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2-HYDROXYESTRADIOL ENHANCES BINGE ONSET IN FEMALE RATS AND REDUCES PREFRONTAL CORTICAL DOPAMINE IN MALE RATS

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Abstract

Women are more likely to suffer from a bingeing-related eating disorder, which is surprising, since estradiol reduces meal size and is associated with reduced binge frequency. This apparent contradiction may involve the estradiol metabolite, 2-hydroxyestradiol. We previously reported that female rats had faster escalations in shortening intake during the development of bingeing than did males, but acute administration of 2-hydroxyestradiol increased the intake of vegetable shortening to a greater extent in male rats once bingeing was established. Here, we report two separate studies that follow up these previous findings. In the first, we hypothesized that chronic exposure to 2-hydroxyestradiol would promote escalation of bingeing during binge development in ovariectomized female rats. In the second, we hypothesized that acute exposure to 2-hydroxyestradiol would enhance dopamine signaling in the prefrontal cortex after bingeing was established in male rats. In study 1, non-food-deprived female rats were separated into 3 groups: ovariectomized (OVX) with chronic 2-hydroxyestradiol supplementation (E), OVX with vehicle supplementation (O), and intact with vehicle (I). Each group was given access to an optional source of dietary fat (shortening) on Mon, Wed, and Fri for four weeks. 2-hydroxyestradiol supplementation prevented OVX-induced weight gain and enhanced escalation of shortening intake over the four-week period ($p < 0.05$). Additionally, in week 4, rats in the E group ate significantly more shortening than I controls, less chow than either the O or I group, and had a higher shortening to chow ratio than O or I ($p < 0.05$). Study 2 indicated that acute injection of 2-hydroxyestradiol abolished shortening-evoked dopamine efflux in the prefrontal cortex of bingeing male rats ($p < 0.05$). Together, these studies indicate that 2-hydroxyestradiol can exacerbate bingeing as it develops and can suppress dopamine signaling in the prefrontal cortex once bingeing is established.

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Keywords

binge eating; 2-hydroxyestradiol; dopamine; sex differences; dietary fat

INTRODUCTION

Approximately 4.5% of people in the United States, or 14 million people, will binge eat at some point during their lifetime. The lifetime prevalence of bingeing is similar in men and women. However, women are nearly three times more likely to suffer from a bingeing-related eating disorder such as bulimia nervosa or binge eating disorder, whereas men are more likely to engage in subthreshold bingeing, which does not meet all of the criteria for an eating disorder (Hudson et al. 2007). The higher prevalence of bingeing-related eating disorders in women is especially surprising, considering that estradiol has been shown to reduce meal size (Eckel 2011) and is associated with reduced binge frequency (Edler et al. 2007; Klump et al. 2008). The explanation for this apparent contradiction may involve the estradiol metabolite, 2- hydroxyestradiol.

We previously reported that acute intraperitoneal administration of 2-hydroxyestradiol increased the intake of vegetable shortening (a semisolid fat used in the production of baked goods) in rats that had learned to binge, but did not change shortening intake in non-bingeing control rats (Babbs et al. 2011). This finding indicates that acute 2-hydroxyestradiol does not cause bingeing, but can exacerbate its severity in animals with binge experience. We also reported that binge intake escalated more quickly during the initial 5-week binge induction period in intact female rats than in intact male rats, which we proposed may have been due to chronic exposure to higher endogenous levels of 2-hydroxyestradiol in the intact females (Babbs et al. 2011). *The goal of Study 1* of the present investigation was to determine if chronic exposure to exogenous 2-hydroxyestradiol enhances escalation of binge intake in ovariectomized female rats, in a manner previously proposed for intact females.

In addition to the above findings, our previous report also showed that acute injections of 2-hydroxyestradiol had a greater stimulatory effect on binge intake in male rats than in female rats with extensive binge experience (Babbs et al. 2011). Again, this may have been due to differences in the levels of endogenous 2-hydroxyestradiol present in the males and females. Acute administration of exogenous 2-hydroxyestradiol may not have had a robust intake stimulatory effect in the females with binge experience because endogenous levels were already high and binge intakes were already large, relative to stomach capacity. Conversely, acute administration of exogenous 2-hydroxyestradiol may have exacerbated intake in the experienced males because endogenous levels were relatively low and the stomach was not yet at capacity. Thus, males appear to offer a better model system for assessing the acute effects of 2-hydroxyestradiol once bingeing is established. Study 2 of the present report exploited these effects in males in order to conduct a preliminary examination of possible alterations in dopamine signaling that may occur during acute 2-hydroxyestradiol-induced exacerbation of binge size.

Dopamine is of interest because it has been implicated in binge-related eating disorders (Bello and Hajnal 2010). Dopamine cell bodies in the midbrain project to several brain regions including the prefrontal cortex, and dopamine signaling in the prefrontal cortex has been associated with reward-based learning and memory (Hyman et al. 2006; Phillips et al. 2008), including food reward (Volkow et al. 2011). Furthermore, neuronal activity within the prefrontal cortex in response to food cues is altered in subjects currently diagnosed with

bulimia nervosa (Uher et al. 2004). Thus, signaling within the prefrontal cortex, and dopamine signaling in particular, may contribute to binge pathology.

2-hydroxyestradiol may alter dopamine signaling via several different mechanisms. For instance, 2-hydroxyestradiol inhibits adenylyl cyclase (Tofovic et al. 2009), a protein which is part of the dopamine receptor signaling cascade, and which also is inhibited by activation of the 2-type dopamine receptor. In addition, 2-hydroxyestradiol has been shown to bind to dopamine 2-type receptors in cultured pituitary cells from female rats (Schaeffer and Hsueh 1979); it is possible that such binding also occurs in the prefrontal cortex, although whether this results in receptor activation or inhibition is not known. Dopamine 2-type receptors are present both presynaptically and post-synaptically. The presynaptic receptor serves as an autoreceptor that limits dopamine release. If 2-hydroxyestradiol blocks dopamine 2-type autoreceptors, then dopamine release would be prolonged and dopamine availability would increase. Alternatively, if 2-hydroxyestradiol stimulates the autoreceptor, then less dopamine would be released and dopamine availability would decrease.

2-hydroxyestradiol has also been shown to have a higher affinity for catechol-*O*-methyltransferase (COMT), an enzyme responsible for degradation of both dopamine and 2-hydroxyestradiol (Ball et al. 1972). This is especially important in the prefrontal cortex, where expression of the dopamine transporter, which removes dopamine from the synapse, is low (Cass and Gerhardt 1995; Sesack et al. 1998). If 2-hydroxyestradiol acts to competitively inhibit COMT, then dopamine availability would be expected to increase. Taken together, these reports indicate that 2-hydroxyestradiol could potentially either decrease or increase dopamine availability. Dopamine availability could potentially decrease if 2-hydroxyestradiol activates the autoreceptor. Alternatively, dopamine availability could potentially increase if 2-hydroxyestradiol inhibits COMT or if it blocks the autoreceptor.

The goal of Study 2 was to determine acute effects of 2-hydroxyestradiol on dopamine efflux within the prefrontal cortex during a binge episode after bingeing was fully established. We hypothesized that 2-hydroxyestradiol would increase dopamine levels.

METHODS-Study 1. Chronic 2-hydroxyestradiol in Female Rats: Food Intake, Body Weight, Binge Escalation

Animals

A total of 56 female Sprague-Dawley rats (Harlan, Indianapolis, IN), 60 days of age, were individually housed in hanging stainless steel wire cages in a temperature- and humidity-controlled environment placed on a 12:12 light:dark cycle. All rats had *ad libitum* access to a nutritionally complete commercial pelleted rodent diet at all times during the study (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of calories as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g) placed in hanging metal food hoppers at the front of the cage. Tap water also was freely available. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

After seven days of adaptation to the vivarium, body weights were recorded and vegetable shortening (Crisco® All-Vegetable shortening, J.M Smucker Co., Orrville, OH) was provided during a single overnight period, in addition to the continuously available chow. Only rats weighing at least 200g were included in the surgical groups, as this was the minimum weight required for minipump implantation, due to the size of the minipump. Rats meeting the weight criterion were divided into three groups (n=16 for each group) that did not differ statistically in either body weight or the amount of shortening consumed during the overnight access period ($p > 0.05$) as assessed by 1-way analysis of variance (see

statistics section). Eight animals weighing less than 200g were assigned to a fourth, non-surgical group. The purpose of this group is described below under “Binge Procedure”.

Ovariectomy (OVX)

Bilateral OVX surgeries were performed on two of the groups of rats, using the following procedure: Rats were anesthetized using 3–5% inhaled isoflurane. The surgical areas were shaved and then cleaned with iodine and 70% ethanol. An incision (~3 cm) was made on each flank of the rat, perpendicular to the spine, and adjacent to the hind limb. Parallel incisions were made in the underlying musculature. The ovaries were removed, and the uterine horns were clamped and tied with a 4-knot ligature. The ovaries were then excised and the clamp was released. The muscle incisions were closed with 2–3 sutures per side. The skin was closed with 2–3 surgical staples per side. The third group of rats was subjected to a sham ovariectomy that included cleaning, incision, and closure techniques performed during the OVX procedure, but left the ovaries and uterine horns intact.

Osmotic Minipump Implantation

After suturing the muscle wall at the end of the OVX surgery, and prior to closure of the skin with staples, an osmotic minipump (model 2ML4, Alzet, Palo Alto, CA) was inserted on the left side through the skin incision and positioned in the scapular region of the rat. Osmotic minipumps were used for the study in order to provide chronic exposure to 2-hydroxyestradiol. This delivery method was selected due to the short half-life of 2-hydroxyestradiol ($t_{1/2}(1)=0.54$ min, $t_{1/2}(2)=10$ min) (Zacharia et al. 2004). The goal was to mimic physiological levels that would normally result from the metabolism of endogenous estradiol, while reducing the stress that would result if multiple daily injections were given. The minipumps inserted into the two groups of OVX rats contained either 2-hydroxyestradiol (10 µg/kg/hr; Steraloids, Newport, RI; E) or polyethylene glycol (PEG-400, 2.5 µL/h; O). This dose was based on a previous report that demonstrated physiological activity of the metabolite and reported the dose as “high physiological” (Tofovic et al. 2005). The minipumps were large enough to deliver 2-hydroxyestradiol or vehicle for four weeks. The rats that underwent the sham ovariectomy were implanted with minipumps containing PEG-400 (2.5 µL/h; I).

Binge Procedure

After surgery, rats were given limited access to shortening in a glass jar clipped to the front of the cage 2–3 hours prior to the start of the dark cycle. Chow was available during the shortening access period and at all other times. In order to maintain normal colony conditions established for our model of bingeing (Corwin et al. 1998; Corwin et al. 2011), the non-surgical group was given shortening every day. Because this group was only used to maintain established conditions (the presence of a daily access group in the colony), the data were not collected. The other 3 groups of rats were given shortening intermittently, i.e. only on Mondays, Wednesdays, and Fridays. These three groups were: I (intact) (Sham surgery + PEG infusion), E (OVX + 2-hydroxyestradiol infusion), and O (OVX + PEG infusion). A group of intact females with 2-hydroxyestradiol infusions was not included for two reasons. First, our previous report indicated that acute exogenous 2-hydroxyestradiol had minimal effects in intact female rats, which we speculated was due to the presence of endogenous 2-hydroxyestradiol. Second, we sought to mimic physiological, rather than pharmacological, levels. Addition of exogenous 2-hydroxyestradiol to rats in which endogenous 2-hydroxyestradiol was already present would raise levels above those that were physiologically relevant.

As previously described shortening was available for 1 hour in week 1, 40 minutes in week 2, and 20 minutes in weeks 3 and 4 (Babbs et al. 2011). This was done in order to mimic the

binge procedure that was used in our previous report. During this 4-week binge induction period, body weights, shortening intakes, and 24-hour chow intakes were recorded.

See Figure 1A for a timeline of the procedures that were used.

Statistics

For all analyses, values were considered significantly different at $p < 0.05$. In order to compensate for the effect of weight gain on shortening intake in Study 1, the intake data that were used in the escalation analyses were normalized to body weight^{0.67} (Heusner 1985). To compare escalation in shortening intake among the groups, data were expressed as percent of normalized intake on the first day (Day 1) of limited shortening access. Percent of Day 1 was used rather than raw intake because the OVX procedure stimulates shortening intake in rats with limited access to optional shortening (Yu et al. 2008). By using percent of day 1, escalations could be assessed independent of initial intake. Escalation data were analyzed using linear regression analyses equivalent to analysis of covariance with GraphPad Prism 4 (GraphPad Software, Inc.). Measures of body weight and intake (not normalized to body weight) were compared among groups at specified time points using 1-way ANOVA with Tukey's HSD post hoc tests.

RESULTS-Study 1

Body weight and cumulative energy intake

Table 1 summarizes the starting and ending body weight of the rats, as well as the cumulative energy intakes (not normalized to body weight) of both shortening and chow over the entire course of Study 1. There were no significant differences in body weight among groups at the beginning of the study [$F(2,47)=0.9394$, p NS]. However, the O group had a significantly higher mean body weight than the I and E groups [$F(2,47)=78.8$, $p < 0.0001$] at the end of the study. The total amount of food consumed during the study reflected this finding, in that the O group had a significantly higher overall energy intake than either of the other groups [$F(2,47)=58.89$, $p < 0.0001$]. This trend did not hold, however, for the shortening intake data. The O group ate significantly more shortening than did the I group [$F(2,47)=4.678$, $p < 0.02$], but the E group's shortening intake did not differ from either of the other groups'. Chow intake did not differ between I and either O or E, but the E group ate significantly less chow than did the O group [$F(2,47)=10.64$, $p < 0.002$].

Binge escalation

Escalation of shortening intake across the study was analyzed as a percentage of the normalized shortening intake on Day 1 of the study (Figure 2). Regression analyses revealed slopes of 0.277 ± 0.038 , 0.160 ± 0.035 , and 0.128 ± 0.043 for the E, O and I groups, respectively. These data indicate that shortening intake escalated more quickly in the E group than it did in either the I or O group [$F(2,522)=4.06$, $p < 0.02$]. This supports the idea that 2-hydroxyestradiol, in the absence of estradiol, can exacerbate binge escalation.

Terminal shortening and chow intake (in grams)

Mean intakes of shortening and chow in grams during the terminal week of the study (week 4) were assessed in order to determine the maximal effects of the treatments on the average mass of food consumed (Figure 3). During the final week of the study, the E and O groups ate significantly more shortening than did the I group, but did not differ from one another [$F(2,47)=5.97$, $p < 0.005$]. This indicates that the rats in the E group ate the same quantity of shortening (not normalized to body weight) as rats in the O group, even though the O rats weighed greater than 20% more than the E group. The average daily chow intake in the final week was lower in the E group than in either of the other two groups [$F(2,47)=13.43$,

$p < 0.0001$], suggesting compensatory behavior in the E group for the relatively high shortening intake. In fact, the ratio of week 4 shortening intake to chow intake (Figure 4) was significantly greater in the E group than in the I group [$F(2,47) = 5.24$, $p < 0.009$]. O did not differ from either of the other groups.

DISCUSSION-Study 1

In this study, two new findings are reported: 1) chronic administration of 2-hydroxyestradiol enhanced escalation of binge intake over time in OVX female rats, and 2) OVX rats exposed to chronic 2-hydroxyestradiol ate significantly more shortening and significantly less chow than did intact vehicle controls.

At the end of Study 1, the O rats weighed significantly more than either the E or I rats. Body weights of rats in the E group, which had also undergone OVX surgery, did not differ from the body weights of rats in the I group. This finding confirms the metabolic activity of the 2-hydroxyestradiol implants and is consistent with the findings of others in male (Tofovic et al. 2001) and OVX female (Munoz-Castaneda et al. 2005) rats. The weight differences are reflected in the total food intakes across the study. Specifically, O rats ate significantly more total energy (not normalized to body weight) than either of the other two groups. Therefore, the 2-hydroxyestradiol implants prevented OVX-induced weight gain by decreasing overall food intake to levels expected in non-OVX rats. This finding also is consistent with findings of others (Munoz-Castaneda et al. 2005). Previous work has shown a similar suppression of food intake in OVX bingeing rats by intraperitoneal supplementation with the parent compound, estradiol, and progesterone (Yu et al. 2008). The present findings suggest that this effect may involve 2-hydroxyestradiol.

Although the overall energy intake of the rats in the E group did not differ from that of rats in the I group, the pattern of intakes from shortening and chow individually were not the same. In week 4 of the study, rats in the E group consumed significantly more shortening (not normalized to body weight) than rats in the I group (Figure 3). Loss of the intake inhibitory effects of the parent compound may have contributed to the stimulation of shortening intake. Estradiol is known to reduce food intake by reducing meal size, which is mediated via interactions with other peptides and neurotransmitters including cholecystokinin (Eckel 2011). Even under the conditions of exaggerated meal size that occur during a binge, estradiol is associated with reduced binge frequency in humans (Edler et al. 2007; Klump et al. 2008), and hormone replacement has been shown to tonically reduce binge size in OVX rats (Yu et al. 2008). In the OVX rats treated with 2-hydroxyestradiol in the present study, minimal endogenous estradiol would have been available to suppress shortening intake. In fact, the E rats' shortening intakes (not normalized to body weight) did not differ from the O rats' intakes, even though the O rats weighed approximately 60 g more than the E rats. Thus, the two groups with no estradiol on board consumed large amounts of shortening, with ceiling effects on gastric capacity likely limiting what could be consumed, particularly in the smaller rats treated with 2-hydroxyestradiol.

In spite of their similar shortening intakes, the E and the O rats differed in some important ways. Specifically, the E rats compensated for the relatively high shortening intake by under-eating chow, whereas the O rats did not. In fact, by week 4 the E rats were consuming significantly less chow than either of the other two groups. As a result, the E rats had a significantly higher ratio of shortening to chow energy intake than the I group. This indicates that loss of the intake inhibitory effects of estradiol cannot fully account for the results reported here. Since endogenous estradiol as well as 2-hydroxyestradiol would have been present in the I group, these findings, coupled with our previous report (Babbs et al. 2011) highlight the apparently different roles that the parent compound and its metabolite

have on ingestive behavior. Specifically, it appears that chronic exposure to 2-hydroxyestradiol can shift intake toward a binge-type pattern with a higher consumption of fat, even though total energy intake remains unchanged. This is similar in some ways to human binge eating, in that intake during a binge is shifted toward higher fat dessert and snack foods and away from vegetables (Hadigan et al. 1989). In addition, in some cases people compensate for the energy consumed during a binge by reducing the consumption of other foods (APA 2000).

In addition to causing a shift in food intake, chronic administration of 2-hydroxyestradiol altered the rate of increase of binge intake. Regression analyses indicated a faster binge escalation (i.e. slope) in the E (2-hydroxyestradiol) group than in the other groups when intake was normalized to body weight. Although escalation in the intact females was slower than that of the 2-hydroxyestradiol rats in the present study, escalation was faster in intact females than in males in our previous report (Babbs et al. 2011). These outcomes may have been due to opposing effects of estradiol and its metabolite on intake. That is, escalation of binge size may have been greater in intact females than in males in the previous report due to the intake stimulatory effect of 2-hydroxyestradiol, even though estradiol also was present and was likely acting to attenuate intake to some extent. In the present study, however, the stimulatory effects of 2-hydroxyestradiol on intake could be seen without the opposing effects of estradiol. Therefore, escalation was greater in the 2-hydroxyestradiol rats than that of the intact females. The faster escalation was not simply due to removal of estradiol, as intake also escalated more rapidly in the 2-hydroxyestradiol rats than in the vehicle infused OVX rats. Again, it appears that estradiol and its metabolite 2-hydroxyestradiol have different and opposing effects on ingestive behavior. These results suggest that 2-hydroxyestradiol, and not anatomical differences or estradiol itself, can account for the faster escalation in females. Together, this and our previous report support the idea that 2-hydroxyestradiol may expedite progression towards more severe forms of bingeing.

The idea that 2-hydroxyestradiol is responsible for increased risk for bingeing in females may seem counterintuitive, given that 2-hydroxyestradiol is highest when estradiol is highest and binge frequency is lowest in cycling females (Edler et al. 2007). However, it is possible that the enhancing effect of 2-hydroxyestradiol is overshadowed by the suppressing effect of estradiol, but that 2-hydroxyestradiol alters the neural physiology in such a way that days later, when estradiol is low, bingeing is exacerbated. In this way, 2-hydroxyestradiol may have a delayed effect on bingeing, i.e. the system is “primed” to respond to a binge episode should one be initiated.

One possible way in which this could occur is that 2-hydroxyestradiol, which would be present at high levels when estradiol is high, may be the mediator of these effects due to effects on dopamine signaling. Others have shown that 2-hydroxyestradiol and estradiol can have opposing effects on behavior mediated by dopamine in female rats, with 2-hydroxyestradiol stimulating the behavior and estradiol reducing it. Furthermore, the behavioral effects of 2-hydroxyestradiol may be mediated by changes in dopamine 2-type receptors that have been shown to occur even 72 hours after the last injection (Clopton and Gordon 1985). This supports the idea that 2-hydroxyestradiol can induce delayed changes in dopamine signaling, which are particularly impressive given its short half-life. Thus, although estradiol acutely decreases meal size and binge risk, its metabolite may increase binge size and risk in a delayed fashion such as occurs during the menstrual cycle (Edler et al. 2007) or when the metabolite would be at constantly high levels, such as would occur during pregnancy. This would explain the contradiction associated with bingeing and pregnancy, which is that binge risk is much higher during this time (Bulik et al. 2007) even though estradiol levels are at their physiologically highest levels. However, the contribution

of progesterone to the increased risk cannot be ruled out (Edler et al. 2007; Klump et al. 2008).

METHODS-Study 2. Acute 2-hydroxyestradiol in Male Rats: Preliminary Investigation of Effects on Shortening-evoked Dopamine Efflux in the Prefrontal Cortex

Animals, Binge Procedure and Microdialysis Methods

Based upon the rationale provided in the introduction, the present study sought to determine if dopamine levels in the prefrontal cortex changed during the exacerbation of binge intake induced by acute peritoneal 2-hydroxyestradiol. Acute injections were used rather than chronic infusions as acute injections allow within-subject comparisons to be made between vehicle and 2-hydroxyestradiol, and permit pre- and post-injection dopamine levels to be analyzed within a test session. Male rats were used for several reasons. First, if no changes in dopamine were seen, stimulation of intake would provide a behavioral indication of drug efficacy. As described in the introduction, our previous research indicated that acute intraperitoneal administration of 2-hydroxyestradiol increased binge size in male rats more so than in females once bingeing was established (Babbs et al. 2011). The present study, therefore, served to follow up our previous report. In addition, estradiol levels are lower in males than in females, which reduces the confounding influence of endogenous metabolism of estradiol to 2-hydroxyestradiol. Intact females or ovariectomized females with hormone replacement were not used for the same reason. Ovariectomized females without hormone replacement were not used, since OVX results in a dramatic increase in food intake and body fat accretion. Under such conditions, intake stimulatory effects of exogenous 2-hydroxyestradiol would be more difficult to detect once bingeing was established. In addition, the accretion of body fat could conceivably interfere with dopamine signaling due to effects of leptin (Grosshans et al. 2011). Based upon all of this, intact males were used for this preliminary investigation. Dialysis samples were collected in rats after bingeing was established, since acute 2-hydroxyestradiol had no effect in non-bingeing rats in our previous report (Babbs et al. 2011). Finally, samples were collected from the prefrontal cortex rather than other dopamine-rich areas, due to the reduced expression of the dopamine transporter in this region (Sesack et al. 1998), and because of ongoing studies in this laboratory suggesting the involvement of dopamine signaling in the prefrontal cortex to binge eating (Babbs and Corwin 2011; Corwin and Babbs 2011).

Six male Sprague-Dawley rats (Harlan, Indianapolis, IN), 60 days of age, were used for this study. A similar number of rats has been used by others for microdialysis studies (Golembiowska et al. 2012; Hillert et al. 2012) and provided sufficient power to detect differences between vehicle and 2-hydroxyestradiol, given the within-subject design of the present investigation (see results, below). Rats were housed as described above and were subjected to the binge protocol, with shortening being provided on Monday, Wednesday and Friday for 1 hour. A 1-hour shortening access period was provided during this study instead of the 20-min period used in Study 1, because of the limitations of the dialysis procedure. Specifically, samples were collected every 15 minutes in order to collect enough dopamine for it to be detectable. The shortening access period needed to be long enough for several samples to be collected. The 1-hour access period has been used in many of our previous studies and readily induces binge-type eating in rats (Babbs et al. 2012). After at least 5 weeks on this protocol, the rats were anesthetized with 4% isoflurane, and the top of the head was shaved and sterilized with betadine and 70% ethanol. After placement on a stereotaxic frame (Stoelting, Wood Dale, IL), the skull was exposed and bregma identified. A hole was drilled through the skull, and a CMA 11 guide cannula was implanted into the prefrontal cortex (coordinates: Anterior = +2.5 mm, Lateral = +0.5 mm, Depth = -1.5 mm).

After 5 days recovery time, rats were moved to the dialysis chamber and a CMA 11 (2 mm) probe was then inserted into the guide cannula. The rats were allowed to adapt overnight with a slow perfusion of artificial cerebrospinal fluid (aCSF; 1.3 $\mu\text{L}/\text{min}$) through the microdialysis probe. The following day, baseline dialysate samples were collected for one hour before intraperitoneal injection with either vehicle (saline with <1% Tween) or 2-hydroxyestradiol (3 $\mu\text{g}/\text{kg}$ body weight). This dose of 2-hydroxyestradiol was the effective dose in our previous report (Babbs et al., 2011). Rats were immediately given one hour of access to a jar of shortening after injection. Collections continued throughout the 1-hr shortening access period, and for at least 30 minutes after the removal of shortening. Each rat was tested on two dialysis sampling days: on one day samples were collected after vehicle and on the other day samples from the same rat were collected after 2-hydroxyestradiol. Injection sequences were assigned using a crossover design with at least 48 hours intervening between test sessions. After completion of the second sampling day, rats were immediately euthanized, and their brains were removed for microscopic examination to verify probe placement.

See Figure 1B for a timeline of the procedures that were used.

High Performance Liquid Chromatography

Dialysate samples (10 μL) were injected every 15 min onto an ESA MD-150 narrow-bore HPLC column 150 $\times$ 2 mm (ESA Inc., Chelmsford, MA) for separation followed by detection by an ESA 5014B microdialysis cell (+300 mV; ESA Coulochem III, ESA, Inc., Chelmsford, MA). A guard cell (ESA 5020) placed in line before the injection loop was set at a potential of +350 mV. The mobile phase consisted of 75 mM sodium phosphate monobasic (EMD Chemical, Gibbstown, NJ), 1.7 mM 1-octanesulfonic acid (EMD Chemical), 25 μM EDTA (Acros, Morris Plains, NJ), 10% acetonitrile (EMD), and 0.01% triethylamine (Sigma Aldrich, St. Louis, MO) in a volume of 1L (pH 3.0). The neurotransmitter and metabolite peak areas were integrated using EZ Chrom Elite software (Scientific Software Inc, Pleasanton, CA) and quantified against known standards of norepinephrine (ESA Inc., Chelmsford, MA), dopamine (ESA Inc.), 3,4-dihydroxyphenylacetic acid (DOPAC; Sigma Aldrich), and homovanillic acid (HVA; Sigma Aldrich).

Statistics

Values were considered significantly different at $p < 0.05$. Microdialysis samples for dopamine, DOPAC, and HVA were converted to a percentage of the mean of the first two baseline measurements (time points –60 minutes and –45 minutes). Area under the curve calculations were conducted for dopamine using Graphpad Prism 4 and were compared with a paired t-test (vehicle vs. 2-hydroxyestradiol). Additionally, a 2-way ANOVA was conducted for dopamine, DOPAC, and HVA obtained at each time point, with time and treatment (vehicle, 2-hydroxyestradiol) as repeated measures. A Bonferroni correction was applied by the software. Paired t-tests were used to compare dopamine levels at each time point, as well as 1-hr shortening intake, between 2-hydroxyestradiol and vehicle treatments.

RESULTS-Study 2

Shortening intake during the microdialysis test session

During the microdialysis procedure, rats consumed significantly more shortening after injections of 2-hydroxyestradiol ($5.3 \pm 1.4\text{g}$) than after vehicle ($1.7 \pm 0.3\text{g}$, $p < 0.03$) [$t(5)=3.29$, $p < 0.05$ paired t-test]. Intake after vehicle was low relative to intake in the home cage ($5.5 \pm 1.2\text{g}$), which may have been due to the stress of the microdialysis procedure. Regardless, 2-hydroxyestradiol still enhanced shortening consumption relative to vehicle.

Dopamine efflux during the microdialysis test session

The dopamine collected from the prefrontal cortex relative to baseline is shown in Figure 5. Area under the curve calculations, analyzed with a paired t-test, revealed a larger Area under the curve after the vehicle treatment than after the 2-hydroxyestradiol treatment ($p < 0.05$). This indicates that 2-hydroxyestradiol decreased dopamine efflux in the prefrontal cortex in bingeing rats. Additionally, the 2-way ANOVA revealed a main effect of time [$F(8,80) = 2.30$, $p < 0.03$], a main effect of treatment (that is, 2-hydroxyestradiol or vehicle) [$F(1,80) = 5.17$, $p < 0.05$], and a time by treatment interaction [$F(8,80) = 2.305$, $p < 0.03$]. Follow-up paired t-tests comparing 2-hydroxyestradiol to vehicle revealed a significant 2-hydroxyestradiol-induced reduction in dopamine at the 30-minute time point ($p < 0.05$). 2-way ANOVAs revealed a main effect of treatment (2-hydroxyestradiol or vehicle) for DOPAC [$F(1,45) = 8.21$, $p < 0.007$] and HVA [$F(1,45) = 13.77$, $p < 0.0006$], indicating that 2-hydroxyestradiol decreased both DOPAC and HVA in the prefrontal cortex (not shown).

DISCUSSION-Study 2

In Study 2, acute intraperitoneal administration of 2-hydroxyestradiol to bingeing male rats completely abolished the dopamine efflux evoked by presentation of shortening. This effect was coupled with a corresponding increase in shortening intake, which confirms our previous report (Babbs et al. 2011). The intake stimulatory effect of 2-hydroxyestradiol was apparent even though rats had 1 hour of shortening access in this study, but only had 20 minutes of access in our previous report, and in Study 1 here. Therefore, behavioral effects of 2-hydroxyestradiol were still detectable even though the intake periods differed. The reduced dopamine efflux is consistent with a previous study showing reduced basal dopamine levels in the medial prefrontal cortex during proestrus, when estradiol (and therefore, 2-hydroxyestradiol) levels are highest. Furthermore, that study also showed that ethanol-stimulated dopamine efflux (which was present during the estrus phase) was inhibited during proestrus (Dazzi et al. 2007). Considering our current findings, it is possible that these reductions in dopamine were mediated by 2-hydroxyestradiol. Though the mechanism by which 2-hydroxyestradiol inhibits dopamine in the prefrontal cortex is currently unknown, there is evidence that could provide clues.

Previous studies have shown that OVX reduces dopamine 2-type receptor number in the striatum, an effect that is prevented with subcutaneous estradiol supplementation (Bosse and DiPaolo 1996). Others have shown that dopamine 2-type receptor number assessed by PET is higher in the frontal cortices of women compared to men (Kaasinen et al. 2001). These findings may be mediated by the metabolite 2-hydroxyestradiol. Administration of 2-hydroxyestradiol has been shown to increase dopamine 2-type receptor binding capacity in the striatum in OVX rats, a result consistent with an increase in receptor number (Clopton and Gordon 1985; but see also Fernandez-Ruiz et al. 1987). An increase in the number of pre-synaptic dopamine 2-type receptors (the autoreceptor) would likely decrease dopamine efflux, such as is reported here. Such an effect in the prefrontal cortex would be especially important in the phenomenon of binge eating, since dopamine signaling has been closely linked to food reward (Volkow et al. 2011), and dopamine in the prefrontal cortex is considered a marker for reward-related learning and memory (Hyman et al. 2006; Phillips et al. 2008).

Whether or not decreased dopamine levels in the prefrontal cortex contribute to binge eating remains to be determined. However, work from other investigators supports this possibility. For instance, long-term decreases in dopamine levels in the nucleus accumbens of monkeys have been associated with postsynaptic dopamine 2-type supersensitivity (Falardeau et al. 1988). If the 2-hydroxyestradiol-associated decrease in dopamine in the prefrontal cortex causes supersensitization of postsynaptic dopamine 2-type receptors, at the same time that 2-

hydroxyestradiol (or the parent compound, estradiol) is increasing the number of these receptors, then 2-hydroxyestradiol could be creating conditions that exacerbate bingeing. Supporting this idea, the phenomenon of simultaneous supersensitivity and increased number of dopamine 2- type receptors has been shown with 6-OHDA lesions in adult animals (Mandel et al. 1993; Kostrzewa 1995). Importantly, activation of post-synaptic dopamine 2-type receptors in the prefrontal cortex inhibits the firing of glutamatergic pyramidal cells (Tseng and O'Donnell 2007), which project to the nucleus accumbens (Brog et al. 1993; Carr et al. 1999). Because inhibition of glutamate receptors in the nucleus accumbens stimulates feeding in sated rats (Stratford et al. 1998), it is possible that post-synaptic dopamine 2-type activation in the prefrontal cortex exacerbates binge intake via inhibition of pyramidal cell firing thereby reducing glutamatergic signaling in the nucleus accumbens. Therefore, a relatively high density of supersensitized postsynaptic dopamine 2-type receptors would “prime” the system to respond to a binge-type episode, due to the long-term effects of chronic exposure to 2-hydroxyestradiol. Non-binge-type eating would not be affected by the “priming”, as the efflux of dopamine upon binge initiation would not be as great.

In addition, 2-hydroxyestradiol has been shown to bind to dopamine 2-type receptors in the pituitary (Schaeffer and Hsueh 1979). Assuming that this binding is activational, 2-hydroxyestradiol could inhibit dopamine release by binding to presynaptic dopamine 2-type autoreceptors, thereby inhibiting dopamine release. In addition, 2-hydroxyestradiol may directly activate postsynaptic dopamine 2-type receptors, which also would inhibit glutamatergic firing to the nucleus accumbens (Tseng and O'Donnell 2007). Another possible mechanism involves 2- hydroxyestradiol's ability to inhibit adenylyl cyclase (Braun 1990; Tofovic et al. 2009), an effect of dopamine 2-type receptor binding by dopamine. In this manner, 2-hydroxyestradiol may either bypass dopamine 2-type receptors altogether and mimic the action of dopamine 2-type binding, or may enhance the activity of dopamine 2-type receptors once they are bound by endogenous dopamine. Based on the present research, it is not likely that the effects of 2- hydroxyestradiol are caused by inhibition of COMT, as this would be expected to increase dopamine levels. Instead, 2-hydroxyestradiol decreased bingeing-evoked dopamine levels in the present study.

GENERAL CONCLUSIONS

Here we have shown that chronic administration of 2-hydroxyestradiol to ovariectomized female rats can exacerbate the induction of binge-type eating. Specifically, we found that 2-hydroxyestradiol attenuates the increases in body weight and food intake associated with OVX, but shifts food intake toward a binge-type pattern, when administration starts prior to binge development and continues during binge development. Additionally, we have shown that 2-hydroxyestradiol increases the escalation of binge size over time, which may reflect increased progression to more severe forms of bingeing. Study 1, therefore, provides suggestive evidence that chronic exposure to 2-hydroxyestradiol facilitates ‘learning to binge’. In addition, we provided evidence that acute 2-hydroxyestradiol inhibits bingeing-evoked dopamine efflux in the prefrontal cortex of male rats that already had extensive binge experience. Given the short half-life of 2-hydroxyestradiol, the results of Study 2 are particularly striking, and lend further support for the possibility that the metabolite may have long-lasting effects on dopamine signaling.

LIMITATIONS

The results reported here were obtained from two distinct studies: one that investigated the effect of chronic administration of 2-hydroxyestradiol in females, and a second that investigated the effect of acute administration of 2-hydroxyestradiol in males. Therefore, it

is currently not known if the mechanisms suggested here apply to both sexes, and additional studies should be conducted before such claims are made. Additionally, the routes of administration that were used in the two studies (minipump vs. acute injection) no doubt resulted in different levels of circulating metabolite. Unfortunately, assays to detect physiological levels of endogenous 2-hydroxyestradiol in rats are not available. In spite of the differences between the two studies, it is tempting to speculate that inhibition of dopamine efflux reported in Study 2 may have contributed to the binge escalation reported in Study 1. However, due to sex differences and differences in exposure time (chronic vs. acute) between the two studies, it is currently not possible to confirm whether or not similar inhibition of dopamine efflux occurs with chronic exposure to 2-hydroxyestradiol or in female rats. If dopamine 2-type receptors are involved, determining effects in females in future studies will be particularly important, due to differences dopamine 2-type receptor activation that have been reported in the prefrontal cortex in male and female rats (Sun et al. 2010). Furthermore, whether or not the reductions in dopamine cause binge exacerbation is not known. In spite of these limitations, the present studies have shown that 2-hydroxyestradiol can exacerbate bingeing as it develops in females and can suppress dopamine signaling in the prefrontal cortex once bingeing is established in males. Together, these studies suggest a novel mechanism that may contribute to binge risk.

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Research Highlights

- Chronic 2-hydroxyestradiol (2OHE2) enhanced binge escalation in female rats.
- Chronic 2OHE2 increased fat intake and decreased chow intake in female rats.
- Acute 2OHE2 abolished dopamine efflux in the prefrontal cortex in male rats.

A. STUDY 1

B. STUDY 2

Figure 1.
Timeline of procedures for Study 1 (A) and Study 2 (B)

Figure 2.

Escalation in normalized shortening intake (percent of Day 1 kcal/body weight^{0.67}) across the 4-week binge induction period. * indicates that the E group's shortening intake escalated significantly faster than did that of either the O or I group ($p < 0.02$). Group designations as described for Table 1.

Figure 3.

Week 4 average shortening and chow intake. * indicates $p < 0.05$ between bracketed groups. Group designations as described in Table 1. (A) The E and O groups ate significantly more shortening during the 20-minute shortening access period than did the I group; E and O did not differ from each other. (B) The E group ate significantly less chow, on average, than did the I or O group; I and O did not differ from each other.

Figure 4. Week 4 shortening to chow ratio (kcal shortening/kcal chow). The E group had a significantly higher shortening to chow ratio than the I group. Significant differences among groups are indicated by different letters.

Figure 5. Shortening-evoked DA efflux in the PFC of bingeing rats. After injection with vehicle, presentation of shortening at time 0 minutes caused an increase in DA efflux that reached maximal levels at 30 minutes. This effect was significantly inhibited after acute injection with 2-hydroxyestradiol (2OHE2). Shortening was removed at time 60 minutes. * $p < 0.05$ vs. 2OHE2.

Body weights and cumulative energy intake (not normalized to body weight) across the four weeks of the study.

Table 1

Group	Start Body Wt (g)	End Body Wt (g)	Total Intake Shortening (kcal)	Total Intake Chow (kcal)	Total Intake All Food (kcal)
I	219.3 (2.1)	252.9 (4.0) ^a	259.1 (26.9) ^a	1235.0 (37.6) ^{ab}	1494.2 (21.5) ^a
E	216.3 (1.8)	251.7 (2.2) ^a	358.4 (26.7) ^{ab}	1099.8 (24.3) ^a	1458.2 (12.3) ^a
O	220.1 (2.4)	307.8 (4.2) ^b	391.2 (40.0) ^b	1374.1 (57.4) ^b	1765.3 (28.7) ^b

Significant differences among groups (at alpha <0.05) are represented by different lower case letters. All data presented as mean and (SEM). I = (Intact) Sham surgery + VEH infusion; E = OVX + 2OHE2 infusion; O = OVX + VEH infusion