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# On the association between nandrolone-mediated testosterone reduction during alcohol intoxication and attenuated voluntary alcohol intake in rats

T.J. Etelälahti, C.J.P. Eriksson *

*Department of Public Health, Hjelt Institute, University of Helsinki, P.O. Box 41, University of Helsinki, 00014 Helsinki, Finland*
*Department of Alcohol, Drugs and Addiction, National Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland*

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ABSTRACT

Human studies have indicated that the use of anabolic androgenic steroids may be associated with the abuse of alcohol and other drugs. Also, experimental animal research has indicated that chronic nandrolone administration subsequently increases voluntary alcohol drinking. The aim of our study was to test our hypothesis that alcohol-induced testosterone elevation, especially associated with stress conditions derived by nandrolone treatment, could be the underlying factor in causing increased alcohol drinking. Male alcohol-preferring AA and low drinking Wistar rats were randomly divided into control and nandrolone decanoate treated (15 mg/kg for 14 days) groups. Basal serum testosterone and corticosterone were determined before the first nandrolone treatment, after 7 days of treatment, and after an additional (7-day) washout period, during which also the acute effect of alcohol (1.5 g/kg) on steroid hormones was determined. Hereafter followed a (5-week) voluntary alcohol consumption period, during the last 2 weeks of which the rats were treated again with nandrolone. Both normal and reversed dark- vs. light-cycle experimental designs were used. Contrary to our hypothesis, nandrolone treatment decreased voluntary alcohol consumption in both AA and Wistar rats. Also, instead of stress causation, elevated basal testosterone and lowered basal corticosterone levels were observed after nandrolone treatment in both AA rats and Wistars. During acute alcohol intoxication the frequency of testosterone decreases was higher in the nandrolone-treated groups compared with control AA and Wistar rats. Present data support the hypothesis that nandrolone-treatment mediated attenuation of alcohol intake in both AA and Wistar rats may be the result of negative reinforcement caused by alcohol-mediated testosterone reduction.



## 1. Introduction

Human studies have demonstrated that the use of anabolic androgenic steroids (AAS) is associated with the abuse of alcohol and other drugs (DuRant et al., 1993; Kindlundh et al., 1999; Lukas, 1996; McCabe et al., 2007). Whether the use of AAS constitutes a risk for excessive alcohol drinking, or vice versa, or whether personality and/or other factors independently promote both forms of abuse, is not clear. Evidence for the direct role of AAS in promoting alcohol drinking has been derived in experimental animal studies, which showed that chronic nandrolone (decanoate) administration subsequently increased voluntary alcohol drinking in rats (Johansson et al., 2000).

The mechanism by which AAS may increase alcohol drinking could be the activation of the hypothalamic–pituitary–adrenal (HPA) axis in conjunction with stress and depression, the states which may promote the reinforcing effects of alcohol and which consequently may be associated with the etiology of alcohol addiction (Ciccocioppo et al., 2006; Gianoulakis, 1998; Pohorecky, 1981, 1991; Roman and Nylander, 2005). Such a mechanism is supported by findings, according to which chronic nandrolone treatment has been found to elevate circulating corticosterone levels in rats (Lindqvist et al., 2002; Matrisciano et al., 2010; Schlussman et al., 2000). However, also opposite results involving nandrolone-mediated suppression of corticosterone levels in rats have been reported (Alsiö et al., 2009). Regarding the human aspect, two early studies report no significant effects of chronic AAS administration on circulating cortisol levels in men (Alén et al., 1985; Bijlsma et al., 1982).

Activated HPA axis, stress and depression have commonly been associated with suppressed testosterone production in humans (Hansen et al., 2009; Hardy et al., 2005; Knol, 1991). Based on studies with alcohol-preferring rats, it has been proposed that underlying stress conditions may constitute situations, which promote the alcohol-mediated testosterone elevation and consequently the reinforcing effects of alcohol (Apter and Eriksson, 2003, 2006).

The aim of the present study was to confirm the previous finding, that chronic nandrolone treatment would increase voluntary alcohol

* Corresponding author at: Department of Public Health, Hjelt Institute, University of Helsinki, P.O. Box 41, University of Helsinki, 00014 Helsinki, Finland. Tel.: +358 9 191 27614, +358 40 7549875.
*E-mail addresses:* tiina.etelalahti@helsinki.fi (T.J. Etelälahti), peter.cj.eriksson@helsinki.fi, peter.eriksson@thl.fi (C.J.P. Eriksson).

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drinking in rats (Johansson et al., 2000), and to test how this effect would be related to the HPA and hypothalamic–pituitary–gonadal (HPG) axes.

## 2. Materials and methods

### 2.1. Animal models

Two different experiments (1 and 2) were made to check whether the timing of experimental procedures in conjunction with the day and night cycle affects later endogenous and alcohol-mediated hormonal changes, and voluntary alcohol drinking. In Experiment 1 lights were on from 7 AM and off at 7 PM. In Experiment 2, which was performed separately 7–8 months later after finishing Experiment 1, the schedule was reversed with lights on at 7 PM and off at 7 AM (habituation time for the reversed schedule was about 3 weeks). The rationale with Experiment 2 was based on the fact that with our normal day and night cycle we got opposite results compared with the previous study (Johansson et al., 2000), in which reversed cycle had been used. In both experiments, two male rat populations, the alcohol-preferring AA (Alko, Alcohol) rats (National Public Health Institute, Helsinki, Finland) and low drinking Wistar rats (Harlan, Horst, The Netherlands) were used ($n = 20$ for each strain). Altogether 80 animals participated in the present study.

At the beginning of the experiments rats were about 2.5–3.5 months of age. Body weights were 311 ± 7 g (mean ± SEM, range 248–357 g) for the AA and 343 ± 3 g (313–377 g) for the Wistar rats, and 259 ± 4 g (231–293 g) (AA) and 377 ± 4 g (347–416 g) (Wistars) in Experiments 1 and 2, respectively. Body weight changes (g) after two weeks of nandrolone treatment plus one week of recovery were (control vs. nandrolone) 19 ± 9 vs. 2 ± 7 ($U = 27.5$, $Z = -1.702$, $P = 0.089$) for the AA and 34 ± 3 vs. 0 ± 3 ($U = 0.0$, $Z = -3.784$, $P = 0.000$) for the Wistar rats, and 13 ± 1 vs. −5 ± 2 ($U = 0.0$, $Z = -3.784$, $P = 0.000$, AA) and 23 ± 2 vs. −15 ± 4 ($U = 0.5$, $Z = -3.643$, $P = 0.000$, Wistars) in Experiments 1 and 2, respectively. These nandrolone effects are in accordance to earlier studies (Johansson et al., 2000; Takahashi et al., 2004).

Animal facilities were air-conditioned, with temperature 20–21 °C, humidity approximately 47.6%, and the rats were housed in plastic cages (Macrolon IV, 56 cm × 34 cm × 19 cm), two animals per cage until the stage of voluntary alcohol intake. The rats had free access to water and standard laboratory pellets (SDS RM1 Witham, Essex, England). The rats had no previous contact with alcohol. 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. Nandrolone treatment and voluntary alcohol intake

AA and Wistar rats were randomly divided into two groups in both experiments, each consisting of 10 rats. One group received daily (at about 8.00 AM) subcutaneous injections (into the loose skin over the neck) of nandrolone decanoate (Organon, Oss, The Netherlands), which was dissolved (50 mg/ml) in sterile oil (*Arachidis oleum*, Yliopiston Apteekki, Finland), 15 mg/kg for 14 days. This dose regimen was used in congruence with earlier studies with Wistar rats (Johansson et al., 2000; Lindqvist et al., 2002; Lindqvist and Fahlke, 2005). The other group of animals was given daily subcutaneous injections of vehicle oil. A second 14 day nandrolone treatment (same dosing regimen) to the same animal groups was made in experimental days 43–56 for studying the acute effect of nandrolone treatment on voluntary alcohol drinking. In the present studies pure nandrolone decanoate was used, because the commonly used commercial nandrolone decanoate product (Deca-Durabol, Organon, Oss, The Netherlands) contained benzyl alcohol (10% vol/vol) as a preservative, which could possibly cause unwanted effects of its own (Nair, 2001). The experimental design is illustrated in Fig. 1.

The first period of nandrolone treatment was followed by one week's washout period. After the washout, on day 22, all rats were placed into single wire mesh cages (21 × 38 × 19 cm) for the rest of the experimental period (weeks 4–8, including day 56). During this time animals had free access to two 100 ml bottles, one with tap water and the other with 10% (vol/vol) alcohol in the water. Fluid consumption was recorded daily and the bottles were cleaned and refilled twice a week. To avoid any place preference, drinking bottle placement was changed two times a week. Animals had free access to food pellets and measurement of food consumption and body weight was carried out once a week.

### 2.3. Alcohol administrations and blood sampling

All rats received an intraperitoneal injection of alcohol (1.5 g/kg, 10% wt/vol, diluted in 0.9% NaCl) approximately at 8 AM on day 22. Blood samples for steroid determinations were taken just before, and 1 and 2 h after the alcohol injection. Additional morning-blood samples for endogenous hormone determinations were taken on days 1 and 8. All first blood samples were taken by puncture from the tip of the tail; consecutive blood samples were taken after removing the coagulated blood plate to minimize handling stress. Samples (200 μl) were immediately diluted with 500 μl saline and centrifuged after coagulation. Serum samples were frozen and kept at −70 °C until the analyses were carried out.

### 2.4. 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). All samples were analyzed in duplicate. Testosterone concentrations were determined from serum using a 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). Basal testosterone determination (after the washout period) failed once in one Wistar rat (control in Experiment 2), which reduced the total number to 9 for this animal group. 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 (Etelälahti et al., 2011).

### 2.5. Statistical analysis

Data were analyzed using SPSS (version 21, Inc., Chicago, IL). The Mann–Whitney U-test was used for all group comparisons, except for frequency comparisons, which were assessed by Pearson's Chi-Square analyses. Bonferroni corrections were used for combined significance of two separate tests. The hormone and alcohol-drinking data, which did not fulfill the requirements of normal distribution, are presented as median ± interquartile range in the figures. In the text, all data are presented as means ± SEM. The figures are made with GraphPad Prism version 4.0, GraphPad Software, Inc. For significant results with a 95% level of confidence, the value of alpha is set to 1–0.95 = 0.05. For trends with 85% level of confidence, the value of alpha is set to 1–0.8 = 0.15. All lower levels of confidence are considered non-significant ($P > 0.05$) or not even trends ($P > 0.15$).