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Serum Estradiol Levels and Ethanol-Induced Aggression

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HILAKIVI-CLARKE, L., M. RAYGADA AND E. CHO. *Serum estradiol levels and ethanol-induced aggression.* PHARMACOL BIOCHEM BEHAV **58**(3) 785–791, 1997.—The biological mechanisms behind ethanol-induced aggression are not known. Because gonadal hormones are linked both to aggression and ethanol, the present study examined relationships among the levels of serum estradiol (E_2), testosterone (T), and aggressive behavior in ethanol-treated male mice. We found that among group-housed male mice, serum E_2 levels were significantly elevated 30 min after a single injection of 0.6 g/kg ethanol. Serum T levels showed a nonsignificant decrease by ethanol. The E_2/T ratio, an index of aromatization of T to E_2, was significantly higher in the ethanol-treated animals when compared with the vehicle-treated animals. We also determined aggressive behavior in the resident-intruder test among isolated male mice at baseline (after a vehicle), and after an injection of 0.6 g/kg ethanol. The mice were grouped accordingly to those that increased, decreased, or remained nonaggressive in response to ethanol administration. We found that at baseline, neither serum T or E_2 levels, nor E_2/T ratio differed significantly between the increased or reduced aggressor mice. In contrast to the increase in serum E_2 levels seen in the nonaggressive mice, ethanol significantly reduced circulating E_2 levels, but did not affect aromatization of E_2 from T in the mice that became aggressive following an ethanol injection. These data suggest that mice who exhibit a paradoxical decrease in serum E_2 levels by ethanol may be particularly prone to ethanol-induced aggression. © 1997 Elsevier Science Inc.

Aggression Estradiol Testosterone Ethanol

ALTHOUGH ethanol is clearly linked to violent acts (6,25), the underlying biological mechanisms explaining this association are not known. We have recently shown that transgenic mice overexpressing transforming growth factor α (TGFα) are highly aggressive and maintain their aggressiveness after treatment with ethanol (13). Similar results were obtained in nontransgenic male mice that were made aggressive by implanting them with pellets releasing estradiol (E_2) (14). The transgenic TGFα mice also have high serum E_2 levels (12), possibly because TGFα stimulates the release of gonadotrophin releasing hormone from the hypothalamus (24). These findings suggest that E_2 may play a role in maintaining high levels of aggression following ethanol administration.

Additional support of the idea that gonadal hormones are important in ethanol-induced aggression is obtained from the findings that alcoholism and aggression are more prevalent among men than women (17), and that treatment with E_2 affects both aggression (11,13,28) and ethanol intake (11,14). Ethanol also affects circulating gonadal hormone levels. Chronic ethanol administration reduces serum testosterone (T) levels, by impairing steroidogenesis of androgens (29) and reducing gonadotrophin release from the pituitary (2,9,15), and paradoxically by elevating the aromatization of androgens to estrogens (29). Alcoholic men often exhibit endocrine dysfunctions, including testicular and prostatic atrophy, as well as impotence, and a loss of sexual interest (29). Due to increased aromatization, ethanol increases plasma estrogen levels (7,8,27), and the number of estrogen receptors in estrogen-responsive tissues (29).

We studied whether serum T and/or E_2 levels as well as the effect of ethanol on these hormones differ among three distinct groups of mice: 1) those who become more aggressive in response to ethanol administration (increased aggressors), 2) those who reduce aggression after ethanol treatment (reduced aggressors), and 3) nonaggressive mice that remain unchanged in reaction to ethanol (nonaggressors). Male mice exhibit significant interindividual variations in the level of aggressiveness. However, an individual mouse maintains the

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same relative level of aggressiveness throughout several encounters with another mouse (19). These baseline levels of aggression can be altered by various pharmacological means, including ethanol (21). The data obtained in this study show that an acute ethanol dose increases serum E_2 levels in nonaggressive mice. In contrast, the increased aggressors' E_2 levels are significantly reduced by ethanol.

METHOD

Seven-week-old male CD-1 mice were obtained from National Cancer Institute (Frederick, MD). They had free access to food and water. The males were housed in a temperature/humidity-controlled room at the Georgetown University Research Resource Animal Facility under a 12-h light–dark cycle. Upon arrival, the mice were kept in cages containing 10 animals (Experiment 1) or housed individually (Experiment 2). Experiments 1 and 2 were started 1 week after the arrival of the mice.

Experiment 1: Serum E_2 and T Levels Following an Acute Ethanol Exposure

The effect of a single ethanol dose on serum estradiol (E_2) and testosterone (T) levels were assessed in group-housed male mice by injecting them IP with 1% PBS vehicle or 0.6 g/kg ethanol (6% v/v) in an injection volume of 0.01 ml/g body weight. The blood was collected by cardiac puncture under methoxyflurane inhalant anesthesia 15, 30, or 60 min following the ethanol injection. The vehicle-treated animals were sacrificed at the same time as the ethanol-treated animals, i.e., 15, 30, and 60 min after the injection (n = 5–11 mice per group).

After the blood was drawn, the mice were sacrificed by cervical dislocation. The blood was placed in vacutainer tubes, centrifuged, and extracted for serum. Total serum E_2 and T concentrations were then determined from the samples by using a radioimmunoassay (RIA) kit from ICN Biomedicals, Inc. (Irvine, CA) according to the manufacturer's instructions. The 17β-estradiol kit has the following specifics: 1) specificity of the antiserum: 100% crossreaction to E_2, 20% to estrone, 1.5% to estriol, 0.68% to estradiol-17α, and less than 0.01% to other compounds; 2) interassay variation, %C.V.: 5.9–11.9%; and 3) intraindividual variation, %C.V.: 4.7–10.6%. The testosterone kit has the following specifics: 1) specificity of the antiserum: 100% crossreaction to testosterone, 3.4% to 5α-dihydrotestosterone, 2.2% to 5α-androstane-3β, 17β-diol, 2.0% to oxotestosterone, and less than 1% to other compounds; 2) interassay variation, %C.V.: female 12.7%, male 7.5%; and 3) intraindividual variation, %C.V.: 4.6–9.1%. The samples were assayed in duplicates, and the intraindividual variability between the samples in our study for E_2 was 0.73–0.75, and for T, 0.99.

Experiment 2: Ethanol-induced Aggression and Serum E_2 and T Levels

Resident-intruder test of aggression. To assess baseline levels of aggression, the male mice, housed singly, were injected IP with 0.01 ml/g of 1% PBS and confronted 30 min later in their home cage with a group-housed male intruder. The intruder and resident had had no previous contact with each other. During a 5-min test period, an observer, using stopwatches, monitored and recorded the duration of various aggressive acts, including lateral threat, tail rattle, biting, attacks, and fighting exhibited by the resident. On the following day, the same animals were injected IP with 0.01 ml/g of 0.6 g/kg ethanol (6% v/v) 30 min prior to the resident-intruder test. The intruder was again unfamiliar to the resident.

To confirm that the change in aggressive behavior following an ethanol injection was caused by this substance, some mice were tested a third time in the resident-intruder paradigm. The third test was performed 2 days after the ethanol-induced aggressiveness was tested and the animals received PBS-vehicle 30 min prior to the test.

Measurement of hormone levels. The levels of E_2 and T were measured from male mice whose aggressive behavior had been assessed following injections of vehicle and 0.6 g/kg ethanol. The males were assigned to three groups based on their behavior in the resident-intruder test: 1) increased aggressors: mice who increased their aggression following ethanol administration; 2) reduced aggressors: mice who responded to ethanol by reducing their aggression; and 3) nonaggressors: mice that were nonaggressive and remained so after an ethanol injection.

The criteria for placing an individual mouse in one of these categories were the following: 1) increased aggressors—the time spent in aggression after vehicle was 0–151 s, and after ethanol 35–279 s. In addition, aggression was increased at least by 50% by ethanol. 2) Reduced aggressors—the time spent in aggression after vehicle was 22–120 s, and after ethanol 0–17 s, aggression was reduced at least by 50%. 3) Nonaggressors—the time spent in aggression after vehicle was 0–18 s, and after ethanol 0–17 s, the time spent in aggression varied less than 50% between vehicle and ethanol injections. Seven days after the last aggression test, representative animals from each group received either vehicle or 0.6 g/kg ethanol 30 min prior to their serum collection for hormone analysis, which was performed as described above.

RESULTS

Experiment 1

Serum E_2 levels were assessed in 31 and T levels in 34 male mice following an acute exposure to vehicle or 0.6 g/kg ethanol. We had three to four vehicle-injected animals at each time point, and because there were no significant differences in either serum E_2 or T levels in the vehicle-treated animals 15, 30, or 60 min after the injection, the vehicle data were pooled. The data shown in Fig. 1 indicate that serum E_2 levels, when compared with the levels seen in male mice that received vehicle, were significantly elevated by ethanol [one-way ANOVA: $F(3, 27) = 5.12$, $p < 0.006$]. The elevation was significant 30 min after the ethanol administration (Fishers' least significance test: $p < 0.05$), and remained high also at 60 min following the exposure. The range of E_2 in the vehicle group was 8.1–31.6 pg/ml (median 19.7 pg/ml), indicating a relative large interindividual variability. The variations in hormone values (and also in the time spent in aggression) are mostly due to genetic variability in the outbred CD-1 mice.

There was a nonsignificant trend for a single ethanol exposure to reduce serum T levels, $F(3, 30) = 2.21$, $p < 0.10$ (Fig. 1). T levels in the vehicle group varied between 0.2 and 35.1 ng/ml, the median being 1.8 ng/ml. Thus, most of the male mice had relatively low serum T content, but there were few mice who exhibited high T levels.

The ratio between E_2 and T, an index of aromatization of E_2, could not be determined in all mice due to inadequate coding (for some serum sample, only group label was used). The E_2/T ratio was significantly increased by an ethanol exposure, $F(3, 18) = 3.22$, $p < 0.05$). Fifteen and 30 min after an in-

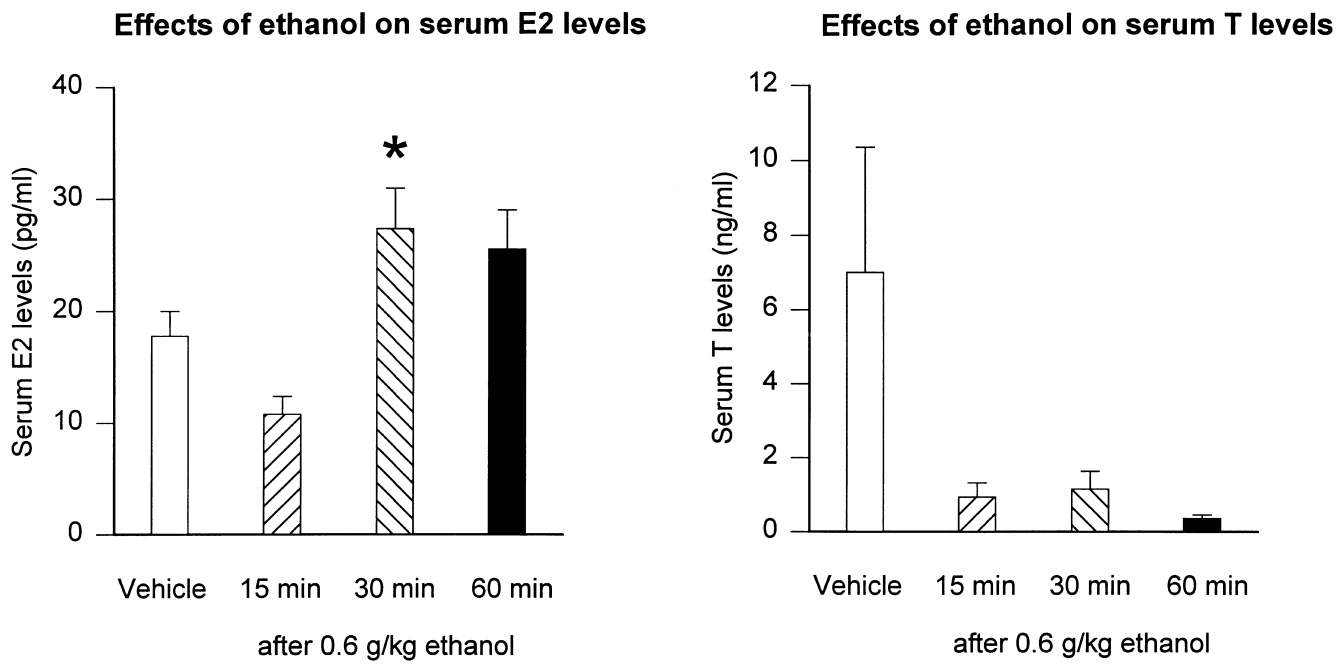

FIG. 1. Serum estradiol (E_2) and testosterone (T) levels in male mice 15, 30, and 60 min after an injection of 0.6 g/kg ethanol. Blood from the mice treated with vehicle also was collected 15–60 after the injection. The means ± SEM of 5–11 mice per group are shown. Asterisk indicates a significant difference between ethanol and vehicle.

jection, the E_2/T ratio was twofold higher in the ethanol-treated (mean ± SEM; 29.1 ± 16.5, $n = 5$ and 26.6 ± 6.2, $n = 6$) than in the vehicle-treated mice (12.0 ± 6.6, $n = 5$). A significant increase was seen 60 min after an ethanol injection (53.3 ± 7.5, $n = 6$, $p < 0.05$).

Experiment 2

Among 97 male mice, response to an injection of 0.6 g/kg ethanol relative to baseline aggression levels was as follows: 18 mice (18%) showed a reduction in aggressive behaviors following the injection of 0.6 g/kg ethanol; 23 mice (24%), an increase; and 41 mice (42%), relatively unchanged, nonaggressive levels. The remaining mice (16%) exhibited high or moderate baseline aggression levels, but did not exhibit alterations in aggressiveness, which fulfilled the criteria described above to be assigned into one of the three aggressor groups. These latter males were not used in this study. The time spent in aggressive acts in mice used for hormone assays are presented in Table 1. Body weights of these groups of mice did not differ from each other (Table 1). To further establish the reliability of our study, we separately analyzed the time spent in aggressive behavior following a vehicle on day 1, an ethanol on day 2, and a second vehicle injection on day 4. These data can be seen in Fig. 2. In this figure, the animals are divided to increased, reduced, and nonaggressor mice. In addition, the times spent exhibiting aggression within each of the aggressor groups also is given separately to those that in the connection of serum assays received either saline or ethanol. The figure shows that male mice that increased their aggressiveness after ethanol administration returned to lower levels of aggression after receiving a second vehicle injection. Male mice that were aggressive after the first vehicle injection, and reduced their aggressiveness after ethanol, did not return to the initial level of aggressiveness following the second vehicle injection. However, the test-retest correlation between the first and second vehicle injection concerning the time spent in aggressive behavior was significant [Pearson's correlation test: $r(31) = 0.48$, $p < 0.01$]. In our previous studies, this correlation has been over 0.90 (19).

Serum E_2 levels were determined following vehicle or ethanol treatments from the three groups as follows: 1) increased aggressors: 11 vehicle-treated and 12 ethanol-treated mice; 2) reduced aggressors: 9 vehicle-treated and 8 ethanol-treated mice (one sample was lost); and 3) nonaggressors: 13 vehicle-treated and 10 ethanol-treated mice (taken randomly from the pool of 41 nonaggressor mice). Assay results showed that circulating levels of E_2 did not significantly differ among the three groups after the animals were injected with vehicle (Fig. 3). However, serum E_2 levels correlated with aggressiveness: the more aggressive a mouse was at baseline, the higher its E_2 levels were, $r(15) = 0.44$, $p < 0.06$. Among the mice that received ethanol, serum E_2 levels did not correlate with ethanol-induced aggression ($r(16) = -0.27$, NS).

The effect of ethanol on serum E_2 levels differed among the groups (two-way ANOVA: among the moderately aggressive mice, which had exhibited an increase in aggressive behavior by 0.6 g/kg ethanol, serum E_2 levels were significantly lower after ethanol administration than at baseline after vehicle administration ($p < 0.05$) (Fig. 3). Ethanol slightly, but nonsignificantly increased serum E_2 levels in reduced aggressors and nonaggressors.

Serum T levels also were determined from the same samples that were used for E_2 assays. However, because many of the samples did not contain enough blood for both E_2 and T assays, the three groups were as follows: 1) increased aggres-

TABLE 1

THE TIME SPENT IN AGGRESSIVE BEHAVIOR DURING A 5-MIN RESIDENT-INTRUDER TEST IN MICE TREATED WITH VEHICLE AND 24 H LATER WITH 0.6 G/KG ETHANOL, AND WHICH RESPONDED TO ETHANOL BY EITHER REDUCING THEIR AGGRESSIVENESS, INCREASING THEIR AGGRESSIVENESS, OR NOT ALTERING THEIR RELATIVELY LOW LEVELS OF AGGRESSION

	Aggression (s)		
	Vehicle	Ethanol	
Mice which reduced aggression b.wt. 30.7 ± 0.4 g $n = 18$	62.8 ± 6.7	5.6 ± 2.2	t = 8.07, $p < 0.0001$
Mice which increased aggression b.wt. 31.1 ± 0.5 g $n = 23$	43.7 ± 9.4	121.6 ± 15.0	t = 4.40, $p < 0.0001$
Non-aggressive mice b.wt. 30.1 ± 0.4 g $n = 25$	5.1 ±1.1	4.1 ± 1.5	t = 0.59, NS

Means ± SEM are given.

sors: seven vehicle-treated and six ethanol-treated mice; 2) reduced aggressors: five vehicle-treated and four ethanol-treated mice; and 3) nonaggressors: six vehicle-treated and six ethanol-treated mice. No significant differences among the groups were seen (Fig. 3). There was a trend for T to be increased by ethanol in the reduced aggressor mice, and reduced in the two other groups. Serum T levels did not correlate with the amount of aggression expressed after a saline injection, $r(18) = -0.07$, NS, or after an ethanol injection, $r(16) = -0.22$, NS.

The E_2/T ratio was significantly influenced by ethanol [two-way ANOVA: $F(1, 25) = 4.3$, $p < 0.05$]. This ratio was similar among the saline-treated groups (mean ± SEM, increased aggressors: 32.1 ± 15.5; reduced aggressors: 44.2 ± 14.9; and nonaggressors: 34.1 ± 22.6), but of the ethanol-treated groups, the reduced aggressors exhibited a significantly reduced ratio (2.2 ± 0.9, $p < 0.05$; Fisher's least significant test), while it was not significantly altered among the increased (12.5 ± 5.3) and nonaggressor mice (13.5 ± 9.8).

DISCUSSION

Efforts to understand the link between ethanol and aggression using animal models have been hindered by the fact that ethanol does not consistently induce aggressive behavior. In accordance with the observations that moderately aggressive male mice and rats often become aggressive by low doses of ethanol (10,18), moderately aggressive male mice in the present study increased their aggressive acts by an ethanol treatment. However, some moderately to highly aggressive mice showed a reduction in aggressiveness postethanol treatment. This variance suggests that the mechanisms determining baseline levels of aggression may be different from the mechanisms that determine the response to ethanol.

Gonadal hormones are linked to aggression (3). Consistent with the observations that E_2 increases aggressive behavior in male mice (11,13,28), we found a positive correlation between aggressive behavior and serum E_2 levels. No correlation was seen between aggressive behavior and T. In addition, there were no significant differences in serum E_2 or T levels at base-

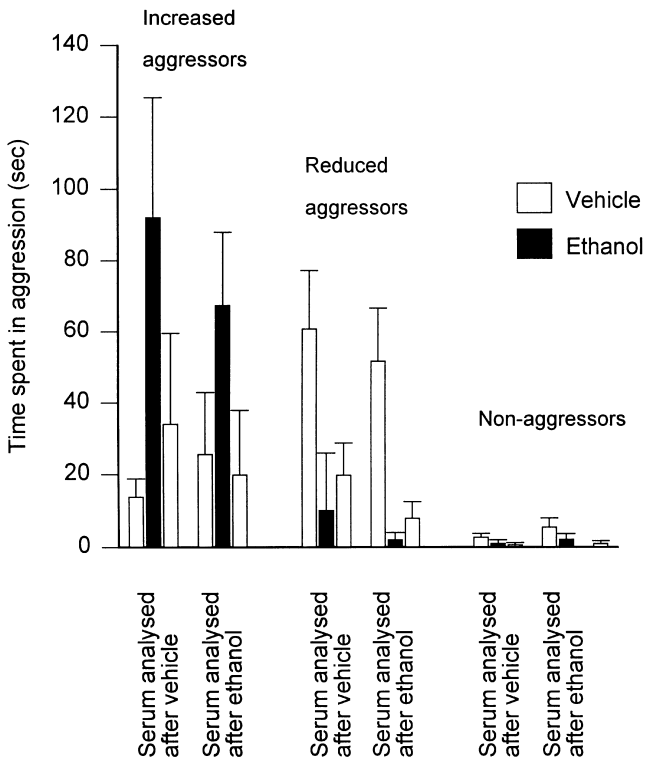

FIG. 2. The time spent in aggressive behavior in the resident-intruder test 30 min after the male mice were treated with vehicle (day 1), 0.6 g/kg ethanol (day 2), and again with vehicle (day 4). The mice were grouped to those that increased aggressiveness following ethanol injection (increased aggressors), reduced aggressiveness following ethanol injection (reduced aggressors), and were nonaggressive both after a vehicle and ethanol injections (nonaggressors). The first three bars in each group (increased, decreased, and nonaggressors) represent the times spent in aggression among those mice that, 30 min prior to the serum hormone assays, were injected with vehicle, and the last three bars represent the data obtained from mice that were injected with 0.6 g/kg ethanol prior to sacrificing them for hormone assays. Means ± SEM of four to five mice per group are shown.

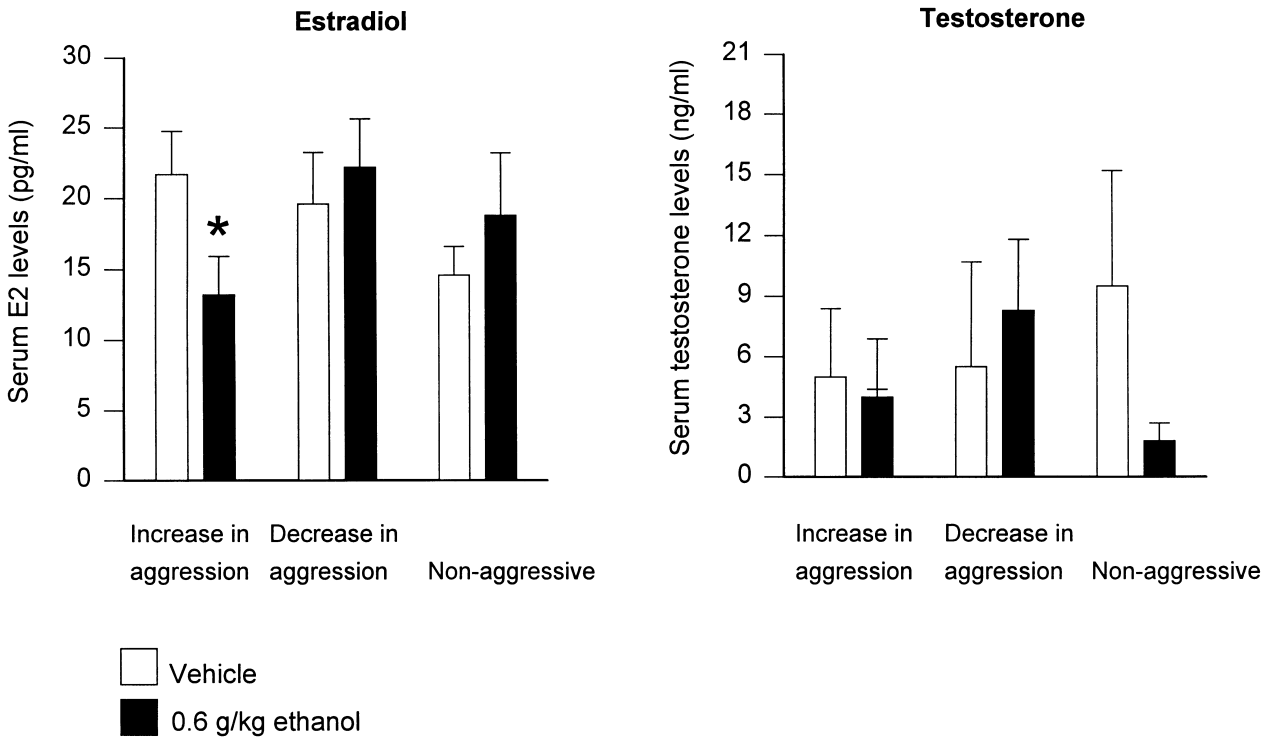

FIG. 3. Serum estradiol (E_2) and testosterone levels in male mice that were previously found to reduce or increase their aggressiveness by 0.6 g/kg ethanol, or in nonaggressive mice. Means ± SEM of 8–13 mice per group are shown in the E_2 study and four to seven mice per group in the testosterone study. *$p < 0.05$.

line among the mice that were observed to increase or reduce their aggressive behavior by ethanol. This may reflect the fact that the mean aggression after a vehicle injection between the increased and reduced aggressors did not significantly differ from each other. Thus, the baseline levels of E_2 or T do not determine which animals exhibit an ethanol-induced increase in aggressive behavior.

Acute and chronic ethanol exposure alters circulating gonadal hormone levels (2,27,29). Plasma E_2 levels are reported to be elevated in men consuming 0.9–2.1 g/kg ethanol, when measured 5–10 h after cessation of drinking (7). After a lower ethanol exposure (0.3 g/kg), unconjugated estrogen levels are not altered (determined 0–180 min subsequent to acute ingestion), while conjugated levels are significantly increased (1). We found a significant increase in circulating E_2 levels 30 min after an administration of 0.6 g/kg ethanol in group-housed male mice that were not tested in the resident-intruder paradigm of aggression. To our knowledge, this is a first time ethanol has been reported to be able to alter circulating E_2 levels and aromatization of E_2 from T, within 30 min after an acute administration. Our observation strongly suggests that E_2 may be involved in controlling behavioral alterations induced by ethanol.

A similar trend for an increase in E_2 levels by ethanol was noted among reduced aggressor and nonaggressor male mice. The lack of significance in the mice used in the aggression tests may reflect complex interactions among hormones, social isolation, and the resident-intruder test. Ethanol appeared to reduce serum T levels, but the effect failed to reach statistical significance. Studies in humans have shown that nonalcoholic men who consume 0.3 to 2.1 g/kg of body weight ethanol during a single drinking session, do not exhibit a consistent pattern of change in plasma T levels (1,7).

In support of the findings obtained after chronic ethanol exposure (29), the index of aromatization of E_2 from T (E_2/T ratio) was significantly increased by an acute ethanol administration among the group-housed mice. A slight increase was seen already 15 min after ethanol, and the difference reached statistical significance 60 min following the ethanol administration. None of the groups whose aggressiveness was evaluated in the resident-intruder test before studying the effect of ethanol on serum gonadal hormones, exhibited ethanol-induced increase in E_2/T ratio. On the contrary, the mice that lost their aggressiveness by ethanol exhibited a lower aromatization index when injected with ethanol than when injected with vehicle. However, the decreased ratio was not caused by a lowered E_2 after ethanol (E_2 levels were slightly increased), but nonsignificant, increase in serum T levels by ethanol.

In contrast to the increase in serum E_2 levels seen in the group-housed male mice, increased aggressor mice had lower serum E_2 levels when treated with ethanol than when treated with vehicle. The mechanisms contributing to this decrease remain unknown. Because E_2/T index was not altered by ethanol in this group, changes in aromatization might not have contributed. An alternate metabolic pathway for T is that of being hydroxylated to dihydrotestosterone (DHT). One possibility is that among the increased aggressor group, ethanol administration favored the DHT pathway of testosterone metabolism. In humans, an acute ethanol administration results in the concentrations of DHT to be reduced (7). DHT is the biologically active androgen ligand that binds to androgen receptors, and the inhibition of androgen receptor activation re-

duces aggressiveness (4). However, we did not measure serum DHT levels in the present study and, thus, the role of this metabolite in ethanol-induced aggression remains to be determined.

It also is possible that the increased aggressiveness, and perhaps the significantly reduced serum E_2 levels, in male mice following ethanol administration are caused by another biological system, besides gonadal hormones, which is activated/inhibited by ethanol. Expression of a variety of genes, including those of neurotransmitters, signaling proteins, molecular chaperones, and oncogenes, is altered by ethanol (22,23). Although most of these alterations cannot occur within 30 min of the ethanol injection, when the increase in aggression is observed, there is evidence to suggest that an increase in the release of neurotransmitters, such as dopamine and serotonin, may happen relatively soon after an ethanol administration (5,30). E_2 also influences the concentrations, turnover, and binding density of these neurotransmitters (16,20,26). It is, therefore, possible that simultaneous changes in circulating levels of E_2 and neurotransmitters could be responsible for the increased aggressiveness in some ethanol-treated male mice. However, the present study did not investigate whether the lowered E_2 levels in increased aggressor mice contributed to the ethanol-induced aggression and, thus, the causality of the events is not known.

In conclusion, a reduction in serum E_2 levels is characteristic of those male mice that increase their aggression by ethanol. These data suggest that mice who exhibit a paradoxical decrease in serum E_2 levels by ethanol may be particularly prone to ethanol-induced aggression.

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REFERENCES

1. Andersson, S. H. G.; Cronholm, T.; Sjovall, J.: Effects of ethanol on the levels of unconjugated and conjugated androgens and estrogens in plasma of men. J. Steroid Biochem. 24:1193–1198; 1986.
2. Bannister, P.; Lowosky, M. S.: Ethanol and hypogonadism. Alcohol Alcohol. 22:213–217; 1987.
3. Beatty, W. W.: Gonadal hormones and sex differences in nonproductive behaviors. In: Gerall, A. A.; Moltz, H.; Ward, I. L., eds. Handbook of behavioral neurobiology. Sexual differentation. New York: Plenum Press; 1992:85–128
4. Brain, P. F.; Simon, V.; Hasan, S.; Martinez, M.; Castano, D.: The potential of antiestrogens as centrally acting antihostility agents: Recent animal data. Int. J. Neurosci. 41:169–177; 1988.
5. Brodie, M. S.; Trifunovic, R. D.; Shefner, S. A.: Serotonin potentiates ethanol-induced excitation of ventral tegmental area neurons in brain slices from three different rat strains. J. Pharmacol. Exp. Ther. 273:1139–1146; 1995.
6. Chermack, S. T.; Taylor, S. P.: Alcohol and human physical aggression: Pharmacological vs. expectance effects. J. Stud. Alcohol 56:449–456; 1995.
7. Couwenbergs, C. J. T.: Acute effects of drinking beer or wine on the steroid hormones of healthy men. J. Steroid Biochem. 31:467–473; 1988.
8. Dorgan, J. F.; Reichman, M. E.; Judd, J. T.; Brown, C.; Longcope, C.; Schazkin, A.; Campbell, W. S.; Franz, C.; Kahle, L.; Taylor, P. R.: The relationship of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). Cancer Causes Control 5:53–60; 1994.
9. Emanuele, M. A.; Tentler, J.; Rada, D.; Kirsteins, L.; Emanuele, N. V.; Lawrence, A. M.: In vivo effect of ethanol on release of LH-releasing hormone and LH in rats. J. Endocrinol. 121:37–41; 1989.
10. Hilakivi, L. A.; Lister, R. G.: Effect of ethanol on the social behavior of group-housed and isolated mice. Alcolol. Clin. Exp. Res. 13:622–625; 1989.
11. Hilakivi-Clarke, L.: Role of estradiol in alcohol intake and alcohol-related behaviors. J. Stud. Alcohol 57:162–170; 1996.
12. Hilakivi-Clarke, L. A.; Arora, P. K.; Sabol, M. B.; Clarke, R.; Dickson, R. B.; Lippman, M. E.: Alterations in behavior, steroid hormones and natural killer cell activity in male transgenic TGFα mice. Brain Res. 588:97–103; 1992.
13. Hilakivi-Clarke, L. A.; Goldberg, R.: The effects of alcohol on elevated levels of aggressive behavior in transgenic mice overexpressing transforming growth factor α. Neuroreports 4:155–158; 1993.
14. Hilakivi-Clarke, L.; Goldberg, R.: Gonadal hormones and aggression maintaining effect of alcohol in male transgenic TGFα mice. Alcohol. Clin. Exp. Res. 19:708–713; 1995.
15. Ho, C.; Emanuele, N. V.; Kirsteins, L.; Lawrence, A. M.: In vivo studies on prolactin and luteinizing hormone in rats and mice. Int. J. Rap. Appl. Instrum. 19:737–740; 1992.
16. Karkanias, G. B.; Etgen, A. M.: Estradiol reduction of the agonist high affinity form of the alpha 2-adrenoceptor in the hypothalamus of female rats: Identification as the alpha 2D subtype. Mol. Pharmacol. 45:509–516; 1994.
17. Kessler, R. C.; McGongle, K. A.; Zhao, S.; Nelson, C. B.: Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from National Comorbidity survey. Arch. Gen. Psychiatry 51:8–19; 1994.
18. Krsiak, M.; Burgesova, M.: Effect of alcohol on behavior of pairs of rats. Psychopharmacologia 32:201–209; 1973.
19. Lister, R. G.; Hilakivi, L. A.: The effects of novelty, isolation, light and ethanol on the social behavior in mice. Psychopharmacology (Berlin) 96:181–187; 1988.
20. Michel, M. C.; Rother, A.; Hiemke, C.; Ghraf, R.: Inhibition of synaptosomal high-affinity uptake of dopamine and serotonin by estrogen agonists and antagonists. Biochem. Pharmacol. 36:3175–3180; 1987.
21. Miczek, K. A.: The psychopharmacology of aggression. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. The handbook of psychopharmacology. New York: Plenum Press; 1987:183–328.
22. Miles, M. F.; Gayer, G.; Sganga, M.: Regulation of neuronal gene transcription by ethanol. In: Allingd, C.; Diamond, I.; Leslie, S. W.; Sun, G. Y.; Wood, W. G., eds. Alcohol, cell membranes, and signal transduction in brain. New York: Plenum Press; 1993:283–298.
23. Nevo, I.; Hamon, M.: Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. Neurochem. Int. 26:305–336; 1995.
24. Ojeda, S. R.; Urbanski, H. F.; Costa, M. E.; Hill, D. F.; Moholt-Siebert, M.: Involvement of transforming growth factor α in the release of luteinizing hormone-releasing hormone from the developing female hypothalamus. Proc Natl Acad Sci USA 87:9698–9702; 1990.
25. Pihl, R. O.; Peterson, J.: Drugs and aggression: Correlations, crime and human manipulative studies and some proposed mechanisms. J. Psychiatr. Neurosci. 20:141–149; 1995.
26. Power, R. F.; Mani, S. K.; Codina, J.; Conneely, O. M.; O'Malley, B. W.: Dopaminergic and ligand-independent activation of steroid hormone receptors. Science 254:1636–1639; 1991.
27. Reichman, M. E.; Judd, J. T.; Longcope, C.; Schatzkin, A.; Clevidence, B. A.; Nair, P. P.; Campbell, W. S.; Taylor, P. R.: Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. J. Natl. Cancer Inst. 85:722–727; 1993.
28. Simon, N. G.; Whalen, R. E.: Hormonal regulation of aggression:

Evidence for a relationship among genotype, receptor binding, and behavioral sensitivity to androgens and estrogen. Aggress. Behav. 12:255–266; 1986.
29. Van Thiel, D. H.; Gavaler, J. S.: Endocrine consequences of alcohol abuse. Alcohol Alcohol. 25:341–344; 1990.
30. Weiss, F.; Lorang, M. T.; Bloom, F. E.; Koob, G. F.: Oral alcohol self-administration stimulates release in the rat nucleus accumbens: Genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267:250–258; 1993.