Pharmacology Biochemistry & Behavior, Vol. 20, pp. 293–299, 1984.

Lack of Acute Alcohol Effects on Estradiol and Luteinizing Hormone in Female Macaque Monkey

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Received 1 June 1983

MELLO, N. K., M. P. BREE, J. ELLINGBOE, J. H. MENDELSON AND K. L. HARVEY. *Lack of acute alcohol effects on estradiol and luteinizing hormone in female Macaque monkey.* PHARMACOL BIOCHEM BEHAV **20**(2) 293–299, 1984.—The effects of alcohol (1.5, 2.5, 3.5 g/kg) on 17-β estradiol and LH were evaluated in adult female Macaque monkeys. Integrated plasma samples were collected prior to and following nasogastric intubation of alcohol or isocaloric sucrose control solutions. Samples were collected at 30 minute intervals over 240 minutes. Each alcohol dose and control was studied at menstruation, the peri-ovulatory and mid-luteal periods and the premenstruum. After low, moderate and high doses of alcohol, blood alcohol levels (BAL) averaged 140, 260 and 344 mg/dl at the peak of the ascending BAL curve. Despite high blood alcohol levels, there was no evidence of alcohol dose-related suppression of LH or 17-β estradiol at any phase of the menstrual cycle. These data are consistent with our findings in human females that acute alcohol intoxication did not suppress LH or estradiol. The apparent resiliency of human and Macaque females to *acute* alcohol effects on reproductive hormones contrasts sharply with data obtained in males that alcohol significantly suppresses testosterone in all species studied.

Alcohol, acute effects of 17-β estradiol LH Macaque monkey Female Macaques

THE effects of alcohol on male reproductive function have been studied extensively (cf., [6,7] for review). Testosterone levels are suppressed following alcohol administration in human males [16, 30, 31, 32] and in rodents [3, 8, 9, 10]. Alcohol induced suppression of testosterone levels is dose-related and the decline in testosterone levels parallels the ascending phase of the blood alcohol curve [8, 31, 32]. Under conditions of chronic alcohol administration, testosterone levels remain suppressed in human males [16, 30, 42] and in rodents [1,15]. Alcoholism in man is associated with a variety of disruptions of sexual function, including impotence, testicular atrophy, gynecomastia and complaints of reduced sexual desire [33, 35, 42].

Given the accumulated evidence of the toxic effects of alcohol on reproductive function in males, it is surprising that there have been so few attempts to examine alcohol effects on ovarian and pituitary hormones which are essential for female reproductive function (cf., [6, 34, 41]). We have recently found that chronic alcohol self-administration by female Macaque monkeys results in amenorrhea and suppression of luteinizing hormone levels [28,29]. These derangements of reproductive function in monkey are comparable to those reported clinically. Alcoholic women often have persistent amenorrhea, infertility and spontaneous abortions [17, 19, 37, 39]. But it has been difficult to determine the relative contribution of alcoholism and related disorders to the spectrum of reproductive system derangements observed in women [19]. Either malnutrition associated with profound weight loss [12] or hepatic dysfunction can disrupt menstrual cycle regularity [19]. Moreover, it is not known if disruptions of reproductive function require sustained alcohol abuse or if high acute doses of alcohol also suppress ovarian and pituitary hormones in females as well as in males [6,7].

This report describes the effects of *acute* alcohol administration on a gonadal steroid, estradiol, and a pituitary hormone, luteinizinig hormone (LH). The female Macaque monkey was studied because its reproductive system is most similar to that of human females [22]. The rhesus monkey has long been the preferred model for studies of basic reproductive physiology (cf., [22, 23, 24, 38]). Since basal levels of LH and estradiol change over the course of the menstrual cycle, the effects of single doses of alcohol were compared with isocaloric doses of sucrose at menstruation, the peri-ovulatory and mid-luteal periods and the premenstruum. Alcohol doses were selected to produce peak blood alcohol levels above the usual legal limit of intoxication (100 mg/dl) and to correspond to levels often seen in chronic alcoholic individuals (200–350 mg/dl) [26,27]. This is the first report of the *acute* effects of alcohol on pituitary and gonadal hormones in female Macaque monkeys.

METHOD

Subjects[1]

Seven sexually mature female Macaque monkeys (4 to 7.8 kg) were housed individually in a cage room with adult male Macaque monkeys. Data are reported for 6 rhesus monkeys (Macaca mulatta) (A345, 10–80, 157–79, T681, B428, Y168) and 1 pig-tail monkey (Macaca nemestrina) [B429]. Six monkeys were alcohol and drug naive; 1 monkey (A345) had a 124 day history of low dose alcohol self-administration, but had been alcohol free for 95 days when these studies began.

Monkeys were maintained on ad lib food and water. Monkey chow was supplemented daily with fresh fruit, vegetables and multiple vitamins. A 12 hour light dark cycle (7 a.m. to 7 p.m.) was in effect. Vaginal swabs were done daily to determine the onset and duration of menstrual bleeding. Once each monkey's menstrual cycle became stable (after 2 to 6 months), acute doses of alcohol were given to coincide with menstruation and the predicted times of ovulation, the mid-luteal period and the premenstruum. However, the accuracy of predictions of cycle phase could not be established until the onset of the next menstruation. The presence of a preovulatory mid cycle surge of estradiol and LH was later confirmed by radioimmunoassay. It was seldom feasible to study the acute effects of alcohol at each of the 4 menstrual cycle phases during a single menstrual cycle. Consequently, it was necessary to study the monkeys over 12 to 18 consecutive menstrual cycles in order to evaluate the acute effects of 3 doses of alcohol and 2 isocaloric control solutions at 4 verified menstrual cycle phases.

Alcohol Administration

Alcohol (1.5, 2.5 and 3.5 g/kg), prepared in a 25% solution was administered through a pediatric grade nasogastric tube. Alcohol effects were compared with equal volumes of sucrose control solutions isocalorically equivalent to 1.5 and 2.5 g/kg alcohol. Monkeys were fasted for 18 to 20 hours to insure uniform absorption of alcohol from the small intestine.

Catheterization Procedures for Integrated Plasma Sample Collection

Since pituitary gonadotrophins are secreted episodically [23, 24, 38], we developed an acute venous catheterization procedure for integrated blood sample collection [4]. An integrated plasma sample collection procedure was used in preference to a discrete bolus sample collection method, since a bolus sample might coincide with either the peak or the nadir of episodic secretory activity. Monkeys were anesthetized with ketamine hydrochloride (5 to 10 mg/kgIM)[2] and the saphenous vein was catheterized using aseptic techniques. A 19 gauge needle containing a 22 gauge Deseret radiopaque intracath was inserted into the vein. After removal of the needle and the internal stylet, the intracath was joined to a sterile, heparin-soaked silicone tube and secured with suture.

The monkey was placed in the standard primate chair and blood was exfused with a Rainin Rabbit Miniature Peristaltic pump into heparinized vacutainer tubes in chipped ice. Blood samples were exfused continuously over 4 hours and 8 samples were collected at 30 minute intervals. Each integrated plasma sample reflects the mean level of each hormone measured for the 30 minute period. Once the required amount of blood was exfused (3.5 ml over 30 minutes), samples were centrifuged, aliquots of plasma withdrawn and frozen at −20°.

The acute catheterization procedure has been performed successfully on over 150 occasions and there have been no problems such as infective thrombosis, thrombophlebitis and phlebothrombosis. Blood samples were usually collected between noon and 5 p.m., a period when estradiol levels tend to be relatively stable [40]. No diurnal fluctuation of luteinizing hormone has been reported in female rhesus monkeys [40].

Pituitary-Gonadal Hormone and Blood Alcohol Analyses

Data are reported for analysis of 17-β estradiol, luteinizing hormone (LH) and blood alcohol levels, at menstruation, the peri-ovulatory and mid-luteal periods and the premenstruum.

17-β Estradiol. Plasma levels of 17-β estradiol were determined in duplicate 0.100 ml samples of plasma, by radioimmunoassay, using a modification of a procedure of Hotchkiss *et al.* [18]. After diethyl ether extraction, estradiol was measured directly, without chromatography. Goat anti-rabbit gamma globulin, rather than dextran-coated charcoal, was used to separate the bound antibody from the free steroid. Antiserum to 17-β estradiol (No. E26-47, Endocrine Sciences, Tarzana, CA) was essentially free of cross reactivity to other estrogens and known plasma steroids; its greatest cross reactivity (1.3%) was with estrone. The labeled steroid used for the assay was the radioiodinated (^{125}I) 7-succinyl tyrosine methyl ester derivative of 17-β estradiol (Cat. No. D-1240, Micromedic Systems, Horsham, PA). 17-β Estradiol (2, 4, 6, 7, 16, 17-^{3}H) (Cat. No. NET-517, New England Nuclear, Boston, MA) was used for extraction recovery corrections. Intra- and interassay CV's were 6% and 19% respectively.

Luteinizing hormone (LH) radioimmunoassay. LH levels were determined in duplicate 0.100 ml plasma samples using a double antibody radioimmunoassay procedure and materials supplied by the Contraceptive Development Branch, Center for Population Research, National Institute for Child Health and Human Development. The assay is based on a method described by Monroe *et al.* [36]. Purified cynomolgus pituitary LH was radioiodinated using the Chloramine-T method, and rabbit antiserum to hCG (R13, Pool D) was employed as the first antibody. The standard was NICHD-rhLH, also known as WP-XV-20. Results are reported as ng NICHD-rhLH/ml plasma. The assay sensitivity was 13 ng/ml. Intra- and interassay CV's were 9.9% and 10.5% respectively.

Blood alcohol analysis. Levels of alcohol in plasma were measured in duplicate 0.020 ml plasma samples using a dye-coupled colorimetric micromethod, based on enzymic oxidation of ethanol to acetaldehyde [25].

[1]Animal maintenance and research was conducted in accordance with the guidelines provided by the Committee on Laboratory Animals Facility and Care, the National Research Council Institute of Laboratory Animals Resources. The Facility is licensed by the U.S. Department of Agriculture. The health of the monkeys was periodically monitored by a consultant veterinarian from the New England Regional Primate Center.

[2]Ketamine was used because it has been shown to have no effect on ovulatory menstrual cycle length, estrogen or progesterone levels in female monkeys observed over 88 menstrual cycles and compared with controls observed over 70 cycles [30]. Moreover, ketamine does not affect the pulsatile release pattern of LH, GnRH and prolactin in rhesus monkey [11].

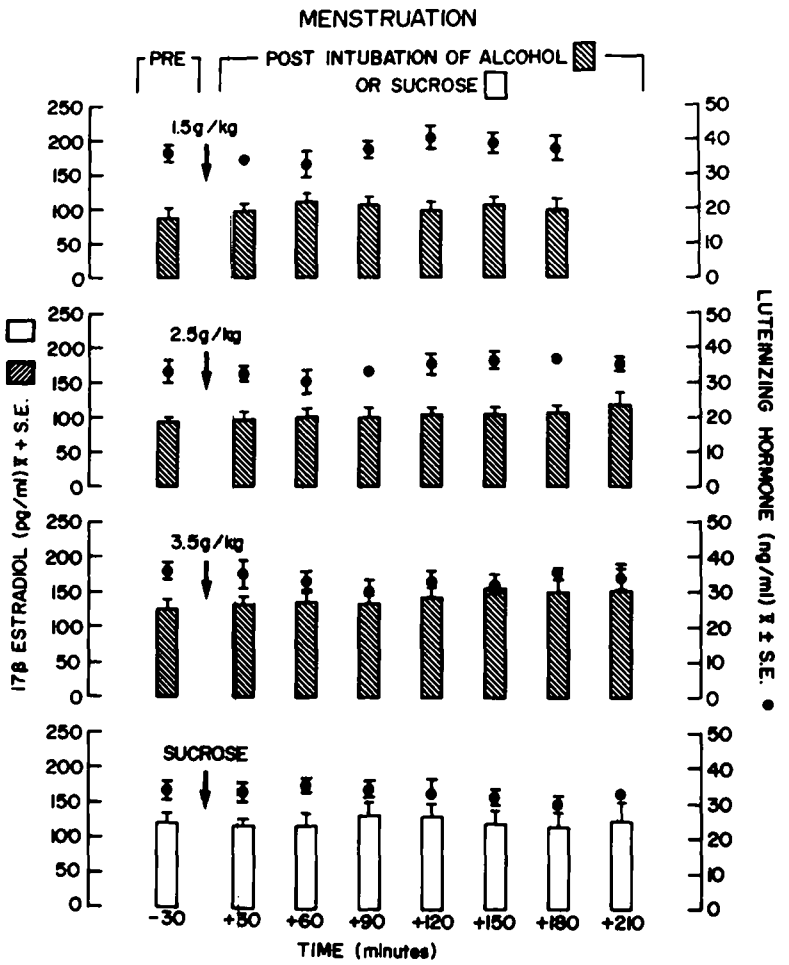

FIG. 1. Menstruation: Group average (±S.E.) levels of 17-β estradiol (pg/ml) and LH (ng/ml) are shown prior to and following administration of alcohol (1.5 g/kg, 2.5 g/kg, 3.5 g/kg) or an isocaloric sucrose solution. Integrated plasma samples were collected over 30 minutes and each value represents the true mean level for that period. Each data point in rows 1 through 3 is an average of 6 samples. Each sucrose control data point is an average of 10 samples. There were no significant differences in values obtained after isocaloric sucrose control solutions equivalent to 1.5 and 2.5 g/kg alcohol so these were combined.

RESULTS

Blood Alcohol Levels

Monkeys appeared intoxicated within 1 hour after nasogastric intubation of alcohol. Blood alcohol levels gradually ascended and reached peak levels within 120 minutes after the lowest alcohol dose (1.5 g/kg) and within 150 minutes after the moderate and high alcohol dose (2.5 and 3.5 g/kg). The average peak blood alcohol levels measured after administration of 1.5 g/kg alcohol were 140 mg/dl (±9.31); 260 mg/dl (±5.59) after administration of 2.5 g/kg alcohol and 344 mg/dl (±16.94) after 3.5 g/kg of alcohol. These blood alcohol levels were comparable to levels measured in alcoholic men during a period of unrestricted alcohol self-administration [26,27]. Since respiratory failure and death can occur at blood alcohol levels of 450 to 600 mg/dl, the administration of alcohol doses exceeding 3.5 g/kg seemed ill advised.

Alcohol Effects on 17-β Estradiol and Luteinizing Hormone

Levels of 17-β estradiol (pg/ml) and luteinizing hormone (ng/ml) prior to and following administration of alcohol or isocaloric sucrose solution at 4 phases of the menstrual cycle are shown in Figs. 1 through 3. Integrated plasma samples collected following alcohol administration were compared to the pre-alcohol control samples and to the temporally equivalent post-sucrose control samples. Data were evaluated with *t*-tests for two means.

Menstruation (Fig. 1). Plasma samples for pituitary and gonadal hormone analysis were collected on the first, second or third day of menstruation as determined by vaginal swabs. Control levels of LH and 17-β estradiol measured before alcohol administration did not differ from post-alcohol levels at any dose or at any phase of the blood alcohol curve.

Following administration of sucrose solutions, calorically equivalent to 1.5 and 2.5 g/kg of alcohol, LH and estradiol

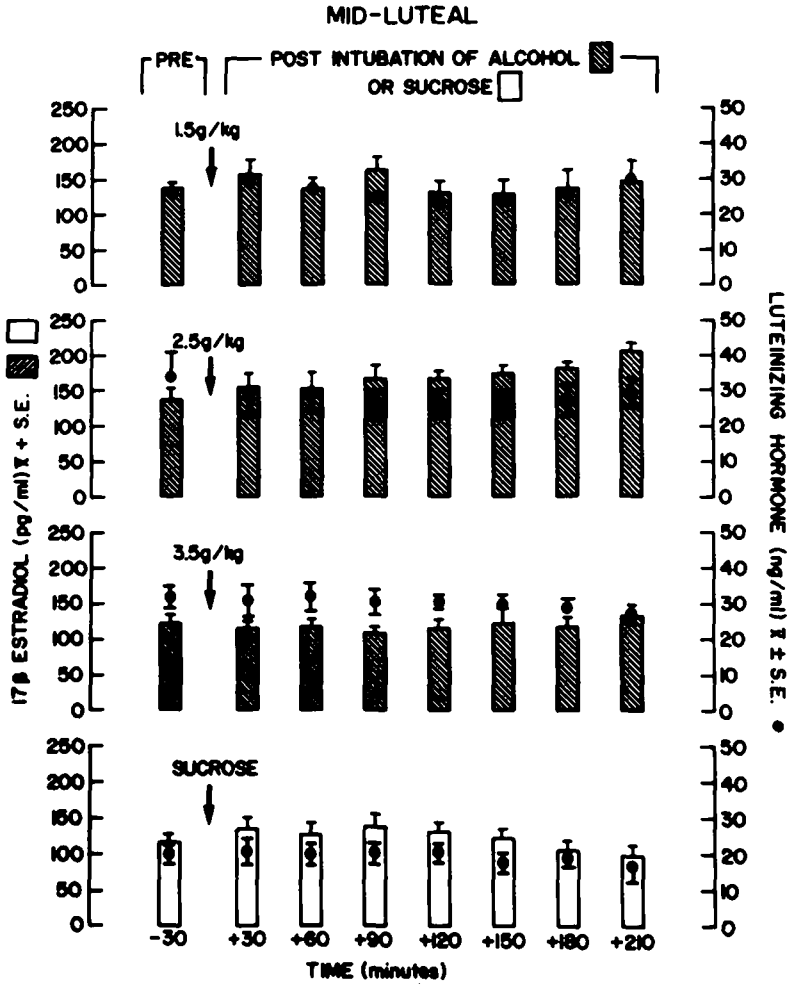

FIG. 2. Mid-luteal Phase: Group average (±S.E.) levels of 17-β estradiol (pg/ml) and LH (ng/ml) are shown prior to and following alcohol administration (1.5, 2.5 and 3.5 g/kg) and isocaloric sucrose administration. Integrated plasma samples were collected over consecutive 30 minute periods and each value represents the true mean level of 17-β estradiol or LH. Each data point in rows 1 through 3 is an average of 5 samples. Each isocaloric sucrose control data point represents 11 samples. There were no significant differences between values obtained after isocaloric sucrose control solutions equivalent to 1.5 and 2.5 g/kg of alcohol so these were combined.

levels were not statistically different, so sucrose control values were combined. There were no significant differences in LH or estradiol between pre-sucrose control samples and each pre-alcohol control sample. Comparison of each post-sucrose sample with the corresponding post-alcohol sample at each alcohol dose also showed no significant changes in estradiol levels. LH values after alcohol administration tended to be somewhat higher than after sucrose administration but these differences were not significant at moderate and high alcohol doses. After the lowest alcohol dose (1.5 g/kg), LH values were higher than after sucrose administration ($p<0.01$).

Mid-luteal period (Fig. 2). Plasma samples were collected 7 to 10 days before the onset of menstruation, i.e., the presumptive luteal phase. After alcohol administration, there were no significant changes in estradiol or LH levels in comparison to pre-alcohol control levels. Sucrose control LH and estradiol levels were combined since levels did not differ significantly after 1.5 and 2.5 isocaloric equivalent doses. Pre-sucrose values did not differ from pre-alcohol values of LH or estradiol. However, the post-sucrose LH values were significantly lower than both the pre-alcohol LH levels at 2.5 and 3.5 g/kg ($p<0.01$) and the post-alcohol LH levels at all three doses ($p<0.001$). After administration of 1.5 and 3.5 g/kg alcohol, estradiol levels were not different from post-sucrose control levels. Estradiol levels were elevated above sucrose control levels at 150, 180 and 210 minutes ($p<0.05$–0.01).

The premenstruum (Fig. 3). Plasma samples were collected 1, 2 or 3 days before the onset of menstruation. Estradiol levels did not change from pre-alcohol control levels after any dose of alcohol. There was no systematic relationship between estradiol levels and the ascending phase of the blood alcohol curve. There were also no significant changes in luteinizing hormone levels in comparison to pre-alcohol control levels at each dose or in comparison to comparable

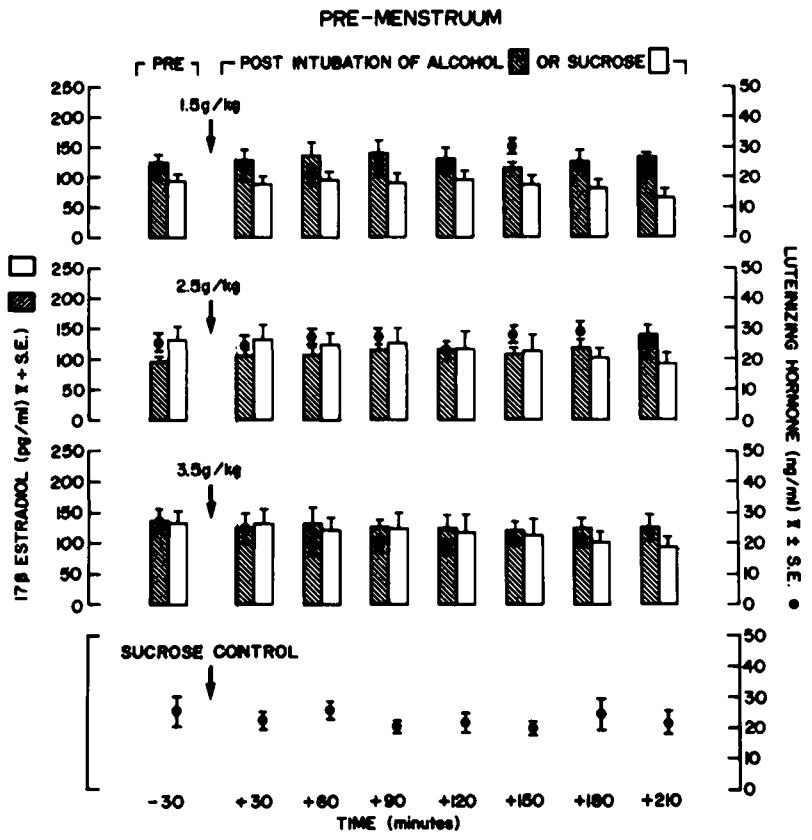

FIG. 3. Premenstruum: Group average (±S.E.) levels of 17-β estradiol (pg/ml) and LH (ng/ml) are shown. Integrated plasma samples were collected prior to and every 30 minutes following administration of alcohol (1.5, 2.5, 3.5 g/kg) or isocaloric sucrose control solution. Each estradiol data point is an average of 5 samples for each alcohol dose (cross-hatched bars, rows 1–3). Each LH data point in rows 1 and 3 represents 6 values and in row 2, 7 values. Sucrose control values of estradiol following administration of the isocaloric equivalent of 1.5 and 2.5 g/kg of alcohol were significantly different so these were not combined. In row 1, each sucrose control value (1.5 g/kg isocaloric equivalent to alcohol) represents 3 samples. In rows 2 and 3, each sucrose control value (2.5 g/kg isocaloric equivalent to alcohol) represents 4 samples. LH values were not significantly different following the 2 sucrose control administrations, so LH values were combined in row 4 and each data point represents 8 samples.

sucrose control values. LH levels after isocaloric sucrose control administration did not differ significantly and were combined.

Estradiol levels were significantly higher after administration of sucrose equivalent to 2.5 g/kg of alcohol than after 1.5 g/kg ($p<0.02$) so these were not combined and are plotted separately. Estradiol levels did not differ significantly from the corresponding sucrose control samples except at 210 minutes after alcohol or sucrose administration. Estradiol levels were higher after alcohol than after sucrose at each dose level but this difference was barely significant ($p<0.05$) only after 2.5 g/kg.

The peri-ovulatory period. Accurate prediction of ovulation proved to be especially difficult. Calendar estimates that later appeared valid (15 to 17 days before the onset of menstruation) were not always confirmed by radioimmunoassay. Our classifications of the peri-ovulatory samples are based on the data of Karsch and co-workers [21] which showed that in rhesus monkey, elevations in estradiol levels in the range of 100 to 200 pg/ml must persist for an average of 42 hours to induce an LH surge. Moreover, initiation of an LH surge was dependent upon the maintenance of high estradiol levels, not on an acute fall in estradiol levels [21].

Of the 26 sample days studied, 3 appeared to be pre-ovulatory since estradiol levels exceeded 200 pg/ml but LH levels had not yet begun to rise and averaged 21–61 ng/ml. Five sample days coincided with the onset of the LH surge since estradiol levels exceeded 200 pg/ml and LH levels were above 100 ng/ml. One sample day coincided with the LH surge (i.e., LH levels were above 500 ng/ml) but estradiol levels had declined to below 160 pg/ml. The remaining 17 samples could not be categorized as peri-ovulatory since both estradiol and LH were well below these criterion levels.

Using these criteria, estradiol levels are reported in three monkeys studied at 1.5 and 2.5 g/kg of alcohol and two monkeys given 3.5 g/kg of alcohol. Alcohol had no effect on pre-ovulatory elevations in estradiol. Control estradiol levels averaged 404 pg/ml before 1.5 g/kg of alcohol and ranged between 343 and 449 pg/ml after alcohol. Control estradiol levels averaged 565 pg/ml before and ranged between 470

and 582 pg/ml after administration of 2.5 g/kg of alcohol. Control estradiol levels averaged 392 pg/ml before and ranged between 383 and 467 pg/ml after administration of 3.5 g/kg alcohol.

Luteinizing hormone levels measured early in the LH surge are reported for five monkeys. LH levels did not increase or decrease systematically after administration of alcohol. One monkey maintained LH levels of 250 ng/ml before and after 1.5 g/kg of alcohol. Two monkeys averaged LH levels of 188 ng/ml before and 207 to 242 ng/ml after administration of 2.5 g/kg of alcohol. Two monkeys had an average LH level of 203 ng/ml before and 169 to 224 ng/ml after administration of 3.5 g/kg of alcohol. Luteinizing hormone levels measured at the midpoint of the LH surge were also unchanged by alcohol (1.5 g/kg) and remained constant at >500 ng/ml.

DISCUSSION

It was surprising to find that acute administration of alcohol had no significant effect on 17-β estradiol or LH. Since alcohol suppresses testosterone in males [6,7] and production of testosterone and estradiol require essentially the same biosynthetic pathways [2], it was predicted that alcohol would suppress estradiol in females. These findings cannot be attributed to limitations in the range of alcohol doses studied since peak blood alcohol levels exceeded 130 mg/ml at the lowest alcohol dose. At intermediate and high doses, peak blood alcohol levels exceeded 250 and 340 mg/dl; levels comparable to those measured in alcoholic men during chronic intoxication [26,27]. In human males, peak blood alcohol levels of only 109 mg/dl were associated with dose dependent decreases in plasma testosterone levels [32]. It could be postulated that the low levels of estradiol and LH measured at menstruation, the premenstruum and the mid-luteal phase might mitigate against a significant alcohol-related change in hormone levels. But, there was also no discernible alcohol effect at the pre-ovulatory phase when basal levels of these hormones were 2 to 3 times higher than at other phases of the menstrual cycle.

These data obtained in female Macaque monkeys are consistent with our recent studies of the effects of a single low dose of alcohol on estradiol, LH and prolactin in human females [34]. Six healthy young women were studied at the mid-follicular phase of the menstrual cycle, 8, 9, or 10 days following the onset of menstruation. Each woman served as her own control during two successive menstrual cycles in which the effects of alcohol and isocaloric sucrose control solutions were evaluated. The average peak blood alcohol level was 88 mg/dl. No significant differences in LH and estradiol levels were detected during any phase of the blood alcohol curve compared to pre-alcohol administration values. No significant differences in LH and estradiol levels were found after isocaloric beverage administration compared to alcohol administration. The comparability of data on both acute and chronic alcohol effects on LH and estradiol in female Macaques [28,29] and human females [17, 19, 34, 37, 39] further extends the generality and validity of the primate model for studies of reproductive function.

One implication of these findings is that sustained alcohol intoxication is necessary to affect female pituitary and gonadal hormones in higher primates. Although acute alcohol intoxication has no obvious consequences, chronic alcohol intoxication is associated with profound disruptions of menstrual cycle regularity in alcoholic women [19, 37, 39] and female Macaque monkeys studied in an operant alcohol self-administration paradigm [28,29]. Similarly chronic alcohol exposure is associated with decreased ovarian mass in alcoholic women [20] and rodents [15, 43, 44] which appears to reflect an absence of corpus lutea rather than ovarian atrophy [41]. Consequently, there is little question that the cumulative effects of prolonged exposure to alcohol lead to reproductive system dysfunctions both in rodents and higher primates. The mechanisms underlying these disruptive effects of alcohol on reproductive function are unknown and the critical dose and duration of alcohol exposure is unclear.

One implication of the contrast between the acute effects of alcohol on reporductive hormones in females and males is that there may be a sex-related difference in resiliency to exogenous toxins. It has been postulated that males and females may be differentially susceptible to the toxic effects of alcohol [13, 14, 41]. Women appear to be more susceptible than men to alcohol-induced hepatic disorders [13] and in rodents, rates of ovarian atrophy are correlated with increases in alcohol concentrations in the diet, whereas the incidence of testicular atrophy in males is unrelated to alcohol dose levels [14]. The findings of this study and our previous observations in young women [34] support the notion that there may be a sex-related difference in responsivity to the acute effects of alcohol. However, in higher primates, female pituitary-gonadal hormones appear to be less vulnerable to *acute* alcohol effects than males.

ACKNOWLEDGEMENTS

This research was supported in part by grant AA04368 from the National Institute on Alcoholism and Alcohol Abuse and by grants from the Committee on Problems of Drug Dependence and Joseph E. Seagram and Sons, Inc. We thank Paul Heffernan and Alice Skupny for technical assistance in the radioimmunoassay and blood alcohol analyses. We are grateful to Dr. Prabhat Sehgal for veterinary consultation.

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