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Acute Ethanol Administration in Diestrus-2 in the Rat on Pulsatile Prolactin and LH Release

A. LAFUENTE,*[1] A. ARCE,† M. LÓPEZ,‡ E. CARRO,‡
J. MARCÓ‡ AND A. I. ESQUIFINO†

*Departamento de Toxicología, Facultad de Ciencias, Sección de Ciencia y Tecnología de los Alimentos, Universidad de Vigo, Campus de Orense, 32004 Orense, Spain
†Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain
‡Departamento de Fisiología, Facultad de Biología, Universidad de Santiago de Compostela, 15706 Santiago, Spain

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LAFUENTE, A., A. ARCE, M. LÓPEZ, E. CARRO, J. MARCÓ AND A. I. ESQUIFINO. *Acute ethanol administration in diestrus-2 in the rat on pulsatile prolactin and LH release.* PHARMACOL BIOCHEM BEHAV **49**(4) 789–794, 1994.—Exposure to ethanol is followed by changes in reproductive function in man and animals, characterized by modifications in the secretion patterns of prolactin and luteinizing hormone (LH). As both hormones are secreted in an episodic fashion, the present work was undertaken to study the effects of acute ethanol administration on pulsatile prolactin and LH secretion patterns in adult female rats. Rats were previously cannulated to allow a continous blood withdrawal to study the pulsatile patterns of prolactin and LH. The mean values of prolactin during the bleeding period and the absolute pulse amplitude of prolactin peaks were significantly increased by acute ethanol administration, whereas a significant decrease of relative pulse amplitude and frequency of this hormone was observed. On the other hand, ethanol administration increased the mean serum LH levels and the absolute and relative amplitudes of LH peaks. Ethanol treatment did not modify either frequency or duration of LH peaks. These data suggest that acute ethanol administration in adult female rats is followed by changes in the pulsatile prolactin and LH secretory patterns, which might be part of the mechanism to explain ethanol effects on the endocrine system.

Ethanol Prolactin LH Pulsatile secretion

SINCE the description of the existence of a pulsatile secretion pattern for LH in ovariectomized monkeys (10), a similar pulsatile secretion for other pituitary hormones, in humans and in laboratory animals, has been described (26).

In the rat, LH (13) and prolactin (26) are secreted in an episodic pattern, with the central nervous system (CNS) involved in the regulation of this process (15,22). Therefore, drugs that affect the CNS activity could modify the pulsatile secretion pattern of a number of hormones.

On the other hand, in the rat both acute and short-term ethanol administration are followed by decreased LH (4–8) and increased plasma prolactin levels (6,8), although the mechanism involved in ethanol effects on LH and prolactin is not fully understood. However, ethanol, which is widely consumed, might act by exerting diverse effects on the CNS activity. In fact, recent data from the literature point towards ethanol effects at the hypothalamic level, changing LHRH and dopamine secretion rates to the portal system (9,23), which might reflect changes in the pulsatile release of specific pituitary hormones.

Therefore, the present study was designed to analyze the pulsatile release of LH and prolactin following acute ethanol administration during the diestrous-2 phase of the estrous cycle in adult female rats.

[1] Requests for reprints should be addressed to Dra. Anunciación Lafuente, Departamento de Toxicología, Facultad de Ciencias, Universidad de Vigo, Campus de Orense, As Lagoas, 32004 Orense, Spain.

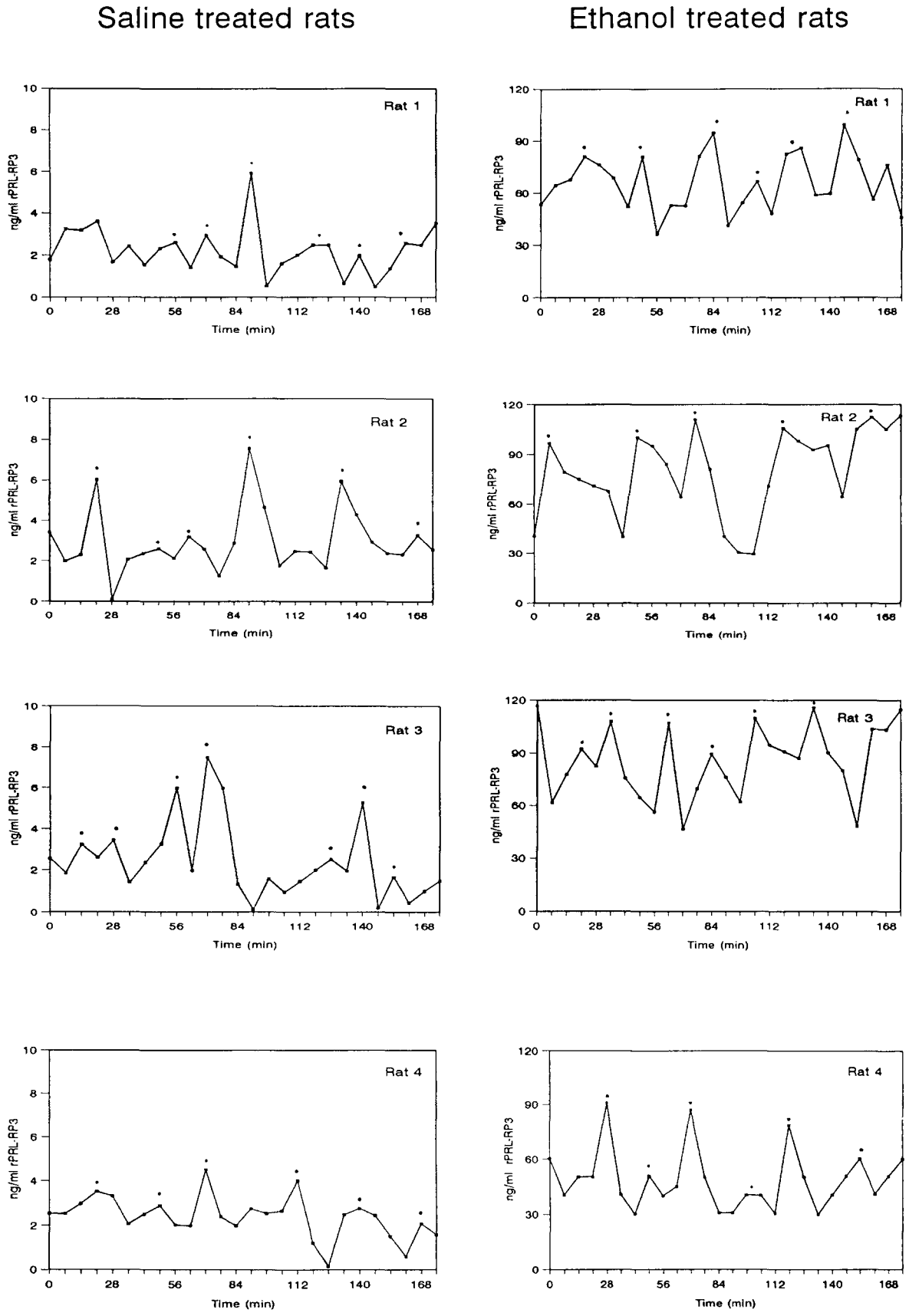

FIG. 1. Individual pulsatile prolactin patterns of four animals from each experimental group: saline or acute ethanol-treated rats. Asterisks indicate the prolactin peaks during the period studied.

METHOD

Animals

Adult female rats of the Sprague–Dawley CD strain, weighing 240–280 g, were used in all experiments. They were maintained in a room with controlled photoperiod (14 L : 10 D; lights on from 0600 to 2000 h), and temperature (22 ± 2°C), and were fed with rat chow and water ad lib. Vaginal smears were taken daily, and only rats demonstrating at least two consecutive 4-day estrous cycles were used in this study. Animals showing diestrous-2 phase of the estrous cycle were used in the study.

Cannula Implantation

Adult rats in the estrous phase of the cycle (40 h before the experiment) were anesthetized with tribromoethanol (2.5% tribromoethanol, 1 ml/100 g body weight) and atrial cannulas were implanted through the external jugular vein according to procedures used in previous studies (17,18). This procedure represents a system to get stress-free blood samples (17,18) and allows the animals to move freely in their cages during the period of bleeding.

Experimental Design and Blood Sampling

Rats were infused with 30% v/v ethanol-saline solution intravenously (IV) at dosage of 1 g/kg, 15 min before starting the bleeding period. Rats infused only with saline were used as a control group.

Half of the animals from each group was used to study the pulsatile pattern of prolactin and the other half were used to analyze the pulsatile pattern of LH. On the day of the experiment, conscious and freely moving rats from each group were continuously infused with 0.9% saline (0.5 ml/h) for 4 h, beginning at 0930 h. One hour after the IV infusion of saline and 15 min after the administration of 300 IU of heparin, rats were bled continuously through a peristaltic pump at a flow rate of 50 μl (for prolactin) or 75 μl every 7 min (for LH). Blood samples were collected in Hamilton microliter syringes every 7 min for 3 h from 1030 to 1330 h. The samples were collected into assay tubes kept on ice and containing phosphate buffer (0.01 mol/l) with 0.1% gelatin. Hematocrits remainded stable with this bleeding protocol (38–42%). Samples were centrifuged at 1500 × *g* for 15 min at 4°C and the serum was kept frozen at −20°C until analyzed.

Hormone Determinations

Prolactin and LH concentrations, in every series of samples from each rat, were measured by specific double antibody radioimmunoassay systems. The reagents were kindly supplied by the National Institute of Health (NIH, Bethesda, MD). Prolactin values were expressed in terms of the rat-NIADDK-PRL-RP-3 and LH in terms of the rat-NIADDK-LH-RP-3 reference preparations, respectively. The sensitivity of these assays were 5 pg/tube for prolactin and 0.5 pg/tube for LH. Samples were analyzed within the same assay to avoid inter-assay variations. The intra-assay coefficient of variation was 8% for prolactin and 7% for LH (18).

Data Analysis

To identify and characterize pulses appearing in the hormonal profile of each rat, a computer program (Ultra-analysis) described by Van Cauter (31) was used. In this program, a pulse is defined as a significant increase exceeding a multiple of the dose-adjusted coefficient of variance (CV), followed by a significant decrease. The intra-assay CVs were calculated from values of five different concentrations of prolactin and LH in their respective standard curves. Thus, the CV and the mean hormone level were determined for all hormone values that comprised the ascending and descending phases of each potential pulse. The pulse was defined when this CV was twice that of the intra-assay CV determined at a comparable mean prolactin level, and three times that of the intra-asays CV determined at a comparable mean LH level. To test the specificity of pulse detection, a series of 26 samples from a pool of serum was analyzed, one false positive being detected.

Pulsatile prolactin and LH secretion patterns were characterized by the mean hormone levels, absolute and relative amplitudes of the peaks, their frequency, and pulse duration. The absolute pulse amplitude was defined as the difference between the hormone level at the maximum of the peak and its level at the preceding nadir. The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Pulse frequency was the number of pulses/3 h. Pulse duration was the time between the beginning of the ascending phase of the peak and the end of the descending phase of the peak.

The mean hormone levels were calculated by the mean of all samples collected from each rat during the 3-h period, and the average for the experimental group from the individual means.

Statical Analysis of the Data

Comparison of values for the pulsatile parameters was done by analysis of variance (ANOVA) followed by Duncan's multiple range test or Student's *t*-test. The results were consid-

TABLE 1

MEAN SERUM PROLACTIN LEVELS, ABSOLUTE AND RELATIVE PULSE AMPLITUDE, AND FREQUENCY AND DURATION OF THE PROLACTIN PEAKS IN FEMALE RATS UNDER ETHANOL OR SALINE ADMINISTRATION

Group	PRL-RP-3 (ng/ml)	Absolute Amplitude	Relative Amplitude	Frequency (pulses/3 h)	Duration (min)
Saline	3.31 ± 0.38	3.38 ± 0.60	3.96 ± 0.87	7.3 ± 0.41	22.7 ± 1.30
Ethanol	59.6 ± 20.9*	33.2 ± 11.3†	1.30 ± 0.22†	5.8 ± 0.61†	25.0 ± 2.03

The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Values are expressed as mean ± SEM. The number of animals per group is eight.

*†Versus saline: $*p < 0.01$, $†p < 0.05$.

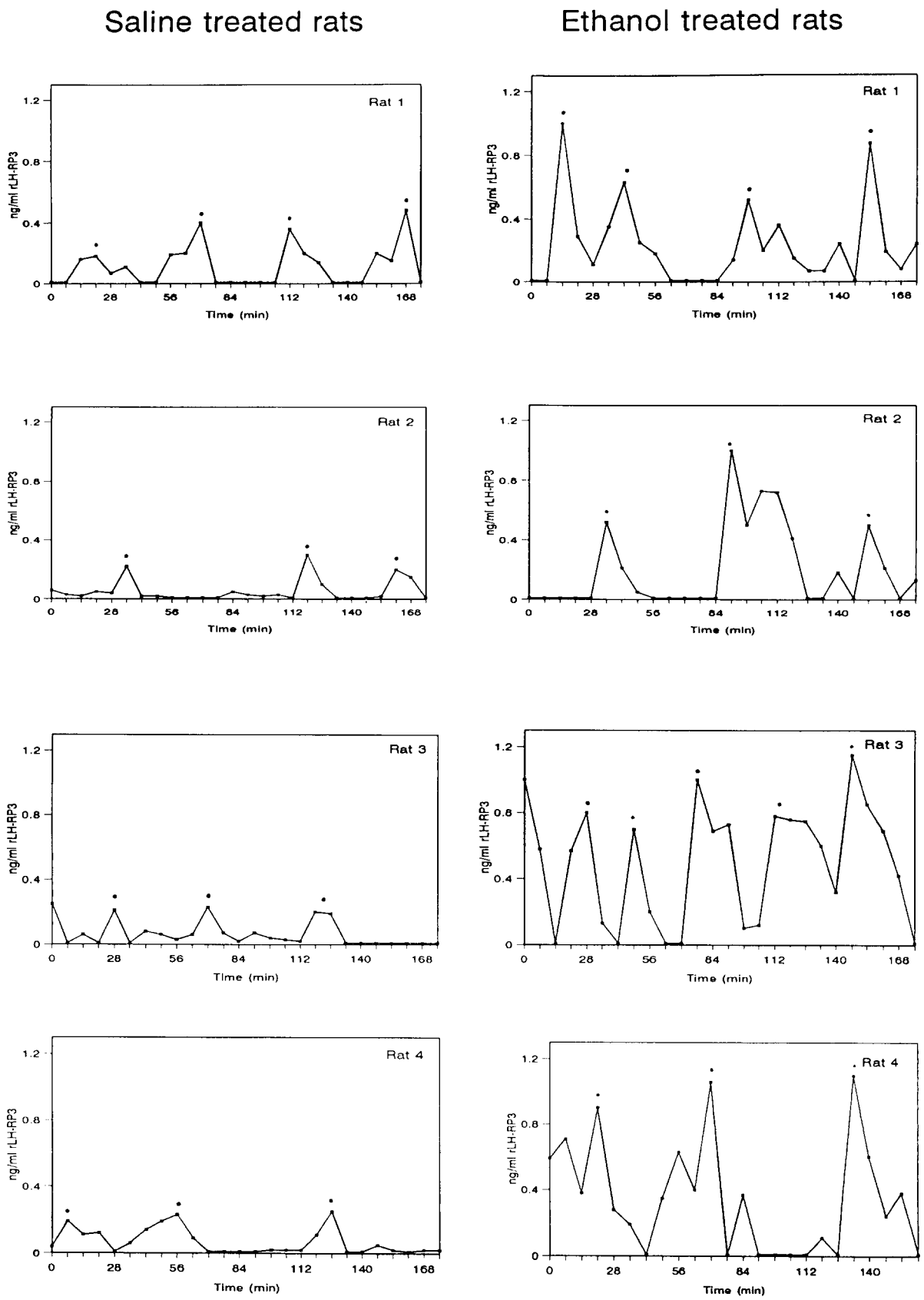

FIG. 2. Individual pulsatile LH patterns of four animals from each experimental group: saline or acute ethanol-treated rats. Asterisks indicate the LH pulses during the period studied.

ered significant at $p < 0.05$. All values represent the mean ± SEM.

RESULTS

Prolactin

Four representative profiles of the pulsatile prolactin secretion in each experimental group are shown in Fig. 1.

Ethanol administration significantly increased mean levels of prolactin and the absolute pulse amplitude of the hormone ($p < 0.01$ and $p < 0.05$, respectively) compared to saline-treated rats (Table 1). The relative amplitude and frequency of the prolactin peaks were decreased by ethanol administration ($p < 0.05$ vs. controls, Table 1) compared to vehicle-treated animals.

Administration of ethanol did not change the duration of prolactin pulses (Table 1) compared to saline-treated rats.

Luteinizing Hormone

Four examples of the pulsatile pattern of LH from each group are given in Fig. 2.

Treatment with ethanol markedly increased the mean values of LH during the bleeding period, and the absolute and relative pulse amplitudes of the hormone ($p < 0.01$, $p < 0.05$, $p < 0.001$, respectively) (Table 2). However, ethanol administration did not change either pulse frequency or duration of the LH peaks (Table 2) compared to rats infused with saline.

DISCUSSION

The data obtained in the present study suggest that acute ethanol administration induced changes in the pulsatile pattern of prolactin and LH. The quickness of the response suggests a hypothalamic-mediated effect of ethanol changing the rate of DA and/or LHRH release. However, changes at the pituitary level cannot be ruled out.

All these changes could be related to modifications in gonadal function, observed in rodents and man during chronic exposure to this drug (12,14). In fact, hyperprolactinemia associated with increased mean levels of LH also produced inhibitory effects on the reproductive function (32) using other experimental models.

Both control and ethanol-treated rats showed an irregular pattern of prolactin secretion that is characteristic of this hormone, in agreement with previous work of our laboratory (18) and from the literature (6).

Ethanol administration was followed by selective changes in some of the parameters that define the pulsatile pattern of prolactin. In fact, ethanol increased the circulating values of the hormone and the absolute amplitude of prolactin peaks together with a decrease in their frequency; all these parameters explain the increase in the mean levels of prolactin obtained in this study and also observed in previous works using a single sample to measure the circulating values of the hormone (1,12,24,25).

The high prolactin baseline observed in alcohol-treated compared to saline-treated rats was not due to stressfull conditions because chronic venous catheters allowed us to get stress-free blood collection, according to previous data from the laboratory (18). Also, as ethanol was administered 15 min before starting the bleeding period, the serum values of the hormone were already increased, as expected from previous works from the literature (6).

The changes in prolactin pulsatility obtained in this work are in agreement with those reported previously in ovariectomized rats (6,8). Ethanol effects in intact rats seemed to be more potent than in ovariectomized animals, thus suggesting that estrogens are modulating the ethanol effects on prolactin secretion. In fact, estrogen levels observed in diestrus-2 are higher than in diestrus-1 and can explain, to some extent, the observed changes in prolactin pulsatility, in agreement with the data reported by Babu et al. (2) for LH. Ethanol effects on prolactin pulsatility could be explained by the increase in hypothalamic DA content (23) shown during the acute stimulation with the drug, together with a decrease of the DA content at the hypophyseal level (23).

Effects of ethanol on other neuromodulators such as VIP or substance P might account for the oberved changes in prolactin pulsatility (16,27,28). Also, ethanol effects might be due to a direct effect of the drug on the lactotrophs, as was previously suggested (11).

The higher mean values of LH after acute treatment with ethanol are explained by the increase in the absolute amplitude of LH peaks. These effects are in agreement with previous data obtained in men after acute ethanol administration (3) and with the effects on LHRH-stimulated LH values in female rhesus monkeys (19,20). Also, ethanol did not inhibit the basal release of LH in women (21). However, opposite effects in man (30) and rodents (12) using a single sample to measure circulating levels of LH were observed. The discrepancies observed could be due to differences in the protocol of blood collection [single samples in previous studies (12) vs. multiple sampling in the present experiment]. Also, in man the timing of bleeding was every 20 min and the half-life of the hormone was estimated over 15 min.

On the other hand, other studies have shown a significant

TABLE 2

MEAN SERUM LH LEVELS, ABSOLUTE AND RELATIVE PULSE AMPLITUDE, AND FREQUENCY AND DURATION OF THE LH PEAKS IN FEMALE RATS UNDER ETHANOL OR SALINE ADMINISTRATION

Group	LH-RP-3 (ng/ml)	Absolute Amplitude	Relative Amplitude	Frequency (pulses/3 h)	Duration (min)
Saline	0.07 ± 0.01	0.22 ± 0.05	10.01 ± 1.60	3.1 ± 0.31	19.33 ± 2.01
Ethanol	0.34 ± 0.09*	0.50 ± 0.12†	56.87 ± 9.04‡	3.4 ± 0.26	17.66 ± 1.13

The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Values are expressed as mean ± SEM. The number of animals per group is eight.

*†‡Versus saline: *$p < 0.01$; †$p < 0.05$, ‡$p < 0.001$.

decline in plasma LH levels following acute intragastric ethanol administration in ovariectomized rats (6), thus suggesting that gonadal steroids may be involved in the effects of ethanol on pulsatile LH release, as was suggested previously for prolactin. However, the changes in LH might accounts for an impairment in gonadal function, as was suggested previously (29).

Although the pulse absolute amplitude of LH peaks was increased, their frequency and relative amplitude or duration were not changed, thus suggesting a pituitary site of action for ethanol to modify the episodic release of LH (11).

In conclusion, all these data suggest that acute ethanol administration in adult intact diestrous-2 female rats is followed by a significant and selective change in prolactin and LH pulsatility, which might be part of the mechanism of the drug to affect the endocrine system.

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