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Alcohol Sensitivity in Female Mice: Effect of Ovariectomy[1]

CARRIE L. RANDALL, ELIZABETH A. LOCHRY, JAMES W. MOSELEY AND PATRICIA B. SUTKER

Veterans Administration Medical Center, Medical Research Service, Charleston, SC 29403 and Department of Psychiatry and Behavioral Sciences Medical University of South Carolina, Charleston, SC 29403

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RANDALL, C. L., E. A. LOCHRY, J. W. MOSELEY AND P. B. SUTKER. *Alcohol sensitivity in female mice: Effect of ovariectomy*. PHARMAC. BIOCHEM. BEHAV. 15(2) 191–195, 1981.—This study tested the hypothesis that decreased estrogen levels accomplished by removing the ovaries affect the response to acutely administered alcohol in female mice. Sensitivity to alcohol was measured in ovariectomized, sham-operated, and non-surgical control C3H/HEN mice. Each animal received an IP injection of alcohol (3.0 or 4.0 g/kg). Core temperature, fall time, sleeptime, and waking blood alcohol levels were the dependent variables. For each of these measures, alcohol sensitivity was found to be a function of the dose of alcohol administered, but not the surgical condition. Additionally, the stage of estrus in control animals was not found to be related to alcohol sensitivity.

Alcohol sensitivity Estrogen Core temperature Ovariectomy Estrous cycle Sleeptime C3H mice Female Alcohol

IN the late 1930's, it was reported that castrated female mice and rabbits were more sensitive to the pharmacologic action of alcohol than intact control females, as measured by the loss of simple reflexes and lethality [12]. However, when the ovariectomized animals were injected with exogenous estrogen, there was a marked reduction in the depressant effects of alcohol. A subsequent experiment demonstrated that the antagonism of the action of alcohol by exogenous estrogen injections in castrated females could not be explained by alterations in alcohol metabolism, since no change was observed [5]. Although the results of these early studies strongly implicated a role for estrogen in sensitivity to alcohol [5,12], the relationship between female sex hormones and the response to alcohol has not been studied systematically in either animal or clinical research. In fact, only recently have clinical studies begun to address this question.

It has been demonstrated among non-alcoholics that gender differences exist in peak blood alcohol levels achieved following ingestion of a moderate dose of alcohol [7]. Additionally, peak levels in women were differentially related to phase of the menstrual cycle. That is, a small sample of women in the premenstrual phase of the sexual cycle reached higher peak blood alcohol levels following oral ingestion of a moderate alcohol dose than they did at other times during the menstrual cycle [7,8]. This finding of increased sensitivity to alcohol associated with the premenstrual phase of the cycle, when estrogen levels were presumably low, complements data reported by the earlier animal work [12].

The purpose of this study was to investigate the effects of decreased estrogen levels, accomplished by ovariectomy, on sensitivity to acute alcohol challenge in C3H mice. The study circumvented many of the design problems associated with earlier animal work [12] by employing two doses of alcohol, large numbers of subjects in each group, multiple measures of alcohol sensitivity, measurement of blood alcohol level, and inclusion of a sham-operated control group. Furthermore, the present investigation attempted to correlate phase of estrous cycle with alcohol sensitivity in control animals.

METHOD

Animals

One hundred female C3H/HEN mice were obtained from Frederick Cancer Research Center (Maryland) at 30 days of age. Upon receipt, the mice were gang housed 18–20 per cage in 23 cm×46 cm stainless steel pans containing Sani-cel bedding and covered with stainless steel lids. Wayne Lab Chow and water were available ad lib. The animals were kept in temperature and humidity controlled rooms (American Association for Accreditation of Laboratory Animal Care approved facility) that were maintained on a 12-hr light-dark cycle with lights illuminated from 0600–1800 hr.

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Surgery

At approximately 18 weeks of age, 20 mice were assigned to a non-surgical control group, while the remaining 80 mice were randomly assigned to one of two surgical conditions, ovariectomized (n=40) and sham control (n=40). Each mouse in the two surgical groups was weighed and given a 90 mg/kg IP injection of pentobarbital sodium (Fort Dodge Laboratories, Inc.) in a volume of 0.025 ml per gram body weight. This dose of pentobarbital was determined from pilot work defining suitable anesthetic levels for this particular strain. Following anesthesia, the coat directly above the right and left ovaries of each mouse was shaved and a 6 mm incision was made. The ovaries of the experimental group (ovariectomized) were then removed while those of the sham control group were left intact. The incisions were closed with stainless steel wound clips (Clay Adams, 9 mm). Following surgery, all animals, including the non-surgical control group, were rehoused with animals of the same surgical group, six per cage in 15.5 cm×26 cm polypropylene cages fitted with zinc-plated lids.

Procedure

Twenty days following surgery, the ovariectomized, sham, and non-surgical groups were tested for sensitivity to alcohol. At approximately 0900 hr on the day of testing, vaginal smears were obtained to confirm success of surgery in the ovariectomized group and to assess the stage of the estrous cycle (diestrus, pro-estrus, early estrus, estrus, and post-estrus) in the sham and non-surgical controls [11]. Immediately prior to testing, core temperatures were measured for baseline levels using a lubricated probe of a Yellow Springs telethermometer that was inserted 2.5 cm into the rectum [10].

Body weight was recorded and indicated that all animals in both surgical conditions had returned to, or surpassed, presurgery weights. From 0930–1030 hr, the animals were injected IP with alcohol (3.0 or 4.0 g/kg) in a volume of 0.025 ml per gram body weight resulting in alcohol concentrations of 12 and 16% (w/v), respectively. Pilot work indicated that a higher dose, 5.0 g/kg, resulted in excessive mortality, irrespective of surgical condition. Immediately following injection, each animal was placed in a holding cage until it was judged to be unable to right itself twice within 30 sec of being placed on its back. The latency from injection to the loss of the righting reflex was recorded as "fall time." The animal was then placed on its back in a V-shaped Plexiglas trough and latency to regain the righting reflex (righting itself twice in 30 sec) was recorded as sleeptime. Core temperatures were taken again at 45, 90, 180, and 240 min post injection. Blood alcohol concentrations (BAC) were determined enzymatically (Ethyl Alcohol Stat Paks, Cal Biochem-Biering) using a 44.7 μl sample of blood obtained from the retro-orbital sinus of each animal when they regained the righting reflex. Blood alcohol concentrations were measured at 340 nm on a Gilford 24002 spectrophotometer. After testing, the animals were autopsied and ovariectomies were further validated by removing and weighing the uteri.

RESULTS

Two ovariectomized subjects, one 3.0 g/kg and one 4.0 g/kg animal, were excluded from the analyses when postexperimental examination revealed incomplete bilateral removal of the ovaries. Uterine horn weights corroborated the successful ovariectomies in the remaining cases. Uterine weights of the ovariectomized mice were significantly less than those of sham controls and non-surgical controls, which were equivalent to each other (mean±SE=0.027±0.008, 0.075±0.023, and 0.078±0.024 g, respectively). One 4.0 g/kg ovariectomized animal died postinjection and was excluded from data analyses, and one 3.0 g/kg non-surgical control animal died of causes unrelated to the experiment and was also excluded. Thus, the number of ovariectomized, sham, and non-surgical control animals was reduced to 19, 20, and 9 in the 3.0 g/kg group and to 18, 20, and 10 in the 4.0 g/kg group, respectively.

A 2×3 analysis of variance with Dose (3.0 and 4.0 g/kg) and Surgical Condition (ovariectomized, sham, and non-surgical) as between-subject factors was performed to compare groups for fall time, sleeptime, and waking BAC. Core temperature measures were subjected to the same type of analysis with the addition of Time (0, 45, 90, 180, and 240 minutes postinjection) as a repeated measures variable.

Fall time was compared across groups and found to be inversely related to the dose of alcohol administered. Six animals (three ovariectomized, two sham-operated, and one non-surgical control), all in the 3.0 g/kg group, failed to lose the righting reflex within 10 minutes and were excluded from this particular analysis. A significant Dose effect was evident, $F(1,84)=4.95$, $p<0.01$, while Surgical Condition and the interaction term were not significant. The mean fall times (±S.E.) for the 3.0 and 4.0 g/kg groups were 141 (±16) and 113 (±9) seconds, respectively.

Sleeptime was significantly influenced by the dose of alcohol administered, $F(1,90)=230.43$, $p<0.001$, however, no effects of Surgical Condition or interaction of Dose with Surgical Condition were apparent. As illustrated in Fig. 1, sleeptime increased according to the dose of alcohol, with animals in the 4.0 g/kg group sleeping longer than those in the 3.0 g/kg group.

Waking BACs were found to vary according to the dose of alcohol. Again, all six 3.0 g/kg animals failing to lose their righting reflex were excluded from this analysis. Waking BACs were significantly higher at the 4.0 g/kg dose, $F(1,84)=16.45$, $p<0.001$, however, neither Surgical Condition nor the interaction of Dose with Surgical Condition approached significance. Mean BAC's (±S.E.) for the ovariectomized, sham operated, and non-surgical controls receiving the 3.0 g/kg dose were 335 (±17), 338 (±16), and 345 (±20) mg/dl, respectively, while those for the same groups receiving the 4.0 g/kg dose were 357 (±30), 364 (±30), and 364 (±32) mg/dl.

Core temperatures were significantly influenced by the dose of alcohol and varied across time. As illustrated in Fig. 2, the magnitude of the alcohol-induced hypothermic response was directly related to dose, with the 4.0 g/kg group showing a more pronounced change in core temperature relative to the 3.0 g/kg animals. A significant effect of Dose was apparent, $F(1,90)=48.89$, $p<0.001$. Again, no effect of Surgical Condition or interaction of Dose with Surgical Condition was evident, indicating that the drop in core temperature for all three surgical conditions was equivalent within the 3.0 and 4.0 g/kg doses. Core temperature was also found to vary as a function of Time, $F(4,360)=388.50$, $p<0.001$. As illustrated in Fig. 2, there was a pronounced drop from baseline levels when temperature was measured at 45 mins postinjection, followed by a gradual rise at subsequent time intervals (90, 180, and 240 mins). Additionally, a significant Dose×Time interaction was evident, $F(4,360)=$

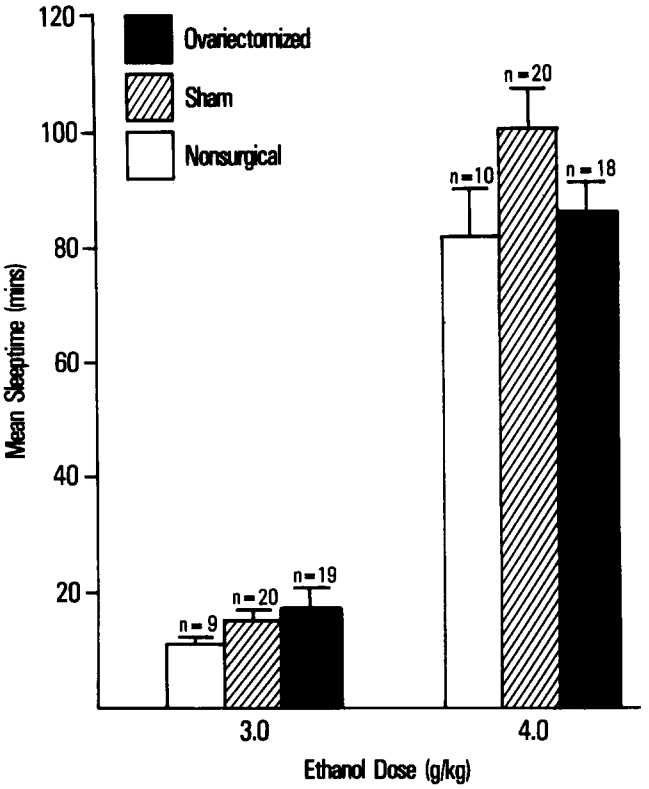

FIG. 1. The mean sleeptime (±S.E.M.) produced by 2 doses of alcohol.

TABLE 1

ALCOHOL SENSITIVITY AS A FUNCTION OF STAGE OF ESTRUS

		Post-estrus	Di-estrus	Pro-estrus	Early estrus	Estrus
		Sleeptime (Mean±SE in min)				
3.0 g/kg	Mean	12.09	11.37	13.25	23.86	10.77
	±SE	2.57	6.11	2.40	6.59	2.94
	N	7	3	6	4	9
4.0 g/kg	Mean	83.67	86.82	109.52	63.66	110.80
	±SE	4.11	13.47	11.36	24.52	10.86
	N	7	9	4	2	8
		Waking BAC (Mean±SE in mg/dl)				
3.0 g/kg	Mean	321	340	346	332	348
	±SE	4	5	2	16	2
	N	6	3	6	4	8
4.0 g/kg	Mean	365	360	365	370	368
	±SE	17	8	14	10	8
	N	7	9	4	2	8
		Core Temperature (Mean±SE in °C)				
3.0 g/kg	Mean	37.1	37.0	36.8	36.6	36.9
	±SE	0.2	0.1	0.2	0.2	0.2
	N	7	3	6	4	9
4.0 g/kg	Mean	36.3	36.4	36.4	36.5	35.7
	±SE	0.2	0.1	0.2	0.3	0.4
	N	7	9	4	2	8

5.96, $p<0.001$, because of the greater initial drop in core temperature and subsequent slower return to baseline levels of the 4.0 g/kg animals. All remaining interaction terms failed to reach significance.

To assess the possibility of estrous cycle influencing alcohol sensitivity, a correlation was computed for each dose between the stage of the estrous cycle and the duration of alcohol-induced narcosis in sham and non-surgical controls, utilizing the information obtained from the pretest vaginal smears. The correlation coefficient for both the 3.0 and 4.0 g/kg dose failed to approach significance ($r=+.05$ and $+.29$, p's<0.05, respectively). Similarly, when the sleeptime scores of the sham and non-surgical controls within each dose were divided into five groups defined by the stage of estrus and subjected to analysis of variance, Estrous Stage did not approach significance, $F<1$. These results indicate that the stage of estrus was not predictive of the duration of sleeptime for the control mice within each challenge dose. When similar analyses were conducted on core temperature of the sham and non-surgical controls, no relationship between stage of estrus and alcohol-induced hypothermia was evident ($r=-.09$, $p<0.05$). The ANOVA revealed only a significant influence of Dose, $F(1,53)=29.22$, $p<0.001$, Time $F(4,212)=209.97$, $p<0.001$, and the Time×Dose interaction, $F(4,212)=3.99$, $p<0.01$, but not of the Estrous Stage or any remaining interactions. The average sleeptime, waking BAC, and core temperature for animals in each stage of estrus is presented in Table 1. Taken together, the results of these analyses indicated that neither the surgical condition of the animal nor the stage of estrus significantly altered any of the three measures of alcohol sensitivity.

DISCUSSION

The results of the present investigation did not support the hypothesis that ovariectomized mice differ in their sensitivity to alcohol from intact control females. Ovariectomized, sham-operated, and non-surgical control mice did not respond differentially to alcohol, regardless of the dose administered (3.0 or 4.0 g/kg) or the measure of sensitivity (sleeptime, core temperature). These results are in contrast to earlier studies in mice and rabbits [12]. While the discrepancy between previous studies and the current investigation might be attributed to strain and/or species differences or to the dosage of alcohol employed, the surgical recovery period and the route of alcohol administration provide a more likely explanation. The present study employed a 20-day surgical recovery period and intraperitoneal injection of alcohol. The former study [12] tested animals approximately 24 hours following surgery, administered alcohol by subcutaneous injection, and did not include a sham-operated control group.

The present findings also do not appear to lend support to more recent clinical reports [7,8] which have linked in-

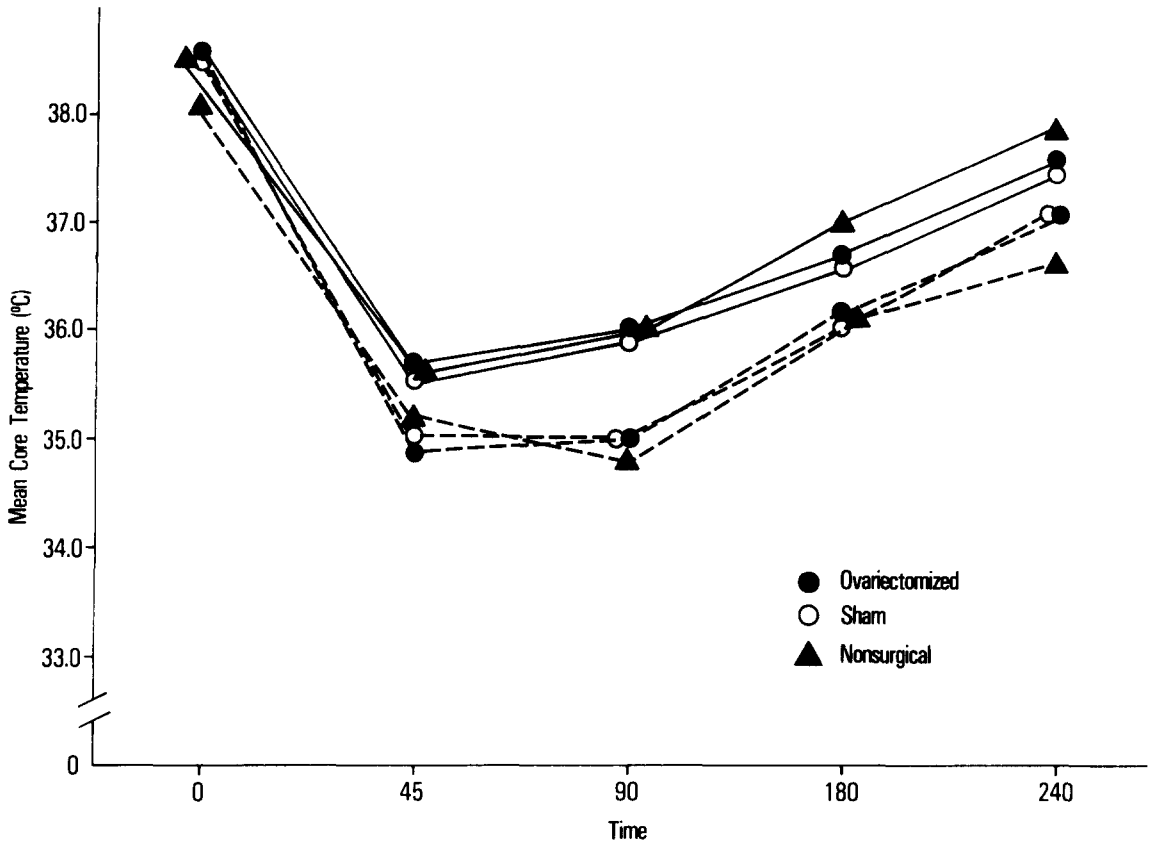

FIG. 2. The mean core temperature just prior to injection and at 45, 90, 180, and 240 minutes following alcohol challenge. The solid line represents the 3.0 g/kg group where group n's=19, 20, and 9, for the ovariectomized, sham, and non-surgical controls. The broken line denotes the 4.0 g/kg group where group n's=18, 20, and 10, respectively. Standard errors were not included for the sake of clarity and did not exceed ±0.33°C.

creased alcohol sensitivity to a specific time of the menstrual cycle when estrogen levels are assumed to be declining. Although the stage of the estrous cycle appeared to be unrelated to the degree of alcohol sensitivity in the present study, this discrepancy between studies may be related not only to species differences, but to the different dependent variables employed by each. The clinical studies [7,8] operationally defined intoxication as the peak blood alcohol concentration achieved after a moderate dose of orally administered alcohol, while the present study utilized alcohol-induced sleeptime and hypothermia as measures of sensitivity. Previous studies [7] attributed the differences observed in peak blood alcohol levels during the menstrual cycle to fluctuations in estrogen and progesterone levels. While hormone levels were not measured in the present study, depressed estrogen levels of the ovariectomized animals were suggested by the significantly decreased uterine weight and diestrous vaginal smears. Similarly, by determining the stage of the estrous cycle in intact controls immediately before alcohol challenge, it was possible to determine that the different stages of the cycle were not significantly correlated with the various measures of alcohol sensitivity.

The findings that latency to fall decreased, sleeptime increased, and core temperature decreased as a function of the alcohol dose were expected. Larger doses of alcohol are associated with higher peak blood alcohol levels which have been found to prolong the loss of the righting reflex [14] and to cause more severe hypothermia [9,10]. Mice injected with 4.0 g/kg slept longer, yet awakened at higher blood alcohol levels than those given 3.0 g/kg. This finding may represent the development of acute tolerance, a phenomenon known to occur in this strain as well as in C57BL mice [13]. If acute tolerance had not developed, one would expect that the 4.0 g/kg animals would have awakened at similar and not higher blood alcohol levels than those in the 3.0 g/kg group. While it may be argued that the sizable anesthetic dose of pentobarbital, a drug known to be cross tolerant with alcohol [6], masked a differential response to alcohol in the two surgical groups, the comparable sleeptimes and hypothermia seen in the non-surgical controls makes this possibility seem unlikely. Hence, the 20-day post-surgical recovery period appears to have been a sufficient length of time to allow any significant amount of pentobarbital tolerance to dissipate. Moreover, when ether was used as the surgical anesthetic in pilot work, differences in the alcohol sensitivity of the ovariectomized, sham, and non-surgical controls, as in the present study, were not apparent.

In summary, the results of this study indicate that sensitivity to alcohol in C3H female mice does not appear to be affected by ovariectomy or stage of the estrous cycle. This

conclusion, however, is limited to the testing conditions as well as to the measures of sensitivity employed. It has been reported, for example, that voluntary alcohol consumption is decreased by ovariectomy [1,4] and is influenced by stage of the estrous cycle [2,3]. It may be that alcohol sensitivity and alcohol ingestion represent completely separate phenomena that are differentially affected by hormones. Obviously, the interaction of alcohol and hormones in females is complex and considerably more research aimed at directly manipulating and measuring hormone levels is needed.

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