

INCREASED LEVELS OF PROGESTERONE IN RATS FOLLOWING ACUTE ETHANOL TREATMENT

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The purpose of this study was to investigate the effect of a single dose of ethanol on reproductive hormone production in female rats. Adult female Wistar rats were injected with alcohol (4g/kg) or equal volume of saline i. p. on diestrus day 1. The animals were sacrificed 1/2 h and 3 h after treatment. Our results showed that acute ethanol treatment had no significant influence on LH and oestradiol levels, but significantly increased progesterone concentration. Since ethanol induced elevated levels of progesterone in male rats as well and caused a significant increase in the level of ACTH, the data obtained indicated that the increased progesterone concentration in female rats might be the result of activation of the adrenal glands.

Key words: ethanol, progesterone, oestradiol, LH, ACTH, female rat

INTRODUCTION

Ethanol has been shown to have an adverse influence on the hypothalamic-pituitary-gonadal axis in humans as well as in experimental animals (Cicero, 1982; Van Thiel et al., 1989). These effects of alcohol have been studied primarily in male subjects, and relatively few studies have been conducted in females. Nevertheless, in women abusing alcohol an increased prevalence of menstrual disorders and amenorrhea has been observed (Van Thiel et al., 1989); while either decreased or unaltered LH levels were reported in female rats after alcohol consumption (Sanchis et al. 1985, Rettori et al., 1987, Emanuele et al., 1986, Eskay et al., 1981).

As in most of the studies the effect of chronic alcohol treatment was investigated, this study was undertaken to ascertain the influence of a single dose of ethanol on LH, oestradiol and progesterone levels in female rats. Bearing in mind that progesterone is also produced by the adrenal glands as well as by the ovaries, the plasma levels of ACTH were also determined. Since the patterns of reproductive hormone secretion are different during the rat ovarian cycle, rats showing the same phase of the oestrus cycle, in this study diestrus day 1, were analysed. Diestrus day 1 is characterized by low levels of LH and FSH, while the

low levels of oestradiol and progesterone begin to rise during the afternoon of this day of the cycle (Freeman 1988).

MATERIALS AND METHODS

Experiment 1. Female Wistar rats 10-12 weeks old were housed under controlled temperature (20-22°C) and light (12:12 hours light:dark). The oestrous cycle was monitored every day by vaginal smear. The rats showing diestrus day 1 were used for the experiments and treated with ethanol or saline intraperitoneally. Ethanol was diluted with sterile saline up to 35% (v/v) and the rats were injected with different volumes to reach a concentration of 4 g/kg body weight. The animals were killed by decapitation 1/2 h and 3 h after alcohol administration and blood samples were collected for determination of plasma LH and ACTH and serum oestradiol and progesterone levels.

Experiment 2. Adult male Wistar rats 10-12 weeks old were treated with ethanol 4g/kg or saline i. p. Thirty minutes later the rats were sacrificed and bled for assessment of serum progesterone concentrations.

Hormone determination. Plasma LH levels were measured by double antibody radioimmunoassay. The assay was formed using rat LH (NIDDK-rLH-RP-1-7) labelled with J-125 (18.5 Mbq/μg), a reference preparation of rLH (NIDDK-rLH-RP-3) in the range: 0.49-250 ng/ml and rabbit anti-rat LH (NIDDK-anti-rLH-s-10) provided by the National Hormone and Pituitary Program, Baltimore, Maryland, USA. Anti-rabbit IgG (INEP, Zemun, Yugoslavia) was used as the second antibody.

Serum progesterone concentrations were determined by a direct progesterone radioimmunoassay kit (INEP, Zemun, Yugoslavia), whereas serum levels of oestradiol were assessed using a direct oestradiol enzyme immunoassay (Serono Diagnostic SA, 1267 Coinsius, Switzerland). Plasma ACTH concentrations were measured using a double antibody radioimmunoassay kit (Vinča, Beograd, Yugoslavia).

Statistical analysis. The data were analysed using Student's t-test.

RESULTS

Experiment 1. The plasma levels of LH in ethanol- and saline-injected rats determined 1/2 h and 3 h after treatment are presented in Figure 1A. No significant differences were found between the values for alcohol-treated and control animals. Similarly to LH levels, the serum oestradiol concentrations, in the same time intervals, were not affected by ethanol administration (Figure 1B). Meanwhile, the acute ethanol treatment significantly increased the level of progesterone 1/2 h post-injection (Figure 1C). Three hours after the treatment progesterone concentration in the ethanol-treated group was still higher than in the controls, although no statistically significant difference was detected. The effect of acute alcohol treatment on ACTH can be seen in Figure 1D. The ethanol-treated animals had significantly higher plasma ACTH concentrations in comparison to the control rats, but, as in the case of progesterone, a statistically significant difference was noted only 1/2 h after treatment.

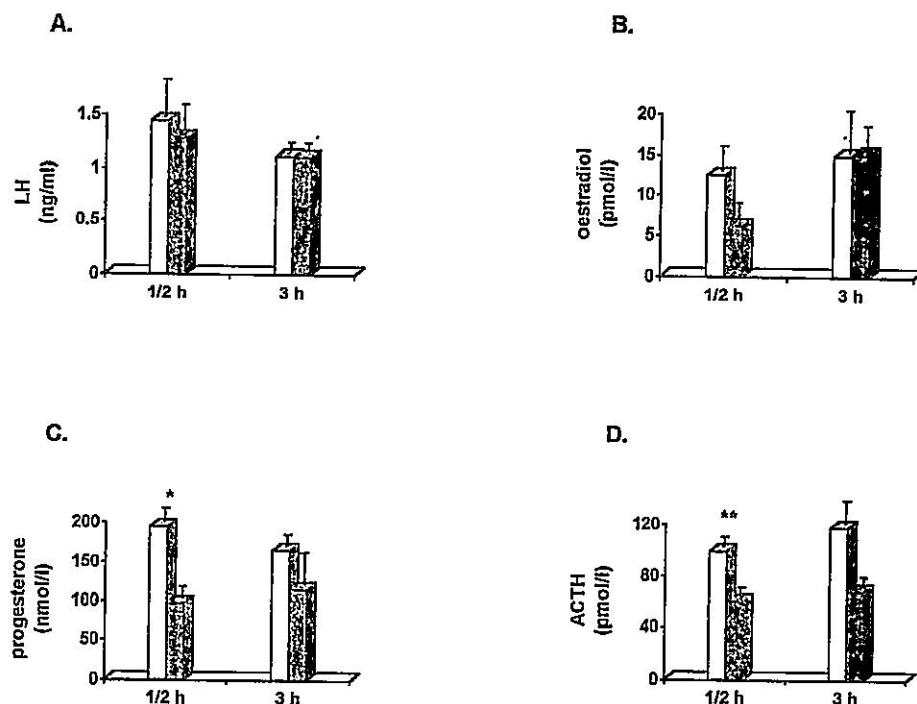


Figure 1. The levels of plasma LH (A), serum oestradiol (B), serum progesterone (C) and plasma ACTH (D) determined 1/2 h and 3 h after ethanol (●) and saline (○) administration to female rats on diestrus day 1. Values are mean \pm SEM; 4-7 rats were used per group for each time point. Statistically significant difference from control, saline-treated animals by t-test: * $p < 0.05$, ** $p < 0.01$.

Experiment 2. In order to establish the origin of the high progesterone level in female rats after alcohol treatment, we investigated the effect of the same dose of ethanol (4g/kg) in male rats. The serum concentrations of progesterone were determined 1/2 h post-injection both in ethanol- and saline-treated rats. Analogous to females, a single dose of ethanol significantly increased the level of circulating progesterone in male rats (28.13 ± 4.34 nmol/l in alcohol-treated v.s. 5.95 ± 74 nmol/l in control rats, $p < 0.01$).

DISCUSSION

The investigations presented in this study examined the effect of acute alcohol treatment on reproductive hormone secretion in female rats on diestrus day 1. The results demonstrated that a single dose of ethanol did not alter LH and oestradiol levels, but markedly increased the level of progesterone.

As regards the influence of alcohol on LH there are diverse reports, since both decreased and unaltered levels were demonstrated. The unaltered plasma concentration of LH following acute ethanol treatment in this study is consistent with the results obtained in ovariectomised rats (Subramanian et al., 1990) and menopausal women (Mendelson et al., 1985), as well as with the study showing that acute administration of ethanol to normal women under controlled conditions does not affect the levels of either sex steroids or gonadotropins (VanThiel et al., 1989). However, there are additional opposite findings, since decreased levels of LH in female (Mancebo et al., 1984) and male rats (Emanuele et al., 1991) after acute ethanol treatment have also been reported. Although LH levels were not affected by the ethanol treatment in the present study, the possibility that ethanol could cause qualitative changes in LH cannot be excluded, considering that in chronic alcohol treatment Emanuele et al. (1986) have shown increased acidic forms of LH, known to be less bioactive than the alkaline forms, in spite of unaltered concentrations of pituitary and serum LH.

In contrast to unaltered LH and oestradiol concentrations, a significant increase in progesterone level was observed after the ethanol administration. It is well known that progesterone is secreted not only by the ovaries, but also by the adrenal glands. Activation of the hypothalamic pituitary adrenal axis by a single dose of ethanol in male rats has been reported by Thiagarajan et al. (1989). The influence of alcohol on ACTH and corticosterone secretion in rats resulting in elevated plasma ACTH and corticosterone levels 1/2 h after treatment has also been shown (Rivier et al., 1993). Previously, with the same experimental design as that used here, we have shown that the action of ethanol on ACTH in female rats was dependent on the phase of the oestrus cycle and was more pronounced on diestrus day 1 compared to proestrus (Budeč et al., 1994). Thus, the elevated progesterone level obtained in this study could be the result of adrenal gland production, since increased ACTH levels were also demonstrated. In order to further confirm if the observed increase in the level of progesterone was of adrenal origin, we applied the same dose of ethanol to male rats and, as expected, ethanol significantly increased the progesterone concentration. The *in vitro* findings obtained by van Weerden et al. (1992) indicated that the adrenals of rats and mice lack the enzyme 17 α -hydroxylase which is necessary for conversion of pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone. This disorder in the ability of rodent adrenal gland steroid hormone production could also contribute to the elevated levels of progesterone obtained in this study.

In summary, unaltered LH and oestradiol levels but elevated progesterone concentrations were found in female rats following acute ethanol treatment. The increased progesterone level was probably the result of adrenal production since the concentration of ACTH was also increased and the same phenomenon was observed in male rats.

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POVEĆANI NIVOI PROGESTERONA U PACOVA NAKON AKUTNOG TRETMANA ALKOHOLOM

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SADRŽAJ

Cilj rada je bio da ispitamo efekat jedne doze alkohola na produkciju reproduktivnih hormona u ženki pacova. Odrasle ženke pacova tretirane su alkoholom 4g/kg ili odgovarajućim volumenom fiziološkog rastvora i. p. prvog dana diestrusa. Životinje su žrtvovane 1/2 i 3 h nakon tretmana. Naši rezultati su pokazali da akutan tretman alkoholom nije značajno uticao na nivoe LH i estradiola u posmatranim vremenskim intervalima, ali je značajno povećao koncentraciju progesterona. Porast u nivou progesterona pod dejstvom alkohola i u mužjaka, kao i zapaženo povećanje koncentracije ACTH ukazuju da je povećani nivo progesterona nakon akutnog tretmana alkoholom u ženki pacova najverovatnije posledica aktivacije nadbubrežnih žlezda.