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Testosterone Increases in Men After a Low Dose of Alcohol

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Background: Heavy acute alcohol drinking decreases blood testosterone in men due to an effect on the testicular level. An acute increase in blood testosterone levels after a low alcohol dose has, however, recently been reported in women. The objective of this investigation was to study the effect of a low alcohol dose on testosterone in men and further elucidate the mechanism behind the effect by using 4-methylpyrazole, an inhibitor of alcohol metabolism.

Methods: A double-blind placebo-controlled interventional crossover trial in random order ($n = 13$).

Results: After intake of alcohol (0.5 g/kg, 10% w/v), an acute increase in plasma testosterone (from 13.5 $\pm$ 1.2 nmol/liter to 16.0 $\pm$ 1.6 nmol/liter, mean $\pm$ SEM; $p < 0.05$), a decrease in androstenedione (from 5.1 $\pm$ 0.4 nmol/liter to 4.0 $\pm$ 0.3 nmol/liter; $p < 0.05$), and an increase in the testosterone:androstenedione ratio (from 2.8 $\pm$ 0.3 to 4.2 $\pm$ 0.4; $p < 0.01$) were observed. The effects were not observed during pretreatment with 4-methylpyrazole (10–15 mg/kg orally), which inhibited the ethanol elimination rate by 37 $\pm$ 3%.

Conclusions: Alcohol intake affects the androgen balance in men through an effect mediated by the alcohol-induced change in the redox state in the liver.

Key Words: Alcohol, Androgen, Testosterone, Androstenedione, 4-Methylpyrazole.

RECENTLY IT HAS been shown that alcohol intake leads to an acute increase in blood testosterone levels in premenopausal women (Eriksson et al., 1994; Frias et al., 2002). This transient increase is independent of the alcohol dose, and it may be observed within 1 hr after intake of one to six standard drinks (Sarkola et al., 2000). The mechanism of the increase seems to involve the liver redox state (Sarkola et al., 2001), which is altered by ethanol metabolism (Forsander, 1970).

Alcoholic men often present with symptoms of decreased sexual function, such as impotence and infertility (Adler, 1992). Decades ago it was recognized that alcohol per se is a testicular toxin, with low testosterone levels in alcoholic men (Van Thiel and Lester, 1979), and that alcohol intake may cause a transient acute decrease in blood testosterone levels in healthy nonalcoholic men (Mendelson et al., 1977; Välimäki et al., 1990; Ylikahri et al., 1974). Later this acute effect of alcohol was shown to be due to an inhibited testosterone synthesis in the testis (Cicero et al., 1980; Orpana et al., 1990) rather than an inhibitory effect on the hypothalamic-pituitary axis (Välimäki et al., 1990). The alcohol-induced acute testosterone decreases in men have, however, mainly been observed during and/or after short-term heavy drinking (Välimäki et al., 1984; Ylikahri et al., 1974).

The objectives of this investigation were to study the acute effect of a single low alcohol dose on blood testosterone levels in men and to further elucidate the mechanism behind the effect by using 4-methylpyrazole (4-MP), an inhibitor of alcohol metabolism. This study is an extension of the authors' previous studies on the acute effect of low to moderate alcohol doses on androgens in premenopausal women (Eriksson et al., 1994; Sarkola et al., 2000, 2001).

MATERIALS AND METHODS

Study Subjects

Thirteen healthy Caucasian men (age, 24 $\pm$ 3 years; body mass index, 23.0 $\pm$ 2.7 kg/m^2) were recruited. None of them had a record of disease or any kind of regular medication. All of them reported a typical alcohol consumption of fewer than 14 standard drinks of alcohol (1 drink = 12 g of ethanol) per week, and they were all classified as light drinkers. The experimental sessions were arranged with the intervening time being at least 1 week; no alcohol was allowed for 1 week preceding each experimental session. The study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki. It was approved by an ethical committee and by the Finnish National Agency for Medicines. Participation was confirmed by obtaining signed informed consent.

Study Design

The study design was a crossover experiment. Each subject participated in four different experimental sessions in random order (placebo plus alcohol, 4-MP plus alcohol, 4-MP plus placebo, and placebo plus placebo)

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starting at 4:00 PM. The different treatments were equally represented at each experimental session. 4-MP or placebo was given per os in a double-blind fashion. The success of blinding the subject and the absence of adverse effects has been reported earlier (Sarkola et al., 2002). 4-MP (or placebo) was given at 4:00 PM. Alcohol (0.5 g/kg, corresponding to three to four standard drinks) or placebo was given per os 2 hr later. Drinking time was 15 min, and subjects remained seated throughout the experiment. Blood samples (10 ml) were collected from the median cubital vein before intake of 4-MP (or placebo), before intake of alcohol (or placebo), and at 75, 150, 225, and 300 min from the start of drinking alcohol or placebo. Subjects were asked to abstain from heavy physical exercise and from intake of food for 4 hr before blood sampling. No smoking was allowed during the experimental sessions.

Reagents and Analytical Procedures

The preparation of the 4-MP (1.0 g of methylpyrazole per subject, corresponding to 10–15 mg/kg orally) and placebo solutions has been reported earlier (Sarkola et al., 2002). The alcohol (10% w/v in lingonberry juice, 0.5 g/kg orally; i.e., 5 ml/kg) and placebo (5 ml/kg juice only) drinks were prepared on the day of use.

Hormone and ethanol measurements were performed from plasma samples stored at −70°C until measurement. Ethanol levels were determined by headspace gas chromatography (Sigma 2000, PerkinElmer Corp., Norwalk, CT). The intra-assay and interassay coefficients of variation were 4.0 and 5.1%, respectively, at the level of 1.5 mmol/liter (n = 10), and the detection limit was 0.03 mmol/liter. Testosterone (4-androsten-17β-ol-3-one) and androstenedione (4-androsten-3,17-dione) levels were determined by standard radioimmunoassay reagent sets (Orion Diagnostica, Helsinki, Finland, for testosterone; Diagnostic Products Corp., Los Angeles, CA, for androstenedione). For testosterone, the within-assay variability (coefficient of variation) was 6.6%, and the between-assay variability was 7.0% at the level of 0.96 nmol/liter (n = 10). The detection limit of the testosterone assay was 0.1 nmol/liter. For androstenedione, the within-assay variability was 8.5%, and the between-assay variability was 9.8% at the level of 5.3 nmol/liter (n = 10). The detection limit of the androstenedione assay was 0.14 nmol/liter.

Statistical Methods

Results are reported as mean ± SEM if not otherwise specified. Due to within-subject differences in steroid levels between treatment sessions (<10% for testosterone and <20% for androstenedione, all sessions), the baseline value (level before intake of 4-MP or placebo) was subtracted from later time points, and the values obtained were used in the figures. The within-subject difference in the steroid ratio between the sessions was negligible. The sample size used was based on the authors' previous work on alcohol and androgens in women (Sarkola et al., 2001). Nontransformed data were used in the statistical analyses. Statistical significance (sphericity assumed) was tested by using analyses of variance for repeated measures with drug (4-MP/placebo), drink (alcohol/placebo), and time as within-group factors. Statistically significant results in the analyses of variance were followed by paired t tests for appropriate time points with no corrections made. Similar results were obtained with the nonparametric Wilcoxon matched pairs test. The ethanol-elimination rate was calculated by extrapolation of the pseudolinear part of the elimination curve to zero ethanol by using time points 150 up to 300 min from intake of alcohol. Data were analyzed with SPSS (version 10.0, SPSS, Inc., Chicago, IL) and GraphPad Prism (version 2.0, GraphPad Software, Inc., San Diego, CA) statistical software.

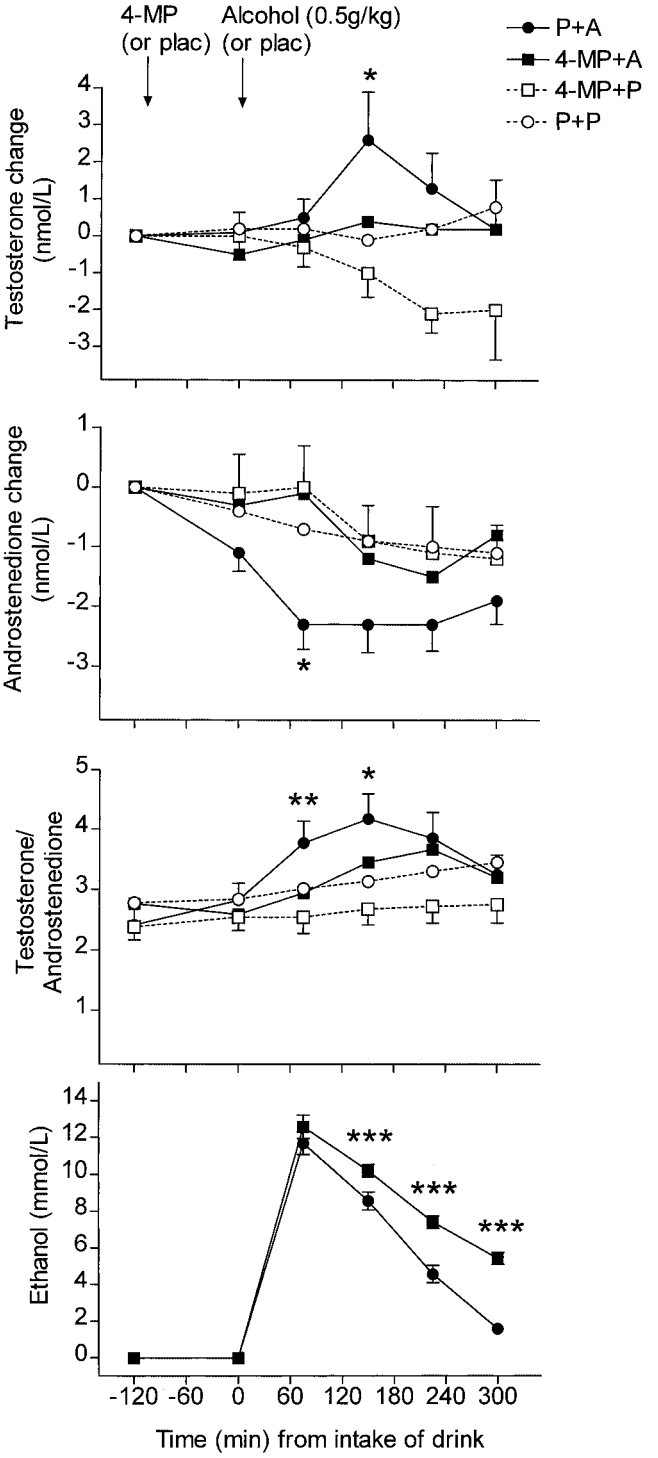

Fig. 1. The acute effect of alcohol (0.5 g/kg orally; solid symbols) and placebo (open symbols) on plasma testosterone, androstenedione, the testosterone:androstenedione ratio, and ethanol (mean ± SEM) during pretreatment with 4-methylpyrazole (squares) and placebo (circles) in 13 men. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared with all other groups. P, placebo; A, alcohol; 4-MP, 4-methylpyrazole.

RESULTS

Plasma Ethanol Levels

The 4-MP pretreatment decreased the ethanol elimination rate by 37 ± 3% (from 0.085 ± 0.003 g/kg/hr to 0.054 ± 0.002 g/kg/hr; $p < 0.001$), with peak concentrations at 75 min from intake (11.7 ± 0.6 mmol/liter versus 12.6 ± 0.6 mmol/liter for placebo and 4-MP, respectively; $p = 0.08$; Fig. 1).

Plasma Testosterone and Androstenedione Levels

An increase in the plasma testosterone levels compared with placebo was observed after intake of alcohol ($F = 2.6$; $p = 0.033$; drink × time interaction; Fig. 1). Testosterone levels increased from 13.5 ± 1.2 nmol/liter before intake to 16.0 ± 1.6 nmol/liter at 150 min from intake. A decrease in the plasma androstenedione levels compared with placebo was observed after intake of alcohol ($F = 4.2$; $p = 0.066$; drink effect; Fig. 1). Androstenedione levels decreased from 5.1 ± 0.4 nmol/liter before intake to 4.0 ± 0.3 nmol/liter at 150 min from intake. An increase in the plasma testosterone:androstenedione ratio compared with placebo was observed after intake of alcohol ($F = 4.1$; $p = 0.003$; drink × time interaction; Fig. 1). The ratio increased from 2.8 ± 0.3 before intake to 4.2 ± 0.4 at 150 min from intake. The transient effects of alcohol on plasma testosterone and androstenedione and the testosterone:androstenedione ratio returned to placebo levels when ethanol decreased to less than 2.0 mmol/liter. No significant effect of alcohol on testosterone, androstenedione, or the testosterone:androstenedione ratio was found during 4-MP pretreatment.

The androstenedione level decreased ($F = 7.1$; $p < 0.001$; time effect) and the testosterone:androstenedione ratio increased ($F = 10.4$; $p < 0.001$; time effect) during the experimental sessions. No time effect was observed in testosterone ($F = 0.8$; $p = 0.6$). No effect of 4-MP per se was observed on testosterone ($F = 1.4$; $p = 0.3$; drug effect), androstenedione ($F = 2.1$; $p = 0.18$; drug effect), or the testosterone:androstenedione ratio ($F = 3.5$; $p = 0.09$; drug effect).

DISCUSSION

An acute increase in plasma testosterone was found after intake of alcohol corresponding to two or three standard alcohol drinks in healthy nonalcoholic men. A concomitant decrease in plasma androstenedione was observed. No significant effects of alcohol were found during pretreatment with 4-MP.

4-MP is a well known inhibitor of alcohol dehydrogenase. Particularly the alcohol dehydrogenase class I isoenzymes, which account for the major part of the oxidation of ethanol (Ehrig et al., 1990), are highly sensitive to the dose-dependent inhibition of 4-MP (Blomstrand and Theorell, 1970). Ethanol oxidation causes an acute increase in the liver ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD^+), and this increase has been reported to be reduced during 4-MP pretreatment (Inoue et al., 1984; Salaspuro et al., 1977, 1978). The 37% decrease in the ethanol-elimination rate observed is similar in magnitude to earlier reported findings (Jacobsen et al., 1996; Salaspuro et al., 1977, 1978) and support the view of a reduced ethanol-induced shift of the liver NADH:NAD^+ ratio during 4-MP pretreatment in this study.

Ethanol oxidation has earlier been shown to be coupled to steroid reduction in the liver (Andersson et al., 1986). More specifically, ethanol oxidation was shown to cause an increased rate of the reduction catalyzed by the liver NAD-dependent 17β-hydroxysteroid dehydrogenase type 2 enzyme (Casey et al., 1994; Wu et al., 1993) with a secondary change in the equilibrium between conjugated 17-hydroxy- and 17-ketosteroids. During normal conditions, testosterone (17-hydroxysteroid) is oxidized to androstenedione (17-ketosteroid), and in the reaction NAD^+ is reduced to NADH. The competitive situation during alcohol intoxication seems, however, to be in favor of alcohol oxidation, during which the increased NADH level leads to an increased 17-ketosteroid to 17-hydroxysteroid reaction. These earlier findings on the conjugated steroids in men (Andersson et al., 1986), as well as on the unconjugated steroids in premenopausal women (Sarkola et al., 1999, 2001), are in accordance with our results. Other investigators have suggested that the shift in the ratio of NAD^+ to NADH in the liver might explain acute increases in estradiol and testosterone as well, although in these reports only an increase in the corresponding 17-hydroxysteroid (i.e., estradiol and testosterone) has been demonstrated in women (Ellingboe, 1987; Mendelson et el., 1987) and in men during gonadotropin stimulation (Phipps et al., 1987). Thus, our results suggest that the increase in the testosterone:androstenedione ratio is the result of an alteration in the steroid metabolism in the liver, i.e., a decreased overall oxidation of testosterone due to the increased reduction of androstenedione mediated by the alcohol-induced increase in the liver NADH:NAD^+ ratio.

That acute alcohol intake leads to decreased levels of testosterone in normal healthy men has been reported in a number of articles (e.g., Mendelson et al., 1977; Välimäki et al., 1990; Ylikahri et al., 1974), and these studies, as well as studies performed in the rat testis (Cicero et al., 1980; Cobb et al., 1980; Orpana et al., 1990), provide evidence that this effect is mainly mediated by an inhibited testosterone synthesis. Our earlier findings of reduced androgen catabolic products in the urine after intake of similar alcohol doses seem to rule out an increased androgen production in the testis during the present conditions (Sarkola et al., 2001). In our view, the acute increase in the testosterone:androstenedione ratio, which is more pronounced in premenopausal women (Sarkola et al., 2001) than in men, is not in contradiction with earlier reports and would rather suggest an additional site in the acute actions of alcohol on unconjugated steroids in humans. Thus, it is proposed that the present effects on androgens in the venous blood are the net effect of an inhibited catabolism in the liver and an inhibited synthesis in the gonads, the result of which will depend on different hormonal, dose, and time conditions. In view of our results, the former effect is not dose dependent and seems to predominate from the beginning of alcohol intoxication (Sarkola et al., 2000). The latter phenomenon may predominate mainly after larger doses, e.g., 1.5 g/kg orally or more, and during the late descending

phase of alcohol elimination or when the alcohol has been completely eliminated (Välimäki et al., 1990; Ylikahri et al., 1974) and the NADH to NAD^+ shift in the liver has recovered.

In conclusion, we observed an acute increase in testosterone and an acute decrease in androstenedione in the peripheral venous blood after intake alcohol in men. The effect was not observed during 4-MP pretreatment. The effect seems to be mediated by the alcohol-induced change in the redox state in the liver.

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