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A Bioassay of Ethanol-Dependence in Rats

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LE MAGNEN, J., P. MARFAING-JALLAT AND D. MICELI. *A bioassay of ethanol-dependence in rats.* PHARMAC. BIOCHEM. BEHAV. 12(5) 707–709, 1980.—A bioassay for the quantitative assessment of ethanol dependence in rat is proposed. It is based upon the alleviation by ethanol of the withdrawal syndrome in previously intoxicated animals. The conditioning by ethanol of a taste preference, linearly related to the duration of the previous chronic intoxication, provides a reliable measure of the level of ethanol dependence. In addition, the results display the neurobehavioural mechanism by which self-maintained intoxication is established in physically dependent rats and humans.

Ethanol dependence Conditioned taste aversion Withdrawal syndrome

ONE of the limits of studying ethanol and its basic mechanisms in animal models is the difficulty to assess the presence and the level of physical dependence. The spontaneous oral intake of ethanol being generally lower than the high daily doses apparently required to induce the symptoms of physical dependence, the latter is established by various means of forced parenteral administration or forced oral intake.

Ethanol exposure is quantified by its various parameters: daily dose, route and periodicity of administration, treatment duration. It is considered to have induced physical dependence when:

(1) A withdrawal syndrome is observed at the cessation of treatment;

(2) Ethanol is shown able to suppress or alleviate the withdrawal state.

The exact brain cellular mechanism underlying ethanol dependence, which could provide a direct measure in the future, is presently unknown. So far, the only index of dependence has been a score, either global or partial, of the various manifestations of the withdrawal syndrome. Several types of scoring have been proposed and used by a number of investigators who have tentatively applied these scores to either the study of relations between the severity of the withdrawal syndrome and parameters of the previous ethanol exposure, or to the study of modifications of ethanol dependence by various drugs [4, 5, 6, 8, 11]. However such measures of the withdrawal syndrome provided only a semi-quantitative assessment. Their diversity and the fact that they are based on subjective observation make them likely not reliable and difficult to compare from one study to another. In the present work, we have used the second index of physical dependence above mentioned i.e. the alleviating effect of acute administration of ethanol on the withdrawal syndrome, to develop a quantitative bioassay of ethanol dependence.

It has been shown earlier that ethanol used as an U.C.S. in ethanol naïve rats in a conditioned taste aversion paradigm induced aversion to a sweet saccharin solution [1, 2, 3, 7, 9].

In the present work, it was demonstrated that, in rats, pretreated with intoxicating doses of ethanol, the same pairing of the intake of a flavored solution and the post-ingestive effects of a moderate dose of ethanol, induced an enhanced saccharin preference linearly related to the duration of the ethanol preexposure. This established response provides a simple and reliable measure of the severity of the withdrawal syndrome and therefore of physical dependence itself. In addition, this positive reinforcement of oral intake by ethanol in dependent rats provides a new insight on the mechanism by which behavioral dependence develops in physically dependent rats and humans.

METHOD

The study was carried out on 42 male, adult Wistar rats. The body weight was 277.7 ± 3 g at the beginning of the experiment. The rats were implanted with a chronic intrajugular catheter according to the technique described elsewhere [10].

After 3 days of recovery from surgery, rats were assigned to one of 4 groups. All rats were submitted to 2 successive phases; in the first phase, the rats were chronically pretreated, according to the group, either with saline or ethanol infusions as follows: they were placed in cylindric Plexiglas cages equipped for periodic and automatically monitored IV infusions via intrajugular catheter. They received, every four hours, six pulses of 2.1 ml of a saline solution or the same volume with 1.5 g/kg of ethanol. During the treatment, rats had free access to their familiar food and water.

In the first group (Ethanol 0, N=12), the rats were injected with saline solution for either 2 days, N=5, 4 days, N=5, or 8 days, N=2.

In the second group (Ethanol 2, N=10), the rats were injected with the ethyl alcohol solution for 2 consecutive days.

In the third group (Ethanol 4, N=10), for 4 days.

In the fourth group (Ethanol 8, N=10), for 8 days.

All rats were then submitted, in a second phase of the experiment, to a conditioning taste response paradigm as follows; On Day 1 (conditioning day), saline or ethanol infu-

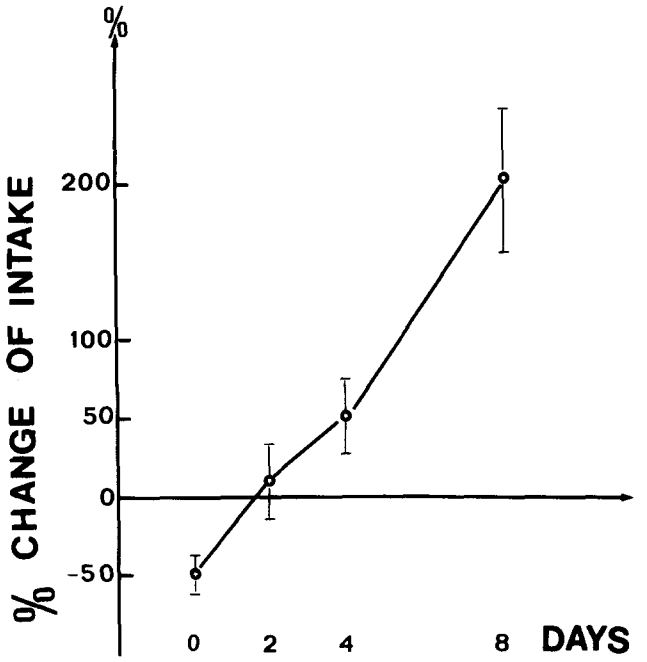

FIG. 1. The preference or aversion towards a saccharin solution (0.1% Na Saccharin) in relation to the duration of ethanol preexposure. These preferences or aversions are measured by the percentage of increase or decrease of fluid intake on the testing day compared to the conditioning day.

sions were interrupted at 6 a.m. The rats, water-deprived overnight, were offered a 30 min presentation of a 0.1% saccharin solution at 4 p.m. Their free intake was immediately followed by an IP administration of 2 g/kg B.W. of ethanol in a fixed volume of 4 ml. From 6 p.m. Day 1 to 6 p.m. Day 2, the rats were allowed free access to their water bottle. On Day 3 (testing day), they were water deprived overnight, and again offered a 30 min oral presentation of the saccharin solution at 4 p.m. The free intake, in the various groups, during this testing trial, assessed the saccharin aversion or preference induced on the conditioning day by the administration of ethanol, paired with the initial oral intake of the saccharin solution.

RESULTS

Rats of the first group chronically treated by saline injections either 2, 4 or 8 days, did not display a statistically different response on the testing day and thus could be combined. As expected, in those rats (EO) a reduced oral intake on the testing day indicated that the pairing of the intake of saccharin with the postingestive effects of ethanol had induced aversion. On the contrary, in the 3 other groups pretreated with the intoxicating dose of 9 g/kg/day, an enhanced intake of flavored solution on the testing day indicated that the same pairing on the conditioning day had induced this preference.

Saccharin intakes in the conditioning trial were: 9.6 ± 0.82; 7.1 ± 0.82; 7.9 ± 0.83; 4.7 ± 0.61 ml, in the 0, 2, 4 and 8 days treated rats respectively. On the testing day, these saccharin intakes shifted to: 4.6 ± 0.87; 7.7 ± 1.118; 11.5 ± 1.57; 12 ± 0.71 ml.

A linear relationship between the percentage of decrease or increase of the 30 min intake on the test day and the duration: 0 to 8 days of ethanol preexposure, was observed (Fig. 1) ($y=31.29\times54.92$, $r=0.738$; $p<0.01$).

DISCUSSION

In an earlier work, a partly similar procedure induced in intoxicated rats the development of a slight preference for a saccharin solution. But various differences with the procedure employed in the present experiment may explain the different result.

The chronic alcohol treatment, administered in the liquid diet presented to the animals, is not comparable to that employed in the present study. Presumably 15 days of free intake of an ethanol containing diet was less intoxicating than six daily acute IV injections which induced high peaks of alcoholemia. In addition, the rats in the present experiment were 24 hr water deprived at the time of the conditioning trial in which they received 2 g/kg instead of 1,5 g/kg.

The objection that the conditioned taste preference could be due to another effect of the ethanol treatment than the induced dependence, such as an induced dehydration, may be ruled out. During the IV chronic treatment, water was available at all times and the rats could compensate for a potential dehydration by their free water intake. In addition, if at the time of conditioning different levels of dehydration had existed as a function of the duration of the prior treatment, there was no reason why the same post-trial injection of a minute volume of the aqueous solution of ethanol might induce the observed differential reinforcement.

It may be assumed that the enhanced intake in ethanol pretreated rats compared to aversion in ethanol naïve rats is due to the property to alleviate the withdrawal syndrome, acquired by ethanol as an effect of pretreatment and therefore as an effect of the established "physical dependence".

The linear relationship between this positive reinforcement of the ingestive response and the duration of the ethanol pretreatment, used here as a variable, validates the test as a reliable bioassay of the established level of ethanol dependence.

As a substitute for the scoring of observed signs of withdrawal symptoms, the bioassay may be proposed as a new tool to investigate so far unanswered problems. The relations between the level of physical dependence and the parameters of the inducing chronic intoxication: doses, periodicity, duration, routes of administration etc. . . could be investigated, as well as the relationship between the initial sensitivity, the acquired tolerance and the level of induced dependence. With the same duration of chronic pretreatment, either 2, 4 or 8 days, the above results indicated in each group a broad-inter-individual variation. After two days of treatment, for example, some rats gave the same response as untreated controls, while other rats gave a response identical to the mean response of 4 day treated rats. Such individual deviations, compared to the mean linear relationship taken as a standard, might be related to the differences both in the initial sensitivity to ethanol and in the acquired tolerance. The same reference to the standard might be used to assess the capacity of various CNS modifying drugs to alter the induction of ethanol dependence when chronically administered with ethanol or to acutely interfere with the manifestations of the withdrawal syndrome.

Beyond the methodological aspect, the results reported here provide a new insight on the mechanism by which the

brain alteration, introduced by chronic ethanol intoxication, induces a behavioral dependence which, in turns, through the high ethanol intake, maintains or increases the brain pathology. It has been shown that in naïve rats, even a moderate dose of ethanol injected after the oral intake of a flavoured solution induced, as other toxic agents (e.g. LiCl), an aversion to the solution [1,7]; this result is replicated in the present EO-saline pretreated group. It explains why when the fluid offered is an ethylalcohol solution, the spontaneous intake in various animal models generally does not exceed the levels able, through cumulative acute intoxication, to introduce physical dependence. However, it is demonstrated here that, when ethanol dependence has been established previously by forced administration in rats, the ethanol induced aversion is replaced by an ethanol induced preference, which leads to self-maintained intoxication through voluntary oral intake.

Extending this notion to the general problem of the conditioning of taste and food preferences, the results also provide new evidence for the role of metabolic (here pharmacological) after effects in the action of oral cues as conditioned stimuli to eat and to drink.

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