Pharmacology, Biochemistry and Behavior 124 (2014) 81–85

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Benzyl alcohol increases voluntary ethanol drinking in rats

T.J. Etelälahti [a,c,*], C.J.P. Eriksson [a,b]

[a] Department of Public Health, Hjelt Institute, University of Helsinki, P.O. Box 41, 00014 Helsinki, Finland
[b] Department of Alcohol, Drugs and Addiction, National Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland
[c] Department of Biosciences, P.O. Box 56, 00014, Helsinki, Finland

ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form 14 May 2014
Accepted 16 May 2014
Available online 25 May 2014

Keywords:
Benzyl alcohol
Voluntary ethanol drinking
Testosterone
Rat

ABSTRACT

The anabolic steroid nandrolone decanoate has been reported to increase voluntary ethanol intake in Wistar rats. In recent experiments we received opposite results, with decreased voluntary ethanol intake in both high drinking AA and low drinking Wistar rats after nandrolone treatment. The difference between the two studies was that we used pure nandrolone decanoate in oil, whereas in the previous study the nandrolone product Deca-Durabolin containing benzyl alcohol (BA) was used. The aims of the present study were to clarify whether the BA treatment could promote ethanol drinking and to assess the role of the hypothalamic–pituitary–adrenal–gonadal axes (HPAGA) in the potential BA effect. Male AA and Wistar rats received subcutaneously BA or vehicle oil for 14 days. Hereafter followed a 1-week washout and consecutively a 3-week voluntary alcohol consumption period. The median (±median absolute deviation) voluntary ethanol consumption during the drinking period was higher in BA-treated than in control rats (4.94 ± 1.31 g/kg/day vs. 4.17 ± 0.31 g/kg/day, $p = 0.07$ and 1.01 ± 0.26 g/kg/day vs. 0.38 ± 0.27 g/kg/day, $p = 0.05$, for AA and Wistar rats, respectively; combined effect $p < 0.01$). The present results can explain the previous discrepancy between the two nandrolone studies. No significant BA effects on basal and ethanol-mediated serum testosterone and corticosterone levels were observed in blood samples taken at days 1, 8 and 22. However, 2 h after ethanol administration significantly ($p = 0.02$) higher frequency of testosterone elevations was detected in high drinking AA rats compared to low drinking Wistars, which supports our previous hypotheses of a role of testosterone elevation in promoting ethanol drinking. Skin irritation and dermatitis were shown exclusively in the BA-treated animals. Altogether, the present results indicate that earlier findings obtained with Deca-Durabolin containing BA need to be re-evaluated.

1. Introduction

The anabolic steroid nandrolone decanoate has been reported to increase voluntary ethanol drinking in Wistar rats (Johansson et al., 2000). However, in our recent study we received opposite results, displaying decreased ethanol drinking after nandrolone treatment (Etelälahti and Eriksson, 2013). The only clear difference in experimental treatments between the two studies is that in the earlier study the solution of nandrolone decanoate in *arachidis oleum* contained benzyl alcohol (BA), but in our recent study only pure nandrolone decanoate oil solution was used. Thus, it is conceivable that the subcutaneous BA treatment may have been the factor causing increased ethanol drinking.

BA is an aromatic alcohol widely used in cosmetic formulations as a fragrance component, in bacteriostatic preservatives and solvents, and as a viscosity-decreasing agent (Nair, 2001). The safe use of BA for all animal species has been limited to a maximum of 125 mg/kg of complete feed by the European Food Safety Authority (European Food Safety Authority (EFSA), 2012). Acceptable Daily Intake (ADI) and the No Observed Adverse Effect Level (NOAEL) have been confirmed to 0.5 mg/kg body weight and 0–0.5 mg/kg body weight, respectively (SCF, 2002). Harmful effects, including acute toxicity, skin irritation, mucous membrane (eye) irritation, skin sensitization, elicitation of phototoxicity, photoallergy, toxicokinetics, reproductive toxicity, genotoxicity and carcinogenicity, which may occur at higher BA exposure, have recently been reviewed (Scognamiglio et al., 2012).

BA and its metabolism interact with ethanol and its metabolism by sharing partly the same oxidizing enzymes, i.e. alcohol and aldehyde dehydrogenases (Messiha, 1992; Messiha et al., 1992). Another commonality between BA and ethanol is the permeability and the "fluidization" effect in cell membranes, which contribute to several toxic effects such as loss of motor function, sedation and dyspnea (McCloskey et al., 1986). These appear at much lower concentration of BA because of its higher lipophilicity compared to ethanol (Ballard et al., 1988).

The aim of the present study was to test the hypothesis that subchronic BA treatment may increase voluntary ethanol consumption, which could explain the discrepancy between our recent study

* Corresponding author at: Department of Public Health, Hjelt Institute, University of Helsinki, P.O. Box 41, 00014 Helsinki, Finland. Tel.: +358 40 7549875.
E-mail addresses: tiina.etelalahti@helsinki.fi (T.J. Etelälahti), peter.cj.eriksson@helsinki.fi (C.J.P. Eriksson).

http://dx.doi.org/10.1016/j.pbb.2014.05.011
0091-3057/

(Etelälahti and Eriksson, 2013) and the previous study by Johansson et al. (2000). An additional aim was to study whether the potential BA-mediated increase in ethanol consumption would be related to our earlier results on stress-induced elevation of ethanol-mediated testosterone and subsequent ethanol drinking (Apter and Eriksson, 2003, 2006; Etelälahti et al., 2011).

2. Materials and methods

2.1. Animals

Two male rat populations ($n = 2 \times 20$), the alcohol preferring AA (Alko, Alcohol) rats (Alcohol Research Centre, National Public Health Institute, Helsinki, Finland) and low drinking Wistar rats (Harlan, Horst, the Netherlands) were randomly divided into control and treatment groups. Final groups consisted of 10 animals, except the Wistar control group, in which one rat did not finish the experiments. At the beginning of the experiment rats were about 90 days of age and the weight of the AA rats was 305–354 g and that of the Wistar rats 336–383 g. Animals were housed in plastic cages (Macrolon IV, 56 × 34 × 19 cm), two animals per cage until measurement of voluntary ethanol intake. The rats had free access to water and standard laboratory pellets (SDS RM1 Witham, Essex, England). The animal facilities were air-conditioned and a 12 h:12 h light:dark cycle was maintained with light onset at 7 AM. The rats had no previous contact with ethanol. All experimental procedures using animals were approved by the Institutional Animal Care and Use Committee at the National Public Health Institute and carried out in accordance with the European Communities Council Directive (86/609/EEC).

2.2. Benzyl alcohol treatment

Benzyl alcohol (BA) is an aromatic preservative with anaesthetic and antipruritic actions. BA is used as a solvent and as a preservative in injectable medications, topical corticosteroids and perfumes. The treatment group in both rat lines received daily subcutaneous BA injections, 30 mg/kg per day for 14 days, (100 mg/ml, mixed in sterile oil, *arachidis oleum*, University Pharmacy, Helsinki, Finland). This dose corresponds to the BA content in the commercial product Deca-Durabolin used by Johansson et al. (2000). The control groups were given daily s.c. injections of vehicle oil (*arachidis oleum*).

The benzyl alcohol treatment caused skin irritation and dermatitis after few days of subcutaneous treatment (Fig. 1). These skin symptoms remained throughout the 2-week treatment period. No such symptoms were expressed in the control groups.

2.3. Voluntary ethanol intake

One week after the last BA injection all rats were placed into single wire mesh cages (21 × 38 × 19 cm) for 3 consecutive weeks. During this time they had free access to two 100 ml bottles, one of which contained tap water and the other 10% (*w*/*v*) ethanol in tap water. Fluid consumption was recorded daily as a change of volume and the bottles were cleaned and refilled twice a week. To avoid any side preference, the placement of the drinking bottles was changed twice a week. The animals had free access to food pellets. Food consumption and body weight were measured once a week.

2.4. Experimental procedure

Basal serum testosterone and corticosterone were determined before the first BA or oil injection, after 7 days of injections, and after a 7-day washout period following the last injection. All rats received an intraperitoneal injection of ethanol (1.5 g/kg, 10% *w*/*v*, diluted in 0.9% NaCl) in the beginning of the voluntary ethanol drinking period. The effect of this injection of ethanol on steroid hormones was determined before and at 1 and 2 h after the injection. All sampling sessions started at 8 AM and were performed in the same order to minimize possible circadian influence.

2.5. Analytical methods

Hormone concentrations were measured using commercially available radioimmunoassay (RIA) kits. The quantifications of the assays were performed by a Wallac Wizard 1470 automatic gamma counter (GMI, Inc., Ramsey, Minnesota, USA). Testosterone concentrations were determined from serum using Spectria testosterone RIA kit (Orion Diagnostica, Espoo, Finland). The inter-assay coefficient of variation (CV) was 8.3% at a testosterone concentration of 18.8 nmol/L, the intra-assay CV was 9.1% at a concentration of 4.8 nmol/L and the minimum detectable concentration was 0.1 nmol/L (Etelälahti et al., 2011). Corticosterone concentrations were determined from serum using an ImmuChem Double Antibody Corticosterone RIA Kit (MP Biomedicals, Orangeburg, NY, USA). The inter-assay CV was 7.2% and the intra-assay CV was 4.9% at corticosterone levels of 100–200 ng/mL and the minimum detectable concentration was 8.0 ng/mL.

2.6. Statistical analysis

Data were analyzed using SPSS (version 19, Inc., Chicago, IL). The Wilcoxon signed-rank test was used for related samples. The voluntary drinking data and the hormonal data were analyzed Mann–Whitney *U* test or Kruskal–Wallis non-parametric analysis of variance followed by the Mann–Whitney *U* test for group comparisons, except

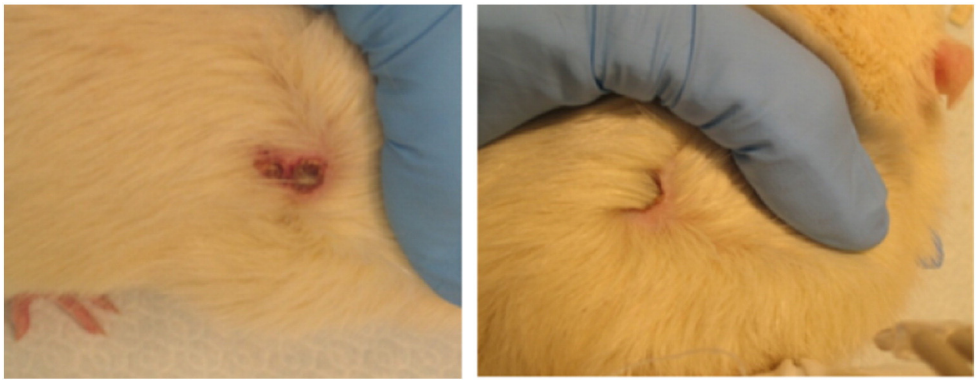

Fig. 1. Examples of skin irritation detected in the BA treatment group.