Bull. Inst. Zool., Academia Sinica **21**(1): 103-111 (1982)

EFFECTS OF ETHANOL ON LUTEINIZING HORMONE-STIMULATED ANDROGEN SYNTHESIS BY DISPERSED INTERSTITIAL CELLS FROM MOUSE TESTIS[1]

J. YUH-LIN YU

Endocrinology Laboratory, Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan 115, R. O. C.

SHEU-YUEH ROAN and ZUEY-SHIN HSU

Department of Physiology, Institute of Medicine, Kaohsiung Medical Coolege Kaohsiung, Taiwan 800, R. O. C.

(Received July 22, 1981)

J. Yuh-Lin Yu, Sheu-Yueh Roan and Zuey-Shin Hsu (1982). Effects of Ethanol on Luteinizing Hormone-Stimulated Androgen Synthesis by Dispersed Interstitial Cells from Mouse Testis. *Bull. Inst. Zool., Academia Sinica* **21**(1): 103-111. The effects of ethanol on LH-induced androgen synthesis in the mouse testis was studied using dispersed interstitial cell preparations. The *in vitro* dose-response relationship was established previously for ethanol on androgen formation (Bull. Inst. Zool. Acad. Sinica, 20(1): 67-74 (1981); low dose of ethanol (0.63%) was stimulatory while median dose (2.5%) and high dose (5%) were partially and completely inhibitory on androgen production, respectively. The present study was essentially based on the response exhibited by low and median doses of ethanol to further demonstrate the possible modes of action of ethanol on the hormone-induced androgen synthesis. Cells were incubated in medium 199 with simultaneous presence of ethanol and rat LH or with ethanol added at various time intervals following LH stimulation. Androgen produced was assayed radioimmunologically using anti-testosterone serum. The results and conclusion are summarized as follows: 1) differential effects of ethanol were obtained on LH-induced androgen synthesis depending on whether ethanol was exposed to the cells simultaneously with or after the hormone stimulation. The interfering action of ethanol was largely reduced or totally abolished when ethanol was added to the cells at certain time intervals following LH stimulation. Such results indicate that the action of ethanol on steroidogenesis is considerably reduced once the androgen synthesis has been evoked by LH; 2) the androgen formation capacity of the cells was similar following exposure to ethanol for either 0.5 hr or 4-6 hrs, indicating the action of ethanol is likely instantaneous; and 3) the inhibitory action of ethanol on androgen formation could not be reversed by additions of increasing or excess amounts of LH, suggesting that ethanol does not compete with LH for the membrane receptors, and thus noncompetitively inhibits the effects of the hormone.

Ethanol affects reproductive functions in humans and animals[2~10,12,13,21]. *In vivo* studies have shown that ethanol lowered gonadotropin and androgen secretions following exposure to either chronic or acute treatment of ethanol[2~7,10,11,13,14,17]. A direct inhibitory action of ethanol on testicular androgen synthesis has

1. Paper No. 229 of the Journal Series of the Institute of Zoology, Academia Sinica.

been demonstrated also under the *in vitro* conditions[4,8,9,25]. The exact mode of the action of ethanol on such changes remain unclear.

In previous paper[25], we reported that ethanol exhibits a direct action on androgen formation by the dispersed mouse testicular interstitial cells; the actions are dual-stimulatory with low dose while inhibitory with high dose. As a series of investigations, we conducted further studies concerning the *in vitro* action of ethanol on the luteinizing hormone (LH)-stimulated androgen synthesis by the dissociated interstitial cells from the mouse tstis. In this communication we report 1) the relationship between ethanol and varying amounts of LH on androgen formation capacity of the interstitial cells; 2) the differential effects of ethanol which was exposed to the cells at different time intervals prior to or post the LH stimulation; and 3) the effects on androgen synthesis of the cells following short time exposure to ethanol. Such *in vitro* data from the present study provide some basic information regarding the mode of actions of ethanol on LH-induced androgen formation in the interstitial cells of the testis.

MATERIALS AND METHODS

Four experiments were conducted to study the effects of ethanol on the *in vitro* androgen formation capacity of the mouse testicular interstitial cells isolated by mechanical dispersion. Each experiment consisted of three separate incubations carried out at different times.

Preparation of Interstitial cells from the mouse testis

Six-wk old mice, ICR, U.S.A., were purchased 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 method in preparation of testicular interstitial cells was similar to that described previously[24,25]. Briefly, after the mice were sacrificed the testes were removed and placed in a plastic Petri dish containing preincubation medium (Medium 199 with Hank's salts, L-glutamine and 25 mM HEPES buffer, penicilin 10,000 units/100 ml, streptomycin 5 mg/100 ml, 0.2% bovine serum albumin, 10% sodium bicarbonate 1.0 ml/100 ml, pH 7.40). The testes were cut into small pieces and were then gently dispersed for 15 min with a magnet stirrer surrounded by an ice-bath; the medium was repeatedly drawn into a Pasteur pipet over several minutes until a homogenous suspension was then filtered through a fine nylon mesh, and preincubated for one hour at 34°C. The cell suspension was cooled in ice-water, and centrifuged. Sedimented cells were suspended in incubation medium (preincubation medium + 0.125 mM methyl-isobutyl-xanthine + sodium heparin, 0.5 ml/100 ml). Incubation was performed in a tightly capped polyethylene tube at 34°C, shaken at 100 cycles/min in a Dubnoff metabolic incubator. The total volume in an incubation tube was 220 μl. Various doses of ethnol and rat LH-RP-1 were incubated with the interstitial cells (approximatey 0.35×10^6 cells per tube). Following incubations (the time period of incubations depending on specific experiments), the tubes were added with 0.01 M PBS and were centrifuged. The supernatant was collected and stored at −25°C until assay for androgen.

Assay of androgen

The radioimmunoassay procedure for androgen was similar to that described previously[24,25]. Such procedure quantified total androgens since a chromatographic separation of androgens was omitted. Briefly, the PBS diluted incubation medium was extracted with diethyl ether (Merck) and allowed to freeze in dry ice-ethanol medium. The ether layer was decanted into another tube and dried under ventillation hood at 38°C. The dried residue was dissolved in PBS containing gelatin and incubated at room temperature for one hour. Triated testosterone (1, 2, 6, 7-^{3}H-testosterone, 88.5 Curries/m mole, Amershan) and testosterone antiserum were added and then incubated for 20 hrs at 4°C. Dextran-coated charcol was employed to separate the antibody-bound from the free steroid. Supernatant containing the bound labaled

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%. Standard and incubation samples produced parallel displacement of triated testosterone.

The specificity of testosterone antiserum was described previously[1]; it cross-reacted with dihydrotestosterone, androstenedione, and androstenediol at 90-, 12-, and 11%, respectively, relative to testosterone (100%). The concentration of androgen in the sample was expressed as testosterone equivalent extrapolated from the standard curve. The data were statistically analyzed using the Least Significance Difference (LSD) to test the difference between controls and ethanol treatments.

RESULTS

Androgen formation capacity as influenced by exposure to ethanol of the interstitial cells that were previously stimulated with LH

In our previous study[25], it was demonstrated that LH-stimulated androgen formation in the mouse testicular intestitial cells were altered by exposure to ethanol; in such study, both LH and ethanol were simultaneously incubated with the cells for 3 hrs. The results revealed that low doses of ethanol (0.63% and 1.25%) increased the LH-induced androgen formation; median dose (2.5%) exhibited a partial inhibition while high doses (5% and 10%) of ethanol completely blocked the androgen synthesis. An experiment was thus conducted to determine whether the ethanol exhibits any actions on androgen formation capacity of the cells after steroidogenesis has began for certain time periods with LH stimulation? If so, what are the quantitative patterns of such influences?

In the present experiment, the androgen produced by the control cells (cells stimulated with LH only) was measured at various time intervals during the 6 hrs incubation period. As indicated in Fig. 1, the androgen formation was essentially increased linearly with time during the incubation period. In the experimental groups the cells were incubated with LH for 0.5, 1, or 2 hrs before additions of different doses of ethanol, and were then incubated for a total period of 6 hrs. The results revealed that cells incubated simultaneously with 12.5 ng of rat LH and low doses of ethanol (0.63%) produced slightly more androgen as compared to the controls; such dose of ethanol, however, did not alter the androgen production when added to the cells that were previously stimulated with LH for one hour. Median dose of ethanol (2.5%) reduced the androgen production by about 60% when simultaneously incubated with LH or added 0.5 hr after the cells treated with LH; the inhibition was less when ethanol was added to the incubation one hour later; if ethanol was added 2 hrs following the cells incubated with LH, no inhibition on androgen production was observed. High dose of ethanol (5%) completely blocked the androgen formation once it was added to the incubations regardless of the timing of LH stimulation.

Androgen formation patterns of the interstitial cells that were exposed to ethanol prior to LH-stimulation

In the experiments described above, the ethanol was added to the cells simultaneously with LH or following the hormonal stimulation. A separate experiment was carried out to examine the influence of ethanol on androgen synthesis of the interstitial cells which were exposed to ethanol for one hour before LH stimulation. As indicated in Fig. 2, the patterns of androgen production, as influenced by different doses of ethanol, were essentially similar to the patterns obtained when the cells were simultaneously incubated with LH and ethanol (Fig. 1). That is, low doses of ethanol increased the androgen formation; meadian dose exhibited a partial inhibition while high doses completely suppressed the androgen synthesis.

Effects of short time exposure of ethanol on the androgen formation capacity of interstitial cells

In all experiments reported so far, the cells were exposed to ethanol for 4 to 5.5 hrs during

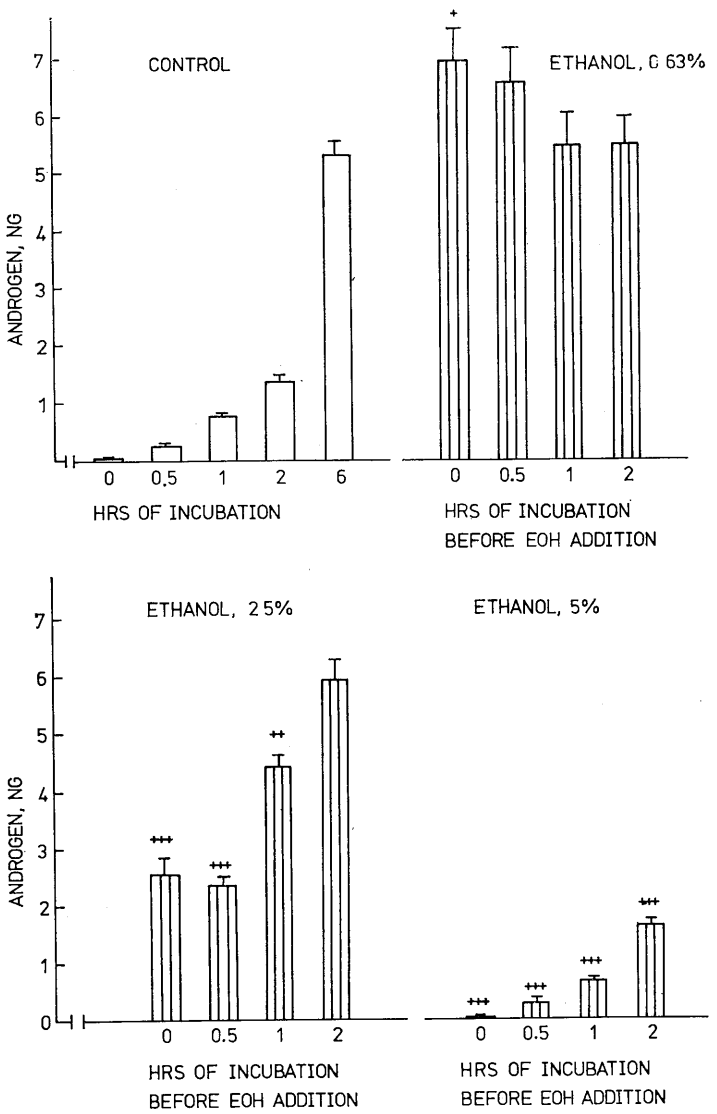

Fig. 1. The effects of ethanol on androgen formation by the dispersed mouse testicular interstitial cells that were previously incubated with 12.5 ng of rat LH-RP-1 for various durations of time. The androgen produced before ethanol added (the controls) was compared with androgen produced in the parallel ethanol-treated tubes. The total incubation period was 6 hrs. The data are expressed as mena± SEM for three different incubation experiments. The symbols +, ++, and +++ denote the significance levels, respectively at $p \leq 0.2$, $p \leq 0.05$, and $p \leq 0.01$ between control (incubated for 6 hrs) and the ethanol treated groups.

the 6 hrs incubation period. An experiment was designed to compare the influence of short time exposure of median and high doses of ethanol to the cells on their androgen formation capacity. The cells were incubated for 0.5 or 1 hr with ethanol which was then removed by repeated washing and centrifuging of the cells with incubation medium. The cells were then

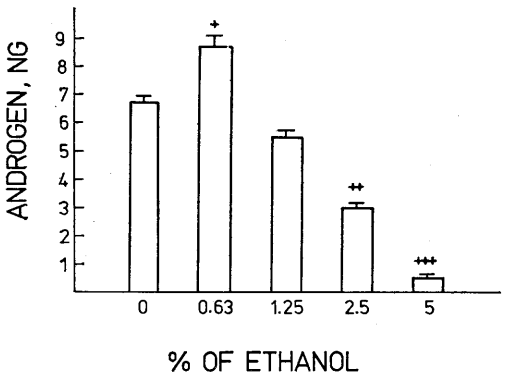

Fig. 2. Androgen synthesis capacity of mouse testicular interstitial cells that were exposed to ethanol prior to LH stimulation. Cells were exposed to various doses of ethanol for one hour before incubation with rat LH-RP-1 for 6 hrs. The values are expressed as mean±SEM from three incubations carried out at different times. The symbols +, ++, and +++ denote the significance levels respectively at $p \leq 0.2$, $p \leq 0.05$, and $p \leq 0.01$ between the controls (without ethanol) and the ethanol treated groups.

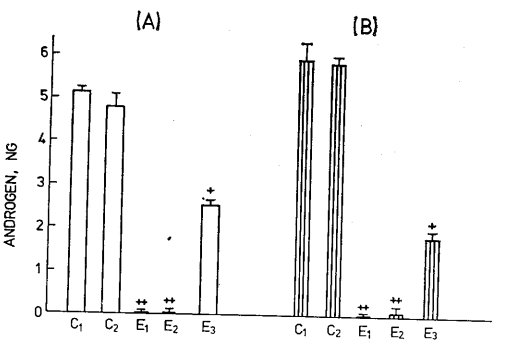

Fig. 3. The effect of short time exposure of ethanol on androgen formation capacity of the dispersed testicular interstitial cells from mice. Cells were preincubated with ethanol for 30 min (A) or 60 min (B). The cells were twicely washed and centrifuged, and then incubated with 12.5 ng of rat LH-RP-1 for 6 hrs. C1 and C2 denote the control cells, respectively, without and with washing and subsequent centrifugation. The values are expressed as mean ± SEM from three incubations carried out at different times. The symbols + and ++ denote the significance levels, respectively at $p \leq 0.05$ and $p \leq 0.01$ between the controls (C1 or C2) and the ethanol treated groups.

incubated with 12.5 ng of LH for 6 hrs; the androgen production capacity of ethanol treated cells were compared to the control cells which were treated identically except without ethanol exposure. As indicated in Fig. 3, wasing and subsequent centrifugation of the cells did not reduce the androgen production when compared to the cells without washing and centrifugation. Cells treated with 2·5% of ethanol for 0.5 or 1 hr produced less amount of androgen as compared to the controls. Exposure of the cell with high doses of ethanol (5% and 10%) resulted in complete inhibition of androgen formation.

Effects of ethanol on androgen formation of interstitial cells as stimulated with increasing amounts of LH

A study was conducted to demonstrate whether the inhibitory action of median or high doses of ethanol on the androgen formation of the interstitial cells can be reversed by additions of increasing or excessive amounts of LH. The cells were incubated for 3 hrs simultaneously with 2 to 125 ng of LH and various doses of ethanol. As indicated in Fig. 4, the androgen production by testicular interstitial cells was essentially linear as stimulated with 4 to 32 ng of rat LH-RP-1, and attained maximum with larger amounts of the hormone. Additions of 1.8% or 2.5% of ethanol partially suppressed the androgen synthesis capacity of the cells as stimulated by varying amounts of LH; the extent of such suppression were parallel to that obtained in the control cells. High dose of ethanol (5%) exhibited a complete inhibitory action on androgen production regardless of the presence of increasing or excessive amounts of LH.

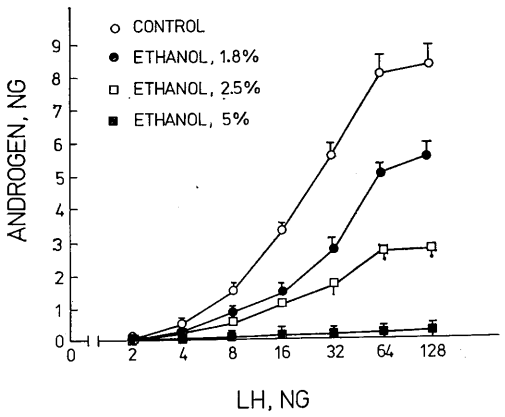

Fig. 4. The effect of ethanol on androgen synthesis by dispersed mouse testicular interstitial cells in response to increasing amounts of rat LH-RP-1. Incubation period was 3 hrs. Data are expressed as mean±SEM from three incubation experiments.

DISCUSSION

Both *in vivo* ad *in vitro* studies have shown that ethanol directly affects the androgen formation capacity of the gonads from humans and animals[2,3,5~11,13,14,19,20,25]. The regulation of LH-stimulated steroidogenesis in the gonad is complex, involving various factors such as receptor binding, cyclic AMP mediation, precursor availability. The mechanism under which the ethanol affects testicular synthesis of androgen is little understood[4,8,12,14]. The acute effect of ethanol on androgen formation of the interstitial cells have been studied mostly by administering ethanol simultaneously with LH[2~5,8,9,11]. By using such pattern of the studies, we have reported that ethanol exhibits dual actions on the androgen synthesis by the mouse testicular interstitial cells under *in vitro* conditions[25]; that is, stimulatory with low dose while inhibitory with high dose. Further studies were undertaken to demonstrate the differential effects of ethanol on androgen synthesis capacity when ethanol was exposed to the cells at certain time intervals following stimulation with LH. The results from the current investigation indicate that the interfering action of ethanol on androgen formation, as exhibited by simultaneous presence of ethanol and LH, was considerably reduced or completely lost when ethanol was added to cells certain time following the hormonal stimulation. These findings indicate that the action of ethanol on LH-induced androgen formation varies with different timing of the hormonal stimulation. Such differential effects of ethanol obtained by *in vitro* studies are probably useful for explanation of the equivocal effects of ethanol on the androgen formation patterns observed in humans and animals under *in vivo* conditions[5,6,23]. It has been illustrated that ultradian rhythm of hypophysial LH secretion occurs in humans and mammals[15,16,18,22]. Consequently, the *in vivo* effects of ethanol on LH-induced steroidogenesis in the testis are likely related to the time sequence of LH secretion at a particular moment. The marked variation observed regarding the effects of ethanol treatment or drinking on the endogenous LH-regulated androgen synthesis in humans and animals may also be concerned with such likelihood[5,6,23].

In general, the *in vitro* actions of ethanol on androgen formation were demonstrated under the conditions where the cells were incubated with ethanol for several hours[3,5,8,9,25]. A study was consequently undertaken to determine the length of time needed for ethanol to exert its action after being exposed to the cells. The results indicated that the androgen formation capacity was affected following exposure to low or median doses of ethanol for as short as 0.5 hr, shortest time interval studied. The extent of stimulation or inhibition on androgen synthesis was, however, as much as that observed for the cells exposed to ethanol for 3 or 6 hrs. Moreover, the androgen production capacity was found to be irreversible following treatment of cells with ethanol for 0.5 hr. Such findings indicate that the action of ethanol, even at low dose, is instantaneous, and the effect is not reversible under the experimental conditions of the present study.

The studies concerned with stimulatory or

partially inhibitory action on LH-induced androgen synthesis exhibited, respectively, by low dose and median dose of ethanol are of physiological significance. As illustrated also in the present study, high dose of ethanol (5% and 10%) completely suppressed androgen synthesis once the agent was exposed to the cells regardless of the amounts or timing of LH stimulation. Such inhibitory action of high dose of ethanol is presumably the general detrimental effect to the cells, and is not specifically destructive to the intracellular steroidogenesis.

In vivo studies have shown that ethanol inhibits the binding of ^{125}I-HCG to rat Leydig cells[12]. However, such effect was not observed by the *in vitro* works where testicular homogenates were incubated with ethanol and ^{125}I-HCG[7]. Consequently, whether the ethano influences the hormone receptor binding capacity remains inconclusive. We have studied, under *in vitro* conditions, to inquire whether the inibitory action of ethanol can be reversed by additions of excess amount of LH. The data obtained in the present investigation indicate that the relative extent of inhibition of androgen synthesis in the cells, as exhibited by median doses of ethanol, was similar regardless of the increasing amounts of the hormone used. Furthermore, the patterns of androgen formation by the cells that were exposed to ethanol prior to LH stimulation, were similar to that obtained when the cells were incubated simultaneously with the hormone and ethanol (Fig. 1). These findings suggest that ethanol does not compete with LH for the membrane receptors; in another words, ethanol noncompetitively inhibits the effects of the hormone. Cicero *et al.*[7] studied the *in vivo* effects of ethanol on plasma testosterone level in the rat, with increasing amounts of HCG. Their results are very comparable to that obtained by our present *in vitro* study. In contrast, it is shown that ethanol can decrease the intracellular level of pyridine nucleotide cofactors which are important in the biosynthesis of steroids[11]. Also, ethanol can inhibit the cyclic AMP-induced androgen formation; the patterns were similar to that obtained with LH-stimulated androgen synthesis. It thus appears that the primary locus of ethanol action is intracellular rather than the plasma membrane receptors of the Leydig cells.

Acknowledgements: We are grateful to National Pituitary Agency, NIAMDD, NIH, U. S. A., for the supply of rat LH, and to the Department of Medicine, University of Washingto, Seattle for supplying anti-testosterone serum. We would also like to thank the Institute of Botany, Academia Sinica for the use of liquid scintillation spectrometer. Appreciations are extended to Dr. W. C.-M. Wan for his assistance during various phases of this work.

REFERENCES

1. ANDERSON, P. H., K. FUKUSHIMA and H. S. SCHILLER (1975) Radioimmunoassay of plasma testosterone, with use of polyethylene glycol to separate antibodybound and free hormone. *Clin. Chem.* **21**: 708-714.
2. BADR. F. M. and A. BARTKE (1974) Effect of ethyl alcohol on plasma testosterone level in mice. *Steroids.* **23**: 921-928.
3. BADR, F. M., A. BARTKE, S. DALTERIO and W. BULGER (1977) Suppression of testosterone production by ethyl alcohol. possible mode of action. *Steroids* **30**: 921-926.
4. CHAPIN, R. E., G. R. BREESE and R. A. MULLER (1980) Possible mechanisms of reduction of plasma LH by ethanol. *J. Pharmacol. Exp. Ther.* **211**: 6-10.
5. CICERO, T. J. and T. BADGER (1977) Effects of alcohol on the hypothalamic-pituitary-gonadal axis in the male rat. *J. Pharmacol. Exp. Ther.* **201**: 427-433.
6. CICERO, T. J., D. BERNSTEIN and T. M. BADGER (1978) Effects of acute alcohol adminstration on reproductive endocrinology in the male rat. *Alcoholism: Clin. Exp. Res.* **2**: 249-254.
7. CICERO, T. J., E. R. MEYER and D. BELL (1979) Effects of ethanol on the hypothalamic-pituitary-luteinizing hormone axis and testicular steroidogenesis. *J. Pharmacol. Exp. Ther.* **203**: 201-215.
8. CICERO, T. J., R. D. BELL, E. R. MEYER and T. M. BADGER (1980) Ethanol and acetaldehyde directly inhibit testicular steroidogenesis. *J. Pharmacol. Exp. Ther.* **213**: 228-233.

9. ELLINGBOE, J. and C. C. VARANELLI (1979) Ethanol inhibits testosterone biosynthesis by directly action on Leydig cells. *Res. Comm. Chem. Patho. Pharmacol.* **24**: 87-103.
10. GORDON, G. G., A. L. SOUTHREN and C. S. LIEBER (1978) The effects of alcoholic liver disease and alcohol ingestion on sex hormone. *Alcoholism: Clin. Exp. Res.* **2**: 259-263.
11. GORDON, G. G., J. VITTEK, A. L. SOUTHREN, P. MUNNANGI and C. S. LIEBER (1980) Effect of chronic alcohol ingestion on the biosynthesis of steroids in rat testicular homogenate *in vitro*. *Endocrinology.* **106**: 1880-1885.
12. GNANAPRAKASAN, M., J. H. CHEN, G. SUTHERLAND and V. K. BHALLA (1979) Receptor depletion and replenishment processes: in vivo regulation of gonadotropin receptor by LH, FSH and ethanol in rat testis. *Biol. Reprod.* **20**: 991-1000.
13. GORDON, G. C. and K. ALTMAN (1976) Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. *N. Eng. J. Med.* **295**: 793-797.
14. MENDELSON, J. H., J. ELLINGBOE, N. K. MELLO and J. KUEHULE (1978) Effect of alcohol on plasma testosterone and LH levels. *Alcoholism: Clin. Exp. Res.* **2**: 255-258.
15. SMITH, K. P., R. K. TCHOLAKIAN, M. CHOWDHURY and E. STEINBEZGER (1974) Rapid oscillations in plasma levels of testosterone, LH and FSH in men. *Fert. Steril.* **25**: 965.
16. STEINER, R. A., A. P. PETERSON, JOHN Y. L. YU, H. CONNER, M. GILBERT, B. TERPMNING and W. J. BERMNER (1980) Ultradian LH and testosterone rhythms in the adult male monkey, macaca fascicularis. *Endocrinology.* **107**: 1493.
17. SYMONS, A. M. and V. MARKS (1975) The effect of alcohol on weight gain and the hypothalamic-pituitary-gonadotropin axis in the maturing male rat. *Biochem. Pharmacol.* **24**: 955-958.
18. VANDE WIELE, R. L. and M. FERIN(1974) The control of pulsatile gonadotrophin secretion. *In* Chronobiological Aspect of Endocrinology Symposia Medicu Hoechst 9, Stuttgart, Schattanet, p. 203-211.
19. VAN THIEL D. H. and J. GAVALER (1974) Ethanol inhibition of Vitamin A metabolism in the testes: Possiblemechanism for sterility in alcoholics. *Science.* **186**: 941-942.
20. VAN THIEL D. H., J. S. GAVALER, C. F. COBB, R. J. SHERINS and G. LESTER (1979) Alcohol-induced testicular atrophy in the adult male rat. *Endocrinology.* **105**: 888-895.
21. WALLGREEN, H. and H. BARRY (1970) In "actions of alcohol". Vol. 1. Chapters 4, 5, 6, and 7. pp. 156-387. Elsevier Pub. Co. New York.
22. YEN, S. S. C., VANDEUBERG, G. and TSAI, C. C. (1974) Ultradian fluctuations of gonadotrophins. *In* Terin, M., Halberg, F., Richart, R. M. *et al.* (Eds). Biorhythms and Human Reproduction. New York. John Wiley, pp. 203-218.
23. YLIKAHRI, R., HUTTUNEN, M., HAKONEN, M. *et al.* (1974) Low plasma testosterone values in men during hangover. *J. Steroid Biochem.* **5**: 655-658.
24. YU, J. Y. L., T. Y. CHANG, H. K. HSU, C. F. LIAO and W. C. M. WAN (1981) Androgen/Testosterone synthesis by the dissociated testicular cells from mice of different ages in response to rat LH stimulation *in vitro*. *Bull. Inst. Zool., Academia Sinica* **20**(1): 57-65.
25. YU, J. Y.-L., S.-Y. ROAN, J.-H. HSY and W. C.-M. WAN (1981) Direct action of ethanol on the luteinizing hormone-stimulated androgen synthesis by the isolated interstitial cells from mouse testis. *Bull. Zool. Acad. Sinica.* **20**(1): 67-74.

酒精對小白鼠睪丸雄性素生成之影響與機轉

余玉林　阮秀月　許瑞信

本研究探討酒精對腦下腺精間激素 (ICSH 或稱黃體生成激素，LH) 所促進之雄性素生成之影響及作用之機轉。本實驗室曾以離體培養法確立酒精劑量與小白鼠睪丸雄性素生成之相互關係（中研院動物所集刊 **20**(1): 67-74, 1981）；卽低劑量酒精 (0.63%) 稍能促進雄性素生成，中等劑量 (2.5%) 有部分抑制作用，而高劑量（5%以上）完全抑制雄性素生成。本研究爲再進一步探討酒精影響雄性素生成之方式與可能之機轉。將 6 週齡之小白鼠睪丸精間細胞分離後，與精間激素及酒精一齊培養於 Medium 199 培養液：再以放射免疫法測定雄性素產生之量。

本研究包含數項實驗，其結果與結論如下：㈠酒精在不同時間加入培養之細胞，對雄性素生成之影響——以 12.5 NG 之 Rat LH-RP-1 與細胞培養 6 小時；其中，在不同時間加入酒精。結果顯示，5%與 10%酒精，一旦加入後使雄性素生成立刻停止；而 2.5%酒精在 LH 與細胞培養 1 小時後加入，對雄性素生成之抑制力，比同時加入大爲減少；如在培養 2 小時後加入該劑量酒精，則不再有抑制作用。而低劑量酒精 (0.63%) 在培養後 0.5 小時內加入稍有促進雄性素生成能力，在此時間後加入則無影響。因此，提示精間細胞已接受精間激素開始生成雄性素，卽不再受低劑量或中等劑量酒精之干擾或干擾程度大爲減低。㈡以酒精短時間培養細胞，觀察對雄性素生成之效應——酒精先與細胞培養 0.5 或 1 小時後，再洗除；然後與 50 NG 之 LH 培養 3 小時。結果顯示，5%酒精處理過之細胞不再有生成雄性素之能力，而 2.5%酒精處理者，仍有部分生成能力。換言之，酒精作用之速度效應可能是立卽的，而其作用效果則是持續的。㈢同量酒精對不同劑量之 LH 之影響——雖然加入大量之 LH，但亦不能克服酒精抑制雄性素生成之能力。此結果提示酒精不與 LH 互相競爭精間細胞 LH 受納器。因此酒精之作用不應在細胞膜受納器，可能直接影響細胞內雄性素之生成。