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ACUTE ETHANOL ADMINISTRATION ENHANCES PLASMA TESTOSTERONE LEVELS FOLLOWING GONADOTROPIN STIMULATION IN MEN

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SUMMARY

Plasma levels of LH, FSH, prolactin (PRL), and testosterone (T) were assessed in six normal men following administration of a pharmacologic dose of gonadotropin releasing hormone (GnRH) (500 μg iv over a one-min period) with concomitant oral administration of either ethanol (0.695 g/kg of body weight over a 15-min period) or ethanol placebo.

Acute ethanol administration had no effect on the response of either LH or FSH to GnRH. PRL levels increased following GnRH and administration of both ethanol and ethanol placebo. Ethanol administration enhanced the T response to GnRH ($p < 0.001$ vs placebo). During the placebo condition, T levels did not rise significantly until 100 min after GnRH administration, at which time the mean increment over baseline was 101 ± 20 ng/dl (± SEM). In contrast, following ethanol intake, T levels were significantly elevated within 30 min after GnRH administration, at which time the mean increment over baseline was 187 ± 42 ng/dl. The mean T increments were 304 ± 62 and 472 ± 77 ng/dl, respectively, 60 and 105 min following GnRH and ethanol administration.

The increase in T levels following acute ethanol intake and concomitant gonadotropin stimulation is in contrast to the well-documented effect of chronic ethanol intake on suppression of testosterone synthesis by testicular Leydig cells.

INTRODUCTION

CHRONIC ETHANOL ABUSE without liver disease or impaired hepatic function may cause testicular atrophy and gynecomastia in adult men (Van Thiel *et al.*, 1974). Furthermore, reductions in plasma testosterone (T) levels have been observed in both chronic ethanol abusers (Mendelson & Mello, 1974) and normal men (Gordon *et al.*, 1976) who ingested ethanol on a chronic basis during controlled research ward studies. Studies on the effects of acute ethanol intake on plasma T levels in normal men have shown either no significant change (Toro *et al.*, 1973; Linnoila *et al.*, 1980; Andersson *et al.*, 1986) or a decrease (Mendelson *et al.*, 1977; Välimäki *et al.*, 1984). In studies with healthy adult women, acute ethanol administration

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produced no significant changes in plasma levels of estradiol (Mendelson *et al.*, 1981; Välimäki *et al.*, 1983). These studies with women, as well as those previously carried out with men, involved basal conditions without exogenous gonadotropin stimulation. However, subsequent studies have demonstrated an increase in estradiol levels in women following acute ethanol intake under conditions of gonadotropin stimulation (Mendelson *et al.*, 1986). The goal of the present study was to determine if acute ethanol administration altered plasma T levels in healthy, normal men following gonadotropin stimulation by a pharmacologic dose of gonadotropin-releasing hormone(GnRH). The effects of ethanol on the response of plasma levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin (PRL) were also measured.

METHODS

Experimental subjects

Six healthy, normal male volunteers, ages 23-33, gave informed consent for participation in this study. All subjects had normal physical examinations and normal hematology, blood chemistry, and urine studies. No subject had any history of mental illness or alcohol or drug abuse. The study was approved by the McLean Hospital Institutional Review Board.

Each subject was studied on two separate occasions, two to seven days apart. Subjects received GnRH (500 μg iv) on both occasions and oral ethanol (0.695 g/kg of body weight) on one occasion and placebo ethanol on the other. To control for expectancy effects, subjects were told they could potentially receive GnRH or placebo GnRH on one or both occasions, and ethanol or ethanol placebo on one or both occasions. The study was conducted under double-blind conditions; half of the subjects were given ethanol on the first study day and the other half on the second study day.

Following an overnight fast, subjects reported to the research facility and on arrival provided a urine specimen for drug screen analysis, all of which were negative. A forearm iv catheter was inserted for continuous blood withdrawal, and a heparin lock was placed in a vein in the other arm for GnRH administration. The subjects were comfortably seated in a light- and sound-attenuated chamber and were instructed to relax and keep their eyes closed, but to remain awake. On each occasion starting at 1000 h, blood was withdrawn continuously for four hours at a rate of 1.0 ml/min. Five-min aliquots were collected, separated, and frozen for subsequent analysis of plasma LH, FSH, PRL, T, and ethanol.

After two hours of blood collection under basal conditions, subjects received GnRH (500 μg iv) through the heparin lock over a one-min period (t = 0), and administration of either ethanol or placebo ethanol was begun. The five-min aliquot collected between t = 0 and t = 5 min was designated as the time 0 specimen. During the drinking session, the subjects were instructed to place a mouthpiece into their mouth, and 350 ml of ethanol solution or placebo ethanol solution was pumped into the mouthpiece over a 15-min period. For both treatments (ethanol and placebo), the first 10 ml pumped into the mouthpiece consisted of 3.0 ml of vodka and 7.0 ml of grapefruit juice to provide a strong initial ethanol taste. The ethanol dose was 0.695 g/kg of body weight diluted in grapefruit juice; the placebo solution contained only concentrated juice after the first 10 ml.

Plasma hormone and ethanol assays

Plasma LH, FSH, and PRL concentrations were determined in duplicate by double-antibody radioimmunoassay (RIA) procedures similar to those described by Midgley (1966; 1967). Antisera and reference preparations (LER-907 and NIADDK-hPRL-RP-1) were provided by the National Hormone and Pituitary Program, supported by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. Results are expressed as ng/ml in terms of the reference preparations. Plasma T concentrations were measured in duplicate by a direct double antibody RIA that did not require solvent extraction, with a kit from Radioassay Systems Laboratories (Carson, CA); results are expressed as ng/dl. Assay sensitivities for LH, FSH, PRL, and T were 3.9, 7.8, 3.1 ng/ml, and 10.0 ng/dl, respectively. Intra- and interassay coefficients of variation were 6.5 and 6.8, 7.6 and 12.4, 6.2 and 15.7, and 5.8 and 13.7%, respectively. For the T assay, at 50% binding of radioiodinated T, there was a 3.40 and 0.56% cross-reactivity for 5α-dihydrotestosterone and androstenedione, respectively. Ethanol concentrations were determined in 20 μl samples of plasma, in duplicate, by a colorometric micromethod (Léric *et al.*, 1970). Assay sensitivity was approximately 10 mg/dl, and intra-assay coefficients of variation averaged 3%. All samples from each subject were analyzed in the same assay.

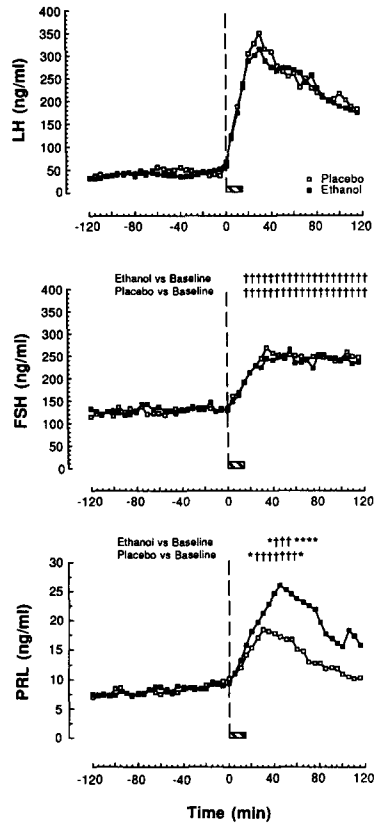

FIG. 1: Mean plasma LH, FSH, and PRL levels in six subjects before and after administration of GnRH (500 μg iv at t = 0) and ethanol (0.695 g/kg po over 15-min period starting at t = 0, as indicated by vertical dotted line and horizontal bar) or placebo (GnRH/ethanol ■ ; GnRH/placebo ❑). By ANOVA, statistical significance of differences were as follows: $P < 0.001$ for GnRH/ethanol vs baseline for LH, FSH, and PRL; $p < 0.001$ for GnRH/placebo vs baseline for LH, FSH, and PRL; NS for GnRH/ethanol vs GnRH/placebo for LH, FSH, and PRL. By Dunnett's follow-up test, differences were $p < 0.01$ for time points marked † and $p < 0.05$ for time points marked *. This analysis is presented on the figure in lieu of standard error bars to enhance clarity.

Statistical analysis

Repeated measures analysis of variance (ANOVA) and Dunnett's follow-up test were used to assess the hormone data to determine if and when differences from mean baseline values occurred under both ethanol and placebo conditions. Repeated measures ANOVA and Dunnett's follow-up test were also performed on the differences between change from baseline under ethanol conditions and change from baseline under placebo conditions. Linear regression analysis, to assess for significance of correlations, and two-sided sign tests were also employed when appropriate.

RESULTS

Fig. 1 shows mean plasma LH, FSH, and PRL levels prior to and following administration of

GnRH and ethanol or placebo ethanol. The LH and FSH responses to GnRH were not affected by the administration of ethanol. PRL levels were increased from baseline following GnRH with or without ethanol. Ethanol increased the PRL response to GnRH; all six subjects had greater mean increases in PRL levels and greater differences between the areas under the curve before and after GnRH when given ethanol compared to placebo ethanol ($p = 0.03$ by sign test). However, the magnitude and the time course of the PRL increases following alcohol were highly variable, so that a statistically significant increase compared to placebo was detectable by repeated measures ANOVA.

Fig. 2 shows mean plasma T levels before and after administration of GnRH and ethanol or placebo ethanol, and mean plasma ethanol levels obtained on study days when ethanol was given. Administration of GnRH with placebo ethanol produced a small rise in T ($p < 0.001$ vs baseline by ANOVA), which was statistically significant for the first time at 100 min ($p < 0.05$ by Dunnett's follow-up test). At that time, the mean increase from the baseline mean of 807 ± 53 ng/dl (± SEM) was 101 ± 20 ng/dl, and no additional increase in the mean occurred subsequently. In contrast, GnRH and concomitant ethanol intake induced a substantially higher increase in T for all six subjects ($p < 0.001$ vs baseline, $p < 0.001$ vs placebo). A statistically significant change compared to baseline was first detected at 30 min following GnRH administration. At that time the mean increase from the baseline mean of 764 ± 45 ng/dl was 187 ± 42 ng/dl. By 60 min, the mean increase from baseline was 304 ± 62 ng/dl; the highest value for the mean increase (472 ± 77 ng/dl) occurred at 105 min. The ethanol-induced accentuations of T elevations were not significantly correlated with baseline T values, mean plasma ethanol levels following ethanol administration, or ethanol-induced accentuations of PRL elevations.

DISCUSSION

The findings obtained in this study of an ethanol-induced enhancement of plasma T levels in men following gonadotropin stimulation is consistent with data obtained in studies of concomitant administration of ethanol and naloxone to women (Mendelson *et al.*, 1986). With concomitant administration of naloxone to normal women during the mid-luteal phase, acute ethanol intake was noted to be associated with a significant increase in plasma estradiol levels accompanying the anticipated increase in LH; this effect on estradiol levels was not seen with placebo ethanol administration. Thus both normal men and normal women have enhanced gonadal steroid levels when ethanol is acutely ingested during stimulated LH release.

We used a large pharmacologic dose of GnRH (500 μg), because smaller doses are less likely to elicit measurable changes in T (Judd *et al.*, 1974). The latency and magnitude of the increase in T following GnRH and placebo ethanol are similar to those observed following GnRH administration alone (Judd *et al.*, 1974; Phipps *et al.*, 1987). The mechanisms underlying the enhanced T response following ethanol remain to be determined. This enhancement was clearly not due to altered gonadotropin responses. Ethanol is thought to decrease testicular T production by Leydig cells both acutely and chronically (Boyden & Pamenter, 1983). Previous studies in normal men have shown either little change (Toro *et al.*, 1973; Linnoila *et al.*, 1980; Andersson *et al.*, 1986) or a decrease (Mendelson *et al.*, 1977; Välimäki *et al.*, 1984) in T levels following the acute administration of ethanol alone. The obvious difference between those studies and the current study relates to concomitant stimulation of the pituitary with GnRH to produce increased LH levels. It appears that, if the testis is sufficiently stimulated, the effect of the levels of ethanol achieved herein is to increase rather than decrease T levels, at least on an acute basis; some other factor(s) appear to be overriding the inhibiting effect of ethanol on T synthesis.

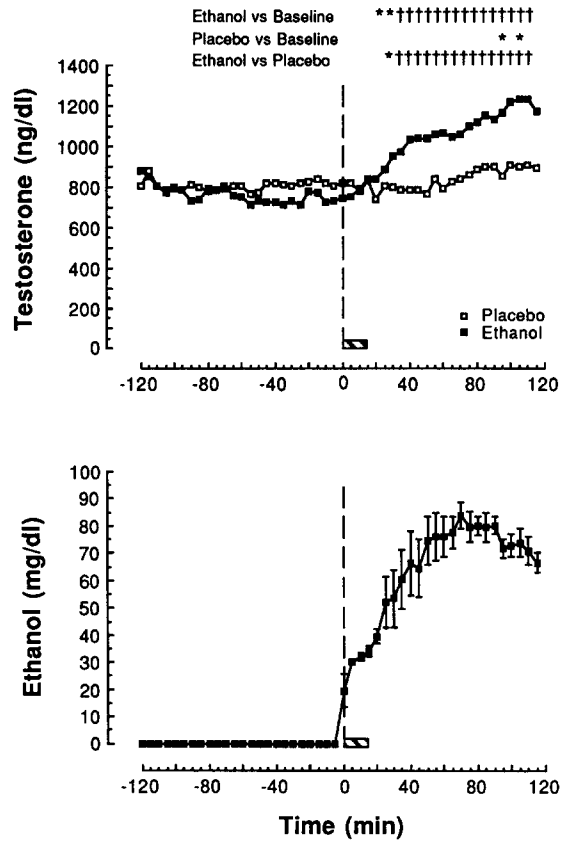

FIG. 2. Mean plasma T levels in six subjects before and after administration of GNRH (500 μg iv at t = 0) and ethanol (0.695 g/kg po over 15-min period starting at t = 0, as indicated by black bar) or placebo (GnRH/ethanol ■ ; GnRH/placebo □), and mean plasma ethanol levels (± SEM) on days of ethanol administration. By ANOVA, statistically significant differences in T levels at the $P < 0.001$ level were present for GnRH/ethanol vs baseline, GnRH/placebo vs baseline, and GnRH/ethanol vs GnRH/placebo. By Dunnett's follow-up test, differences were $p < 0.01$ for time points marked + and $p < 0.05$ for time points marked X. This analysis is presented in the figure in lieu of standard error bars to enhance clarity.

One possible mechanism for the ethanol-induced increase in T levels is the increase in PRL levels that occurred in all six subjects following ethanol compared to placebo. Previous work has shown variable but generally small increases in PRL levels following ethanol administration alone to normal men (Ellingboe *et al.*, 1979), so this effect of ethanol was not surprising. Since under certain conditions elevated PRL levels may enhance T production by the testis (Rubin *et al.*, 1978a), it is possible that the effect of ethanol on T is mediated by PRL. However, in our subjects the effect of ethanol on PRL did not correlate with its effect on T; furthermore, elevated PRL levels do not appear to further increase T levels when the latter are already elevated as a consequence of exogenous LH administration (Rubin *et al.*, 1978b).

Another possibility for the enhanced T levels following ethanol is a facilitation by ethanol of Leydig cell LH receptor response, since it has been shown that exposure to ethanol may initially

activate rat Leydig cells (Bhalla *et al.*, 1983). However, these studies may not be relevant to the human testis *in vivo* (Cicero & Bell, 1980).

Altered hepatic metabolism of T and related steroids may in part account for the ethanol-induced enhancement of T levels. Acute ethanol administration may increase hepatic blood flow (Mendeloff, 1954; Castenfors *et al.*, 1960; Stein *et al.*, 1963), and ethanol catabolism causes a prompt and dramatic increase in the hepatic NADH/NAD ratio (Forsander *et al.*, 1958; Slater *et al.*, 1964). Cronholm & Sjövall (1968) found a significant increase in the ratios of 17 ß-hydroxy/17-ketosteroid pairs in the monosulfate fractions of plasma steroids obtained from normal men who were given an acute oral dose of ethanol. Acute alcohol administration also increased the ratio of urinary 17 ß-hydroxy/17-ketosteroid pairs (Axelson *et al.*, 1981). In addition, Murono and Fisher-Simpson (1985) have shown that ethanol concentrations commonly found in men following acute alcohol intake increase steroid reductive metabolism in rat liver as a direct consequence of an increased NADH/NAD ratio. Taken together, these findings suggest that increased T levels following ethanol and concomitant gonadotropin stimulation may be due in part to increased hepatic conversion of precursor steroids such as androstenedione to testosterone as a consequence of increased NADH/NAD ratios during intrahepatic ethanol catabolism. Why such a mechanism may override other effects of ethanol only in the setting of increased LH levels and consequent increased T production is not known.

The significance of an ethanol-induced increase in plasma T levels in normal men for behavioral phenomena commonly associated with acute alcohol intoxication (increased sexual and aggressive behavior) remains to be determined.

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