THE ROLE OF SOCIAL ISOLATION IN THE EFFECTS OF ALCOHOL ON CORTICOSTERONE AND TESTOSTERONE LEVELS OF ALCOHOL-PREFERRING AND NON-PREFERRING RATS

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(Received 29 April 2005; first review notified 7 July 2005; in revised form 14 September 2005; accepted 15 September 2005; advance access publication 10 October 2005)

Abstract — Aims: Alcohol has been reported to affect the hypothalamic-pituitary-gonadal axis (HPG-axis) and hypothalamic-pituitary-adrenal axis (HPA-axis) as expressed by increased or decreased corticosterone and testosterone levels. Both hormones have also been related to the aetiology of alcohol drinking and the development of alcoholism. Our aim has been to study these interrelations in animal models of alcohol drinking by using social isolation as a model of anxiety. Methods: The effects of alcohol on serum testosterone and corticosterone concentrations were investigated in alcohol-preferring (AA) and alcohol non-preferring (ANA) rat lines. Animals were tested in mornings and afternoons with 0.75 and 1.5 g alcohol/kg. Half of the animals were kept in single cages, while the control animals were housed in groups of four individuals. Results: The group-caged ANA rats displayed higher control corticosterone levels than the corresponding AA rats during morning sessions (P = 0.007). The AA rats displayed elevated corticosterone levels (A: P = 0.047) and the ANA rats displayed reduced control corticosterone levels (rm: P = 0.016) in the single cage situation compared with the group-cage situation. Corticosterone concentrations were not affected by low doses and increased (P < 0.05) by high doses of alcohol in all test groups except for isolated AA rats during afternoon sessions. In general, more significant reductions in testosterone levels following alcohol administration were found in the ANA line. In group-caged AA rats, alcohol reduced testosterone levels, while no such effect was observed in isolated AA rats. Conclusions: We suggest that social isolation, representing stress, may constitute a situation in which the HPA and HPG axes are connected together in promoting alcohol drinking.

INTRODUCTION

Stress and corticosteroids have previously been associated with alcohol drinking in humans (Dai et al., 2002; Pohorecky, 1991) and experimental animals (Fahlke and Eriksson, 2000; Gianoulakis, 1998; Tanaka, 1998). Many attempts have been made to explain the stress/alcohol drinking relationship, resulting for example in the development of the tension reduction and stress response dampening theories (Pohorecky, 1991). According to these explanations, individuals will tend to treat themselves with alcohol when outside pressure causes anxiety and tension. Herewith, the unsolved question remains, why alcohol on one hand may be used to relieve stress, and on the other hand has generally been shown to elevate cortisol/corticosterone levels.

In addition to the above-mentioned hypothalamic-pituitary-adrenal axis (HPA-axis), also the hypothalamic-pituitary-gonadal axis (HPG-axis) has been suggested to play a role in alcohol drinking. In an early study, testosterone administration was found to increase alcohol drinking in castrated male rats (Lakoza and Barkov, 1980). We have recently reported higher basal testosterone levels in alcohol-preferring (AA) than alcohol non-preferring (ANA) male rats (Apter and Eriksson, 2003). In humans, traits associated with high testosterone concentrations—low harm avoidance, high sensation seeking, impulsiveness, and antisocial behaviour—have been described in type 2 alcoholics (Devor and Cloninger, 1989). Furthermore, higher testosterone levels have been reported in sober alcoholics (King et al., 1995) and in alcoholic impulsive offenders with antisocial personality disorder than in control subjects (Virkkunen et al., 1994).

As high testosterone levels have been associated with an increased alcohol preference, alcohol drinking also has been shown to have an affect on testosterone levels. In male rats the acute effect of a high dose of alcohol (1.5 g/kg) has generally been a decrease in testosterone levels (Apter and Eriksson, 2003; Cicero and Badger, 1977; Eriksson et al., 1983). However, in one recent study (Alomary et al., 2003), testosterone elevations were observed after a high (2.0 g/kg) dose of alcohol. Low doses of alcohol (<1 g/kg) have in male rats been reported to elevate (Cicero and Badger, 1977), to reduce (Apter and Eriksson, 2003), or not to alter testosterone levels (Apter and Eriksson, 2003; Eriksson et al., 2003). We recently suggested that an alcohol-mediated testosterone surge may, through an increasing effect on the feedback regulation, elevate β-endorphin concentrations and thus promote further alcohol drinking (Apter and Eriksson, 2003). An association between a highly ethanol-responsive endogenous opioid system and an increased risk of developing alcoholism has previously been suggested in literature (Gianoulakis, 2001).

The effects of stress on behaviours and physiological responses of animals have been examined using various types of stressors. In rat studies, commonly used stressors include immobilization, electric foot shock, fasting, and immersion into cold water (Tanaka, 1998). However, also structural and social aspects, such as housing conditions, can influence behavioural and biological responses (Brown and Grunberg, 1995; Esquifino et al., 2004; Rivier and Rivest, 1991; Serra et al., 2003). The non-physical nature of social isolation minimizes discomfort yet reveals stress responses in animals. Thus, social isolation can, perhaps with even greater validity than physical, potentially painful stressors, be compared with the social and psychological stressors, which are of particular interest in humans. Furthermore, social isolation is a suitable test condition for measuring the combined effect of stress and alcohol on the AA and ANA rat lines since the development of the two rat lines differing in their alcohol preference was carried out by measuring the alcohol preference of isolated animals (Eriksson, 1971).
This study is an extension of our previous investigation (Apter and Eriksson, 2003). The aim of the present study was to clarify the possible role of stress and/or corticosterone levels in the effects of alcohol on testosterone. The investigation is related to the question whether alcohol-induced changes in testosterone and/or corticosterone can be associated with the causality of alcohol drinking in rat lines with and without a disposition for alcohol addiction.

MATERIALS AND METHODS

Animals

Male rats of two rat lines differing in their alcohol preference were used in the study (Eriksson, 1971). AA and ANA rats belonging to the F_{80} generation were housed in either single cages (N = 12 for AA and N = 10 for ANA) or in groups of four individuals (N = 12 for both rat lines) throughout the entire experimental time period (12 weeks). The single-caged animals were placed in isolation 1 week prior to the beginning of the experimental period.

The AA rats weighed in average 348 ± 5 g and the ANA rats 398 ± 8 g at the beginning of the experiment (P = 0.000 for difference between rat lines). The rats were ~2 months old when the experiments were started. During the experiments the rats were given water and a standard laboratory chow (SDS RM1, Witham, Essex, England) ad libitum. In the animal facilities, air temperature was set at 20–21°C, humidity was kept at 47.6% ± 2.1, and a 12 h light 12 h dark cycle was maintained (lights on at 6 AM). Rats were housed in macrolon cages (transparent polycarbonate, dimensions: 595 × 380 × 200 mm, group housing and 210 × 380 × 190 mm, single housing). Aspen chips were used as bedding. The rats had had no previous contact with alcohol.

Experimental procedures

The study consisted of six treatment conditions (at least 1 week between the treatments), differing by the alcohol dose (0, 0.75, and 1.50 g ethanol/kg of body weight) and time of the day (AM and PM). The two doses of ethanol were used because of the earlier reported dose-dependent variability of the effect of alcohol on testosterone and corticosterone levels. The alcohol dose was administered as a 10% ethanol intraperitoneal injection (w/v, diluted in 0.9% NaCl, i.e. final volumes of 7.5 and 15.0 ml/kg). The control dose consisted of 0.9% NaCl (10 ml/kg). Both morning sessions (starting ~8 AM) and afternoon sessions (starting ~3 PM) were performed in order to assess a possible circadian influence. All rats were subdivided to all above-mentioned treatment conditions in a random order. However, for one ANA rat the data on the treatments with the low alcohol dose are missing.

During each experimental session, four tail blood samples (à 200 µl) were collected, from which alcohol, testosterone, and corticosterone concentrations were determined. The first blood sample was taken after excising 1 mm of the tip of the tail. Consecutive blood samples were taken after removing the coagulated blood plate. This procedure has according to our experience caused less handling stress than repeated puncture methods. Blood samples were taken prior to the alcohol/saline injection and 1, 2, and 3 h after the injection. The blood samples were immediately diluted with 500 µl saline and then centrifuged. Serum samples were frozen and kept at −70°C until the analyses were carried out.

The study was approved by the County Administrative Board of Southern Finland and the ethical committee of the Finnish National Public Health Institute.

Analytical methods

Hormone concentrations were measured using commercially available radioimmunoassay kits. The quantifications of the assays were performed by a Wallac Wizard 1470 automatic gamma counter.

Testosterone concentrations were determined using Orion Diagnostica’s (Espoo, Finland) Spectria testosterone radioimmunoassay kit. The inter-assay coefficient of variation (CV) was 7% at a testosterone concentration of 1.2 nmol/l, the intra-assay CV was 7.5% at a testosterone concentration of 1.6 nmol/l, and the minimum detectable concentration was 0.1 nmol/l.

Corticosterone concentrations were measured from serum using an ImmunoChem Double Antibody Corticosterone RIA Kit from ICN Biomedicals (Costa Mesa, CA). The inter-assay CV was 7.2% and the intra-assay CV was 4.9% at corticosterone levels of 100–200 ng/ml.

Serum ethanol concentrations were determined using head-space gas chromatography (Perkin Elmer Sigma 2000) (Eriksson et al., 1977). Maximum ethanol levels of ~30 mmol/l (1.5 g/kg dose) were detected 1 h after the alcohol administration (results earlier published; Apter and Eriksson, 2003).

Statistical analysis

Data were analyzed using SPSS (version 11.5, Inc., Chicago, IL). Only non-parametric tests were used as the hormone data did not even after logarithmic transformations fulfill the requirements of parametric tests, such as normal distribution. Differences within lines, i.e. the effects of social isolation (separate animals) and alcohol administration compared with the control situation (the same animals) were analyzed using the Mann–Whitney and Wilcoxon tests, respectively. Differences between the lines (i.e. differences in control hormone levels) were analyzed using the Mann–Whitney test. The differences in hormone levels during control treatments were analyzed by comparing the averages of the four measuring points within sessions, while the effects of alcohol (alcohol-control values) were measured only at relevant time points.

P-values ≤ 0.05 were considered statistically significant. Data in the text are presented as mean ± standard error of the mean, while data in the figures are owing to their non-parametric nature presented as median ± interquartile deviation (GraphPad Prism version 4.0, GraphPad Software, Inc., San Diego, CA).

RESULTS

Control corticosterone and testosterone levels in AA and ANA rats

The group-caged ANA rats displayed significantly higher corticosterone levels than the AA rats during morning sessions (basal levels of AA: 36 ± 6 ng/ml; ANA: 98 ± 19 ng/ml, P = 0.007, Fig. 1, white bars). No significant line differences...
were found during afternoon sessions, when corticosterone levels were significantly higher than during morning sessions (see above for AM levels, PM AA: 143 ± 18 ng/ml, P-value for the difference between morning and afternoon sessions = 0.003; ANA: 170 ± 15 ng/ml, P = 0.008).

Overall differences in testosterone levels have been reported previously (Apter and Eriksson, 2003), with significantly higher testosterone levels (P < 0.05 both in mornings and afternoons) in the AA compared with the ANA line.

**Social isolation and corticosterone concentrations**

The isolated AA rats displayed elevated levels (AM 66 ± 15 ng/ml, i.e. 83% higher levels, P = 0.047) and the ANA rats displayed reduced corticosterone levels (PM: 89 ± 18 ng/ml, i.e. 48% lower levels, P = 0.016) compared with the group-caged animals. Within both subgroups of the AA rats, as within the group-caged ANA rats, corticosterone levels were higher during afternoon than the morning sessions (P-values < 0.010). However, this diurnal difference in corticosterone levels was lost in the isolated ANA rats (P = 0.173).

**Social isolation and testosterone concentrations**

Splitting the animals into single-caged and group-caged blocks did not change the overall picture, i.e. trends for higher testosterone levels in AA compared with ANA rats were observed regardless of housing condition and time of day (Fig. 2). However, these line differences were only apparent following the administration of the saline injection. Social isolation did not significantly affect basal testosterone levels within either of the rat lines.

**The effect of alcohol and social isolation on corticosterone levels**

The lower dose did not significantly alter corticosterone levels in any test group. The higher dose (Fig. 1) increased corticosterone concentrations under all conditions in both lines (P < 0.05) except for isolated AA rats during afternoon sessions, where a similar trend, however, was found (P = 0.071). In the group-cage situation, alcohol-induced corticosterone elevations were significantly higher in the AA rats compared with the ANA rats during morning sessions (AA: +182 ng/ml, ANA: +152 ng/ml, P = 0.028). In the afternoon, the alcohol effects were smaller in proportion and no significant line difference was observed.

**The effect of alcohol and social isolation on testosterone levels**

Under group-cage conditions, both alcohol doses significantly decreased testosterone levels of ANA rats, while this effect
was only apparent following administration of the larger doses in the AA line (Fig. 3, data not shown for the lower dose). During single-caged conditions, however, the animals were less sensitive to the effects of alcohol on testosterone, as a significant decrease was found only during afternoon sessions with the higher dose in the ANA line ($P = 0.009$).

Moreover, during the morning conditions, involving single-caged AA animals, the median alcohol effect seemed to be an increase, in contrast to median decreases in all other conditions (Fig. 3).

**DISCUSSION**

The major finding of the present study (Fig. 3) was that social isolation seemed to change the way alcohol affected testosterone levels in AA rats during morning sessions. These conditions were also associated with elevated underlying corticosterone concentrations, implying some degree of stress by the social isolation in this line of animals.

In rats, adrenalectomy has been shown to attenuate ethanol intake (Fahlke et al., 1994, 1996; Lamblin and De Witte, 1996; Morin and Forger, 1982) and the effect has been reversed by corticosterone treatment (Fahlke et al., 1994, 1995). However, in studies on the AA and ANA rat lines, only the AA line has responded in this manner to the manipulation of the HPA-axis (Fahlke and Eriksson, 2000). In ANA rats, in contrast, no shift in alcohol intake was seen due to adrenalectomy or the subsequent corticosterone treatment and thus it was concluded that corticosterone stimulates alcohol intake in animals with high alcohol preference. Further support to this theory was presented by Piazza and Le Moal (1997), as the authors reported a higher sensitivity to the positive reinforcing effects of glucocorticoids in individuals with a predisposition to develop drug intake.

Earlier data relate corticosterone and HPA-axis activity directly to increased alcohol drinking (Fahlke and Eriksson, 2000; Gianoulakis, 1998; Tanaka, 1998). Such a direct relation does not explain the present line differences, with the higher corticosterone levels being found in non-drinking ANA rats (group-caged animals during morning sessions). Here, the present results differ from two previous studies, where no corticosterone differences were observed between the two rat lines (Fahlke et al., 1993; Gianoulakis et al., 1992). However, differences in handling effects may explain these discrepancies. Such handling effects were quite apparent in the present study.

The corticosterone concentrations of our two rat lines were differently affected by social isolation. In AA rats social isolation increased corticosterone levels during morning sessions. On the other hand, social isolation reduced corticosterone levels in ANA rats during afternoon sessions. Thus, it seems that social isolation has only been stressful in the AA rats. Interestingly, the selective breeding of our rat lines is based on the alcohol preference of isolated rats. Hence, the association between alcohol drinking and corticosterone may, in fact, be enforced through a change in hormone levels, that is, the increased corticosterone levels in isolated AA rats and decreased levels in ANA rats in the isolated situation.

The present alcohol-induced changes in corticosterone levels are in line with previous studies (Rivier, 1996). High doses of alcohol increased while low doses did not affect corticosterone levels. In general, higher relative stress-induced increases in corticosterone levels were seen during morning sessions, when the corticosterone levels were lower than
during afternoon sessions, which also is in accordance with other investigations (D’Agostino et al., 1982). The only exceptions to this were the isolated ANA rats, higher relative increases in corticosterone levels were seen during afternoon sessions than during morning sessions.

The present study showed that the AA rats generally, regardless of social isolation or diurnal factors, had higher control testosterone levels than the ANA line. These findings together with reported human data (Devor and Cloninger, 1989; Vinkkonen et al., 1994) support the role of the HPG-axis as one factor determining alcohol drinking. The fact that an activated HPA-axis, with subsequently elevated corticosterone levels, may attenuate testosterone levels (Orpana et al., 1990; Rivier and Rivest, 1991) and that control corticosterone levels, if anything, were higher in ANA compared with AA rats during control conditions in the present study suggest that the basic role of the HPA-axis has been to modulate the HPG-axis in the present AA/ANA selection.

Earlier, seemingly contradictory results regarding the effect of alcohol on testosterone levels may be explained by the present results, i.e. the degree of stress may modulate the effect of alcohol on the HPG-axis. The general alcohol-induced effect seems to be an attenuation of testosterone levels. However, as shown here, the only exception was observed with the AA rats during social isolation, i.e. a mildly stressful situation. The previous exceptional report of alcohol-induced testosterone elevation in rats after a high dose of alcohol (Alomary et al., 2003) may also be explained by stressful experimental conditions.

Previously, we have proposed a theory that alcohol-induced testosterone elevations could, through an increasing effect on the feedback regulation of testosterone biosynthesis, elevate the concentrations of hypothalamic β-endorphin and thus promote alcohol drinking (Apter and Eriksson, 2003). Now, we extend our theory by suggesting that stress, at least in AA rats, could be a further promoting factor for the testosterone-mediated reinforcement of alcohol drinking. This theory may explain earlier findings of positive associations between isolation-rearing and alcohol-mediated reinforcement (Hall et al., 1998a) and increased alcohol consumption (Hall et al., 1998b) in rats. Future investigations concerning the relation between the HPA-axis and alcohol drinking ought to consider the possible interactions with the HPG-axis. Furthermore, future studies should be carried out in order to evaluate the relevance of the present findings in the human context.

Acknowledgements — This study has been supported by the Ella and Georg Ehrnrooth’s Foundation as well as the Finnish Foundation for Alcohol Studies. The authors wish to thank Mrs Hilkka Salohalla for the excellent technical assistance.

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