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Induction of High Voluntary Ethanol Intake in Dependent Rats

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MARFAING-JALLAT, P. AND J. LE MAGNEN. *Induction of high voluntary ethanol intake in dependent rats.* PHARMAC. BIOCHEM. BEHAV. **17**(4) 609–612, 1982.—The experimental conditions under which oral high intake may be induced in previously intoxicated rats have been investigated. Seventeen rats were administered intragastrically with 10 g/kg/day of ethanol for 15 days. At cessation of treatment, they were presented a single bottle of alcoholic solution (10% v/v) during 24 hr. For the following 6 days, they received either an ethyl alcohol solution or water in alternation for 8 hours each. Ethanol treated rats exhibited a high oral intake of ethanol equivalent to the previously injected doses. Controls displayed a significantly lower intake of ethanol. It is concluded that the suppression of the withdrawal state by an initial priming oral intake of ethanol in physically dependent rats is a condition for the development of a conditioned taste preference for ethanol as a basis for the behavioral dependence.

Ethanol dependence Withdrawal syndrome Conditioned taste preference

MUCH evidence exists that the spontaneous low intake of ethanol in rats and mice is due to a genetically determined taste and olfactory aversion or, after a first exposure, to the toxic consequences of ingestion. Indeed, the ethanol aversion threshold in individual rats is correlated with the aversion to a bitter solution [18,21]. Intake is dependent on concentration, that is on the sensory activity of the solutions [3, 27, 31]. It is enhanced in rats and in some non-preferring strains of mice by removal of the olfactory bulbs [14,29]. Sweetening the solution with a non caloric sweetener or with a highly palatable saccharine-glucose mixture induces immediately an elevated intake of ethanol [15,22].

Various procedures have been used to overcome the sensory barrier and to develop oral consumption of alcohol in rats. Rats offered an alcohol solution as only source of fluid, food restricted rats or rats with VMH lesion increase their daily oral intake of ethanol [23, 32, 37]. The same increase is obtained with intragastric self-administration [1, 5, 24, 33].

However, sensory aversion is only reduced by means of these procedures. An ethanol preference over water is not observed. Most of all, induced elevated intake does not exceed 7 to 8 g/kg body weight per day. In rats, this intake level corresponds to the daily oxidative capacity. By maintaining its rate of oral intake below the rate of ethanol elimination from the blood, the rat is prevented from becoming chronically intoxicated through oral intake.

This suggested that post-ingestive toxic effects of ethanol limit oral intake in naïve rats. This suggestion has been fully confirmed by the "conditioned taste aversion" paradigm. A learned aversion to a flavored solution develops when oral intake of the solution is followed by a parenteral administration of ethanol [9, 16, 20, 25]. When the flavored solution is an ethanol one, the post-ingestive effects of oral intake act both acutely and through conditioned aversion to limit present and subsequent intakes to infra-toxic doses [7].

In rats, physical dependence is easily and rapidly obtained by various methods of forced administration. Such chronically intoxicated rats were expected to exhibit an elevated oral intake able to maintain a continuous high blood ethanol level. This expectation was based upon the notion—clearly supported by clinical observations in human alcoholism—that during withdrawal, alcohol alleviates withdrawal signs rather than inducing acute neural disturbances. Therefore, ethanol could then act as a rewarding, rather than an aversive, unconditioned stimulus to reinforce oral intake. Such a "rewarding" effect of ethanol in physically dependent rats has been recently demonstrated with a "conditioned taste preference" paradigm [35]. Preference for a sweet solution was enhanced in dependent rats by post-ingestive administration of a low dose of ethanol [19,25]. This elevated taste preference for a sweet solution has been shown to be a reliable measure of brain reward [10,11]. Thus, a moderately high blood alcohol level, aversive to naïve rats, is indeed rewarding to dependent rats.

Nevertheless, many investigators have failed to obtain the maintenance of chronic intoxication by inducing high oral intake of ethanol. Rats highly intoxicated by forced administration and exhibiting severe signs of withdrawal, maintained a low ethanol consumption either in single bottle or free choice presentation [4, 5, 12, 28, 36]. At the best, the elevated intake was transient [35]. However, Le Bourhis [17] obtained a significant rise and somewhat persistent oral intake in withdrawn rats, previously intoxicated with ethanol inhalation. Other investigators have obtained a comparable results after intragastric (IG) intoxication [6,8]. These conflicting results suggested that oral intake of ethanol, in physically dependent rats, could be observed only under the peculiar experimental conditions that allow the development of ethanol-induced taste preference.

In the experiment reported here, we have studied these

conditions of inducing and testing the self-maintained intoxication in rats rendered physically dependent by chronic IG administrations.

METHOD

Seventeen male adult Wistar rats were used. Body weight was 324±4 g at the beginning of the experiment. All rats were implanted with a chronic IG catheter according to a technique described elsewhere [30].

After 6 days of recovery from surgery, all rats were submitted to a chronic treatment with periodic and automatically monitored IG infusions. For 15 consecutive days, rats received from 2 a.m. to 7:30 p.m. 5 pulses of 2 g/kg dose prepared from 95% ethanol diluted in physiological saline (3.36 ml). A gap of 6 1/2 hr, without injection, allowed the rats to eat and to drink without the disturbance due to the acute effects of ethanol.

During the treatment, the rats had free access to their familiar powdered food and water.

Twelve hr after cessation of treatment, all rats—24 hr water deprived—were offered from 6 p.m. an ethylalcohol solution (10% v/v) as the only source of fluid for 24 hours.

The rats were then offered for 6 days alternate presentation of 8 hr duration of a 10% v/v ethyl alcohol solution or water. Starting with ethyl alcohol presentation, the 3 alternate daily presentations began at 6 p.m., 2 a.m. and 10 a.m. Then, the next day, water was presented from 6 p.m., alcohol from 2 a.m. and water from 10 a.m. By this schedule, it was possible to compare for the same rat, in successive day pairs, the 8 hr intake of alcohol and water at the same period of the diurnal cycle.

Eight control rats were submitted to the same schedule of presentation of ethyl alcohol solution and subsequent alternate presentation of alcohol and water without a previous chronic treatment.

RESULTS

At the cessation of the IG chronic treatment all rats exhibited the classical signs of physical dependence (hyperexcitability, tremors, etc). During the initial day of ad lib single presentation of alcohol solution, ethanol-treated compared to untreated control rats exhibited a higher consumption of alcohol 41.4 ml ±2.3 corresponding to 11.1±0.6 g/kg body wt. Control rats ingested 29.7±1.7 ml (7.6±1.1 g/kg body wt.). The difference is highly significant. (Student $t=4.85$, $p<0.01$).

Figure 1 illustrates the large interindividual differences of previously treated rats in their volume intakes during the 24 hr presentation. On the contrary, in untreated controls, this volume intake was relatively constant (29.7±1.7 ml). In treated rats the intake was, in some rats, in the range of untreated rats and in others considerably higher.

During the 3 subsequent couples of days of alcohol and water—8 hr alternate presentation—pretreated rats, compared to controls, exhibited indeed a higher preference for the ethyl alcohol solution versus water. The intake of alcohol solution was, in average, during 24 hours presentation in 2 days: 43.8±3.2; 44.8±3.7 and 44.3±3.8 ml in experimental rats versus 23.1±3; 27±26 and 28.9±2.7 ml in controls for the 3 couples of days, respectively. The difference is highly significant, $p<0.01$ (mean±SEM, Student t-test). However, a strong interindividual variability of the responses on alcohol was again exhibited by the pretreated group and is apparent in FIG 1. Statistically, among the 17 treated rats, 4 were

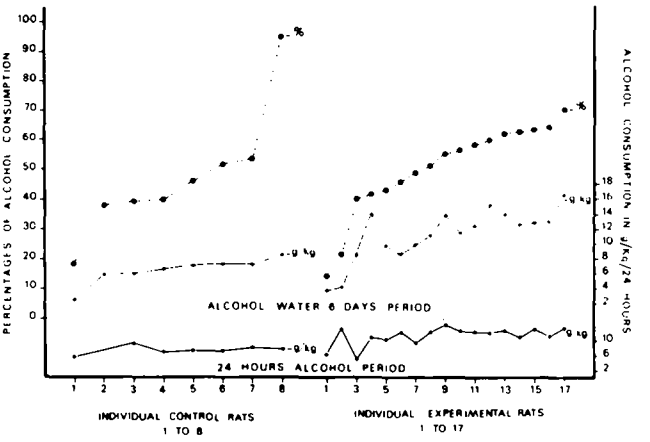

FIG. 1. Bottom: individual consumption in g of alcohol/kg during the 24 hr of alcohol (10% v/v) presentation. Top: individual percentages of alcohol preference of total fluid intake and consumption in g of alcohol/kg during the 6 days of alternate 8 hr presentation. The rats have been ordered according to increasing percentages of alcohol preference along the abscissa.

not significantly different from the 8 untreated controls (Student t).

Figure 1 shows that 2 of the 4 rats which were not different from controls during the 6 days presentation also consumed little during the single bottle presentation on the initial day.

DISCUSSION

Investigations designed to obtain in the rat an experimental model of human alcoholism have been developed in 2 steps. The first one is represented by studies of techniques or procedures of forced chronic alcohol administrations or intakes aimed to realize in rats the state of "physical dependence." This state was identified at the cessation of treatments and tentatively measured by withdrawal symptoms. Tentatively also, scores of these neurologic withdrawal symptoms, considered as a measurement of the severity of the physical dependence, were correlated to parameters of the previous ethanol treatment: initial and acquired tolerance, doses and duration of the treatment [13]. Due to the lack of reliability in the observational scoring of withdrawal signs, such attempts of quantifying "physical dependence" were generally unsuccessful.

Another and important step of the investigation was to obtain behaviorally dependent rats, that is rats which following a chronic ethanol treatment, drink voluntary high doses of ethanol above the threshold of acute and chronic ethanol intoxication. Only such an experimental model of self-maintained intoxication can allow experimenters on the one hand to try the antagonizing effects of drugs on this alcohol hyperconsumption, and on the other hand, to correlate the acquired ethanol preference both to parameters of the chronic treatment and to constitutional or genetic characteristics.

However, unexpectedly, many difficulties have been encountered in obtaining rats which, after an ethanol treatment and exhibiting obvious withdrawal symptoms drink

larger quantities than ethanol naïve rats and hence maintain their physical dependence. For the first time, Amit *et al.* [2] obtained behaviorally dependent rats following presentation of ethanol with 30 min of forced daily lateral hypothalamic electrical stimulation. After 30 days of treatment, high ethanol consumption was exhibited in a free choice of alcohol solution versus water and assessed as behavioral dependence by various means.

Using the technique of ethanol inhalation a high and persisting ethanol preference was obtained by Le Bourhis [17] after 31 days of keeping rats in an atmosphere containing 15–22 mg/l of alcohol. At the end of the treatment the exhibition of the high alcohol intake in a free choice of ethyl alcohol solution and water seemed to be primed by an initial 24 hr presentation of the ethanol solution as the only fluid available to 24 hr water-deprived rats.

In the present study, we have used the initial 24 hr presentation and confirmed its value in priming the latter and sustained response to ethanol. As early as the first day, the daily intake of ethanol-treated rats demonstrates that 82% (14/17) of them, by drinking 10 g of ethanol per kg B.W. or more, maintained through their oral intake the dose level of the previous forced intragastric administration while untreated controls drunk in average only 7.5 g/kg body wt. In the subsequent period of alternate presentations, 13 among 17 treated rats maintained a 24 hr ethanol intake of 12.80±0.34 g/kg.

This procedure of alternate single presentations of ethyl alcohol solution and water during successive periods of 8 hr was different from the ad lib choice between alcohol and water used by Le Bourhis. It was designed both to refer the alcohol intake in a single bottle presentation to the intake of water at the same time, the subsequent day, and to permit in further works to test the effect of a drug during a chosen daily period of 8 hr alcohol intake. It could not be anticipated whether such alternating pattern of alcohol and water presentations would permit the persistent manifestation of the high voluntary alcohol intake apparently established during the initial 24 hr single bottle presentation. In a preliminary study we failed to obtain the persisting exhibition of behavioral dependence during 4 (6 hr apart) 30 min alternating presentations of ethanol solution and water.

Using in this study the 8 hr presentations and alternations, 13 among 17 preintoxicated rats, compared to controls, exhibited a persisting high alcohol intake. This is assessed both in terms of alcohol solution intake to total fluid intake during 48 hr and of ethanol intake during 24 of the 48 hr. Such a procedure of alternate 8 hr presentations in behaviorally dependent rats has been used successfully in another study to test the effect of short acting drug on alcohol consumption [26].

Another aim of the study was to relate the acquired behavioral dependence with parameters of the previous chronic ethanol treatment with the hope to get a measure of the individual severity of the established state of physical dependence by the level of acquired preference for ethanol. In the present study, most of treated rats manifested a clear-cut behavioral dependence. However a small proportion of them are identical to controls and despite the treatment continue to accept the same doses of ethanol as naïve rats. Periodicity, duration and doses of IG administrations being identical, it is possible that the observed interindividual differences of this acquisition of a high preference for ethanol is related to a difference in the initial sensitivity to ethanol, or initial nervous tolerance. Further study will be needed to test this suggestion. But this exhibition of interindividual differences through this procedure proves that it is "discriminative." Thus, this method could be usable in a general study of the parameters that induce "chronic alcoholism" in rats following forced administration.

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