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THE EFFECT OF ACUTE ETHANOL ADMINISTRATION[1] ON SERUM LUTEINIZING HORMONE AND ANDROGEN LEVELS IN ADULT MALE MICE[1]

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Sheu-Yueh Roan and John Yuh-Lin Yu (1983) The effect of acute ethanol administration on serum luteinizing hormone and androgen levels in adult male mice. *Bull. Inst. Zool., Academia Sinica* **22**(2): 201-207. The acute inhibitory effects of ethanol on pituitary luteinizing hormone (LH) release and testicular androgen synthesis were studied in 10-wk old mice. Ethanol (2.0 g/kg, 20% V/V in 0.9% saline) was injected i. p. into animals concomitantly with or 0.5 hr prior to i. v. injection of gonadotropin releasing hormone (GnRH) [des-Gly^{10}, D-Ala^{6}-LRH ethylamide, 100 ng]. The controls received GnRH only. Blood samples were taken immediately before, and 1-, and 3 hr following GnRH injection. LH and total androgens in the serum were quantified by radioimmunoassays. The results revealed that the GnRH evoked considerable increases in serum LH and androgen levels at 1- and 3 hr intervals following its stimulation. Concomitant administrations of ethanol and GnRH significantly lowered the GnRH-induced elevations of serum LH and androgen levels at both time intervals examined. Ethanol, however, did not alter the hormonal levels when it was administered 0.5 hr prior to GnRH stimulation. It is concluded that ethanol also exhibits a direct action on pituitary release of LH in the hypothalamo-pituitary-testis axis. The findings from the present study also suggest that ethanol is rapidly turnoverd in the body, and consequently the inhibitory action of ethanol is likely to vary with the endogenous rhythmic releases of GnRH/LH.

Ethanol affects reproductive functions in humans and animals (Gordon and Southren, 1977). *In vivo* studies have shown that ethanol suppressed gonadotropin and androgen secretions following exposure to either chronic or acute treatment of ethanol (Badr *et al.*, 1974; Chapin *et al.*, 1980; Cicero *et al.*, 1979; Gnanaprakasan *et al.*, 1979; Gordon and Altman, 1976; Mendelson *et al.*, 1978; Symons and Mark, 1975; Van Thiel *et al.*, 1979). Evidences from both *in vivo* and *in vitro* studies indicated that ethanol exhibits a direct inhibitory action on androgen synthesis in the testis (Badr and Bartke, 1974; Cicero *et al.*, 1980; Ellingboe and Varanelli, 1979; Gordon *et al.*, 1978, 1980; Van Thiel *et al.*, 1981; Yu *et al.*, 1981b, 1982). The effect of ethanol on hypothalamo-pituitary axis, however, remains equivocal (Cicero *et al.*, 1978, 1979; Symons and Mark, 1975; Van Thiel and Lester, 1978; Van Thiel *et al.*, 1979). Whether ethanol exhibits a direct effect on pituitary gonadotropin secretion or not, requires further clarification.

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A study was thus conducted to illustrate the acute action of ethanol on pituitary release of LH into circulation in the mice. Ethanol was administered to the animals prior to or concomitantly with GnRH stimulation, and the serum levels of LH and androgen were subsequently quantified. The findings from the current investigation support that ethanol exhibits an acute and direct inhibitory action on GnRH-induced hypophysial LH secretions.

MATERIALS AND METHODS

Ten-week old male mice, ICR, U. S. A. were obtained from the National Laboratory Animal Resources, Taipei. The animals were raised in a temperature controlled room (22± 2°C), fed *ad libitum* with Purina Chow; the lighting schedule was 12L: 12D.

The animals were anesthetized with ether, and ethanol (2.0 g/kg of body weight, 20% V/V in 0.9% saline) was injected i. p. immediately or 0.5 hr prior to injection through tail vein of GnRH agonist (des-Gly^{10}, D-Ala^{6}-LRH ethylamide, M. W.: 1168, Beckman) of 100 ng/mouse in 10 μl of saline). Blood samples were taken through the heart puncture immediately before, and 1- and 3- hrs after injection of GnRH. Sera were prepared from the samples and stored at minus 20°C until analyses for LH and androgen contents.

LH radioimmunoassay

The radioimmunoassay kit was supplied by the U. S. National Institute of Health. LH was iodinated with ^{125}I by use of chloramine T and were purified through use of Sephadex G-75 column. The reference praparation was NIAMDD LH-RP-1. Double antibody assay was carried out. The detection limit of the assay was from 0.5 ng through 200 ng per assay tube.

Assay of androgen

The radioimmunoassay procedure for androgen was similar to that described previously (Yu *et al.*, 1981a). Such procedure quantified total amount of androgens since chromatographic separation of androgen was omitted. Briefly, the serum samples were extracted with diethyl ether (Merck) and allowed to freeze in dry ice-ethanol medium. The ether layer was dried under ventillation hood at 38°C. The dried residue was dissolved in PBS containing gelatin and then incubated at room temperature for one hour. Tritiated testosterone (1, 2, 6, 7-^{3}H-testosterone, 93.9 Curries/m mole, New England Nuclear) and testosetrone antiserum were added and then incubated for 16–20 hrs at 4°C. Dextran-coated charcol was employed to separate the antibodybound from the free steroid. Supernatant containing the bound labelled steroid was counted in a liquid scintillation spectrometer. The assay was sensitive to 10 pg of testosterone per assay tube. The between-assay coefficient of variation was 14.2% and the within-assay coefficient of variation was 5%.

The specificity of testosterone antiserum was described previously (Anderson *et al.*, 1975); it crossed-reacted with dihydrotesterone, androstedione and androstenediol at 90- ,12-, and 11%, respectively, relative to testosterone (100%). The concentration of androgen in the serum was expressed as testosterone equivalent extrapolated from the standard curve.

Statistical analysis

The data were statistically analyzed using paired student t-test (Mcarthur and Colton, 1970) to test the difference between controls and the ethanol treated group.

RESULTS

The LH and androgen levels in the serum of normal untreated mice are indicated in Table 1. Both values were low and remained relatively constant at the 3-hr experimental period. Following injection of GnRH analog, the serum LH levels increased considerably at one hour after the hormonal stimulation, being 2900% greater than the values before treatment (Fig. 1). At 3-hr interval, the LH values declined, but remaining at much higher levels than those before GnRH injection, Concomitant administration of both GnRH and

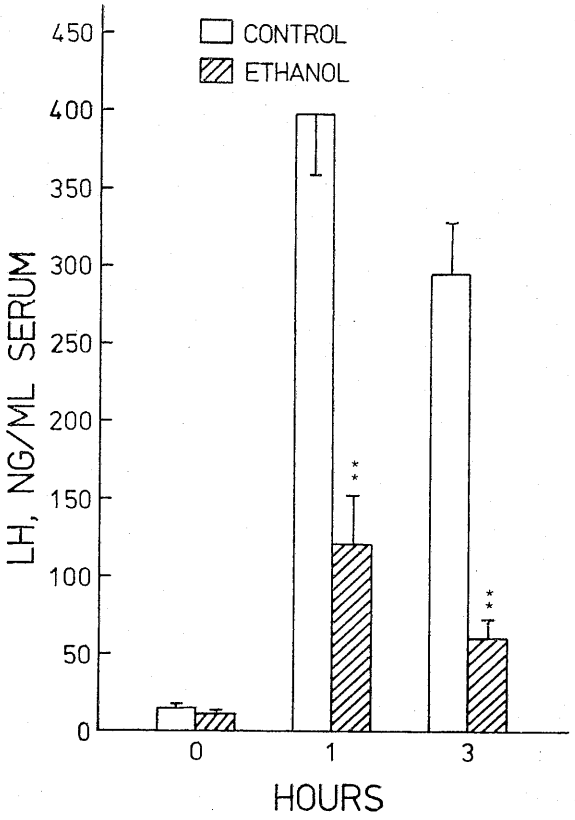

Fig. 1. Acute effect of ethanol on GnRH-induced changes in serum LH level of the mice following concomitant administration of ethanol and GnRH. The data are expressed as mean ±SEM (N=4). ** denotes the significance levels at $p \leqq 0.01$ between controls and ethanol group. The control animals received GnRH only.

TABLE 1
Serum LH and androgen levels of normal control 10-wk old mice during 3 hrs period*

Hormones	Hours		
	0	1	3
LH	8.62±0.27	12.68±1.37	14.23±1.82
Androgen	0.35±0.02	0.29±0.00	0.48±0.01

* Blood samples were taken from 12:00 through 15:00. The data are expressed as ng/ml serum ±SEM from 4 animals.

ethanol, however, remarkably lowered the serum LH levels at all time intervals examined; the extent of decreases was 30% and 20% at 1-hr and 3-hr intervals, respectively, as compared to those treated with GnRH only (contrd=100%).

As indicated in Fig. 2, the serum androgen levels were also elevated greatly at 1-hr and 3-hr intervals following GnRH administration, being parallel to the patterns observed for LH levels as shown in Fig. 1. When ethanol was administered to the animals in conjunction with GnRH, the GnRH-stimulated elevation of serum androgen levels were considerably decreased as well; the extent of decreases was 55% and 34% at 1-hr and 3-hr intervals, respectively, as compared to those treated with GnRH only (contrd=100%) (Fig. 2).

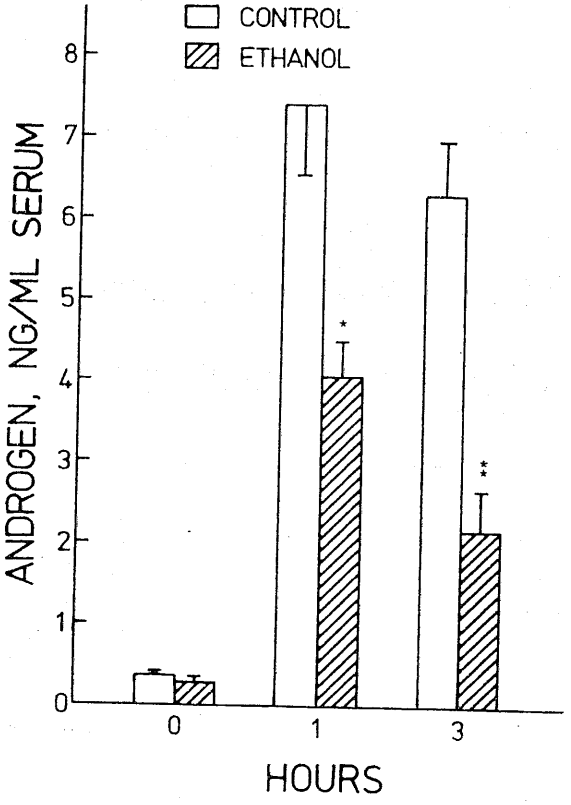

Fig. 2. Acute effect of ethanol on GnRH-induced changes in serum androgen levels of the mice following concomitant administration of ethanol and GnRH. The data are expressed as mean ±SEM (N=4). * and ** denote the significance level at $p \leqq 0.05$ and $p \leqq 0.01$, respectively, between controls and ethanol group.

Separate experiments were conducted to observe the effect on the changes of serum LH and androgen levels when ethanol was administered to the animals some time before the stimulation of GnRH, in comparison to the changes obtained by concomitant administration of both GnRH and ethanol. As shown in Fig. 3, when ethanol was given to the animals 0.5 hr prior to GnRH treatment, it did not alter the patterns in GnRH-induced elevation of serum LH levels at all time intervals examined, as compared to those treated with GnRH only. Similarly, the serum androgen levels were not affected when ethanol was injected 0.5 hr before GnRH stimulation (Fig. 4). The androgen levels were subsequently parallel to LH patterns, remaining

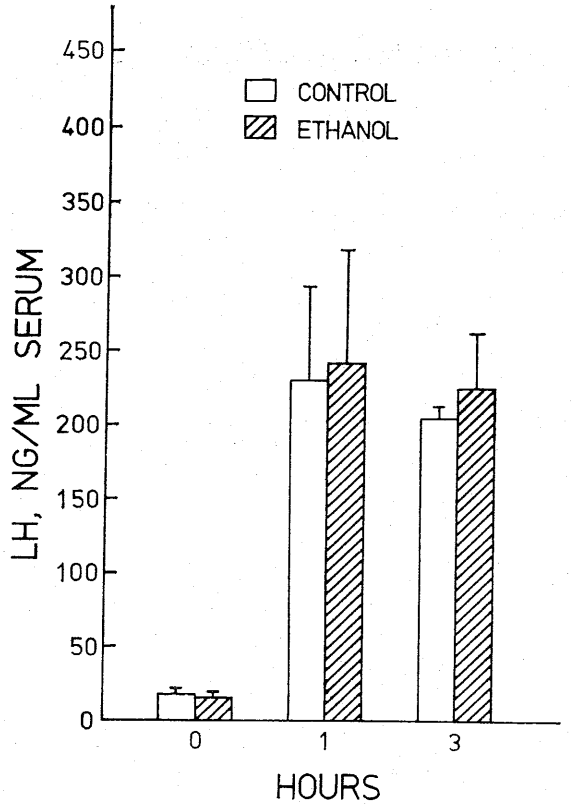

Fig. 3. The effect on serum LH levels of ethanol administered to the mice 0.5 hr prior to GnRH treatment. The data are expressed as mean ±SEM from 4 animals. The controls received GnRH only.

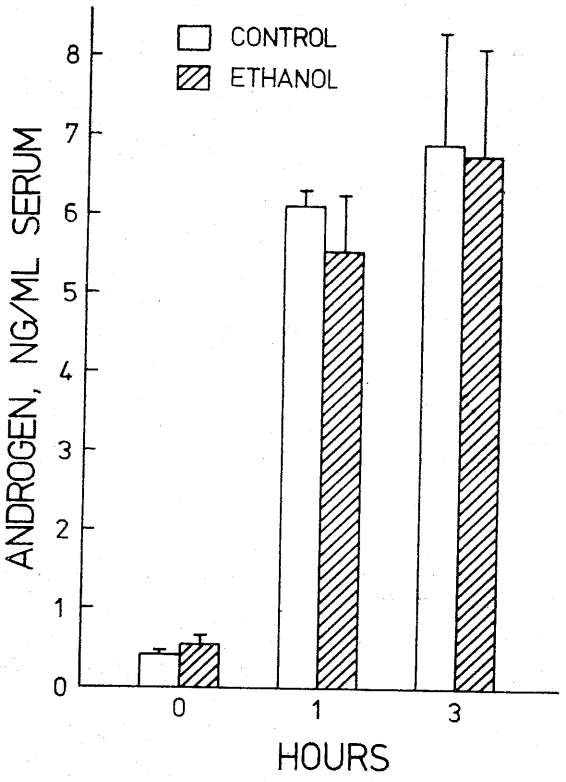

Fig. 4. The effect on serum androgen levels of ethanol administered to the mice 0.5 hr prior to GnRH treatment. The data are expressed as mean ±SEM from 4 animals.

elevated for an extended period of time following stimulation with the GnRH agonist.

DISCUSSION

It has been equivocal regarding the action of ethanol on pituitary gonadotropin secretions (Cicero *et al.*, 1978, 1979; Gordon *et al.*, 1978; Seymons and Mark, 1975; Van Thiel and Lester, 1978; Van Thiel *et al.*, 1979). Symons and Marks (1975) reported that the sensitivity of the anterior pituitary to exogenous luteinizing hormone-releasing hormone was the same in ethanol-fed and control rats, suggesting that the ethanolic effect on the reproductive endocrine system is at the hypothalamic or higher neural centers, since ethanol was capable of lowering circulating LH levels within one hr after being intubated. Cicero *et al.* (1978;

1979) subsequently demonstrated similar findings. They were unable to detect any effect of ethanol on the ability of GnRH to evoke a release of LH from male rat pituitary; instead, they observed that the blockade of castration-induced increase in LH levels by ethanol was completely reversed by systematically administered GnRH, and thus concluded that ethanol acts at the hypothalamus rather than at the pituitary. Other researchers indicated also that the major effect of ethanol on testicular androgen synthesis is at the peripheral (testicular level) rather than central (hypothalamo-pituitary) situ (Mendelson *et al.*, 1978; Gordon *et al.*, 1978). However, it was illustrated that continuous infusion of ethanol into castrated rats exhibited an inhibitory effect on GnRH-induced pituitary LH secretion (Van Thiel *et al.*, 1979). Such divergency is likely related to the pharmacological problems in the design of studies concerning the acute action of ethanol as tremendous variations exist among different laboratories in the doses and timing of administration of ethanol/GnRH employed in their studies. The results from the present study indicated that ethanol exhibited an acute inhibitory effect on serum LH levels when administered concomitantly with GnRH stimulation. Such findings thus support the proposal that ethanol directly blocks GnRH-induced LH secretions from pituitary, but without excluding the possibility that it also inhibits hypothalamic release of GnRH.

As illustrated in the current investigation the interfering action of ethanol on serum LH and androgen levels, exhibited by concomitant administration of GnRH and ethanol, was completely lost when ethanol was administrered one-half hr prior to GnRH stimulation. Such findings would indicate firstly that ethanol was rapidly turnovered following injection into the animals—being metabolized to inactive forms or virtually disappeared before GnRH stimulation, and secondly that the exposure of the animals to the toxic dose of ethanol one-half hr prior to GnRH administration did not affect the subsequent capability of the hypophysial secretion of LH and testicular synthesis of androgen. Such observations provide an explanation of the equivocal actions of ethanol obtained in humans and animals under *in vivo* conditions (Chapin *et al.*, 1980; Cicero and Badger, 1977; Cicero *et al.*, 1978; Mendelson *et al.*, 1978). It has been demonstrated that ultradian rhythm of hypophysial LH secretions and testicular androgen synthesis occurs in humans and mammals (Gordon *et al.*, 1978; Smith *et al.*, 1974; Steiner *et al.*, 1980; Vande Wiele and Ferin, 1974; Yen *et al.*, 1974). Consequently, differential effects of ethanol on LH and androgen secretions are likely to exist depending on endogenous rhythmic releases of hypothalamic GnRH and pituitary LH at the particular physiological conditions during the experimental period.

Evidences have also shown that ethanol affects androgen formation capacity of the gonads from humans and animals (Bard and Bartke, 1974; Cicero *et al.*, 1980; Ellingboe and Varanelli, 1979; Gordon *et al.*, 1978; Van Thiel *et al.*, 1981; Yu *et al.*, 1981b, 1982). *In vitro* studies in animals demonsrated that ethanol acutely inhibits testicular androgen synthesis in the dispersed interstitial cells. Although the exact mode of ethanol action on the testis remains unclear, evidences from the *in vitro* studies suggest it directly interferes the intracellular steroidogenesis (Bard *et al.*, 1977; Cicero *et al.*, 1980; Ellingboe and Varanelli, 1979; Mendeson *et al.*, 1977; Van Thiel *et al.*, 1981; Yu *et al.*, 1981b, 1982). The testicular androgen formation is regulated principally by LH stimulation, and the serum androgen levels are thus fluctuating along with the pre-existing LH levels in the circulation. As observed in the present study, the serum androgen levels were conspicuously lowered when ethanol was concomitantly administered with GnRH; the patterns being parallel to those of LH levels. Since ethanol can act directly on both pituitary LH release and testicular androgen synthesis, the inhibition of androgen formation observed in the present study represented the combined effects of ethanol-induced decrement in LH

secretions and of the direct ethanolic inhibition of testicular intracellular formation of androgen.

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酒精對小白鼠腦下腺及睾丸性腺激素分泌之作用

阮 秀 月　　余 玉 林

本研究探討酒精對 10 週齡雄性小白鼠腦下腺黃體生成激素 (LH) 及睾丸雄性素 (Androgens) 分泌之急性抑制作用。實驗組：酒精 (2.0 g/kg, i. p.) 與合成之腦下視丘性釋激素 (GnRH) [des-Gly^{10}, D-Ala^{6}-LRH, 100 ng, i. v.] 同時注射，或注射 GnRH 前半小時注射酒精。在 GnRH 注射前，及注射後 1 及 3 小時採血以作爲放射免疫法定量黃體生成激素及雄性素。

結果指出，性釋激素于注射後 1 及 3 小時，顯著提高血清 LH 及 Androgen 之濃度。如同時注射酒精，則使 GnRH 提升之血清 LH 及 Androgen 濃度顯著降低；但如酒精先於 GnRH 注射前半小時給予，則不改變血清 LH 及 Androgen 之濃度。以上結果顯示酒精直接作用於腦下腺，抑制黃體生成激素之分泌。亦提示酒精在體內迅速代謝，因此其抑制作用極可能須視當時體內所分泌 GnRH/LH 量之多寡而異。