Ethanol consumption in the female Long–Evans rat: a modulatory role of estradiol

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Abstract

The examination of various gonadal hormone manipulations on ethanol intake in human subjects and in rodent models has resulted in disparate findings. In the present study, we examined the effects of ovariectomy and subsequent estradiol (E₂) replacement on ethanol intake in a within-subject design, as well as assessed the relevance of reproductive status on the efficacy of an E₂ stimulus in eliciting consumption. Female Long–Evans rats (n = 24) were given access to 10% ethanol and water in a continuous-access paradigm. After establishment of baseline intake values, rats were divided into four groups: sham/placebo (Shm/P), sham/estradiol (Shm/E₂), ovariectomized/placebo (Ovx/P), and ovariectomized/estradiol (Ovx/E₂). Rats in the Ovx/E₂ group were found to have a large and permanent decline in ethanol intake that persisted more than 3 months postsurgery. Administration of E₂ to Ovx/E₂ rats was associated with restoration of ethanol consumption to baseline levels. When Shm/E₂ and Ovx/E₂ groups were compared, reproductive status was found to be a determining factor in the efficacy of E₂ to elicit ethanol intake. Together, these findings provide evidence that ovarian hormones, particularly estradiol, exert activational effects on estrogen-responsive substrates to modulate ethanol consumption in the adult female rat. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Ethanol self-administration; Continuous access; Female rat; Ovariectomy; Estradiol replacement

1. Introduction

The majority of studies with human subjects that have addressed ethanol–gonadal hormone interactions have focused primarily on the effects of moderate ethanol consumption on gonadal hormone levels and hypothalamic-pituitary-gonadal (HPG) axis integrity. Ovarian hormone modulation of ethanol consumption and the effects of these hormones on the pharmacological and behavioral sensitivity to ethanol, on the other hand, have remained understudied. Methodological constraints have made it difficult to assess ethanol consumption patterns across the menstrual cycle in human beings (Gill, 1997). Individual variation in the hormonal profiles exhibited in women across the menstrual cycle necessitates the use of a within-subject design, which few studies have incorporated (Gill, 1997). In one recent study, a within-subject design was implemented. However, there was no detectable difference in ethanol consumption across the menstrual cycle (Holdstock & de Wit, 2000). The investigators advocated that several limitations hindered their results; that is, variable drinking histories of the women studied, insufficient sample size, and the absence of a test phase when estradiol levels were elevated (Holdstock & de Wit, 2000).

Animal models may offer a means for arriving at a precise understanding of ovarian hormone action on ethanol intake by avoiding many of the pitfalls inherent to studies with human subjects. The female rat provides a useful model of ethanol self-administration because it exhibits both spontaneous and cyclic ovulation (Yen et al., 1999), possesses cyclic hormone profiles that resemble those of the human menstrual cycle (with the exception of a truncated luteal phase), and is thought to have similar neural circuitry mediating the neurobiological effects of ethanol (Koob, 1992; Koob et al., 1998). In addition, rats can be investigated within an experimenter-controlled environment, thereby enhancing the probability that the underlying effects of an endogenous hormonal state on ethanol consumption are delineated (Roberts et al., 1998).

Sex differences in ethanol consumption have been observed in several rat strains, with females typically consuming more than males (Juárez & Barrios de Tomasi, 1999; Lancaster & Spiegel, 1992; Li & Lumeng, 1984). It is be-
lieved that these gender differences in consumption can be attributed, at least in part, to an assembly of sexually dimorphic neural substrates whose sensitivity to ethanol varies in relation to cyclic ovarian hormone levels (Almeida et al., 1998; Kelly et al., 1999; Lancaster, 1994; Lancaster et al., 1996). Lancaster et al. (1996) have documented an increase in ethanol intake throughout a peripubertal transition period that encompasses the establishment of estrous cycles. The reports of an enhancement of operant responding for ethanol during diestrus day (Roberts et al., 1998) and an attenuation of ethanol intake on proestrus day (Forger & Morin, 1982) are additional pieces of evidence that indicate that ovarian hormone environment and the estrous cycle may be important determinants of ethanol consumption in the female rat.

Several studies have been conducted to assess the effects of ovariectomy (ovex) and estradiol replacement on ethanol consumption in rodents. Hilakivi-Clarke (1996) reported that outbred CD-1 mice that were 30 days postovex showed a small decrease in intake of a 5% ethanol solution in comparison with findings for sham controls. In this same study, 60 days of 17β-estradiol pellet treatment in ovariectomized mice did not alter ethanol intake from levels observed in ovariectomized controls (Hilakivi-Clarke, 1996). In a separate study, ovex (1–2 weeks postsurgery) in Wistar rats was not associated with a change in intake of a 12% ethanol solution, whereas ovariectomized rats receiving daily injections of 17β-estradiol had significantly decreased ethanol intakes when compared with findings for rats with intact ovaries (Almeida et al., 1998). A large dose (300 μg) of ethinyl estradiol was shown to be associated with suppressed intake of a 5% ethanol solution in intact female Sprague–Dawley rats (Messina, 1981). Similarly, results of additional studies have shown that administration of large doses (5 or 100 μg) of estradiol benzoate led to an attenuation of ethanol intake in ovariectomized rats (Aschkenasy-Lelu, 1960; Sandberg et al., 1982). Forger and Morin (1982) observed that ovariectomized Sprague–Dawley rats had greatly attenuated intake and preference for 4% ethanol. In contrast, Cailhol and Mormède (2001) concluded that ovex failed to alter the maintenance of ethanol self-administration in the three rat strains studied.

The disparity of findings among studies of ovex and estradiol replacement may be due, in part, to the species and strain of rodent selected, to the dose and route of hormone administration chosen, to the reproductive status (intact ovaries, days postovex, or weeks to months postovex) of the test animals studied, or to the concentration of ethanol solution presented. Importantly, none of the aforementioned studies determined a baseline ethanol intake before the assignment of animals to experimental groups, introducing the possibility that intake variability was not evenly distributed among groups. Collection of an intake baseline would also have supported a within-subject analysis of the experimental manipulations. The purpose of the present study was to examine the effects of various gonadal hormone manipulations on ethanol intake by female rats in a within-subject design, to delineate further the modulatory role that ovarian steroids play in ethanol consumption. Furthermore, estradiol replacement regimens were administered to rats with intact ovaries as well as to ovariectomized rats to determine whether reproductive (hormonal) status is associated with an altered efficacy of estradiol stimulus on ethanol consumption.

2. Materials and methods

2.1. Animals

Twenty-four female, Long–Evans rats, each weighing between 190 and 220 g, were acquired from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The rats were housed singly in hanging wire cages with artificial lights on between 0500 and 1900 and given ad libitum access to rat chow and water (except where noted). The rats were acclimated to home cages and handled daily for at least 6 days before the start of the experiment. The Animal Care and Use Committee of Wake Forest University School of Medicine approved all procedures in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

2.2. Experimental design

Before the initial preference test, 10% ethanol (10E) was presented as the only fluid for 3 days. Experimental phase I involved the presentation of 10E and tap water in a two-bottle-choice, continuous-access paradigm for 14 days, as previously described (Tolliver et al., 1988). Bottle positions were alternated daily between left and right sides to minimize the effects of side preference on ethanol consumption. All bottles were removed daily at 0830 and replaced at 1030. During this time-out period, fluid volumes were measured and the rats were weighed. Water and 10E were replenished every other day. At the conclusion of the preference test, rats were assigned to either a sham-operated or an ovariectomized group (n = 12 for each) on the basis of their mean baseline ethanol intakes (grams per kilogram of body weight per day). Intake baseline values were ranked from largest (#1) to smallest (#24). Odd-ranked drinkers were assigned to the ovariectomized pool, and even-ranked drinkers were designated to the sham pool. After surgery, all rats were allowed a 3-day recovery period without access to 10E to prevent any potential interaction between ethanol and the surgical anesthetic used.

Experimental phase II included a 24-day evaluation of postsurgical ethanol intake with access to both 10E and water. After this assessment, sham-operated and ovariectomized rats were divided further into subgroups (n = 6) on the basis of their postsurgical ethanol intakes. A similar
ranking procedure was implemented as described above to create the following experimental groups: sham/placebo (Shm+/P), sham/estradiol (Shm+/E2), ovariectomized/placebo (Ovx+/P), and ovariectomized/estradiol (Ovx+/E2). At the beginning of phase III, each rat received either a placebo or a 17β-estradiol pellet implant (0.25 mg; 21-day release). All rats were allowed a 2-day recovery period in the absence of 10E before being reevaluated in a two-bottle-choice paradigm for an additional 64 days. Phase IV of the experiment encompassed the period that elapsed during and after the washout of implant-supported estradiol activity.

2.3. Surgery: ovariectomy and implants

The rats were anesthetized with halothane (4.0%) and maintained on this inhalant (2.0%) throughout surgery (20–25 min) by means of a fitted nose cone. All instruments were autoclaved before use, and the procedure was conducted in a sterile environment. Bilateral (1.5-cm) incisions were made in the flanks. Ovaries were excised after ligation at the tip of the uterine horn. Sham operations were conducted under identical conditions, but without ligature application or ovary removal. The presence or absence of ovaries was confirmed during autopsy. The subcutaneous implantation of pellets at the nape of the neck was conducted under similar anesthetic conditions used for ovariectomy.

2.4. Drugs

Ethanol (95% [vol./vol.]) was diluted in tap water to a concentration of 10% (vol./vol.). Placebo and 17β-estradiol pellets (0.25 mg; 21-day release) were purchased from Innovative Research of America (Sarasota, FL). The pellet was selected for its zero-order-release kinetics and ability to maintain 17β-estradiol plasma concentrations in the mid- to upper-physiological range (30–40 pg/ml). Halothane was purchased from Halocarbon Laboratories (River Edge, NJ).

2.5. Data analysis and presentation

Phase I baseline measures are reported as 8-day means ± standard error of the mean (S.E.M.) (representative of the last 8 days of preference testing before surgery). The baseline body weights and all parameters during phases II–IV are presented as 4-day means ± S.E.M. These 4-day periods are referred to as intervals in the subsequent text. The results are depicted in figures as four experimental groups throughout all phases, despite that these groupings were not empirically determined until the end of phase II. This was done to facilitate the analysis of across-phase effects for each group.

All experimental parameters were analyzed by using the GB-STAT version 6.5 software package (Dynamic Microsystems, Inc., Silver Spring, MD). A two-way repeated measure analysis of variance (ANOVA) (factors day and group) was carried out to assess differences in baseline values among the experimental groups. A two-way repeated measure ANOVA (factors interval and surgery) was used to detect significant differences among groups during phase II. Similarly, differences among groups throughout phases III and IV were analyzed by three-way repeated measure ANOVA (factors interval, surgery, and pellet). Within-group analyses across phases were examined by two-way repeated measure ANOVA (factors interval and group). The Tukey multiple comparisons procedure was implemented to determine pair-wise differences if a significant main effect occurred. In all cases, intervals 1 and 7 (corresponding to the 4-day periods immediately after surgery and pellet implantation) were excluded from statistical analyses to isolate the gonadal hormone effects from the deprivation-like effect that was apparent at these intervals.

3. Results

3.1. Phase I: baseline

The baseline ethanol intake (Fig. 1) and the baseline ethanol preference ratio (Fig. 2) for all rats averaged 4.86 ± 0.39 g/kg/day (range, 1.61–8.88 g/kg/day) and 0.47 ± 0.04 (range, 0.16–0.79), respectively. There was a statistically significant main effect of day for both baseline intakes [F(7,140) = 2.54, P < .05] and baseline preference ratios [F(7,140) = 2.88, P < .01]. The baseline water intake (Fig. 3) and the baseline body weight (Fig. 4) for all rats averaged 15.73 ± 1.11 ml/day (range, 5.88–24.67 ml/day) and 235 ± 2 g (range, 214–254 g), respectively. A statistically significant main effect of day was found for water intake baselines [F(7,140) = 3.62, P < .01] and body weight baselines [F(3,60) = 12.19, P < .001]. There was no statistically significant group effect detected for any parameter evaluated.

3.2. Phase II: surgery (ovex vs. sham)

Three and a half weeks after surgery (interval 6), the mean ethanol intake in sham rats (Shm+/P and Shm+/E2 groups) was 3.28 ± 0.39 g/kg/day (range, 1.11–6.28 g/kg/day). At the same postsurgical period, ovariectomized rats (Ovx+/P and Ovx+/E2 groups) exhibited a mean ethanol intake of 2.34 ± 0.20 g/kg/day (range, 1.42–3.74 g/kg/day). A statistically significant main effect of surgery [F(1,55) = 11.28, P < .01] was found for ethanol intakes during this phase. In an across-phase analysis, a statistically significant main effect of interval [F(20, 400) = 4.83, P < .001] was also determined. The Ovx+/P and Ovx+/E2 groups had significantly attenuated ethanol intakes as early as 2 weeks postovex (intervals 4–6; P < .05) when compared with findings for their respective baseline intakes (Fig. 1). By three and a half weeks after surgery (interval 6) the sham and ovariectomized rats had mean ethanol preference ratios of 0.37 ± 0.04 (range, 0.12–0.65) and 0.33 ± 0.03 (range, 0.16–0.56), respectively.

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The absence of a statistically significant main effect of surgery on preference ratio may have been attributable to two ovariectomized rats whose preference ratios were consistently greater than their baseline values. Ethanol preference ratios measured in the remaining majority of ovariectomized rats averaged 62% of baseline.

Water intake in sham and ovariectomized rats averaged $18.08 \pm 1.38$ ml/day (range, 12.00–26.00 ml/day) and $18.25 \pm 1.36$ ml/day (range, 11.00–27.00 ml/day), respectively, at the conclusion of phase II (interval 6). At three and a half weeks after surgery (interval 6), the mean body weight of sham and ovariectomized rats was $252 \pm 6$ g (range, 230–280 g) and $292 \pm 6$ g (range, 253–321 g), respectively. A main effect of surgery [$F(1,55) = 123.43, P < .001$] was found for body weights during phase II. The body weights of ovariectomized rats throughout the latter half of phase II were statistically greater than those of the sham rats (intervals 3–6; $P < .05$). Furthermore, an across-phase analysis revealed a statistically significant main effect of interval [$F(20, 400) = 184.60, P < .001$] on body weight. Statistically significant postsurgical increases in body weight (Fig. 4) occurred earlier in the Ovx+P and Ovx+E$_2$ groups (interval 2; $P < .01$) than in the Shm+P and Shm+E$_2$ groups (interval 5; $P < .05$).

### 3.3. Phase III: pellet implant (placebo vs. estradiol)

A statistically significant surgery x pellet interaction [$F(1,40) = 11.80, P < .01$] was present for ethanol intakes during the hormone implant phase. In an across-phase analysis, a statistically significant main effect of interval [$F(20, 400) = 4.83, P < .001$] on ethanol intake was determined. Ethanol intakes in the Ovx+P group (Fig. 1) were less than baseline values throughout this phase (intervals 8–11), a statistically significant finding ($P < .05$). A statistically significant main effect of surgery [$F(1,40) = 4.82, P < .05$] and a surgery x pellet interaction [$F(1,40) = 5.25, P < .05$] were found for the ethanol preference ratios within phase III (Fig. 2).

Ethanol intakes in the Ovx+P group (Fig. 1) were less than baseline values throughout this phase (intervals 8–11), a statistically significant finding ($P < .05$). A statistically significant main effect of surgery [$F(1,40) = 46.59, P < .001$] and pellet [$F(1,40) = 24.78, P < .001$] on body weights were found during this implant.
phase. In addition, a surgery x pellet interaction \([F(1,40) = 6.12, P < .05]\) was detected for body weight. Estradiol replacement was associated with reduced body weights in the Ovx/E2 group (Fig. 4).

3.4. Phase IV: postimplant

Statistically significant main effects of surgery \([F(1, 110) = 13.63, P < .001]\) and pellet \([F(1, 110) = 27.25, P < .001]\) were found for ethanol intakes during phase IV. A statistically significant surgery x pellet interaction \([F(1, 110) = 8.37, P < .01]\) also occurred for this measure. In an across-phase analysis, a statistically significant main effect of interval \([F(20, 400) = 4.83, P < .001]\) on ethanol intake was determined. Ethanol intakes by the Ovx+P group (Fig. 1) were significantly attenuated throughout phase IV (intervals 12–22; \(P < .05\)). Statistically significant main effects of interval \([F(10,110) = 2.77, P < .01]\), surgery \([F(1,110) = 137.85, P < .001]\), and pellet \([F(1,110) = 51.47, P < .001]\) were observed for body weights during phase IV. A statistically significant surgery x pellet interaction \([F(1,110) = 7.14, P < .01]\) was also found for this measure.

3.5. Patterns of ethanol intake

Although identifiable estrous cycle-like (4- to 5-day) patterns of ethanol intake were not consistently observed in rats with ovaries intact, occasional periods of cyclic drinking were exhibited: throughout phase II for the Shm+E2 rat whose drinking profile is depicted in Fig. 5B and throughout phase I for the Ovx/P rat whose drinking profile is shown in Fig. 5C. Ovex (as early as 2 weeks postsurgery) was associated with decreases in daily intake variability when daily ethanol intakes were compared with baseline profiles. This phenomenon was apparent in the Ovx+P rat whose drinking profile is represented in Fig. 5C when comparing intake during phases II through IV to baseline patterns and in the Ovx+E2 rat whose drinking profile is de-
picted in Fig. 5D when comparing intake during phase II to baseline profiles. In contrast, the Shm+P rats exhibited consistent variability in ethanol intakes across all experimental phases (as exemplified in Fig. 5A).

4. Discussion

In the current study, ovex was associated with a statistically significant decline in ethanol intake by 3 weeks after surgery, and this effect was found to be permanent in rats that remained in a hormone-deprived state. This finding contradicts results of some previous reports (Almeida et al., 1998; Cailhol & Mormède, 2001) in which no change in ethanol intake was observed after ovex. The differences in experimental approach among studies may help explain the disparity of findings. The collection of intake baselines before ovex in our study supported a within-subject design, whereas in previous studies these measures were not obtained. In most cases, we observed that rats with the largest baseline ethanol intakes exhibited the greatest reductions in intake after ovex. The converse was true in rats with low baseline intakes. This apparently graded-consumption response to ovex within our rat cohort underscores the importance of properly balancing ethanol intakes among experimental groups before surgical manipulation. It is possible that the experimental groups compared by a between-subject design in previous studies (Almeida et al., 1998; Cailhol & Mormède, 2001) were insufficiently balanced to detect an ovex effect. Also, in the present study the quality of the ethanol stimulus (10% [vol./vol.] solution) remained constant throughout acquisition and maintenance periods of exposure. In both the study by Cailhol and Mormède and the study by Almeida and colleagues, the acquisition of ethanol consumption was conducted while the concentration of the ethanol solution was increased in a step-wise fashion. The absence of stable conditions throughout this acquisition procedure may have similarly confounded the detection of an ovex effect on ethanol consumption.

Ovariectomy was not associated with statistically significant alterations in water intake within the Ovx+P and Ovx+E groups across phase II. Furthermore, the consistent discrepancy in water intake between Ovx+P and Shm+P groups during phases III–IV mirrored initial baseline differences (Fig. 3), indicating that the lack of statistically significant changes in water intake held true for ovariectomized rats more than 3 months postsurgery. Consistent with findings in previous reports (Mohamed & Abdel-Rahman, 2000; Tarttelin & Gorski, 1973), ovex led to significant body weight gain over sham control values. This change in body weight, however, did not influence the postovex decline in the milliliters of ethanol consumed (data not shown).
During E2 replacement to the Ovx+E2 group, ethanol intakes were restored to presurgical baseline levels (Fig. 1; phases III–IV). In contrast, results of other studies in rats have shown decreases in ethanol intake of ovariectomized rats administered either 5 μg of E2 for 17 days (Almeida et al., 1998) or 5 μg of estrogen benzoate for 20 days (Sandberg et al., 1982). However, the findings of these two studies were somewhat ambiguous. In the study by Almeida and colleagues, E2 treatment began before ethanol presentation, making it necessary to compare the consumption by the ovariectomized group to that of controls with ovaries intact while the ethanol solution concentration was incrementally manipulated. Although Sandberg and collaborators administered hormone to ovariectomized rats that had several months of drinking experience, the treatment was associated with an initial suppression of ethanol intake. However, intake that followed repeated administration of estrogen benzoate was shown to return to levels observed in untreated ovariectomized rats. The disparity of results among these earlier reports and the current study could also be due to the route of administration or to the estrogen dose selected. In our laboratory, we have found that daily E2 injections of 5 μg/kg support supraphysiological plasma concentrations of this hormone, as well as that a distinctive rise and subsequent decline in E2 concentrations are exhibited after administration (unpublished observations, M. M. Ford, J. C. Eldridge, & H. H. Samson, 2001). In the current study, pellet implants were used to provide stable plasma E2 concentrations within a physiological range.

The efficacy of E2 administration on ethanol consumption was decidedly dependent on the reproductive status of the rat (Fig. 1; phase III). A hormone stimulus that had little to no effect on Shm+E2 rat ethanol intakes was shown convincingly to be associated with restoration of ethanol consumption of Ovx+E2 rats to their baseline intake levels. This finding would support the suggestion that estrogen-responsive substrates facilitating this behavior were sensitized owing to hormone deprivation. Dependency on reproductive status in the determination of the responsiveness to an E2 stimulus has been demonstrated previously in the control of luteinizing hormone release and in the activation of lordosis behavior (McGinnis et al., 1981). McGinnis and colleagues purport that the magnitude and duration of alterations in nuclear estrogen receptors (ERs) determine the physiological or behavioral efficacy of an estrogen stimulus. Augmentation of ER mRNA levels has been demonstrated within several hypothalamic brain regions of rats after ovariectomy (Shughrue et al., 1992). It is possible that the sensitization of Ovx+E2 rats to the E2-invoked ethanol consumption may have been attributable to an elevated nuclear ER state within the neuronal circuitry mediating this behavior. In addition, the enactment of an autoregulatory process by which E2 up-regulates or down-regulates ER levels in a concentration-dependent manner (Mohamed & Abdel-Rah-
man, 2000) may help to explain the underlying ramifications of the prevailing endogenous hormone environment on the sensitivity of this ethanol consumption response.

Estradiol may alter ethanol consumption through several mechanisms of action. Estradiol has been shown to exert immediate (seconds to minutes) effects by altering neuronal excitability, modulating cAMP signal transduction cascades, and interacting with G-protein-coupled receptors (McEwen & Alves, 1999). Prolonged (hours to days) effects of E2, through a classic genomic mechanism, may result in transcript expression changes that are relevant to ethanol consumption. Alterations in taste reactivity (Wade & Zucker, 1970), neurotransmitters and their respective receptors (Lancaster et al., 1996; McEwen & Alves, 1999), ingestive behavior regulation (Kalra et al., 1999), and energy metabolism (Wade et al., 1985) may be some of the potential mechanisms by which E2 exerts its modulatory effects on ethanol consumption.

The elevated ethanol intakes rapidly achieved after E2 implants to the Ovx+E2 group persisted throughout the first half of the hormone washout period (Fig. 1; phase IV). The persistence of ethanol consumption may have resulted from the accumulation of E2-inducible gene products within the target neurons that control this behavior (McGinnis et al., 1981). In the absence of further hormonal support, these E2-inducible products would degrade over time, and only when declining below a set threshold would a corresponding change in the affected behavior be apparent. Similarly, this type of delayed response to the discontinuation of hormonal support may help explain why sizeable decreases in ethanol consumption were not observed until after the hormone washout period.
consumption were not found until more than 2 weeks postovx.

Interestingly, the finding that E2 elicited augmented water intakes in both Shm+E2 and Ovx+E2 groups (Fig. 3) seems to indicate that this hormone perhaps acts through distinct mechanisms in its modulation of water as opposed to ethanol intake. Mohamed and Abdel-Rahman (2000) have demonstrated that autoregulation of ER by E2 is region and tissue specific. This finding is substantiated by the observation that mRNA expression of ERα in rats 12 weeks postovx is up-regulated in some tissues and remains unchanged in others, as well as that E2 treatment is associated with the restoration of ERα mRNA levels to sham rat (intact ovaries) levels in only those regions previously demonstrating an up-regulation. It is possible that the substrates regulating water consumption possess ER populations that are not subject to the same degree of autoregulatory control as those affiliated with ethanol intake modulation. The differential distribution and regulation of ERα and ERβ isoforms, which likely alter brain function and behavior in distinct manners (Österlund et al., 1998), may also help explain this dichotomy. In addition, water turnover has been shown to decrease when taking into consideration that ethanol consumption induces diuresis (Eisenhofer & Johnson, 1982).

In this study, female rats with ovaries intact consumed twice as much ethanol as that of several cohorts of male rats (4.71 ± 0.27 vs. 2.24 ± 0.18 g/kg/day, respectively) previously examined in this laboratory under similar experimental conditions (Files et al., 1994; Samson et al., 1991, 1992). This observation is in agreement with results of earlier studies that have shown similar gender differences across numerous rat strains (Juárez & Barrios de Tomasi, 1999; Lancaster & Spiegel, 1992; Li & Lumeng, 1984). The ethanol intake of female rats 3 months postovx (2.33 ± 0.39 g/kg/day; interval 22) closely matched historical male intake values. Lancaster and Spiegel (1992) have reported gender differences in the daily patterns of beer (10% ethanol) intake in Long–Evans rats, with female rats exhibiting larger and more variable intakes compared with those of male rats. Five of six Ovx+P rats in this experiment expressed decreased amounts of variability in daily ethanol intakes as early as 2 weeks postovx (refer to Fig. 5C). Thus, in the current study, ovex was associated with a reduction in both ethanol intake and daily intake variability in female rats. These findings are consistent with results of earlier studies (Findlay et al., 1979; Tarttelin & Gorski, 1973), documenting a reduction of variability in fluid drinking patterns after ovex. This altered consumption pattern arguably resembles more closely the reported drinking patterns of male rats (Lancaster & Spiegel, 1992) than the patterns of female rats with ovaries intact, supporting the suggestion that reproductive status and fluctuations in ovarian hormones may contribute greatly to gender differences in ethanol consumption by the Long–Evans strain.

Ethanol and water intakes were expressed as 4-day means ± S.E.M. to reduce the potential effects of estrous cycle on the variability in these measures. Although it has been documented extensively that female rats normally exhibit estrous cycles of 3–5 days in length, the 4-day cycle is considered the most prevalent variant (Everett, 1989). This precaution was adopted in consideration of results of a previous study in which a cyclic regulation of ethanol consumption across the estrous cycle was observed, with intake and preference ratio being lowest on proestrus (Forger & Morin, 1982). In the current study, consistent cyclical drinking patterns were not observed in female rats with ovaries intact (Fig. 5A–B). The lack of such patterns is in agreement with results of a recent study that showed no change in ethanol consumption across various menstrual cycle phases in human subjects (Holdstock & de Wit, 2000). It is possible that the detection of discernible differences in intake across the estrous cycle is reliant on the concentration of ethanol used, as well as that the 10% solution used in the current study was too aversive (and consumed as too small of a volume). When a 4% ethanol solution was used, which was found to be optimally preferred across a wide range of concentrations, estrous cycle patterns were observed (Forger & Morin, 1982). Furthermore, some rats in our study exhibited obvious side preferences, which resulted in alternating days of binge and abstinence behavior as the ethanol solution was moved daily from side to side. The development of side preferences may have confounded the observation of cyclic drinking patterns in this study.

The peaks in ethanol intakes that occurred at intervals 1 and 7 may be attributable either to a deprivation-like effect resulting from the imposed postsurgical periods of ethanol abstinence or to surgical stress. The ethanol deprivation effect is not typically observed after this short of an abstinence period (Heyser et al., 1997), but it has been shown to occur after 3 days of abstinence in a continuous-access procedure (Spanagel et al., 1996). Abstinence periods were applied to prevent potential ethanol interactions with the surgical anesthetic used. Recent evidence from our laboratory has shown that these transient peaks in ethanol intake still occur after surgery when continuous access to 10E is maintained throughout the perioperative period (unpublished observations, M. M. Ford, J. C. Eldridge, & H. H. Samson, 2001), supporting the suggestion that these peaks are related more to surgical stress than to ethanol deprivation. Regardless of the cause, the changes in ethanol and water intake between intervals 1 and 2 and between intervals 7 and 8 are likely due to a reduction of the surgical stress, the ethanol deprivation effect, or both.

Recent survey estimates indicate that approximately 25% of postmenopausal women receive some form of estrogen...
replacement therapy (Wysowski et al., 1995). A single ethanol challenge has been shown to elevate estradiol levels as much as threefold (of the intended target range) in women receiving hormone replacement therapy (HRT) (Ginsburg et al., 1996), thereby increasing the potential risk of breast cancer (Purohit, 1998). Although not yet addressed in studies with human subjects, HRT in postmenopausal women may raise an important health issue in women who consume alcoholic beverages. The results of this study seem to indicate that HRT augments ethanol intake to preestablished baseline levels and above. In the human condition, this may translate to postmenopausal women receiving HRT drinking levels of ethanol that resemble their premenopausal levels. It is possible that the same level of moderate ethanol consumption throughout subsequent life stages (premenopause followed by postmenopause) may have considerably different health consequences for women (Gill, 2000).

In summary, ovex was associated with a significant decline in ethanol intake, and subsequent E2 administration was associated with a restoration of ethanol consumption to sham levels. Reproductive status was found to be a determining factor in the responsiveness of an ethanol drinking response to E2. Together, these findings provide evidence that ovarian hormones, particularly E2, exert activational effects on hormone-inducible substrates to modulate ethanol consumption in the adult female rat. The latency of ethanol intake changes after hormone removal (either through ovex or discontinuation of E2 replacement) seems to indicate that E2 has prolonged effects on this behavior, perhaps through a genomic mechanism of action. Additional studies will be required to dissect the mechanisms by which E2 regulates ethanol intake in the female rat.

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