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A pharmacological dose of estradiol can enhance appetites for alcoholic beverages

Meta L. Reid*, Christopher L. Hubbell, Larry D. Reid

Laboratory for Psychopharmacology, Rensselaer Polytechnic Institute, Room 302, Carnegie Hall, Troy, NY 12180-3590, USA

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Abstract

Each of 30 female Sprague–Dawley rats were given 2 mg of estradiol valerate (EV), 30 others were given placebos. EV is a preparation that delivers estradiol for more than 12 days, but probably less than 20. Fifteen days later, the females had the opportunity to take sweetened alcoholic beverage 24 h a day across 25 days. Subsequently, they could self-administer other alcoholic beverages, including one of only alcohol and water. After a period of abstinence, rats had another opportunity to take sweetened alcoholic beverage (94 to 96 days after the single injection of EV). With every measurement, rats given EV consumed significantly more ethanol than controls. For example, mean of measurements representing daily intake for the fourth week of availability of palatable alcoholic beverage for placebo-treated = 5.29 grams of ethanol per kilogram of bodyweight (g'E/kg); for EV-treated = 8.13 g'E/kg; P=.003. The data support the conclusion that pharmacological doses of estradiol can induce marked, enduring changes in appetite for alcoholic beverages.

Keywords: Ethanol; Alcohol; Alcoholic beverages; Alcoholism; Alcohol abuse; Alcohol dependence; Alcohol use disorders; Estradiol; Estradiol valerate; SERMs

1. Introduction

Estradiol valerate (EV) is converted slowly into 17-β-estradiol and valeric acid. The 17-β-estradiol, produced by the conversion, has the same pharmacodynamics and pharmacokinetics as the endogenous steroid (Dusterberg and Nishino, 1982). An intramuscular injection of EV can release estradiol for 12 days or more (Dusterberg and Nishino, 1982). Even though the released estradiol is the same as endogenous estradiol, the events following injections of EV are those of a pharmacological dose, because the level of estradiol is sustained at supraphysiological levels for days. There are indications that a single injection of 2 mg of EV to female rats sets events that are toxic to the β-endorphin neurons of the hypothalamus (Brawer et al., 1993; Desjardins et al., 1990, 1993).

A single injection of EV modifies rats' intakes of alcoholic beverages. While estradiol is being released, rats' intakes of saccharin-sweetened alcoholic beverages are reduced (Reid et al., 2002). Those results are concordant with those of Sandberg and Stewart (1982) and Sandberg et al. (1982). They found that daily injections of estradiol benzoate (5 μg) to ovariectomized female rats reduced intakes of an alcoholic beverage (10% ethanol and water) across a number of days. Juarez et al. (2002) observed similar findings. The results are also concordant with those of Hilakivi-Clarke (1996) who measured ethanol intake in ovariectomized mice implanted with pellets that released about 2 or 4 μg of estradiol daily. The measures of ethanol intake among females occurred 29 to 36 days after the surgery of implanting the pellets designed to release estradiol for as many as 60 days.

With the end of release of estradiol by a single dose of EV (1 or 2 mg/female), rats' intakes of saccharin-sweetened and nonsweetened alcoholic beverages are enhanced (Marinelli and Gianoulakis, 2000; Marinelli et al., in press; Reid et al., 2002). Further, the aftereffects of a single dose of EV were remarkably enduring; they sustained enhanced consumption of ethanol for months after the injection (Marinelli and Gianoulakis, 2000; Reid et al., 2002). The finding that pharmacological dosing with estradiol could instate enduring changes that would manifest themselves

* Corresponding author. Tel.: +1-518-276-8270; fax: +1-518-275-8268.
E-mail address: reidl@rpi.edu (M.L. Reid).

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postdosing as enhanced self-administration of alcoholic beverages is novel and seems particularly relevant to issues of alcohol abuse and alcoholism (AAA). Each of the initial experiments involved rather small numbers of subjects and there were instances when the mean difference between EV- and placebo-treated females, although on average large, did not meet standards for statistical significance.

Collectively, the available evidence supports the idea that after pharmacological doses of estradiol, an enhanced appetite for alcoholic beverages will likely emerge with opportunities to take alcohol (Reid et al., 2002). Nevertheless, further assessment is desirable. In particular, there is a need to assess whether the effect is not only statistically significant (reliable), but also whether the effect is clinically significant.

Clinical significance is clearly related to the amount of ethanol consumed. An increment in intake of ethanol when baseline intake is very small (as is usually the case when only ethanol and water are presented to rats) can have little meaning, in terms of clinical significance, because of the rats' ability to efficiently metabolize ethanol and, thereby, avoid toxic doses of ethanol. On the other hand, even a modest, and particularly a large, increment in intake when the procedures are arranged for large, probably toxic, intakes at baseline is more apt to be of interest in terms of clinical significance. To assess the potential for clinically meaningful effects, therefore, it seems reasonable to use a palatable alcoholic beverage that does induce large, probably toxic, self-administered doses of ethanol. In addition, flavored alcoholic beverages are those most often marketed to people (Reid, 2002).

The available evidence is not sufficient to determine whether the particular procedures used here are the optimal ones for enhancing baseline intakes of ethanol. The evidence does support, however, the conclusion that the procedures by themselves (providing a palatable alcoholic beverage 24 h a day for a number of days) induce high levels of intake among placebo-treated, female rats (Reid et al., 2002). Consequently, we assessed the effects of EV treatment using a larger number of subjects per group than have been used in the past and using, throughout most of the procedures, a palatable alcoholic beverage. We reasoned that if EV treatment enhanced consumption of ethanol more than that induced by procedures instigating large intakes by themselves that such a finding would be of considerable interest to those building theory of AAA.

We presented the alcoholic beverage at a time other data (Reid et al., 2002) indicated would be near, or at the time when the enhanced appetite for alcohol emerges. The changes in bodyweight that follow a single injection of EV (Reid et al., 2002), as well as direct assessments of estradiol (Dusterberg and Nishino, 1982), indicate the estradiol is being released for a period of at least 12 days, but probably not more than 20 days. During the time estradiol is being released by EV or being provided by daily injections of estradiol benzoate (Sandberg and Stewart, 1982; Sandberg et al., 1982), intakes of alcoholic beverages are reduced. There is also a reduction in bodyweight with the onset of the chronic dosing (Reid et al., 2002; Sandberg and Stewart, 1982; Sandberg et al., 1982). The rats adapt to the supraphysiological doses of estradiol and, within a few days, again gain weight. They also gradually return to taking alcoholic beverage at levels seen predosing (Reid et al., 2002; Sandberg and Stewart, 1982; Sandberg et al., 1982). The previously collected data indicate that the enhanced appetite for alcoholic beverages emerges after pharmacological doses of estradiol (Reid et al., 2002). Unspecified effects of estradiol induce changes that outlast dosing itself and that, with opportunity to self-administer alcoholic beverages, manifest themselves as large intakes of ethanol. Here, we introduce the alcoholic beverage after the changes that we believe are salient to the modification in appetite. This time of introduction also avoids any conditioned aversion to the alcoholic beverage that might develop when the beverages are presented with the onset of dosing.

2. Method

2.1. Subjects

The subjects were 60 female Sprague–Dawley rats purchased from Taconic Farms (Germantown, NY) when they weighed about 150 g (the group that eventually received EV weighed 152 g and the controls 148 g, a statistically insignificant difference). Upon arrival at the laboratory, they were housed individually in standard cages with food and water always available. The windowless room housing their cages was maintained at about 22 °C and was lighted by incandescent lamps for 12 h a day beginning at 0800 h. These procedures, as well as the general care and use of the animals, was approved by Rensselaer Polytechnic Institute's institutional review committee, which, in turn, adheres to the Guide for Care and Use of Laboratory Animals (National Academy of Sciences, 1996).

2.2. Estradiol valerate

The doses of EV (from Sigma Aldrich) were 2.0 mg/rat given intramuscularly by way of a 0.2-ml injection of EV and carrier. The carrier of EV was sesame oil. An injection of the same volume of oil served as the placebo. EV was given 15 days before the rats were given their first opportunity to take alcohol. This is the time when pharmacological dosing with estradiol is waning. The initial days of this period is also a time of no significant gain in weight. This experiment, which extends considerably beyond 20 days, therefore, is not an assessment of the direct effects of EV itself, but an assessment of postpharmacological doses of estradiol.

2.3. Procedure

A few days after arrival at the laboratory, rats were injected (EV or placebo). Then, 15 days later, they were given their first opportunity to drink alcoholic beverage (the first data-point is 16 days after injection). Their first alcoholic beverage was a 12% ethanol solution sweetened with 0.25% saccharin, i.e., 100.00 g of the solution contained 12.00 g of ethanol, 87.75 g of tap water and 0.25 g of saccharin. For 25 days, rats were presented this beverage. Food, water and beverage were always available (except for brief periods necessary to refill bottles).

Intakes of fluids were measured for the first 3 days of availability (Block 1). Although fluids continued to be available, intakes were not measured for the next 4 days. This routine (3 days of measurement, 4 days of no measurement) was repeated across a period of 24 days (Blocks 2–4). The scores, therefore, represent 4 weeks of measurement. The scores conform to an analysis of variance (ANOVA) having a factorial design for repeated measures with factors of groups (EV- or placebo-treated) and repeated measures (scores summarizing each week's intake for 4 weeks) (the statistical package was Statistica).

Following 25 days with an alcoholic beverage with 0.25% saccharin, the concentrations of saccharin were reduced. The subjects were left with each concentration for 1 week. Intakes were measured the last 4 days across 3 weeks with each of three concentrations of saccharin, 0.125%, 0.0625%, and none. The mean of each of those 4 days was taken as the index of intake for that concentration. These means were combined with the last scores associated with 0.25% saccharin to provide data on the effects of decreasing concentration of saccharin on intake of ethanol. These data correspond to a 2×4 ANOVA for repeated measures with factors for groups (EV- or placebo-treated) and the four concentrations of saccharin.

Subsequent to the measures with zero saccharin and 12% ethanol solution, the rats continued to be presented with unsweetened alcoholic beverage for 12 days. During this time, some rats were taking little or no ethanol. To make conditions uniform, we then suspended all presentations with alcoholic beverage for 21 days. After the period of abstinence from alcohol, rats were again exposed to 0.0625% saccharin–12% ethanol solution for 3 days. The 3 days postabstinence spanned 94 to 96 days postinjection and correspond to an ANOVA for repeated measures with factors for groups and days.

The intakes of fluid were measured by weighing the bottles (equipped with ballpoint sipping tubes) used for presentation of the fluids before and after their presentation for the measurement period (24 h). The differences, corrected for spillage (by subtracting mean reduction in weight from putting bottles up and down on empty cages), were the raw data. Bodyweights were taken just before measurements of intake. Using the data of intake of alcoholic beverage and bodyweights, grams of ethanol per kilogram of bodyweight (g'E/kg) were calculated.

3. Results

These subjects' changes in bodyweights are very similar to those previously described in some detail (Reid et al., 2002). As a consequence of the expected changes in bodyweights, during the time of measurements of intakes, rats of EV are, on average, lighter than the placebo controls: mean bodyweight of placebo controls across 24 days of first opportunity to take alcoholic beverage = 221.3 g (S.E.M. = 3.45); mean of EV-treated = 187.6 g (S.E.M. = 2.00).

As noted, there is a loss or decline in bodyweight gains with the onset and withdrawal of estradiol. The period of no weight gain with withdrawal of estradiol is, however, only a matter of days (the end of the period is probably during the first week of availability of alcoholic beverage). The placebo controls gained about 8% of their bodyweight from measures during the second week of availability of alcoholic beverage to the measures of the fourth week. The EV-treated rats also gained about 8% of their weight across the corresponding period. It follows that the intakes in alcoholic beverage during the second to the fourth week are from rats that would be generally considered healthy in terms of gaining weight at a steady rate.

Fig. 1 presents the data of intake of alcoholic beverage in terms of g'E/kg. On the first day of availability of the beverage, all subjects sampled it. Many took considerable amounts of the beverage. Among the placebo controls, there were some subjects that took very little ethanol and some that took large amounts. Notice from Fig. 1 that the placebo controls, on average, took considerable amounts of ethanol and generally increased their intakes from the amounts taken initially. The large intakes of placebo controls make the intakes of the EV-treated rats seem particularly large.

An ANOVA of the data used to derive Fig. 1 (each rat's mean intake for a 3-day block of measures with each of the four blocks representing a week of presentation of alcoholic beverage) yields the following: (a) for the group effect (EV or placebo), $F(1,58) = 13.5$, $P = .0005$ and (b) for the block effect (weekly intakes across 4 weeks), $F(3,174) = 13.1$, $P = .0000001$. The interaction term was not a reliable source of variance ($P = .33$). The ANOVA confirms the general impression that EV induced a marked increase in intakes of the alcoholic beverage.

Given the fact that rats of EV do not weigh as much as controls, and given that rats of EV take more g'E/kg, a question may be asked whether the greater g'E/kg are merely an artifact of the EV-treated rats' lighter weight. On every occasion in which intake of alcoholic beverage was tabulated, EV-treated subjects took, on average, more alcoholic beverage than controls. Fig. 2 presents a summary of those data. An ANOVA of the data of grams of alcoholic beverage of Fig.

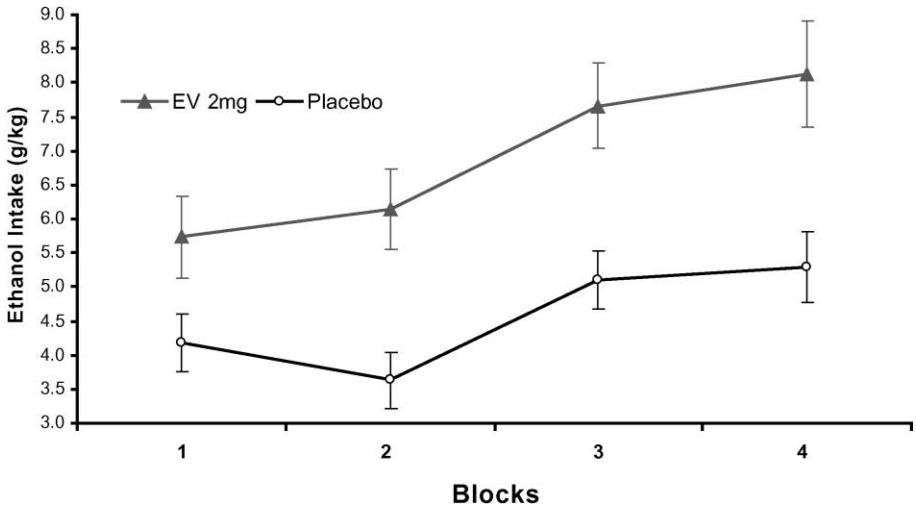

Fig. 1. A summary of amount of ethanol taken by rats treated with EV ($n=30$) and placebos ($n=30$) is presented as mean daily intake of ethanol per kilogram of bodyweight. Each block is a mean representing a week of intake (a mean of the 3 days during which measurements were made for that week). The n for each group is 30. The bars are standard errors of the mean. An ANOVA indicates that the scores of the rats receiving EV are reliably larger ($P<.001$) than those of rats receiving placebo.

2 yields, for the group effect, $F(1,58)=5.4$, $P=.02$ (mean of EV-treated rats = 10.9 g; mean of placebo-treated = 8.4 g). The F value for the block effect is $F(3,174)=21.3$, $P<.0000001$. The interaction term was not a reliable source of variance ($P=.38$). Thus, g'E/kg are not merely an artifact of lighter weights among EV-treated rats, but rather the best measure available to index intake of ethanol.

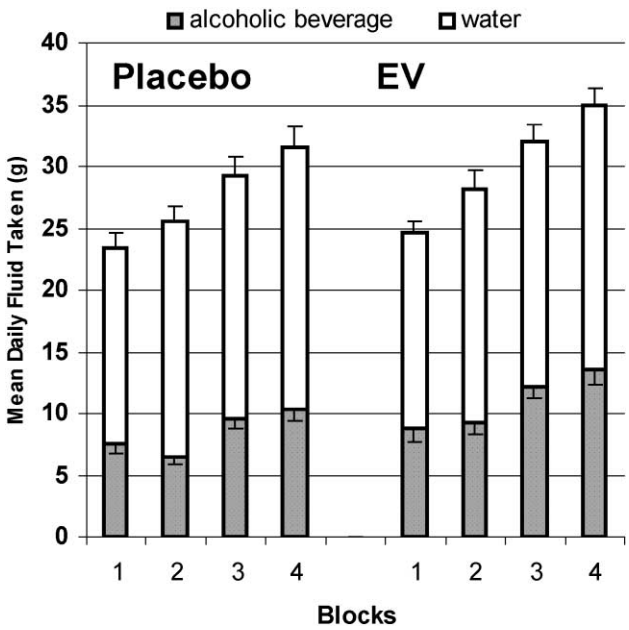

Fig. 2. The data are a summary of daily intake of fluids for the four periods of opportunity to take alcoholic beverage for the same data-points of Fig. 1. The four bars to the right are scores of rats receiving EV treatment. The four to the left are those of placebo controls. The filled bars represent mean consumption alcoholic beverage in terms of grams self-administered. The open bars represent intake of water. The n for each measurement is 30. The bars are standard errors of the mean. The total fluid intake is the sum of the two. The intake, for example, of the first week for the placebo-treated rats is a mean of 7.5 g of alcoholic beverage and 15.9 g of water for a total intake of 23.4 g of fluid daily.

Fig. 2 also presents data of water intake in terms of grams taken a day. Notice that the rats of EV and placebos take about the same amount. An ANOVA of the data of water intake of Fig. 2 yields, for the group effect, $F(1,58)=0.02$, $P=.89$. The interaction term (Groups × Blocks) yields $F(3,174)=0.52$, $P=.66$. There was a general increase in intakes across blocks, $F(3,174)=6.7$, $P=.0003$.

Fig. 2 also is a summary of the amount of total fluid taken daily for each of the four measurement periods. With the passage of time, both placebo- and EV-treated groups increased intake of alcoholic beverage and water, hence, total fluid taken. The increase in water intake is probably merely a function of the rats' increasing size. It is also possible, however, that the large intakes of ethanol are disturbing fluid balances that are corrected by more drinking.

Fig. 3 presents the data of intakes of ethanol when the concentration of saccharin is reduced. Notice that there is a strong relationship between the amount of saccharin in the alcoholic beverage and the amount of ethanol taken by both the placebo- and the EV-treated subjects.

ANOVA of the data used to derive Fig. 3 yields: (a) for the group effect (EV or placebo), $F(1,58)=17.8$, $P=.00009$, (b) for the effect associated with decreasing concentrations of saccharin, $F(3,174)=53.5$, $P<.0000001$, and (c) for the interaction, $F(3,174)=3.3$, $P=.02$. Tests for simple main effects comparing the two groups at each concentration of saccharin indicates that rats of EV took reliably more g'E/kg at each concentration (all P's $<.05$). The t test comparing intakes at the zero concentration of saccharin, for example, yielded $t(58)=2.92$, $P=.005$.

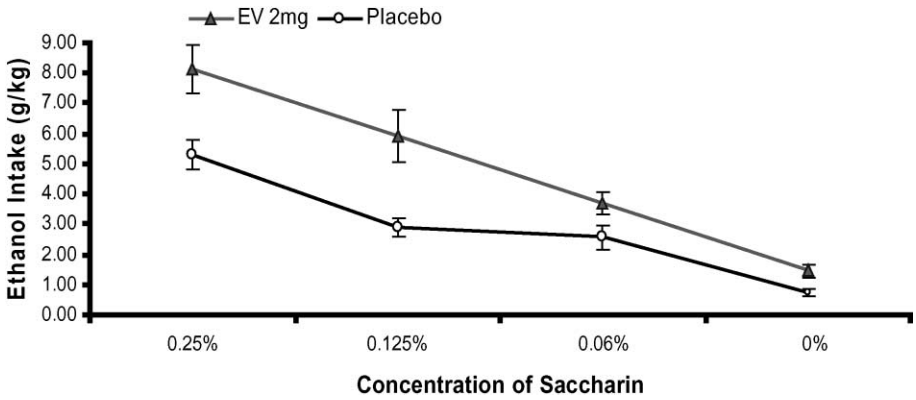

Fig. 3. Mean daily intake of ethanol per kilogram of bodyweight is depicted. The leftmost data-points are the same as those of last data-points of Fig. 1. The *n* for each group is 30. The bars are standard errors of the mean.

The intakes of sweetened alcoholic beverage (0.0625% saccharin, 12% ethanol) after the period of no opportunity to drink alcoholic beverages are summarized in Fig. 4. The leftmost data-points are intakes of the beverage before the period of no opportunity and are the same data as that associated with 0.0625% saccharin in Fig. 3. Notice that intakes on the first day following the period of abstinence are somewhat larger than intakes of that same solution before the period, but the rats' differences, for each group, are not consistent enough to be statistically significant (P's>.17). An ANOVA of the data of the 3 days postabstinence yields for the group effect (EV or placebo), $F(1,58) = 6.34$, $P = .02$. The rats took, on average, more alcoholic beverages on some days than others, $F(2,116) = 21.0$, $P < .0000001$. The interaction term of the ANOVA was not a reliable source of variance ($P = .94$). Further analyses indicated that intakes on the last 2 of the 3 days postabstinence were not reliably different from one another, but were reliably different from the first day after abstinence (P's < .000002).

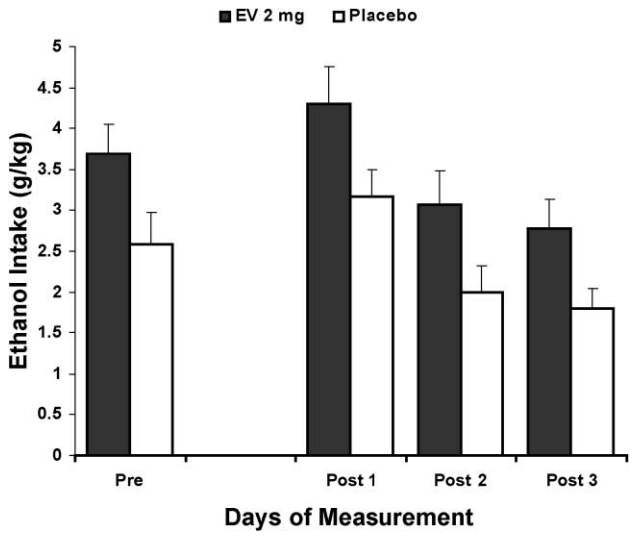

Fig. 4. Mean intakes of ethanol, in terms of grams per kilogram of bodyweight, are depicted. The leftmost pair of bars is the mean daily intake across a block of days and is the same data as in Fig. 3 for the alcoholic beverage having 0.0625% saccharin and 12% ethanol. The right bars are the first 3 days' intakes of the same beverage after an intervening period, the last 21 days of which were without any alcoholic beverage. The *n* for each group is 30. The bars are standard errors of the mean.

4. Discussion

The results support the conclusion that EV given about 2 weeks before opportunity to take alcoholic beverage establishes a state manifest as the development of enhanced intakes of alcoholic beverage. The enhanced propensity to take alcoholic beverage is seen across a number of concentrations of saccharin-flavored, 12% ethanol solutions and a 12% ethanol solution flavored only by its ethanol and water. The results strongly support the conclusions derived from the initial experiments (e.g., Reid et al., 2002).

The control group drank large amounts of ethanol when it was part of a palatable beverage (about 32 g'E/kg a week across the first weeks of availability). Yet, the rats given EV took considerably more (about 48 g'E/kg a week). Of the 30 females that got EV, 11 of them consumed, on average, more than 10 g'E/kg a day during the last week of the initial presentations of the palatable beverage compared to 3 of 30 of those of placebo. The value, 10 g'E/kg a day, was chosen as a cutoff for tabulating extra large intakes, because it is round figure at the upper end of the range of published reports of rats' intakes of ethanol. For example, the intake of high alcohol drinking (HAD) male rats, i.e., rats bred to be high consumers of alcoholic beverages, in one experiment (Kiefer et al., 1995), took on average 5.35 g'E/kg a day after 20 days of opportunity to take 10% ethanol, 90% water solution. The 10 g'E/kg cutoff score is more than this mean plus 5 standard errors of that mean. We believe that average intake of EV-treated rats, as well as the incidence of extra large intakes, indicates that the effect induced by EV is of clinical significance.

A remarkable feature of the enhanced appetite for ethanol is that once it is present, it seems to persist. Here, we have indications that enhanced intakes are apparent from

about 15 to about 100 days after EV injections, with no indication of enhanced intake waning. The enduring effects may be due to the interaction of estradiol's effects in producing a high intake and adaptation to the high intake itself. Marinelli et al. (in press), however, found enhanced intakes some months after EV treatment and after only a brief exposure to alcoholic beverage. Thus, the enduring effects are probably more directly related to EV treatment than a history of alcohol intake.

Initial intakes after a period of abstinence are greater than the following 2 days. This finding might be ascribed to abstinence leading to an enhanced motivation to take alcoholic beverage. The intakes, however, are not larger, in terms of statistical significance, than comparable intakes preabstinence. This kind of data might also be ascribed to the possibility that the rats took about the same alcoholic beverage as before, but, having lost some ability to rapidly metabolize ethanol, took sufficient amounts of ethanol to induce a mild sickness that, in turn, might lead to some moderation of intakes on subsequent days. In brief, the enhanced intakes postabstinence can be explained a number of ways and there are no data to guide speculation as to which of the reasonable explanations might be correct. Although theorizing about the effects seen postabstinence might be of some interest, what we find more interesting is that previous EV treatment enhanced intakes and that, the enhanced appetite was not diminished by a history of changing concentrations of saccharin in the beverage or a period of abstinence.

Female rats are avid consumers of alcoholic beverages taking amounts that produce signs of behavioral toxicity, a conclusion supported by these data and, recently, by those of Le et al. (2000) and Adams et al. (2001). The conclusion is so strongly supported (see, e.g., Lancaster and Spiegel, 1992; Lancaster et al., 1996; Li and Lemung, 1984) that it needs to be taken into account when developing theories of AAA. There are differences between males and females germane to AAA. There is nothing inherent to being female, however, that can account for women's smaller rates of AAA. Any reduced amounts of alcoholic beverages taken by women are, therefore, likely to be a product of social conventions. This conclusion and the inherent implications do not address the issue of any potential differential toxicity between males and females that might be associated with equal intake of g'E/kg of ethanol during an early history of drinking. It follows, from our findings and our general understanding, that only mild social sanctions against women's consumption of alcoholic beverages (which seems to becoming extant in the USA) and an increased likelihood that women will receive pharmacological doses of estradiol (which is the case in the USA) are conditions that will lead to very high incidences of AAA among women.

The strongly supported conclusion that rats' intake of alcoholic beverages is linked to the flavor of the beverage also needs to be taken into account in modern theories. As manufacturers of alcoholic beverages add to the palatability of their products, it seems reasonable to predict that there will be greater instances of chronic toxic dosing than otherwise. Relatedly, it is no longer tenable, if it ever was, to conclude that rats are not avid consumers of alcoholic beverages. Although ordinary laboratory rats may not be initially avid consumers of only water and ethanol, they, particularly females, are surely avid consumers of many other alcoholic beverages.

Drinking alcoholic beverages is, obviously, an ingestive behavior. So, it follows that variables (e.g., palatability) that affect ingestion, in general, also should affect ingestion of alcoholic beverages. This understanding, however, is only a beginning toward understanding the specifics of intake of alcoholic beverages. We presume that the postingestive effects of ethanol are complex and some of those effects are salient to developing strong appetites for alcoholic beverages that are seen among some individuals. One postingestive effect of daily intake of at least some alcohol is a change in the hedonics of alcohol as manifest in reactivity to its taste. Among rats, a history of drinking some alcoholic beverages changes the taste of alcohol solutions from one generally rejected (similar to bitter solutions) to one acceptable (similar to sucrose solutions) (Bice and Kiefer, 1990; Kiefer et al., 1994, 1995). For rats, as well as people, initial acceptability is often merely a matter of presentation of alcoholic beverages that are palatable by virtue of being a mixture of alcohol and palatable tastants (sweet, fruit flavor, grain flavor, vanilla flavor derived from aging in charred oak barrels, etc.). Given the changes in the hedonics of taste (Bice and Kiefer, 1990; Kiefer et al., 1994, 1995), the issues of AAA devolve, therefore, from one of initial acceptability to one of explaining the differences among intakes of palatable alcoholic beverages. Factors that may be relevant to such explanations, among females, are events following pharmacological dosing with estradiol.

Although it seems clear that issues of AAA devolve into differing explanations of intakes of palatable alcoholic beverages, there might still be concerns that the very large appetites for saccharin-flavored alcoholic beverage might be a product of a restriction in presented solutions for the rats to drink. For example, the females might have an enhanced appetite for sweets, in general, as well as unsweetened and sweetened alcoholic beverage. So, if EV-treated females were presented sucrose solutions or cookies (sucrose and fat), they might also manifest an enhanced appetite for these substances as well as, or even rather than, an enhanced appetite for alcoholic beverages. Rats would prefer, probably, many alternatives to unpalatable alcoholic beverages when initially presented, but would also, with experience with the effects of ethanol, probably, develop a taste for a wide variety of alcoholic beverages. Such outcomes would not detract from the potential clinical significance of our findings, because enhanced appetite for sweets and fats covary, clinically, with AAA. It has been known for sometime (e.g., Jonas, 1990) that bulimia (manifest as an excessive appetite for

sweets and fat), binge eating disorder, obesity, and AAA often occur together. Pharmacological doses of estradiol could induce a state that might be manifest in some women as excessive intake of alcoholic beverages and in other women as bulimia. Which disorder might emerge as the dominant one could be a product of the surrounding social sanctions. A finding that EV enhanced intakes of saccharin or sucrose solutions among female rats would not detract from these findings, in terms of their clinical significance, but would rather support the idea that EV induces a state that might be problematic.

Although the germane experiments (Ford and Samson, 2001; Ford et al., 2000, 2002; Marinelli and Gianoulakis, 2000; Marinelli et al., 2001, in press; Reid et al., 2001, 2002) have been done only recently, there is an impressive array of evidence supporting the conclusion that changes inherent to pharmacological doses of estradiol can induce a large appetite for alcoholic beverages. The findings are supported by results from four strains of rats (Lewis, Long–Evans, Sprague–Dawley and Wistar). The enhanced intakes are seen across procedures: (a) with 2 and 24 h daily availability of alcoholic beverage, (b) with a number of different solutions of ethanol including (i) saccharin-flavored ethanol solutions with a variety of different concentrations of saccharin and (ii) no flavoring other than ethanol and water with different concentrations of ethanol, and (c) across experimenters using slightly different procedures in different laboratory conditions. These findings provide strong support for the generalization that treatment with EV enhances appetite for alcoholic beverages. Further, once the enhanced intakes are observed, they seem to persist for an extended period (Reid et al., 2002; this experiment), even after a period of abstinence (this experiment).

In terms of intake of alcoholic beverages, there are two effects from the administration of pharmacological doses of estradiol, an effect associated with the administration of estradiol (a direct effect) and an aftereffect. The reductions in intakes seen with direct administration of estradiol are associated with a number of events any one of which might be casually related to a reduced appetite for alcoholic beverages. The weight loss may index sickness due to a variety of potential disturbances in homeostasis and, generally, sick rats are not avid consumers of alcoholic beverages. Relatedly, the loss of appetite for alcoholic beverage may be associated with a loss of appetite for food (e.g., Wade, 1975). Daily doses of estradiol are associated with a reduction in dopamine in the subcortical forebrain (Dupont et al., 1981) and disturbances in functioning of the arcuate nucleus as indexed by changes in β-endorphin (Reid et al., 2002). Dopaminergic (Salamone, 1994) and endorphinergic processing (Reid et al., 1982) have both been linked to appetitive behaviors. Daily dosing with estradiol changes breast tissue in a similar fashion to the onset of gestation (Rahkumar et al., 2001). In brief, daily doses of estradiol produce a wide variety of effects that in turn have led to the idea that pharmacological doses of estradiol (or other agonists at the estrogen receptor) and selective estrogen receptor modulators (SERMs) are useful medicines to treat a wide variety of conditions.

In terms of self-administration of ethanol, the effects seen during estradiol dosing are probably not a function of the estradiol significantly modifying the pharmacokinetics of ethanol. Sandberg and Stewart (1982) demonstrated that neither estradiol benzoate nor MER-25 (an antiestrogen) reliably modified rate of elimination of ethanol from blood. They also found that treatment with estradiol did not affect the extent of conditioned taste aversions induced by large doses of ethanol.

Using newly developed techniques, Robinson et al. (2002) have assessed the pharmacokinetics of ethanol as a function of gender and estrous cycle. Their microdialyses allowed them to measure ethanol levels in specific regions of the brain. Of particular interest to appetite for alcoholic beverages, they measured ethanol levels in the extracellular fluid of the nucleus accumbens. They concluded that gender was a significant factor in the pharmacokinetics of ethanol (females achieving greater levels of ethanol in the brain just after administration of ethanol). They also concluded that any small differences associated with changes in the estrous cycle "...are unlikely to play a role in ethanol-induced behaviors, such as ethanol intake. Our findings suggest that any variation in ethanol ingestion across the estrous cycle is due purely to differences in the pharmacological response to ethanol, rather than pharmacokinetics" (Robinson et al., 2002, p 171). Given the results from Sandberg and Stewart (1982) and Robinson et al., it is reasonable to conclude that variations in estradiol levels do not significantly modify the pharmacokinetics of ethanol. Processing of ethanol, however, may affect the pharmacokinetics of estradiol (Purohit, 1998).

The posteffects of chronic dosing with estradiol have been studied less. There is, of course, the finding of the emergence of a large appetite for alcoholic beverages. The enhanced appetite is significant in terms of the reliability of the finding as well as in terms of the magnitude of the effect (clinical significance). Another enduring effect, of a period of sustained estradiol, is resistance to the development to carcinogenesis of breast (Rahkumar et al., 2001). There are the findings of enduring effects with respect to the arcuate nucleus of the hypothalamus and, relatedly, modification of the levels of β-endorphin (Brawer et al., 1993; Desjardins et al., 1990, 1993). These enduring effects obviously follow from the direct effects of EV, but may be adaptations to those effects or follow from toxic effects of chronic estradiol and may be very different than, even opposite to, those seen following acute dosing. How these changes are related to one another is unknown, but given the relationship between opioids, ingestion in general, and ingestion of alcoholic beverages (see chapters in Reid, 1990), it is likely that chronic dosing with estradiol enhances appetite for alcoholic beverage by way of modification of endogenous opioider-

geric mechanisms related to ingestion. Having just established the significance of the observation that pharmacological doses of estradiol induce a large appetite for alcoholic beverage, however, sufficient data have not been collected to confidently direct theorizing on how EV might lead to the development of a female with a large appetite for alcoholic beverages.

Because estradiol and SERMs are being used as pharmacological interventions to treat a wide variety of conditions (i.e., changes attendant to menopause, heart disease, breast cancer, osteoporosis, and age-related cognitive decline) and because there are a number of ongoing clinical trials assessing these interventions (for a partial listing, see the various chapters of Anthony et al., 2001), and because excessive intake of alcoholic beverages have been shown to modulate many germane parameters of the diseases in question, it is probably a reasonable suggestion that investigators tabulate the alcoholic beverages consumed by the subjects in the ongoing clinical trials of agents affecting the estrogen receptor. Many of the potential effects that might be attributed to the direct effect of ligands at the estrogen receptors might be an indirect effect of modifications in intakes of alcoholic beverages.

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