INTRODUCTION

Acute and chronic alcohol misuse have been shown to cause reproductive function derangements in humans and experimental animals (Mendelson et al., 1977, 1981, 1987; Little et al., 1992). Studies carried out in our laboratory on adolescent humans revealed that alcohol ingestion produces a decrease in plasma testosterone levels in males, a great increase in testosterone levels in females and no significant changes in either follicle-stimulating hormone (FSH) or luteinizing hormone (LH) levels in males or females (Frias et al., 2000). Preliminary studies on rats (Little et al., 1992) demonstrated that administration of alcohol to prepubescent males stimulated testosterone secretion with no changes in serum LH, whereas alcohol administration to adult male rats led to decreases in both LH and testosterone levels. These data suggest that the effects of alcohol on pituitary–gonadal axis hormones may depend on gender and sexual maturation of the subjects. Intervention studies have demonstrated that alcohol ingestion elevates pituitary–adrenal axis hormone (Aguirre et al., 1995), β-endorphin (Gianoulakis et al., 1996) and prolactin (Ida et al., 1992; Sarkola et al., 1999), which may disrupt the reproductive function (Yen, 1991). The hypersecretion of prolactin, β-endorphin and pituitary–adrenal axis hormones may also be involved in alcohol-induced dysfunction of the pituitary–gonadal axis hormones in human adults.

The present paper represents a cross-sectional study on the effects of acute alcohol intoxication (AAI) on pituitary–gonadal axis hormones in adult men and women and the possible relationship to changes in β-endorphin, prolactin and pituitary–adrenal axis hormones.

SUBJECTS AND METHODS

Subjects

A total of 21 subjects (12 men and nine women) with AAI aged 20–27 years were studied. They arrived at the emergency department with evident behavioural symptoms of drunkenness (slurred speech, unstable gait). We could not determine the exact time period between alcohol ingestion and arrival at the emergency room, although their clinical symptoms suggested that this interval was not very long. According to those who accompanied them, the drinking sessions had commenced 4–6 h earlier and no other drugs had been consumed by the subjects. After symptoms had remitted, all subjects confirmed that they had been drunk on their arrival at the emergency department after drinking for 4–6 h and most admitted to being habitual drinkers, although, on the basis of questionnaire responses and tests (MAST, CAGE and MALT) none could be classified as alcoholics (Selzer, 1971; Paton and Saunders, 1981; Rueff et al., 1989). All subjects confirmed that they had taken no drug other than alcohol.

A total of 27 healthy volunteers (11 men and 16 women) aged 20–27 years whose alcohol consumption was nil were studied as controls. These volunteers were not habitual drinkers and none of them had consumed any alcohol during the 48 h prior to withdrawal of blood samples for assay. The controls arrived at the Emergency Department with mild trauma (contusions, sprains, etc.) in all cases.

All participants gave their informed consent to take part in this study, which was carried out in accordance with the Helsinki Declaration. None of the subjects studied had apparent endocrine disorders and none was taking any medication at the time of the study. None of the women was pregnant. The study design and situation of the AAI women and controls made it difficult to establish the ovarian cycle phase or use of the contraceptive pill.
Biochemical assays

To avoid circadian variations in the hormone study, blood samples were drawn from both AAI and controls at the same time of day, during the 3-h period from 12.00 to 03.00. For hormone assays, the serum was frozen at –20°C until its analysis. For alcohol determinations, the blood was refrigerated at 4°C until the assays were performed.

\[\beta\text{-endorphin} \text{ was measured using Nichols Institute (San Juan Capistrano, CA, USA) radioimmunoassay kits and corticotropin (adrenocorticotropic hormone, ACTH) was measured using immunoradiometric CIS Biointernational (Gif-sur-Yvette, France) kits. Prolactin, FSH, LH, testosterone, oestradiol (E2) and progesterone were measured by electrochemiluminescence immunoassay using the Boehringer Mannheim Elecsys 2010 immunoassay analyser using the Boehringer Mannheim Elecsys 2010 immunoassay analyser (Roche Diagnostics, Mannheim, Germany). Dehydroepiandrosterone-sulphate (DHEAS) and cortisol were measured by chemiluminescent enzyme immunoassay using the Immunolite Automated Analyzer (Diagnostic Products Corporation Los Angeles, CA, USA). Intra-assay and inter-assay coefficients of variation were 4.1 and 7.7% for \[\beta\text{-endorphin}; 2.9 and 4.8% for ACTH; 2.8 and 3.8% for prolactin; 1.8 and 5.3% for FSH; 1.8 and 5.1% for LH; 2.7 and 5% for E2; 1.5 and 4.1% for progesterone; 6.8 and 8.1% for DHEAS; and 6.8 and 9.9% for cortisol. The detection limits were 10 pg/ml for \[\beta\text{-endorphin}, 2 pg/ml for ACTH, 0.47 ng/ml for prolactin, 0.10 mIU/ml for FSH, 0.10 mIU/ml for LH, 0.02 ng/ml for testosterone, 10 pg/ml for E2, 0.03 ng/ml for progesterone, 2 \mu g/dl for DHEAS and 0.2 \mu g/dl for cortisol. The blood-alcohol concentrations were determined by the gas-chromatographic head-space method using a Perkin–Elmer Sigma 300 FID chromatograph with Hewlett Packard HP 390A integrator. The limit of detection (LOD) was 0.01 g/l and the limit of quantification (LOQ) 0.1 g/l.

Statistical analysis

Results are expressed as means ± SEM. Student’s t-test or Welch’s t-test was employed where appropriate to examine statistically significant differences. A linear correlation between all hormones was determined, and \( P < 0.05 \) was considered significant. The regression models were constructed with a forward stepwise procedure. A variable was included when \( P < 0.05 \).

RESULTS

Effects of AAI on the pituitary–gonadal axis

Serum testosterone levels were very significantly lower in AAI men versus control men (Table 1); in contrast, testosterone levels were significantly higher in AAI women versus control women. Serum LH and FSH levels were not significantly different between AAI women and control women. In AAI men, the serum FSH was not different but LH was significantly decreased versus control men. Serum E2 did not differ but progesterone was higher in AAI men than in control men; in contrast, neither progesterone nor E2 levels differed in AAI women versus control women.

Effects of AAI on the pituitary–adrenal axis

Serum ACTH and cortisol levels were significantly increased in AAI men and women (Table 2). The responses of ACTH and cortisol to AAI were higher in women than in men. ACTH and cortisol were 11- and 2.2-fold higher, respectively, in AAI women (versus control women), compared with 6.2- and 1.5-fold higher in AAI men (versus control men). Serum DHEAS levels were significantly higher in AAI females, compared to control females, but were not different between AAI males and control men.

Effects of AAI on other hormones

Serum prolactin levels were significantly increased in AAI men and women, compared to their respective controls (Fig. 1).

Table 1. Pituitary–gonadal axis hormones in acute alcohol-intoxicated (AAI) subjects and controls

<table>
<thead>
<tr>
<th></th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Oestradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
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<tr>
<td>AAI (n = 12)</td>
<td>5.03 ± 0.6</td>
<td>5.4 ± 0.6a</td>
<td>3.5 ± 0.24c</td>
<td>1.6 ± 0.4b</td>
<td>24 ± 2.9</td>
</tr>
<tr>
<td>Controls (n = 11)</td>
<td>5.41 ± 0.7</td>
<td>7.4 ± 0.5b</td>
<td>6.3 ± 0.12c</td>
<td>0.39 ± 0.06b</td>
<td>25 ± 2.6</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AAI (n = 9)</td>
<td>6.89 ± 0.7</td>
<td>7.15 ± 1</td>
<td>2.76 ± 0.37d</td>
<td>3.75 ± 2.4</td>
<td>114 ± 18</td>
</tr>
<tr>
<td>Controls (n = 16)</td>
<td>6.61 ± 0.6</td>
<td>7.20 ± 0.9</td>
<td>0.56 ± 0.07d</td>
<td>3.98 ± 1.8</td>
<td>115 ± 21</td>
</tr>
</tbody>
</table>

Values are means ± SEM. \( aP < 0.05; bP < 0.01; cP < 0.0005; dP < 0.0001 \).

FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Table 2. Pituitary–adrenal axis hormones in acute alcohol-intoxicated (AAI) subjects and controls

<table>
<thead>
<tr>
<th></th>
<th>ACTH (pg/ml)</th>
<th>Cortisol (\mu g/dl)</th>
<th>DHEAS (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAI (n = 12)</td>
<td>156 ± 41a</td>
<td>28.1 ± 2.2a</td>
<td>2249 ± 224</td>
</tr>
<tr>
<td>Controls (n = 11)</td>
<td>25 ± 8.8a</td>
<td>19.2 ± 1.6a</td>
<td>2409 ± 231</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAI (n = 9)</td>
<td>286 ± 61b</td>
<td>4.1 ± 1.9b</td>
<td>2606 ± 190b</td>
</tr>
<tr>
<td>Controls (n = 16)</td>
<td>26 ± 10b</td>
<td>19.3 ± 1.8c</td>
<td>2094 ± 186a</td>
</tr>
</tbody>
</table>

Values are means ± SEM. \( aP < 0.05; bP < 0.01; cP < 0.003 \).

ACTH, adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone-sulphate.
The response of prolactin to AAI was higher in women than in men. Thus, prolactin was 5.8-fold higher in AAI women than in men. Plasma DHEAS was 3.5-fold higher in AAI men. Thus, prolactin was 5.8-fold higher in AAI women than in men. The response of prolactin to AAI was higher in women than in men.

**Linear regression analysis**

The multiple linear regression analysis between the hormones studied showed a positive correlation in females for prolactin–ACTH ($r = 0.57$), prolactin–DHEAS ($r = 0.59$), prolactin–testosterone ($r = 0.61$), prolactin–cortisol ($r = 0.56$), ACTH–cortisol ($r = 0.62$), ACTH–DHEAS ($r = 0.58$), ACTH–testosterone ($r = 0.59$), testosterone–DHEAS ($r = 0.54$), testosterone–cortisol ($r = 0.57$), and cortisol–DHEAS ($r = 0.52$), and a positive correlation in males for ACTH–progesterone ($r = 0.65$), ACTH–cortisol ($r = 0.61$), cortisol–progesterone ($r = 0.50$), and testosterone–DHEAS ($r = 0.49$).

**Alcohol concentrations**

Blood-alcohol concentrations in the AAI subjects were $196 \pm 1$ mg/dl in the men and $210 \pm 14$ mg/dl in the women.

**DISCUSSION**

Our results clearly demonstrate that AAI produced a large increase in ACTH, β-endorphin, cortisol and prolactin in our adult men and women, in agreement with the findings of our previous studies on adolescent humans (Frias et al., 2000). Our present data are also consistent with those reported by other authors for ACTH (Aguirre et al., 1995), β-endorphin (Gianoulakis et al., 1996) and prolactin (Ida et al., 1992; Sarkola et al., 1999) in spite of the fact that our experimental design, representing a cross-sectional approach, was different from that of the others, which represented intervention studies. Alcohol may modify central levels of corticotropin-releasing hormone (CRH), opioid peptides, catecholamines and gamma aminobutyric acid, substances involved in prolactin, ACTH and β-endorphin secretion (Widdowson and Homan, 1992; Harris et al., 1995).

The response of prolactin and pituitary–adrenal axis hormones to AAI was greater in women than in men. Alcohol metabolism is different in women and men (Mishra et al., 1989; Ammon et al., 1996; Kwo et al., 1998); however, this does not explain our results, as there were no significant differences in blood alcohol between men and women with AAI. Our results are, however, consistent with the findings by Ogilvie and Rivier (1997) that alcohol administration results in activation of the pituitary–adrenal axis, with female rats secreting more ACTH and corticosterone than males in response to the same dose of alcohol.

In the present study, we found reduced LH in AAI men versus controls. In our previous studies on adolescents, we found no significant differences in LH between AAI males and controls (Frias et al., 2000). One explanation for these discrepancies could be the varied sexual maturity of the adolescent. Another possibility is that in the fully sexually mature male, alcohol affects both the testes and the central component of the hypothalamic–pituitary–gonadal axis, i.e. the release of luteinizing hormone-releasing hormone (LHRH)/LH, as has been described in rats (Little et al., 1992). Alcohol increases CRH at the central level, and this elevated CRH may decrease serum LH by a mechanism probably mediated by LHRH (Rivier et al., 1986; Barbarino et al., 1990; Frias et al., 1990, 1997). In accordance with previous reports (Mendelson et al., 1981; Välimäki et al., 1983), we found no significant differences in LH levels among AAI and control women. Earlier studies (Sarkola et al., 1999) found no significant alcohol effects on LH levels among subjects not using oral contraceptives, and observed a decline among subjects using oral contraceptives at mid-cycle. The lack of a significant difference in LH, E, and progesterone among the women we studied could be attributed to differences in the ovarian cycle phase or contraceptive pill. Unfortunately, in the present study, neither the ovarian cycle phase nor use of the contraceptive pill was assessed.

We found an increase in circulating testosterone among the AAI females, in accordance with an earlier report (Sarkola et al., 2000). The higher values of testosterone in women were not associated with significant changes in LH or FSH. Although the effects of alcohol on the hepatic metabolism of testosterone should be borne in mind (Karila et al., 1996), the high levels of ACTH and prolactin and the correlations of prolactin and ACTH with cortisol, DHEA-S and testosterone...
in the women we studied suggest that prolactin and ACTH could have contributed to stimulated adrenal androgen production in our AAI women. The role of prolactin has been earlier suggested (Higuchi et al., 1984; Glasow et al., 1996).

The increase in DHEAS in blood and perhaps in the brain of our women (Corpechot et al., 1981) could be of physiological significance. In fact, neurosteroids influence sexual behaviour, mood, memory and aggressiveness (Robel et al., 1995).

In contrast to the findings in women, serum testosterone levels were very significantly decreased in our AAI men, in agreement with earlier findings that demonstrated the inhibitory effect of alcohol on testosterone in adult males (Mendelson et al., 1977). A comparison between the testosterone values obtained in the present study (AAI adult men) and those in our earlier study (AAI adolescent males) (Frias et al., 2000) shows that the decrease in testosterone levels was greater in AAI adults (44.5%) than in AAI adolescents (20%). Several hypotheses can be proposed to account for this finding: (1) testosterone values are higher in adult controls versus prepubertal controls (6.3 ± 0.1 vs 4.7 ± 0.39), and the decrease in testosterone values could be more evident at high values than at low values of testosterone; (2) the adolescents studied (both AAI and controls) could have varied degrees of sexual maturity, which could mask the effects of alcohol on testosterone levels in these subjects; (3) the amount of alcohol consumed by the adults was greater than that taken by the adolescents (Frias et al., 2000); (4) another possible explanation could be differences in adrenal/testicular contribution to circulating testosterone. In fact, alcohol could increase circulating testosterone in prepubertal males (as in females), since testicular contribution to circulating testosterone could be minimal (at least in cases of less sexual development). In contrast, in sexually mature adult men, the adrenal contribution to circulating testosterone is minimal compared with the testicular contribution. With respect to the effects of ethanol on the testes, it has been well established that alcohol decreases testicular testosterone production through modifications in the [NAD+]/[NADH] ratio (Emanuele et al., 1993), the arginine–NO synthase (Adams et al., 1993), the opioid system (Cicero et al., 1989; Emanuele et al., 1998), and a neural adrenergic-dependent pathway between the brain and the testes (Rivier, 1999).

As we mentioned in the introduction, Little et al. (1992) studied the effects of alcohol on the pituitary–gonadal axis in sexually mature and immature rats, controlling for age and dose of alcohol consumed. Alcohol reduced testosterone levels in the sexually mature rats and increased testosterone levels in the sexually immature ones. This finding would support hypothesis 4 above.

To summarize, we have demonstrated that AAI produces an increase in β-endorphin, prolactin and pituitary–adrenal axis hormones among human adults of both sexes. AAI produces a decrease in LH in men, but not in women, and an opposite change of pattern in testosterone according to gender, with an increase in plasma testosterone in women and a decrease in plasma testosterone in men. This opposite behaviour of testosterone could explain, as other authors have suggested (Erikkson et al., 1994), the opposite effects of acute alcohol ingestion on the subjective feeling of sexual arousal, excitement and desire in men and women. Finally, DHEAS increased significantly in women, but not in the men, after alcohol ingestion. DHEA, a neurosteroid, may also increase sexual excitement in women. Neurosteroids are thought to influence cerebral functions that control mood, memory and sexual behaviour in animals. Based on our previous and present data, we believe that the gender and the age of the individual may modify the response of pituitary–gonadal axis hormones to alcohol consumption, at least in part through modifications in the testicular/adrenal contribution to the circulating testosterone. Our study suggests a possible additional biochemical mechanism to explain the opposing effects of acute alcohol ingestion on plasma testosterone levels in men and women. In one gender, there is the hyper-response of pituitary–adrenal axis hormones, including DHEA, and, in the other, there is a direct effect on the testes.

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REFERENCES


