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Estradiol valerate and intake of sweetened water

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Abstract

Recently, it has been shown that female rats receiving very large doses (e.g., 2 mg) of estradiol valerate (EV) take considerably more alcoholic beverage than placebo controls. The question asked, with these procedures, is whether the enhanced appetite for alcoholic beverages was specific to those beverages or was a reflection of a general increase in appetite. Female rats were provided with various sweetened beverages. In one experiment, they were provided a palatable saccharin solution (0.25% solution) and a less palatable one (2% saccharin solution). EV treatment led to more intake of the palatable saccharin solution and reduced intake of the less palatable solution. EV induces changes leading to enhanced appetite for some ingesta (including palatable saccharin solutions and alcoholic beverages), but surely not all ingesta.

Keywords: Estradiol; Ingestion; Saccharin; Female rats; Alcohol; Estradiol valerate; Estrogen; Appetite

1. Introduction

This research was prompted by observations that a single dose of estradiol valerate (EV) given to female rats enhanced their appetite for several kinds of alcoholic beverages (Marinelli et al., 2003; Reid et al., 2002, 2003). Many interventions disrupt rats' ordinary intake of alcoholic beverages. Only a few increase intakes. Since an enhanced appetite for alcoholic beverages is the salient variable for alcohol abuse and alcoholism (AAA), a finding that a hormone can increase intake of alcohol seems particularly interesting. It is even more interesting when it is realized that compounds inducing continuous estrogenic activity are widely used as medicines by women. The interest is honed by observations that the enhanced appetite, in rats, was induced after a single administration of EV and that the enhanced appetites were sustained for many days post dosing (Marinelli et al., 2003; Reid et al., 2003).

EV is a preparation designed to produce sustained release of estradiol across an extended period (e.g., 3 or more weeks). The released estradiol has the same pharmacokinetic and pharmacodynamic effects as endogenous estradiol, except for those effects that accrue from large doses and continuous administration (Dusterberg and Nishino, 1982).

The dose used in the demonstrations of EV leading to enhanced appetites for alcoholic beverages is very large, 2 mg a female. This dose was chosen because it produces a selective lesion within the arcuate n. of the hypothalamus (Brawer et al., 1993; Desjardins et al., 1990, 1993). The lesion is supposedly limited to the neurons that produce β-endorphin. If EV did, indeed, reduce the functioning of the neurons of arcuate n., the findings of Marinelli et al. (2003) and Reid et al. (2002, 2003) lead to the conclusion that reduced functioning of the arcuate n. leads to an enhanced appetite for alcoholic beverages. The question addressed with this research is whether the changes in appetite for alcoholic beverages are specific to alcoholic beverages. To address that question, we provided female rats with solutions of saccharin or sucrose after they had received

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the same dose of EV that enhanced intakes of alcoholic beverages.

2. General methods

There were two experiments. They shared a number of common features summarized in this part of the report.

2.1. Subjects

The subjects of the first experiment were 20 female Sprague–Dawley rats purchased from Taconic Farms (Germantown, NY) when they weighed about 200–225 g. The subjects of second experiment were another 40 Sprague–Dawley females purchased from the same supplier when they weighed about 100–125 g. Upon arrival at the laboratory, they were housed individually in standard cages with food and water always available. The windowless room in which they lived was maintained at about 22 °C and was lighted for 12 h a day. The institutional review committees of Siena College and RPI, both of which adhere to the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, 1996), approved the procedures of this study, as well as the general care of the animals.

2.2. Estradiol valerate

EV, from Sigma Aldrich, was administered in a dose of 2.0 mg/rat intramuscularly by way of a 0.2-ml injection of EV and carrier. The carrier was sesame oil. An injection of the same volume of oil served as a placebo.

2.3. Procedure

After a few days to habituate to the living conditions of the laboratory, the procedures of the individual experiments began. Each experiment involved presentation of sweetened water. During the first experiment, two test solutions were presented to the females in their home cages, a sucrose and a saccharin solution. During the second, the presented solutions were two concentrations of saccharin solution.

The test solutions were presented in bottles with ballpoint sipping tubes. The bottles were weighed before and after their presentation to measure the amount drunk. The ballpoint sipping tubes prevent evaporation and spillage. Nevertheless, there is some spillage. By putting bottles up and down on empty cages, and measuring loss of fluids, the amount of spillage was indexed. The differences (to the nearest 0.1 g) in bottle weights, corrected for spillage, are the raw data. The rats were weighed just before the test solutions were presented for a day's trial.

In every trial, a sweetened solution was presented with water, giving the subjects the opportunity to choose which solution to drink. In every trial, the test solution and the water were presented for a limited period (2 h/day). With these trials, as well as numerous similar trials with alcoholic beverages, the total fluid intake (test solution plus water intake) remains rather constant, and similar to control groups, subsequent to the first 5 or 6 days of opportunity to adjust to the schedule of presentations. When the amount taken of a test solution increases or decreases, intake of water is decreased or increased. Consequently, the data of intake of test solution adequately reflects the intake of water. Since intake of test solution is of primary interest, we present only intakes of test solutions.

3. Experiment 1

When female rats are given an opportunity to take sweetened alcoholic beverage and water for 2 h a day, the females gradually increase their intakes of the alcoholic beverage until they take over 2 g of ethanol/kg of body weight; an amount sufficient to produce signs of behavioral intoxication (e.g., reduced righting reflexes). After about 3 weeks of opportunity to drink alcoholic beverage, intakes of ethanol (in terms of g of ethanol/kg) asymptote and will remain rather constant for months provided there is no change in the procedure (Reid, 1996). When an injection of EV is given during this daily regimen of limited access to fluids, there is a marked reduction in intake of saccharin sweetened alcoholic beverage. With continued opportunity to take alcoholic beverage, there is a gradual increase in intakes. Eventually (in about 2 to 3 weeks), the intakes of ethanol become greater than the intakes seen before injections and greater than the intakes of controls that received placebos (Reid et al., 2002).

With this experiment, we asked if the same, large doses of EV that initially reduced and then increased intakes of alcoholic beverages also similarly modified intakes of a sucrose solution and a saccharin solution that were being presented on a similar limited access schedule as alcoholic beverages.

3.1. Procedure

The two groups of subjects of this experiment (n=10 a group) were placed on the limited access schedule of sweetened water and water for 2 h a day. The 2-h presentation of fluids was during the light phase of the light–dark cycle. For one group, the sweetened water contained sucrose (5% by weight) and for the other group saccharin (0.25% by weight). After 10 days, one-half of each group received an injection of EV and the other half placebo. Seven days later, the other females received EV.

We were surprised by the results seen across the first days after the injections of EV; the subjects taking saccharin dramatically reduced intakes whereas those taking sucrose did not. To determine if the results were specific to the kind of sweetened solution, we gave those getting sucrose

solutions the saccharin solution; and, to those getting saccharin, we gave the sucrose solution. The solutions were switched 8 days after EV injections.

The intakes of rats receiving placebos initially remained stable indicating that the effects of any change in intakes were likely due to the EV injections. To simplify presentation of the data, we present the data as if all subjects received their EV injection on the same day.

The data collected a few days subsequent to the switch in intakes is confounded. Any change in intakes might be due to the continuance of testing or to the switch in intakes. Consequently, we focus on the data just following injections.

3.2. Results and discussion

The results are summarized in Fig. 1. The data (mean intakes for each of the two groups) of the first 5 days of the figure are the intakes of the two groups just before their EV injections. The intake amounts of both solutions are very similar. The data of the 6th day are intakes 24 h after EV injections. The data after the 13th day are the data after the solutions were switched.

The results are clear (compare intakes on Days 5 and 6). The EV produced a dramatic decrease in intakes of the saccharin solution (a reduction of 59% of mean baseline, p-value of dependent t-test=0.001). There was only a marginal decrease in intakes of the sucrose solution (a reduction of 12% of mean baseline, p-value of dependent t-test=0.18).

When the presented solution was changed from sucrose to saccharin (Days 13 to 14 of the procedure, Fig. 1), there was a dramatic decrease in amount of test solution taken, i.e., the saccharin solution was not taken in large amounts. A t-test for dependent scores comparing intakes on Days 13 and 14 indicates that the decrease is not likely to be a chance finding ($p<0.00001$). This marked reduction could be due to neophobia, but sweet tastes were not novel and usually neophobia to sweet solutions is no longer manifest on the 2nd day of presentation (note the change in intakes from saccharin to sucrose).

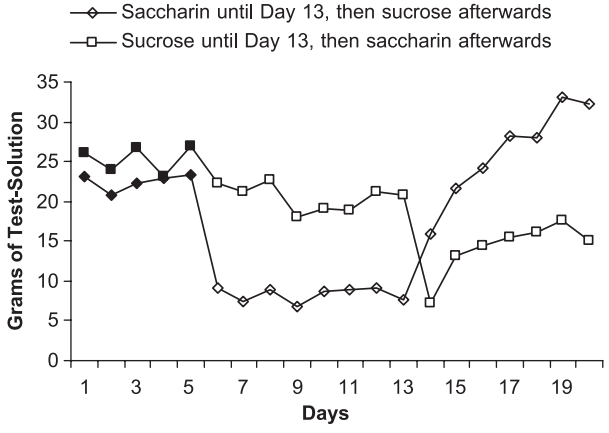

Fig. 1. Intakes of two sweetened solutions on days before and after an injection of estradiol valerate are depicted for two groups of 10 females each. One solution was sweetened with sucrose the other with saccharin. The closed data points indicate intakes before injections; the open data points indicate intakes after injections. One group (diamond data points) received saccharin solution until the 13th day of the procedures, then sucrose solution subsequently. The other group received sucrose solution until the 13th day, then saccharin solution. Note that the injection sharply reduced intakes of the saccharin solution, but only marginally reduced intakes of sucrose solution. When solutions were switched, the females changed their intakes.

When the presented solution was changed from saccharin to sucrose, there was an increase in intakes. The increase on the first day of opportunity to take sucrose was not as large as on subsequent days, but the difference was clearly large enough to meet standards for statistical significance (t-test for dependent means, Days 13 to 14, yields a p-value of 0.0003). The data associated with the switch in solutions confirms that EV-treated females' appetite for saccharin is markedly reduced following an EV injection.

4. Experiment 2

Experiment 1 provided some unexpected information. The immediate effects of EV were dependent on the kind of sweetened beverage presented. Because of the unplanned change in procedure to assess the finding that EV markedly reduced intake of saccharin solution and not sucrose solutions, the experiment did not address the issue of whether the enhanced intakes of alcoholic beverage seen previously were unique to alcoholic beverages. This experiment is another attempt to address that issue.

4.1. Procedure

Five days after their arrival at the laboratory, 20 females were injected with EV and 20 with placebo. Five days after injections, rats received their first opportunity to drink a saccharin solution. Two concentrations of saccharin were used: (a) a high concentration solution (2.00%), i.e., 2.0 g of saccharin for every 100.0 g of water and (b) a low concentration (0.25%), i.e., 0.25 g of saccharin for every 100.0 g of water. Ten rats from each group (EV or placebo) were assigned at random to receive either the high or the low concentration. The body weights of all four groups of rats were comparable (no statistically significant differences existed among the groups at the time of injection).

The low concentration of saccharin is thought to be very palatable as indexed by the large intakes it usually engenders (e.g., pre-injection intakes in Experiment 1). The high concentration is not so palatable and is initially taken in only small amounts. To the human taste, it is bittersweet.

Immediately after lights went out (1230 h), bottles containing saccharin solution were presented. This time of presentation, just as the rats are aroused from sleep, ensures that they focus on the presented solutions due to

the fluid deprivation inherent to sleep. Test solutions were removed 2 h later, a time corresponding to the time that alcoholic beverages were presented in the previous experiments. Rats were given opportunity to drink saccharin solution during periods of four consecutive days. Over approximately 3 months, rats experienced seven 4-day blocks. During most days when saccharin solution was not given, weights were taken at a comparable time. Water was always available.

There are procedural differences among the studies of EV's effects on intake of alcoholic beverages, Experiment 1 and this experiment. In the previous experiments, EV was often given when females were taking the test solution. By way of pilot studies and the data of Reid et al. (2002, 2003), the conclusion was reached that EV, at the doses used, induces weight loss across the first 5 days after the injections (see Fig. 2). This weight loss probably signifies a malaise that could easily establish a conditioned taste aversion that would modulate appetite for the test solution (de Beun et al., 1991; Flanagan-Cato et al., 2001). Since the interest here are not the reduced intakes just following injections, but rather the increased intakes seen subsequently, the decision was made to introduce the test solution 5 days after injections.

4.2. Results

The effect of EV on body weights can be seen in Fig. 2, and is similar to effects observed previously (Reid et al., 2002, 2003). At the time of injection, mean weights of rats receiving EV (142.8 g) were very similar to the mean of rats receiving placebo (141.8 g). For about 5 days after injection, the EV-treated rats did not gain weight, while the placebo-treated rats showed a consistent increase (a mean gain of 17.4 g, or a 12% increase over weight at time of injection). After these 5 days, the EV-treated rats began to gain weight at a rate comparable to the placebo-treated rats. EV-treated rats maintained lower weights than the placebo controls throughout the course of the study. There were no reliable differences in body weights between the two groups getting EV or the two groups getting placebos, i.e., the presence of saccharin solution did not significantly affect body weight or body weight gain.

Fig. 3 presents the data of intake of saccharin solutions. A factorial ANOVA for repeated measures considering drug treatment (EV or placebo), saccharin concentration (high or low) and days (28 opportunities to drink) verified an overall greater intake of the low concentration of saccharin, $F(1,27)=95.6$, $p<0.0000001$. The variable of saccharin concentration interacted with drug treatment, $F(1,27)=10.5$, $p<0.0000001$.

The ANOVA yielded a significant interaction of drug, saccharin concentration and days, $F(1,27)=3.1$, $p=0.0000002$. Referring to Fig. 3, this interaction is explained by noting (a) the levels of consumption of the less palatable solution are relatively stable over days, (b) the mean amount of the less palatable solution taken by placebo control rats was always greater than the amount taken by EV-treated rats and (c) the levels of consumption of the palatable solution were similar for both groups of rats initially (during the first two blocks) but subsequently diverged, with EV-treated rats taking greater amounts than placebo control rats in the later sessions. The interactions are exemplified by an inspection of the scores of 41–44 days after injections. The mean intake of the sums of the more palatable solution across these days for the subjects of EV=68.4 g compared to 40.2 g for the subjects of placebo, i.e., EV reliably ($p=0.04$) increased intakes (Fig. 4). Mean intake of less palatable solution across these days for EV subjects=8.7 g compared to 15.4 g for placebo-subjects, i.e., EV reliably ($p=0.02$) reduced intakes.

Given that subjects weighed somewhat differently, scores of intakes might look differently if intakes were expressed in terms of grams of intake per kilogram of body weight. The mean intake the more palatable solution, in terms of grams per kilogram, across Days 41–44 was for the subjects of EV=337.8 g/kg compared to 176.9 g/kg for the subjects of placebo, i.e., EV reliably ($p=0.008$) increased intakes. Mean intake of less palatable solution across these days for EV

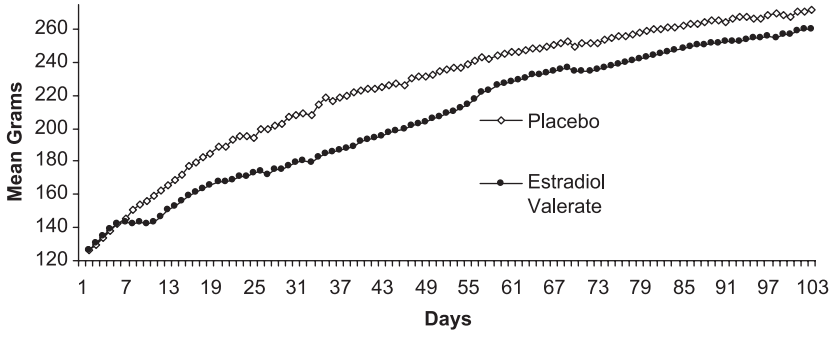

Fig. 2. Daily mean body weight for the subjects that received EV (2 mg a female) or received placebo. The injections were given on Day 6. Day 7 reflects the first effects of EV. Notice that subjects receiving EV did not gain weight, as their counterparts did, across the few days subsequent to injections. Subjects receiving EV gained weight at roughly the same rate after the initial few days after injection, but remained a few grams lighter throughout the balance of experiment. The difference between groups is statistically significant everyday from Day 7 (p's<0.05) until the last few days of testing. The average standard error of the mean of subjects (n=20) receiving placebos is 4.0, the average for subjects (n=20) getting drug is 6.8.

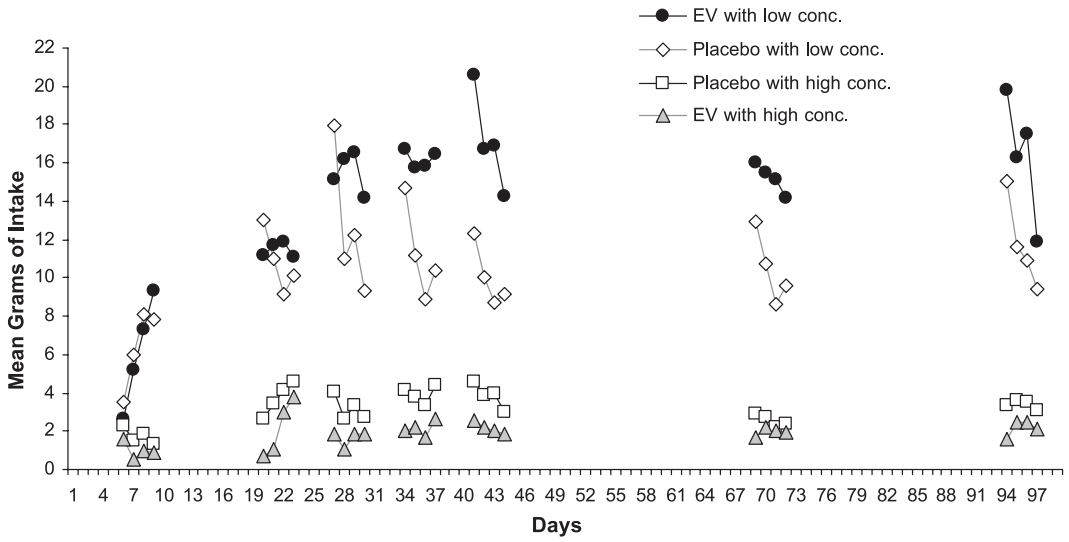

Fig. 3. Intakes of the four groups of subjects are depicted as mean intakes on each day the solutions were presented. Notice that intakes of the two kinds of saccharin solutions (low concentration, the more palatable solution; and high concentration, the less palatable) are remarkably different. Notice that EV treatment eventually led to greater intake of the more palatable solution whereas just the opposite happened with the less palatable solution.

subjects, in terms of grams per kilogram, 43.2 compared to 67.7 g/kg for placebo-subjects, i.e., EV reduced intakes (p=0.09). Although probably values shift a bit with scores expressed in terms of g/kg compared to g of intakes, the conclusion remains the same, EV enhances intakes of the more palatable solution and does not enhance intakes of the less palatable solution and, on average, tends to decrease intakes of the less palatable solution.

An interesting feature of the data for low concentration solution is that (after the first block) consumption levels for the first day of each block are, in general, markedly higher than for the remaining 3 days of the block; this pattern was observed for both EV-treated and placebo control rats.

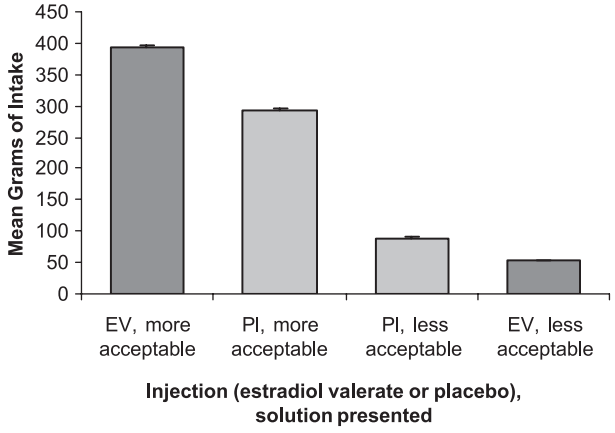

Fig. 4. The values depicted here are the sums of mean intakes across the 28 days when test solutions were presented (standard error bars are barely perceptible). For example, the females (n=10) that were given EV took an average of 392 g of the more acceptable saccharin solution during the experiment, i.e., 34% more than females given placebo (n=10). By contrast, the EV-treated females took 41% less of the less acceptable solution than their placebo-treated counterparts (n's=10). Every comparison between any two groups of scores, by way of t-tests, yields values of $p<0.002$, p-values sufficiently small to conclude that the scores are statistically significantly different from one another and to take into account any problems that might accrue from multiple comparisons.

4.3. Discussion

As expected, the low concentration of saccharin was taken more avidly than the high concentration. EV induced an enhanced appetite for the low concentration and the contrary for the high concentration.

The presentation of the palatable saccharin solution some days after injections led to an increase in intakes sooner than was apparently developing with the saccharin solution in Experiment 1. The likelihood of a conditioned taste avoidance developing during the time of weight loss (de Beun et al., 1991; Flanagan-Cato et al., 2001), thereby, masking what otherwise might be an enhanced appetite for some ingesta, seems reasonable.

A noteworthy aspect of the data for the low concentration solution is the enhanced intakes seen for both EV and placebo rats on the first day of each block, after block 1. If this study had involved alcoholic beverages, those who subscribe to the idea of an alcohol deprivation effect would undoubtedly see these data as strong evidence for such an effect. However, it is difficult to conceptualize the present data as a deprivation effect, in that saccharin is nonnutritive and not a substance one can "need" or on which dependence is apt to develop. Furthermore, since the effect is not observed for the high concentration solution, it would not seem that motivation for saccharin itself is involved. In the barren context of a laboratory rat's existence, the presentation of a palatable substance following abstinence could provide reward from novelty as well as palatability and the rats' avidity on the first day of each block might be understood from that perspective.

5. General discussion

When EV, at these large doses, is given after intakes are well established, there is a marked reduction in intakes of saccharin sweetened alcoholic beverages (Reid et al., 2002) and certain saccharin solutions, even usually palatable saccharin solutions (Fig. 1). There is no corresponding reduction in sucrose intake, although there is a small reduction in intakes (Fig. 1). Just after injections of EV, there is a loss of body weight or no gain in body weights across about 5 days post injection (see Fig. 2). This effect on body weight probably signifies a mild malaise that leads to conditioned taste aversions. These conditioned aversions, in turn, may mask heightened appetite for saccharin solutions and alcoholic beverages that become manifest when there is no pairing of the purported malaise with a test solution. EV, just after injections, apparently induces more finickiness toward some ingesta than other ingesta, since the EV-injected females did not reduce intakes of sucrose solution to the same degree as seen with saccharin solution and alcoholic beverage. Although the term "finickiness" is not usually applied to rats' intakes of ingesta, the term's colloquial meaning may be appropriate, i.e., an approach to food similar to that of a pregnant woman who reacts adversely to food previously taken avidly while craving food otherwise taken without gusto.

From some observations, the reasonable conclusion to be drawn is that continuous estradiol reduces appetite for alcoholic beverages (e.g., Ford et al., 2002, 2004; Reid et al., 2002; Sandberg et al., 1982; Sandberg and Stewart, 1982). The reasonable conclusion to be drawn from other observations is that continuous estradiol enhances appetite for alcoholic beverages. Both conclusions are reasonable from a limited perspective, but are overgeneralizations. The initial days of continuous estradiol (whether by way of EV or daily injections of estradiol) is characterized by weight loss, or a lack of weight gain (Reid et al., 2002; Fig. 2) and accompanied by a reduction in intake of alcoholic beverages and other ingesta (e.g., Fig. 1). When alcoholic beverages are presented continuously for many days after the initiation of continuous estradiol (Ford et al., 2004; Marinelli et al., 2003; Reid et al., 2002, 2003), or are presented after there is some adjustment to continuous estradiol (Marinelli et al., 2003; Reid et al., 2003), there are enhanced intakes of alcoholic beverages. By taking into account the possibility that initial effects of a regimen inducing continuous estradiol produces a malaise and conditioned aversions to tastants presented during that malaise, the results from the relatively small literature on the effects of estrogenic drugs on alcohol intake which appeared not to be concordant with one another are, in fact, concordant. Estradiol reduces intakes initially, but can enhance intakes of some substances subsequently. With the provisioning of continuous estrogenic dosing, the same differential effects may hold for ingesta of many kinds, an initial finickiness (e.g., Wade, 1975; Experiment 1) followed by enhanced intakes of some substances (particularly, palatable saccharin solutions and alcoholic beverages).

The interesting observations (Ford et al., 2004; Marinelli et al., 2003; Reid et al., 2002, 2003) are that continuous estradiol can induce a state of heightened appetite for alcoholic beverages. A question then emerges: Is the enhanced appetite for alcoholic beverages a manifestation of a general increase in appetite or a manifestation of a more specific increase in appetite?

AAA can be considered special cases of ingestive disorders (Reid, 1985, 1990). The salient variable in AAA is ingestion of ethanol, a substance that provides calories and taste, as well as, it is supposed, reinforcement by way of a mechanism somewhat different from the reward of other sources of calories and taste. Variables known to modify ingestive behavior in general also modify ingestion of alcoholic beverages (e.g., deprivation of calories or fluids and palatability). Given the lack of features specific to ingestion of alcoholic beverage, it was unlikely, at the outset, that changes in appetite following EV treatment would be specific to alcoholic beverages. EV treatment did modify intake of saccharin solutions.

Subsequent to its initial effects, EV enhanced intakes of one of the saccharin solutions, but decreased intakes of the other one. The long-term effects of EV did not apparently enhance appetite for ordinary rat chow (notice the lack of weight gain depicted in Fig. 2). The long-term effects of EV are to enhance intakes of alcoholic beverages and highly palatable saccharin solutions. Although these data argue against the idea that EV's effects are specific to alcoholic beverages, they also argue against the idea that EV's effects generally increase appetite. At this stage of understanding, it appears that large, continuous doses of estradiol can enhance appetites for ingesta producing uncomplicated, immediate gratification.

There is an interesting correlation between propensity to take highly palatable saccharin solutions and propensity to take alcoholic beverages. For example, rats bred to be consumers of alcohol are also avid consumers of these saccharin solutions (for a review, see Kampov-Polevoy et al., 1999).

There are data from women that are concordant with the idea that enhanced estrogenic activity will enhance appetite for alcoholic beverages. Muti et al. (1998), for example, have data to indicate that premenopausal women with higher estrogenic tone were characterized by a significantly greater alcohol intake (92.8 g/week) compared to those with lower tone (31.6 g/week).

In the Introduction, the point was raised if EV reduced the functioning of the neurons of arcuate n.; the conclusion to be drawn is that reduced functioning of the arcuate leads to an enhanced appetite for alcoholic beverages. Note that the premise of the statement is reduced functioning of the arcuate n. neurons. These neurons produce a variety of neuromodulators and neurotransmitters. Although some of the initial research was prompted by theories of opioidergic

functioning in relationship to appetite for alcoholic beverages, the potential reduction in β-endorphin may not be what is critical. Other modulators produced by the proopiomelancortin gene may be critical. The theory that a surfeit of opioidergic functioning (rather than a deficit) is related to enhanced appetites for alcoholic beverages (Reid et al., 1991) is not contradicted by the finding that reduced functioning of the arcuate n. neurons leads to an enhanced appetite. Any reduction in β-endorphinergic functioning could easily be offset by events associated with actions of the other modulators produced.

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