Ethanol Decreases Nocturnal Plasma Levels of Thyrotropin and Growth Hormone But Not Those of Thyroid Hormones or Prolactin in Man*

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ABSTRACT

Previous studies on the effects of ethanol on circulating pituitary hormones have been carried out mostly during daytime when the secretion of these hormones is generally at a nadir. Therefore, we studied the effects of ethanol on the nocturnal secretion of GH, PRL, TSH, and thyroid hormones (protocol I, nine healthy subjects, five women) and on the TSH and PRL responses to synthetic TRH (protocol II, healthy subjects, four women). Ethanol was given in doses of 0, 0.5, or 1.0 g/kg of BW (protocol I) and 0 or 1.0 g/kg (protocol II) and ingested po at 1900-1945 h. In protocol I, plasma GH rose from 0.6 ± 0.2 µg/L (mean ± SE) at 2200 h to 25.0 ± 4.3 µg/L at 0100 h in control subjects and was almost completely inhibited at 4.5 ± 1.7 µg/L at 0100 h in subjects receiving 1.0 g/kg ethanol (P < 0.01). In subjects receiving 0.5 g/kg ethanol, the inhibition was also significant (P < 0.01), plasma GH being 8.2 ± 2.5 µg/L at 0100 h. Plasma GHRH was measured after solid phase separation in RIA, but it did not show any ethanol-related changes. Plasma PRL exhibited a clear diurnal rhythm in control subjects and rose from 77 ± 26 µg/L at 1800 h to 214 ± 62 µg/L at 0700 h (P < 0.01). The plasma PRL profile was not affected by ethanol. Plasma TSH was 1.4 ± 0.2 mU/L at 1800-2200 h and rose to 2.3-2.4 mU/L for 0100-0700 h (P < 0.001) in the control subjects. Ethanol 1.0 g/kg suppressed plasma TSH to 1.4 ± 0.2 mU/L (P < 0.05 at 0100 h and P < 0.01 at 0200 h). According to the area under the curve analyses, the suppression in the nocturnal TSH was 32% in the 0.5 g/kg group and 45% in the 1.0 g/kg group (P < 0.05 for both cases). Circulating free or total T3 and T4 did not show any statistically significant changes that could explain the ethanol-induced inhibition in the nocturnal TSH peak. In protocol II, synthetic TRH (1 µg/kg BW) was given intravenously, and blood samples were collected before, at 20 and 60 min. TRH significantly stimulated plasma TSH and PRL, but ethanol (1.0 g/kg BW) had no effect on these responses.

In conclusion, small amounts of ethanol have unexpectedly great effects on nocturnal surges of TSH, and especially on those of GH, that are apparently mediated by suprapituitary mechanisms. On the other hand, ethanol did not affect the nocturnal PRL surge. These inhibitory effects of ethanol may have unfavorable effects on growth and metabolism in adolescent drinkers. (J Clin Endocrinol Metab 81: 2627–2632, 1996)

Serum concentrations of pituitary hormones exhibit well-known diurnal rhythms in adult man. The secretion of GH peaks at night, approximately 1.5–2 h after falling asleep, and the total amount of GH secreted during the night is several times greater than the amount released during the daytime (1–3). PRL shows an initial rise 60–90 min after falling asleep and reaches maximal levels at 0500–0700 h (4). Circulating TSH also presents a diurnal rhythm with elevated nighttime levels (5–7); the mean TSH pulse amplitude increases at night by 82% and 92% in men and women, respectively (8). It is evident that the rhythmic patterns in the release of pituitary hormones depend on environmental cues and on endogenous oscillators in the brain where various transmitters and releasing hormones bring about these physiological rhythms.

Administration of ethanol results in deleterious effects on physical and mental performance, and these effects are believed to be mediated by ethanol-induced interference with cell membrane functions and synaptic transmission in the central nervous system. For example, ethanol is known to increase γ-aminobutyric acid activity and to decrease the affinity of β-receptors in the brain (9, 10). We have recently shown that the administration of small or moderate doses of ethanol delayed the nocturnal melatonin surge but stimulated the secretion of noradrenaline and β-endorphin during early evening and night (11, 12). Most previous studies on the effects of ethanol on other pituitary hormones have been carried out during the daytime. In these studies, ethanol has been found to suppress the daytime secretion of GH, but the changes have been small (13, 14), or no changes have been observed (15). Instead, a substantial reduction in nocturnal GH levels by ethanol has been reported in one study in which ethanol was given in the evening and blood samples taken during night (16). Data on the effects of ethanol on PRL secretion are inconsistent and mostly daytime levels have been measured. It has been demonstrated that ethanol stimulates PRL secretion (17–19), but contradictory findings have also been reported (14, 20–22). The inhibitory role of ethanol on PRL secretion was emphasized in studies in which ethanol decreased the PRL response to TRH when the test was performed 14 h from the beginning of alcohol intake (15) or to breast stimulation (23).
The acute effects of ethanol on TSH secretion are poorly understood, with two previous studies suggesting no changes in circulating daytime TSH levels or in TSH responses to TRH after ethanol intake (13, 15). In another previous study, ethanol was ingested late in the evening and blood samples were taken every 20 or 30 min during the following night (24). No effects of ethanol on plasma TSH were, however, observed.

At the time when we started these series of experiments (11, 12) there were no detailed data available about the effects of ethanol on the nyctohemeral secretion of other pituitary hormones than GHRH (16) and PRL (14). In the case of 1STH, this was unexpected because the major part of its secretion occurs at night. Also the usual social ingestion of ethanol in the evening would imply that studies on the effects of ethanol on the secretion of pituitary hormones should be carried out through the night. Therefore we wanted to study the effects of ethanol on nighttime levels of circulating TSH, GH, and PRL. In addition, we measured circulating thyroid hormones and TSH and PRL responses to synthetic TRH to explore mechanisms by which the possible effects of ethanol are mediated.

Subjects and Methods

Subjects

The trial took place at the Department of Physiology. Fifteen healthy medical students who gave written informed consent (five women and four men in protocol I and four women and two men in protocol II, aged 21–23 yr) were chosen as test subjects. A detailed history was taken, and a routine medical examination was performed before commencing the trial. None of the subjects were excessive drinkers, because their consumption (as stated on their informed consent form) was less than 100 g ethanol per week (see also below). The protocol for the study was approved by the Ethical Review Board of the University of Oulu Medical School and the National Agency for Medicines, Helsinki, Finland. The subjects were not allowed to use any alcoholic beverages for 1 week before or during the 2-3 test weeks. The consumption of caffeine-containing products was also prohibited during the test days, and was limited to a maximum of 200 mg/day during the test weeks. The use of any medications and smoking was also prohibited during the test weeks.

Study design

Protocol I comprised a dose-dependent, double-blind, randomized, cross-over experiment in which the subjects received single oral doses of 0, 0.5, and 1.0 g ethanol/kg BW mixed in a sugar-free carbonated drink (600 mL) at 1-week intervals. The amount of ethanol of the 0.5-g/kg dose is 35 g in a 70-kg subject and represents approximately the amount of ethanol in 35 g in a 70-kg subject and represents approximately the amount of ethanol present in a half liter bottle of wine with a concentration of 7%. The subjects arrived at the site of the trial at 1700 h, at which time a cannula was inserted in a dorsal vein of the hand. Heparin 5000 IU/100 mL (Medica, Helsinki, Finland) in physiological sodium solution (200 μL) was used to keep the cannulae open. The drinks were given at 1900 h and were to be consumed at a constant pace by 1945 h. The blood sampling was started at 1800 h (before intake of alcohol or vehicle) followed by samples at 2000, 2200, 2400, 0100, 0200, 0300, 0400, and 0700 h. Lights were turned off at 2300 h, at which time the subjects were asked to go to bed. The blood samples were thereafter taken in the dark (under 2 lux). The subjects had been fasting since 1500 h and received a standardized snack containing bread, butter, cheese, 0.2 L of orange juice, and a banana at 2100 h.

Protocol II was an open, randomized experiment carried out after the information of protocol I was available. Each of the six subjects received 0 or 1.0 g ethanol/kg BW mixed in a drink as in protocol I. The 0-min blood sample was taken between 2250 and 2310 h from the subjects, and immediately thereafter 1 μg of synthetic TRH/kg BW (Relafen TRH, Hoechst, Frankfurt am Main, Germany) was given iv. The following blood samples were taken at 20 and 60 min from the injection of TRH. All subjects completed the study and no serious adverse effects or differences in sleep habits between the control and experimental subjects were observed.

Biochemical assays

Blood was taken into EDTA tubes for GH, GHRH, PRL, TSH, T4, free T4 (FT4), T3, and free T3 (FT3) measurements, and into glass tubes for serum ethanol measurements. The tubes were centrifuged within 30 min and the plasma and sera stored at −70°C. GHRH from the solid-phase plasma extracts was analyzed by RIA (25). Plasma GH was analyzed by a kit from Pharmacia (Uppsala, Sweden). Plasma PRL and TSH were analyzed with Gamma-BCT PRL IRMA and TSH IRMA kits (IDS, Tyne & Wear, Boldon, UK), respectively, according to the instructions of the manufacturer. Plasma concentrations of T2 and T3 (Oxim Diagnostica, Turku, Finland) as well as FT4 (Diagnostic Products Corp., Los Angeles, CA) were determined by RIA and FT3 levels by a chemiluminescence system (Ciba-Corning ACS:180 Analyzer, Medfield, MA) following the instructions of the manufacturer. Serum ethanol was determined by gas chromatography. The intra- and interassay variation in GHRH RIA were 11% and 18%, respectively, and in the other assays <4% and <9%, respectively. Detection limits were 0.1 mL/L for TSH, 5 μg/L for PRL, 0.3 μg/L for GH, 5 ng/L for GHRH, 5 nmol/L for T4, 1.5 pmol/L for FT4, 0.2 nmol/L for T3, and 0.3 pmol/L for FT3.

Statistical analyses

Results are expressed as means ± SEM. Series of measurements of each hormone formed a complete 3 × 9 in protocol I and 2 × 3 in protocol II (doses × time points) block with 9 or 6 replicates and were analyzed with one-way repeated measures ANOVA, followed by the Newman-Keuls test to assess the significance of differences between doses and various time points. Calculations for area under the curve (AUC) were carried out using the FigP graphics program (Biosoft, Ferguson, MO) and analyzed as explained above.

Results

Protocol I

The serum ethanol concentrations (data not shown) peaked at 2000 h, 1 h after the start of the ethanol intake, i.e. 13.1 ± 1.1 mmol/L (mean ± se, n = 9) and 26.8 ± 1.8 mmol/L in subjects receiving 0.5 and 1.0 g of ethanol/kg BW, respectively (P < 0.001 between the groups). Thereafter serum ethanol decreased to undetectable levels (<0.4 mmol/L) in both groups within 5 h and 11 h, respectively. Subjects from the control sessions also had undetectable serum ethanol levels.

In the control group, plasma GH was 8.4 ± 1.6 μg/L at 1800 h and decreased to 0.6 ± 0.2 μg/L at 2200 h. These relatively high GH levels at 1800 h most probably present a small GH peak in the evening in the wakeful state as reported earlier (25). After midnight, plasma GH presented an expected large peak, 25.0 ± 4.3 μg/L at 0100 h. Ethanol had no significant effects on plasma GH at 2000 h or at 2200 h until at 0100 h and 0200 h, when the higher dose (1.0 g/kg) almost completely inhibited the nocturnal GH peak (P < 0.01, Fig. 1A). The lower dose also decreased nocturnal GH levels at 0100 h and 0200 h, but to a lesser degree (P < 0.01, Fig. 1A).

In the subjects receiving the lower ethanol dose, plasma GH was significantly higher (P < 0.05) than the control value at 0400 h, possibly because of a rebound phenomenon.

In the control subjects plasma immunoreactive GHRH showed a diurnal variation, so that the levels at 0200, 0400, and 0700 h were significantly (P < 0.01, Fig. 1B) lower than
those at 1800 h in the control subjects. No significant changes were found in plasma GHRH (Fig. 1B) after ethanol administration.

Plasma TSH (Fig. 2A) showed a significant \( P < 0.01 \) nyctohemeral rhythm in the control group. It was \( 1.4 \pm 0.2 \) mU/L at 1800 h, and the nocturnal rise began after 2200 h, reaching maximal levels of \( 2.4 \pm 0.4 \) mU/L at 0100 h and remaining high until morning \( (2.1 \pm 0.3 \) mU/L at 0700 h). We were able to show that the higher dose of ethanol inhibited the nocturnal TSH increase. The inhibition was significant at 0100 \( (P < 0.05) \) and 0200 h \( (P < 0.01) \) with plasma TSH concentrations of \( 1.4 \pm 0.2 \) mU/L. Although there were decreases after the lower dose, these were not significant.

Plasma PRL levels (Fig. 2B) in the control subjects were at the same level at 1800–2200 h, \( 60–80 \) pg/L, whereafter they gradually increased to 105 at midnight and to \( 248 \) pg/L at 0700 h \( (P < 0.001) \). Interestingly, ethanol had no significant effect on plasma PRL in our study.

When we observed that ethanol resulted in a significant inhibition of plasma TSH after midnight, we wanted to know if this inhibition was preceded by an increase in the plasma levels of thyroid hormones. Because of the much longer half-lives, measurements on 2–4 time points in each test group were carried out. Plasma \( T_4 \) (Fig. 4A) measured at 1800 and 0700 h or \( FT_4 \) (Fig. 4A) measured at 1800, 2200, 0300, and

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**Fig. 1.** Effect of ethanol ingestion on plasma immunoreactive GH (A) and GHRH (B) in nine healthy volunteers (mean \( \pm \) SE) after intake of 0 (○ and solid line), 0.5 (● and dashed line), and 1.0 g/kg BW (■ and solid line) of ethanol in 600 mL of beverage between 1900 and 1945 h. *, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.001 \) vs. controls. #, \( P < 0.05 \) between ethanol groups.

**Fig. 2.** Effect of ethanol ingestion on plasma TSH (A) and PRL (B) in nine healthy volunteers. For explanations see legend of Fig. 1.

**Fig. 3.** Effects of graded doses of ethanol on PRL, TSH, and GH secretion as analyzed by the AUC method in nine healthy volunteers (mean \( \pm \) SEM). The level of inhibition (%) is given on the y-axis and ethanol doses on the x-axis. Asterisks indicate significance as in legend of Fig. 1.

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Fig. 4. Effect of ethanol ingestion on plasma T₄ (A), free T₄ (B), T₃ (C), and free T₃ (D) in nine healthy volunteers (mean ± SEM) after the intake of 0 (open column), 0.5 (single-hatched column), and 1.0 g/kg BW (cross-hatched column) of ethanol in 600 mL of beverage between 1900 and 1945 h. *, P < 0.05 vs. controls.

0700 h were similar and showed no significant changes between the test groups. Plasma T₃ (Fig. 4C) at 2200 h did not change significantly from the values at 1800 h but exhibited a statistically significant decrease (P < 0.05) at 0700 h in the 0.5 g ethanol/kg BW group (1.64 ± 0.13 vs. 1.97 ± 0.2 nmol/L), most possibly a random event. It should be noted that plasma FT₃ (Fig. 4D) measured at 1800, 2200 and 0700 h were similar and showed no changes between the test groups.

Protocol II

After we observed that the changes in plasma thyroid hormones most possibly do not explain the reduced plasma TSH after ethanol intake, we performed a TRH stimulation test and measured TSH and PRL (Table 1). Synthetic TRH (1 μg/kg BW, totaling 55–88 μg/subject) led to a 5.5-fold increase at 20 min and a 4.2-fold increase at 60 min in the control subjects, and to 5.4- and 4.4-fold increases in the ethanol-treated subjects, respectively (P < 0.01 in both cases). There were no significant changes between control and ethanol-treated subjects. Synthetic TRH led to significant increases in plasma PRL but no significant differences were found between control subjects and ethanol-treated subjects (Table 1).

Discussion

Ethanol administration has been shown to result in diminished circulating GH levels in studies carried out in the evening or at night. For example, an earlier study from this laboratory demonstrated that large doses of ethanol (1.5 g/kg BW) inhibited the small evening surge of GH (13) but no nighttime measurements were performed. Later, acute and chronic effects of ethanol (0.8 g/kg) on sleep pattern and GH secretion were studied and it was found that ethanol decreased nighttime GH secretion by 70% (16). We observed in this study that ethanol caused a significant and dose-dependent decrease in plasma GH levels during 4 h after midnight and, what is more important, the abolition of the nocturnal GH surge was almost total (80% after the dose of ethanol 1.0 g/kg BW). The lower dose also led to a substantial (63%) inhibition at 0100 h when ethanol had disappeared from circulation. Thus, our present results agree with those of Prinz et al. (16) and show, in addition, that a relatively small ethanol dose (0.5 g/kg) corresponding to the amount of ethanol present in half a bottle of wine, also effectively suppresses nocturnal GH secretion. We wish to emphasize that the suppression was evident although there was no
longer any ethanol detectable in serum, indicating that ethanol had caused a long-lasting (up to 8 h) interference with a cellular mechanism related to the nocturnal GH surge.

We have previously found that in peripubertal children the majority of nocturnal GHRH pulses precedes or coincides with GH pulses (26). Therefore we also measured plasma GHRH and observed that plasma GHRH levels before the nocturnal GH surge were stable. Evidently the long sampling interval in our present study (1–4 h) did not make it possible to detect the pulsatile secretion of GHRH. Anyway, plasma GHRH in subjects receiving vehicle only was significantly lower at 0400 and 0700 h than between 1800–0100 h, indicating the presence of a diurnal rhythm in the secretion of GHRH. We found no changes in circulating immunoreactive GHRH levels after ethanol administration, suggesting that the GH suppressing effect of ethanol could be mainly mediated by factors other than hypothalamic GHRH. However, small changes in portal GHRH caused by ethanol could be lost when peripheral GHRH is monitored as in our present study. According to previous studies carried out in experimental animals, ethanol seems to have multiple effects: it inhibits GH and GHRH gene expression (27, 28) and signal transduction in the pituitary somatotrophs (29). In man, GH response to synthetic GHRH was similar in vehicle- and ethanol-treated subjects (30), indicating a suprapituitary mechanism for the ethanol-induced inhibition of GH.

The effects of ethanol on the GHRH-GH axis may have important clinical implications on growth and development in children. It is known that maternal alcohol abuse leads to fetal alcohol syndrome associated with intrauterine growth retardation and slow postnatal growth (31). This phenomenon might be caused by the inhibitory effect of ethanol on GH secretion that we describe in this study, although other factors such as nutrition are also important. The main evidence comes from animal studies: animals fed with ethanol-containing diets exhibit decreased growth even when food intake and other nutritional factors are controlled (32).

In our control subjects (both females and males) plasma PRL was low between 1800 and 2200 h, but thereafter it gradually increased, reaching its maximum (a 4-fold rise) the next morning. Our results are in accordance with previous studies on a diurnal rhythm in circulating PRL: the peak response to synthetic TRH. These results differ from those in alcoholics (34) are reduced after ethanol intake. Taken together, these findings suggest that ethanol appears to have an inhibitory effect on PRL secretion, but the inhibitory effect cannot be seen during nighttime.

To our knowledge the present study is the first to show an acute inhibitory effect of ethanol on the nocturnal TSH surge in healthy volunteers as judged from the plasma TSH measurements and AUC analyses. There are previous human studies that partly describe the effect of ethanol on nocturnal TSH secretion (13, 15). However, in both studies TSH was monitored only until midnight and no changes were observed. Therefore the reason for these negative results may be that TSH levels were not followed long enough. In another previous report, nighttime levels of plasma TSH were measured after ethanol intake, but all the samples were pooled and no significant differences were observed between control night and first ethanol night (24). In the aforementioned study ethanol (0.8 g/kg) was ingested late in the evening, which also explains why it did not have any effects.

If the regulation principles of TSH secretion are taken into account, the decrease in plasma TSH by ethanol may be caused by elevated levels of thyroid hormones or caused by central mechanisms. Therefore we studied the effects of ethanol on the serum levels of free and total thyroid hormones and found no significant changes before plasma TSH was inhibited. We also performed a TRH stimulation test by using low TRH doses. We reasoned that the usual TRH dose (200 µg/subject) may be too high, so that the possible ethanol-induced inhibition at the pituitary thyrotrope level may not be detected. Therefore we used TRH doses that were 55–88 µg (adjusted for BW). These TRH doses led to greater than 5-fold increases in plasma TSH, but the TSH response did not change after ethanol intake. Thus our present results suggest that there is a central mechanism behind the ethanol-induced TSH decrease.

The adenohypophysial secretion of TSH and PRL are under the control of hypothalamus, principally of dopamine, but also of TRH. Based on bromocriptine tests, Iida et al. (19) have proposed that the stimulation of PRL by ethanol may be mediated by ethanol-induced inhibition of hypothalamic dopamine. Because we observed no changes in plasma PRL but a decrease in plasma TSH, the role of the dopaminergic mechanism remains unproven.

In conclusion, we have demonstrated that ethanol in low doses inhibits nocturnal TSH and GH secretion, and that the inhibition occurs in a dose-dependent manner, disturbing the normal diurnal rhythms of TSH and GH. The inhibition is most probably mediated by a suprapituitary mechanism. It is of interest that the same ethanol doses have no significant effect on plasma PRL. Thus the nocturnal secretion of TSH and especially that of GH are extremely sensitive to alcohol, which may have undesired effects on growth and metabolism, especially in adolescents, even if ingested in small amounts.

References


