight CTA, but it should be considered that this dose is voluntarily ingested in 2 h, while in the CTA paradigm it was given in a single IG injection. In the CPP paradigm, ethanol-naive msP rats increased, but not significantly, the time spent in the ethanol-paired compartment (5).

On the other hand, CTA was observed following IP injection of 0.7 g/kg, and a very pronounced CTA was observed in response to IP injection of 1.5 g/kg. These findings are similar to those reported in ethanol-naive P rats, in which CTA was induced by IP injection of 1 g/kg and a very marked CTA was evoked by 1.5 g/kg of ethanol (9,21). In the CPP paradigm in ethanol-naive msP rats neither 0.7 nor 1.5 g/kg evoked CPP (5) by IP injection.

Further studies are needed to evaluate the reasons accounting for the results of the present study. It has been suggested that the aversive properties of ethanol occur during the decreasing portion of BAL (17,19). However, the slopes of the BAL curves for the doses that evoked CTA, 0.7 and 1.5 g/kg, were not significantly different following each method of administration. Nurmi et al. (16) reported a different brain distribution of ethanol in the first few minutes following the IP vs. the IG route of administration; differences in brain ethanol levels could contribute to a different evaluation of the amount of ethanol administered. However, in our previous study (5) CPP was elicited following ethanol administration by IG catheter, but not by IG gavage, implying that administration of ethanol into the stomach cannot be the only determinant of the behavioral differences. Discomfort and stress, which may occur during the administration by gavage or by IP injection, may also contribute to determine the differences observed in the CTA and CPP paradigms. Finally, also local effects of IP injected ethanol should be taken into account; for instance, IP ethanol administration might result in a more pronounced activation of peripheral opioid receptors in the gut, which have been proposed to be involved in the aversive effects of ethanol (3,8).

In conclusion, the amount of ethanol (0.7 g/kg) that msP rats voluntarily ingest in a short drinking episode induces CPP and does not evoke CTA, when it is administered by means of an indwelling IG catheter. The same amount of ethanol, given by IP injection, does not evoke CPP, but elicits CTA. These findings suggest that the IG administration by catheter may reveal more faithfully than the IP injection the motivational properties of amounts of ethanol, that alcohol-preferring rats voluntarily ingest; thus, it may represent an appropriate method of ethanol administration in studies investigating the biochemical and neurochemical correlates of voluntary ethanol drinking.

ACKNOWLEDGEMENTS

This work was supported by grant from the University of Camerino (MC) Italy. The authors wish to thank Mr. M. Cippitelli for determination of blood alcohol levels.

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