ALCOHOL DEPRIVATION EFFECT (ADE) INDUCED BY SHORT TERM ETHANOL EXPOSURE IN RATS

Prunell Tudurí, M. (†), Alvarez García, C.M., Borges, R., Boada Juárez, J.N.

Unidad de Farmacología. Facultad de Ciencias de la Salud (Medicina). Universidad de La Laguna

Author for correspondence:
J.N. Boada. boadajuarez@gmail.com

Abstract

An increase in ethanol intake is observed after a period of forced abstinence in rats, a phenomenon known as "Alcohol Deprivation Effect" (ADE). Most of data confirm that the robustness of this phenomenon depends on the duration of ethanol exposure, habitually several weeks being necessary to obtain a clear response. Nevertheless, in the present study we tried to determine whether a shorter ethanol exposure is also able to induce the phenomenon. A free choice ethanol (20% v/v)/water test was offered to Sprague-Dawley rats for one week which was followed by two weeks of ethanol deprivation; when the free choice test was reintroduced a clear-cut transitory increase in ethanol preference was observed. Therefore, the ADE may be induced in rats by a short exposure to moderate concentration of ethanol voluntary drinking.

Introduction

In rats that have previously been consuming ethanol, a transient increase in ethanol intake is observed after a period of forced abstinence. This phenomenon, first described by Sinclair and Senter (1967) four decades ago in Long Evans rats, was called the "alcohol deprivation effect" (ADE). The same authors (1968) also reported that the ADE develops as a function of the duration of previous ethanol consumption, 21 days of exposure to ethanol, but neither 1 nor 7 days induced the phenomenon after two weeks of deprivation. Following these first reports, a series of modifications were assayed by varying rat strains, ethanol concentration, duration of both ethanol exposure and deprivation, as well as number of deprivation periods (Rodd-Hendricks et al 2000; Koros et al 1999). It is now known that long term, concurrent exposure to various ethanol concentrations, with repeated deprivation periods, causes the animals to develop robust and reproducible ADE, a phenomenon which is even used to evaluate new ametistic drugs (Fredriksson et al, 2023).

Therefore, a long-term exposure to ethanol is apparently necessary to induce the ADE. However, by revisiting the data reported by Sinclair and Senter (1968), contrariwise to the authors' conclusions, it is noticeable that, despite being statistically non-significant, 7 days of exposure produced a transient clear-cut increase in ethanol consumption when the supply was re-established. On revising the material and methods described in their report, we identified at least two reasons which could have contributed to this non-significance of results. Firstly, the "n" of animals tested was too low (only 12); and, secondly, ethanol intake was expressed in terms of "per animal" instead of "per kg". Therefore, further experiments to ascertain whether this short-term ethanol exposure is able to induce the ADE, are required.

Thus, the aim of this study was to determine whether one week of ethanol exposure can induce the ADE in rats after a deprivation of two weeks. To this end, as compared with procedures carried out by Sinclair and Senter (1968), we increased the number of animals, moreover ethanol consumption was measured by using standardized units (g/kg/day and ethanol/water ratio) and, finally, ethanol blood concentration was determined Ethanol was offered as a 20% ethanol/water free choice test, instead of 7% employed by those authors. Two reasons suggested to use this concentration: i) it represents a mean point between those of 5% to 30% concurrently employed by other authors, and ii) it is a concentration commonly used

by many people initiating ethanol consumption.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats weighing 275-290 g, bred in the Laboratory Animal Centre of the University of La Laguna, were used. During ten days before the beginning of the experiments, the animals were housed in groups of four to six in standard Makrolon cages (60x40x20 cm) under regulated conditions (light period from 08:00 to 20:00 hours) with free access to food (Diet D04, PanLab S.L., Barcelona) and water until the beginning of the experiments.

Experimentation ethics

We adhered to Directive 86/609/EEC (updated by Directive 2010/63/EU), ''European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes'', 1986, and the ''Guiding Principles in the Use of Animals in Toxicology'', adopted by the Society of Toxicology in 1989, for the use of experimental animals.

Free choice ethanol/water tests

A first group of 42 animals was transferred to individual cages with food ad libitum to adapt them to single housing. During the following days the animals were left with food and only tap water delivered from two bottles provided with stainless steel sipper tubes. On day eight, the content of one bottle was replaced by a solution of ethanol (20% v/v) and its position changed alternatively every day for one week; ethanol solution was then replaced by water for two weeks. Immediately after the second week of deprivation, ethanol supply was re-established following the same protocol used previously, i.e., a two-bottle free-choice 20% ethanol/water for one week.

Ethanol and water consumption was measured daily. Measurements were performed by weighing the bottles at 11 a.m. Ethanol consumption was expressed as ethanol intake (g/kg/day); ethanol/water ratio was also calculated, which represents the percentage of the volume of ethanol solution ingested daily from the total volume of liquid consumed.

Body weight was recorded daily from the start of the experiments. The ethanol solution was prepared with pure 99.5% ethanol obtained from Panreac, Barcelona.

Blood ethanol concentration (BEC)

Blood ethanol concentration was determined in samples obtained by heart punction in four animals belonging to the first group; the samples were obtained 24 hr after starting the free-choice test. In this manner, we tried to assess whether the animals drank an amount of the ethanolic solution enough as to produce measurable blood ethanol concentrations. These animals were then withdrawn from any further experiments. Ethanol concentration was determined by using the AxSYM REA Ethanol assay (Abbot Laboratories).

Statistical calculations

ANOVA for repeated measures with post-hoc Tukey's test was performed to analyse the time course of water and ethanol consumption throughout the first and second phase of the free choice test. Graph Pad Prism 4.0 application was employed to perform all calculations; graphic presentation was performed by using Excel application.

RESULTS

As shown in Figure 1, water intake was significantly higher during the first three days than the following ones, volume consumed stabilized from the 4th day onwards. The presentation of 20% ethanol was accompanied by decrease in the amount of water simultaneously ingested although statistically non-significant; ethanol was consumed in a slightly higher amount during the first day, blood ethanol concentration measured 24 hr after starting the free choice test being 45.4 ± 11.3 mg/dl (mean ± SD). Through ought the period of ethanol discontinuation, water drinking returned to base line values. When 20% ethanol was re-introduced a significant twofold increase in both intake and ethanol/water ratio, as compared with the previous baseline values, was noted. This phenomenon sharply disappeared on the day after and was accompanied by a slight, non-significant, decrease in water consumption.

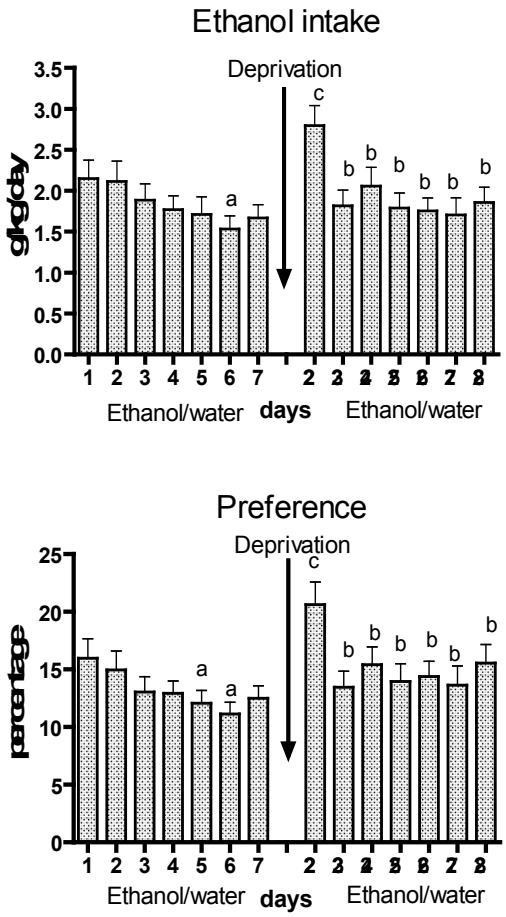

Figure 1. Time course of ethanol intake and preference measured in a free-choice 20% ethanol/water test in rats.

Upper panel. a = Statistically significant difference (ANOVA for repeated measures, F(6, 41) = 9.57, p<0.0001); post hoc Tukey's test p<0.05 between the 1st and the 6th days.

Upper panel: b = Statistically significant difference (ANOVA for repeated measures, F(6, 41) = 15.71, p<0.0001); post hoc Tukey's test p<0.0001 between the 22nd and the remaining days.

Upper panel: c = Statistically significant difference (ANOVA for repeated measures, F(7, 41) = 10.20, p<0.0001); post hoc Tukey's test p<0.05 at least between the 22nd and 1st to 7th days.

Lower panel: a = Statistically significant difference (ANOVA for repeated measures, F(6, 41) = 3.49, p<0.0001); post hoc Tukey's test p<0.05 between the 1st and the 5th days; p<0.01 between the 1st and the 6th days.

Lower panel: b = Statistically significant difference (ANOVA for repeated measures, F(6, 41) = 16.31, p<0.0001); post hoc Tukey's test p<0.01 between the 22nd and the remaining days.

Lower panel: c = Statistically significant difference (ANOVA for repeated measures, F(7, 41) = 8.78, p<0.0001); post hoc Tukey's test p<0.05 at least between the 22nd and the 1st to 7th days.

DISCUSSION

The results of this study allow us to conclude that short-term ethanol drinking may induce a clear-cut ADE. Our findings suggest that the ADE-like phenomenon seen after seven days exposure by Sinclair and Senter (1968) could have been a real ADE.

The fact that water intake was higher during the first few days may be explained by the stress caused by shifting animals from group to individual housing (Scalera et al, 1993). Interestingly, our animals showed more ethanol intake during the first day, as observed by Henniger et al (2002). We cannot provide a satisfactory explanation for this observation; perhaps the attractiveness of a new liquid in one of the bottles, in spite of its concentration, might be a plausible interpretation, although merely speculative.

It must be emphasized that the ADE was induced by offering a 20% ethanol concentration. Although at this concentration animals habitually avoid drinking, in our case measurable BECs were observed 24 hr after starting the ethanol/water free choice test, which suggests that animals experienced ethanol effects.

Whether or not the ADE here described is related to mechanisms of ethanol "craving" cannot be directly inferred from our observations, since specific ethanol-seeking behaviour tests were not carried out. Moreover, there are authors who interpret ADE as hedonic rather than seeking behaviour (Wolffgramm et al 1999; Agabio et al 1999). Clarifying this point could help not only to interpret our findings but also to evaluate the usefulness of the ADE as a tool in research into alcoholism.

In conclusion, a single short-term period of ethanol exposure followed by a single period of ethanol deprivation caused our rats to develop significant ADE. Thus, these results complement those obtained by Sinclair and Senter (1967) and suggest that Sprague Dawley rats are highly susceptible to develop the ADE.

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Addendum

Este artículo ha sido preparado para honrar a su primera firmante, la Doctora Margarita Prunell, recientemente fallecida, quien tenazmente trabajó en la psicofarmacología del alcoholismo (q.e.p.d).