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Effects of Alcohol Dependence on Shock-Induced Fighting: Action of Muscimol and Homotaurine

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ROUHANI, S., E. EMMANOUILIDIS, C. PAYAN, G. TRAN, A. CASTRESANA, A. SOULAIRAC AND S. POENARU. *Effects of alcohol dependence on shock-induced fighting: Action of muscimol and homotaurine.* PHARMACOL BIOCHEM BEHAV **41**(1) 49–51, 1992.—We have applied the electroshock-induced fighting behavior to the study of experimental alcohol dependence. Adult Wistar rats were intoxicated chronically with ethanol (10 g/kg/24 h) for 13 days. Electroshock-induced fighting behavior was studied during chronic intoxication and withdrawal in comparison with normal rats receiving a water-carbohydrate solution isocaloric to ethanol. Rats were divided into groups receiving respectively muscimol (0.25 mg/kg), a GABAA agonist; homotaurine (140 mg/kg) a GABA mimetic; and physiological saline (10 ml/kg), intraperitoneally. During chronic intoxication, rats showed an increase in defensive-fighting behavior. Withdrawal accentuated the aggressive behavior and muscimol and homotaurine inhibited it. These results confirm the relevance of the electroshock-induced defensive fighting behavior test in chronic intoxication with alcohol, but to show the involvement of GABAergic transmission in the behavioral effects of alcohol withdrawal, additional experiments with other GABA mimetics and with GABA antagonists should be considered.

Alcohol Chronic intoxication Dependence Withdrawal GABA Shock-induced fighting Muscimol Homotaurine Rat

ALCOHOL intoxication determines a dysfunction of the GABAergic system (4, 5, 17, 20). During the period of chronic intoxication a decrease in the activity of GABAergic neurones (24) with a decrease of GABA turnover in various cerebral structures (16) has been shown. However, a possible decrease in the affinity and number of GABA receptors has also been suggested (8,12). During the period of withdrawal the GABAergic transmission was found less efficient (4) and properties of the GABA-benzodiazepine-chloride-ionophore receptor altered (5). In fact, most authors are in agreement on the decrease in the number (19) and affinity (21) of GABA receptors in the mouse and in the rat (8, 10, 18, 22, 23) during withdrawal.

The electroshock defensive fighting behavior test is a model for studying aggressive behavior (3,14). To our knowledge, no study on electroshock-induced defensive fighting behavior in the alcohol-intoxicated rat has been performed. Such a technique is justifiable because: 1) it is a quantifiable method that is well codified (3,14); 2) the behavior studied involves the GABAergic mechanisms (14). For these reasons we have applied this method to the study of experimental alcohol dependence.

METHOD

Animals

Eighty male Wistar rats, weighing 260–280 g obtained from Iffa Credo (Saint Germain sur l'Arbresle, France), were housed in individual cages equipped for automatic and programmed administration of ethanol.

Rats were able to move around freely with water and food (standard complete chow, Iffa Credo) available ad lib. The animal room was maintained at a constant temperature (21 ± 1°C) and humidity (60%) with a 12-h light-dark cycle (light from 8:00 a.m.).

Alcoholism Model

All animals were fitted with an indwelling intragastric (IG) catheter under general anaesthesia (sodium pentobarbitone, 25 mg/kg, IP) with the distal end cemented to the exterior of the skull (13).

Ethanol was infused by intragastric administration one week after surgery using electronically controlled pumps that were programmed to deliver regular divided doses of 3 ml of solution each administered over 6.5 min during the 24-h period, at 4:30 a.m., 8:30 a.m., 2:00 p.m., 7:00 p.m. and 11:30 p.m.

Ethanol infusions were 21 ± 2% v/v ethanol-water solutions. The animal was permanently connected to the pump, freedom of movement being assured by a rotating watertight joint inserted between the pump and the infusion catheter. The infused dose represented 10 g absolute ethanol per kg body weight per day (416 mg/kg/h). This amount exceeds the ceiling of metabolic oxidation situated around 300–400 mg/kg/h (15) or 8 mg/kg/24 h (11). Administration in divided doses permits, moreover, a

relatively constant and durable blood alcohol level to be maintained, thus improving the effectiveness of chronic intoxication (7). The duration of intoxication was 13 days, a period sufficient for the development of physical dependence (7,9). To measure the alcohol concentration, blood was sampled from the tail vein on the second day of intoxication, one hour after infusion of ethanol at 2:00 p.m. The ethanol infusions were stopped on the 13th day at 11:30 p.m.

Normal rats received a water-carbohydrate solution isocaloric to ethanol.

Apparatus and Behavioral Procedure

The electric shocks for inducing defensive and fighting behavior, delivered by an electronic scrambler (Campden Instruments Ltd.) at a rate of 50 shocks per session, were of an intensity of 1.20 mA and a duration of 0.5 second at 6-second intervals. The testing area was a $22 \times 22 \times 26$ cm Plexiglas cage, placed on a floor-grid via which the electric footshocks were given.

Each pair of animals matched for weight was maintained during the whole of the experiment and underwent conditioning for a period of 9 days, starting on the fourth day after the start of the infusions. During this period, electric shock sessions were held each day at a fixed time between 3:00 p.m. and 5:00 p.m.

After each electric shock, reactions of the animal were noted as follows according to the following criteria (3,14): no reaction; shock avoidance reaction: jumping or/and climbing up the sides of the cage; threatening reaction: the animals rear up and exchange blows, the forelegs touch the adversary's body and/or head; attack: the rat is placed over its opponent which lies on its back.

At the end of each test session, the number (N) of attacks and fights were calculated for each pair of animals.

A baseline of N was also calculated as the average of last three days of infusion.

Administration of Products

On the 14th day (withdrawal day) either physiological saline (10 ml/kg), muscimol (0.25 mg/kg) or homotaurine (140 mg/kg) dissolved in physiological saline (0.9%) were administered intraperitoneally (IP). These IP administrations took place ½ hour before the last shock-induced fighting test, between the 14th and 16th hours of withdrawal. This is the period when maximum signs of physical dependence appear (9).

Rats were divided into six groups receiving: ethanol and physiological saline, 6 pairs (EP); ethanol and muscimol, 7 pairs (EM); ethanol and homotaurine, 7 pairs (EH); water + carbohydrate isocaloric to ethanol (W) and physiological saline, 6 pairs (WP); W and muscimol, 7 pairs (WM); W and homotaurine, 7 pairs (WH).

Statistical Analysis

The results of shock-induced fighting were evaluated using the analysis of variance (ANOVA), to study changes occurring during conditioning period and on the day of withdrawal. Paired Student's *t*-test was used to compare the value of N on the day of withdrawal with baseline values. Differences were considered significant at $p \leq 0.05$.

All the results are expressed as the mean ± SEM.

RESULTS

Blood alcohol levels were 93 ± 5 mg per 100 ml. The mode of administration ensured constant blood alcohol levels between the periods of infusion (7).

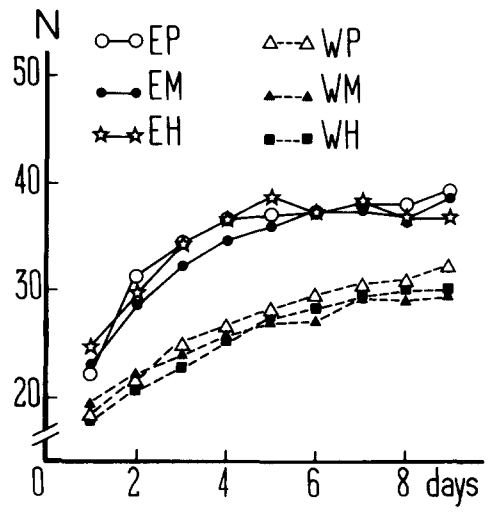

FIG. 1. Changes in electroshock-induced fighting behavior during the conditioning period in ethanol and water rats, before intraperitoneal injections of products. An increase of the number (N) of attacks and fights is observed in alcohol-intoxicated rats ($p<0.0001$, ANOVA).

During the conditioning period of 9 days with the water-ethanol infusion there was an increase in shock-induced fighting behavior. At the end of this period, the values of N stabilized (Fig. 1), and were higher in rats intoxicated with alcohol (EP, EM, and EH). The differences compared to nonintoxicated rats were significant ($p \leq 0.0001$, ANOVA).

On the day of withdrawal, maximum signs of physical dependence appeared 14 to 16 hours after the end of infusion. Under the effect of IP injection of muscimol or homotaurine N decreased in the EM, EH, WM, and WH groups ($p \leq 0.0001$, ANOVA). Table 1 shows these values compared to baseline N.

In the case of muscimol and homotaurine-treated groups the changes are parallel in intoxicated and nonintoxicated animals. In the case of rats receiving IP injection of saline, no difference was observed between day 9 and the day of withdrawal in nonintoxicated group (WP), whereas a significant increase of fights and attacks was observed in the intoxicated group (EP).

DISCUSSION

Our results in the chronically intoxicated rat show that though this behavior is increased during the conditioning period in both

TABLE 1

EFFECT OF INTRAPERITONEAL INJECTIONS OF PHYSIOLOGICAL SALINE, MUSCIMOL, OR HOMOTAURINE ON THE DAY OF WITHDRAWAL (MEAN ± SEM)

	Baseline	Day of Withdrawal
EP	38.47 ± 0.73	43.43 ± 0.75†
EM	37.80 ± 0.52	27.43 ± 2.58*
EH	37.55 ± 0.85	25 ± 2.29*
WP	31.22 ± 0.48	32.17 ± 1.11§
WM	29.19 ± 1.48	19.61 ± 1.85‡
WH	29.39 ± 0.56	22.29 ± 0.89†

*$p<0.01$, †$p<0.001$, ‡$p<0.0001$, §NS (day of withdrawal vs. baseline; paired Student's *t*-test). Data are compared with mean values calculated for the last three days before withdrawal (baseline).

normal and intoxicated groups the number of attacks and fights are higher in intoxicated rats. Withdrawal accentuates this increase in the alcohol-dependent rat, whereas muscimol and homotaurine decrease the number of attacks and fights both on the alcohol-dependent and normal rats.

Muscimol is a GABAergic agonist acting on the GABAA receptors (1) and homotaurine, a structural analogue of GABA is considered as an active GABA mimetic (2). It acts on the GABA receptors, the sites of which are characterized by their affinity for the agonist isoguvacine and their sensitivity to the action of the antagonist bicuculline (6).

The inhibition observed in the normal rat with muscimol and homotaurine confirms the results of Rodgers and Depaulis (14) concerning the implication of GABAergic mechanisms in the inhibition of defensive fighting behavior. Bearing in mind the doses used, this action cannot be imputed to motor impairment.

In the alcohol-intoxicated rat, the increase of fights and attacks during the period of chronic intoxication and during withdrawal could be primarily related to a dysfunction of GABAergic system: a decrease in the activity of GABAergic neurones or a less efficient GABAergic transmission. Nevertheless, in this study, the muscimol and the homotaurine present similar effects in both intoxicated and nonintoxicated groups; there is no significant difference when comparing the evolution of the curves between the 9th and 10th days in normal rats versus alcohol-dependent rats.

Although our study confirms the relevance of the electroshock-induced defensive fighting behavior test in chronic intoxication with alcohol, we can not assert there is a GABAergic involvement in behavioral effects of alcohol withdrawal manifested through this test. Other complementary experiments with other GABA agonists and GABA antagonists would be needed.

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