Effects of Lisdexamfetamine and Haloperidol on a Binge-Like Eating Model & Preliminary Investigations of the Dopaminergic Mechanism Underlying Binge Eating Disorder (BED)

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Abstract

Binge eating disorder (BED) is a psychophysiological disorder defined as the excessive intake of high-caloric, palatable foods within a short span of time, accompanied by feelings of distress. Animal models of binge-like eating behavior have been developed that give intermittent, limited access to a highly palatable food. Presby et al. (2020) used chocolate as the highly palatable food to induce binge-like eating behavior in rats. Lisdexamfetamine (LDX), a d-amphetamine prodrug and dopamine (DA) uptake inhibitor, is currently used to treat BED in human. In rats, binge-like eating of chocolate was induced by exposure to unpredictable and limited chocolate access over several weeks. After training, rats were tested for the effects of LDX and the DA D2 antagonist haloperidol in one-hour chocolate exposure sessions. Consistent with previous results, the highest doses of LDX (0.75 and 1.5 mg/kg IP) suppressed chocolate intake. In contrast, doses of haloperidol (0.05-0.1 mg/kg IP) did not significantly affect chocolate consumption. Furthermore, haloperidol partially reversed the LDX-induced decrease in chocolate consumption. These results have implications for understanding the role of DAergic mechanisms in the regulation of binge-like eating. One hypothesis for this reduction involves increases in mesolimbic DA but more classic literature regarding the anorexic effects of amphetamines, such as LDX, identifies the perifornical lateral hypothalamus (PFH) as a critical brain region responsible for driving a reduction in food intake. This study observed the effects of LDX and haloperidol on the binge-like consumption of chocolate as well as the DAminergic mechanism of LDX in the PFH through microdialysis and high-performance liquid chromatography. These results have implications for the further understanding of mechanisms underlying binge eating behavior as well as improved knowledge of treatment for BED patients.
1. Introduction

Binge eating disorder (BED) is a psychophysiological disorder prominent in today’s society. BED is defined as the excessive intake of high-caloric, palatable foods within a short span of time and is generally characterized by feelings of distress and a lack of self-control during the consumption of food. The current diagnostic and statistical manual from the American Psychiatric Association (DSM-5) recognizes BED as one of three specified eating disorders, thereby distinguishing it from overeating (DSM-V; APA, 2013). Individuals with BED are often obese, which can lead to further health complications such as diabetes and cardiovascular disease (National Institute of Mental Health, 2017). According to the National Institute of Mental Health, 1.2 percent of adults in the United States were diagnosed with BED between 2001 and 2003, affecting twice as many females than males (NIMH, 2017). Most strikingly is that only 43 percent of people suffering from this disorder have received treatment (NIMH, 2017). Thus, it is important to explore novel animal models to find reliable forms of treatment as well as understanding their underlying mechanisms.

Rodent models of binge-like feeding behavior have been developed and are commonly used for the study of BED (Hagan et al., 2002; Colantuoni et al., 2001; Avena & Hoebel, 2003; Rada et al., 2005; Boggiano & Chandler, 2006; Corwin & Wojnicki, 2006; Avena et al., 2004, 2006; Heyne et al., 2009; Vickers et al., 2015; Smith et al., 2015; Small-Crevier et al., 2018; Presby et al., 2020). These models typically involve induction of excessive eating of a highly palatable food. Vickers et al. (2015) used a chocolate intake procedure and reported that a common treatment for BED, lisdexamfetamine (LDX), reduced chocolate intake in a dose dependent manner during binging sessions. More recently, Presby et al. (2020) observed that
LDX not only reduced chocolate intake using procedures similar to those employed by Vickers et al. (2015) but also intake of standard lab chow (Presby et al., 2020).

The induction of binge eating in animals has been reported to cause changes in several neurochemical systems, especially the mesolimbic DA system, which innervates the nucleus accumbens. Avena et al. (2008) noted several changes in DA transmission in the nucleus accumbens that are induced by exposure to high sugar foods and suggested that many of these changes are similar to those that are associated with drug addiction. The suggestion that DA transmission is involved in binge consumption of food is important, particularly in view of the fact that LDX, which is approved for treatment of BED (as known under the trade name Vyvanse), acts as a pro-drug (i.e., slow release formulation) of d-amphetamine. Amphetamines, a class of drug to which LDX belongs, increase the release and block the uptake of catecholamines such as DA, norepinephrine (NE) and epinephrine (Rowley et al., 2012) and has been shown to induce anorexigenic effects in animal models as well as in human clinical studies.

Although there is considerable research demonstrating that LDX is an effective treatment for moderate to severe BED, the specific brain areas that LDX is targeting to produce the suppression of intake of highly palatable foods in rodent models of BED are unclear. It is possible, and is supported by existing literature, that the critical brain region mediating this effect is the perifornical region of the lateral hypothalamus (PFH), and not the nucleus accumbens. Leibowitz (1975a&b) demonstrated that amphetamine administration in the lateral hypothalamus induced anorexic behavior, and later studies conducted by Leibowitz & Rossakis (1976) localized these targeted catecholaminergic neurons to the PFH specifically and demonstrated that injections of exogenous DA and epinephrine into the lateral perifornical region of the rostral and middle hypothalamus significantly suppressed the food intake of hungry rats (Leibowitz &
Rossakis, 1978). Furthermore, intercranial injections of DA into the PFH had demonstrably greater anorexigenic effects than administration of NE (Leibowitz & Rossakis, 1979). These anorexigenic effects induced by catecholamine transmission in the lateral hypothalamus have been shown to be blocked via administration of DA antagonists such as haloperidol, a DA D2 competitive antagonist (Leibowitz & Rossakis, 1979), which demonstrated that the feeding-suppressant effects of amphetamine were 90% blocked upon coadministration (Leibowitz & Rossakis, 1978). Leibowitz et al. were the first to formally offer the underlying hypothesis that hypothalamic neurons in the PFH, affected by amphetamine-induced elevations in extracellular DA, inhibit feeding in rats through a DAminergic mechanism. However, they did not provide any direct measurements of DA release in their papers.

Given these observations reviewed above, it may be possible that, in binge eating models, LDX is not acting on mesolimbic DA levels to suppress chocolate intake, but rather via a hypothalamic mechanism. However, the hypothesis that LDX is suppressing appetite by increasing extracellular DA in PFH is dependent on the assumption that doses of LDX that suppress chocolate intake are actually dependent upon increased PFH DA transmission. This study aimed to observe the effects of pharmacological manipulation of chocolate intake through administration of LDX and the DA D2 receptor antagonist haloperidol, either alone or in combination, on binge-like eating behavior. This study also conducted a preliminary investigation of the DAergic mechanisms of LDX, at doses that suppress chocolate consumption in binge eating rats, within the PFH. Extracellular DA concentrations were measured through microdialysis probe sampling and subsequent high-performance liquid chromatography.
2. Materials/Methods

2.1. Subjects

23 adult male Sprague Dawley rats purchased from Envigo, (Indianapolis, IN) were utilized for the completion of these experiments. These rats were not previously exposed to any drugs prior to the experiments and were singly housed in controlled environmental conditions of 23 °C and a 12-hour light/dark cycle. Water and food were available ad libitum throughout experimentation. All animal procedures were in accordance with NIH guidelines and were approved by the university institutional animal care and use committee.

2.2. Pharmacological Agents

LDX and haloperidol (HAL) were used to observe the effects of pharmacological manipulation of DA transmission on chocolate consumption during binge eating sessions. LDX, a catecholamine uptake inhibitor and a d-amphetamine prodrug, was dissolved in a 0.9% saline solution. HAL, a DA D2 antagonist, was dissolved in 0.3% tartaric acid. The LDX used in this study was donated by SHIRE and the HAL was obtained through Sigma Aldrich.

2.3. Behavioral Procedure- Acquisition of Binge Eating Behavior

Similar to the study of Presby et al. (2020), ground Cadbury’s Dairy Milk Chocolate was utilized in the acquisition and maintenance of the binge-like eating behavior. All rats in this study were trained on an acquisition schedule that mirrored that of Presby et al. (2020). Rats received chocolate in ceramic bowls during 12 one-hour sessions over the course of 4 weeks with chocolate exposure occurring on days 1, 2, 4, 6, 7, 9, 12, 14, 15, 18, 23, and 28. The chocolate
was weighed prior to and after the one-hour exposure session. Chow was weighed and recorded to monitor daily intake.

2.4. Effect of LDX on Chocolate Intake

This experiment utilized 7 drug-naïve adult male Sprague Dawley rats. Upon completion of the acquisition phase, one baseline chocolate exposure session was conducted earlier in the week followed by the chocolate exposure session with administered LDX later in the week. IP injections of 0.1875, 0.375, 0.75, or 1.5 mg/kg LDX or vehicle (saline) were administered in a randomly varied order once per week with a lead time of 60 minutes prior to the start of the chocolate exposure session. IP doses of LDX were based on Presby et al. (2020). The chocolate was given to the rats in ceramic bowls for a one-hour session. The chocolate was weighed prior to and after the one-hour exposure session.

2.5. Effect of Haloperidol on Chocolate Intake

This experiment utilized the same 7 adult male Sprague Dawley rats used in the above LDX study. Upon completion of the LDX experiment, a washout week where no drugs were administered to the rats took place. After the washout week, IP injections of 0.05 or 0.1 mg/kg haloperidol or vehicle (0.3% tartaric acid) were administered in a randomly varied order once per week with a lead time of 50 minutes prior to testing. The chocolate was given to the rats in ceramic bowls for a one-hour session. The chocolate was weighed prior to and after the one-hour exposure session.
2.6. LDX/Haloperidol Reversal

A separate group of 15 drug-naïve adult male Sprague Dawley rats were utilized for this experiment. IP injections of 1.5 mg/kg LDX or vehicle (saline) were administered 60 minutes prior to the chocolate exposure session. Those same rats were administered an IP injection of 0.05 or 0.1 mg/kg haloperidol or vehicle (0.3% tartaric acid) 50 minutes prior to the chocolate exposure session. The chocolate was given to the rats in ceramic bowls for a one-hour session. The chocolate was weighed prior to and after the one-hour exposure session.

2.7. Surgical Implantation of Dialysis Cannula

This procedure utilized 1 adult male Sprague Dawley rat. The rat was anesthetized with a 1.0-mL/kg intraperitoneal (IP) injection of a solution containing 10.0mL of 100 mg/mL ketamine plus 0.75mL of 20.0 mg/mL xylazine (Phoenix Scientific, Inc., St. Joseph, MO). While in the stereotax (Kopf, Tujunga, CA; incisor bar 5.0mm above interaural line), the rat received unilateral implantations of a 10.0-mm probe guide cannula (Bioanalytical Systems, Indianapolis, IN). The tip of the guide cannula was implanted 2.0mm above the PFH (anterior/posterior: - 0.7 mm from bregma, medial/lateral: ± 1.3 mm from the midline, dorsal/ventral: - 7.2 mm from bregma; counterbalanced left vs right) and secured to the skull with stainless-steel screws and cement. A stainless-steel stylet was inserted into the cannula to maintain patency. The rat was singly housed and allowed 7 days postsurgical recovery.

2.8. Microdialysis and High-Performance Liquid Chromatography (HPLC)

After the allotted postsurgical recovery period, the rat with an implanted cannula was habituated in a Plexiglas chamber (28×28×23 cm³) the day before sampling for 8 hours with the infusion
pump running. The following day, a probe was inserted through the cannula, set to extend 2.0 mm beyond the guide cannula, and artificial cerebrospinal fluid was pumped through at a rate of 2.0 μL/min by a syringe pump (Harvard Apparatus, Cambridge, MA). Two hours post-insertion, sampling began and continued for 6 hours. After sampling for two hours more, the animal received an IP injection (1.0 ml/kg total volume) of 1.5 mg/kg LDX (n=1). This dose was selected because it had been shown to suppress chocolate intake in a BED model (Presby et al. 2020). After four more hours of dialysis sampling, the session was completed, the probe was removed, and after euthanasia, histological analysis was performed to verify placement. Samples were frozen and analyzed for DA content using reverse-phase HPLC with electrochemical detection (ESA, New Bedford, MA; Segovia et al. 2011; Nunes et al. 2013). Each liter of mobile phase contained 27.6g sodium phosphate monobasic monohydrate, 750μL 0.1M ethylenediaminetetra acetic acid, and 2,200μL 0.4M sodium octyl sulfate dissolved in dH2O (pH = 4.5). DA standards were assayed before, during, and after the dialysis samples. At the completion of the microdialysis experiment, the animal was anesthetized with CO2 and then perfused intracardially with physiological saline followed by a 3.7% formaldehyde solution. The brain was removed, stored in formaldehyde, and then sliced with a vibratome in 60μm sections, which was mounted on glass microscope slides. After mounting, slides were stained with cresyl violet for microscopic observation by an observer who was unaware of the experimental treatment.

2.9. Data Analyses

All drug experiments used a repeated measures designs; all animals received all treatments in a randomly varied order. A repeated measure analysis of variance (ANOVA) was used to evaluate
the effects of LDX and haloperidol on the amount of chocolate intake. A regression analysis was conducted on the reversal of the effects of LDX by haloperidol on chocolate consumption due to an incomplete dataset (collection was interrupted by the COVID crisis). For analysis of the microdialysis data, DA levels (calculated first in nanograms, based upon comparisons with standard solutions of DA) were analyzed as percentage change from baseline, with the mean of the 2 samples immediately preceding the injection serving as the 100% baseline level.

3. Results

3.1. Acquisition of Chocolate Intake

Analysis of the binge eating acquisition phase was performed using a repeated measures ANOVA. Overall, there was a significant effect on chocolate consumption due to binge eating session \([F(11,143) = 28.269, p < 0.001]\) Fig. 1]. There was an observed increase in chocolate consumption over the course of majority of binge sessions; the average chocolate consumption increasing from 1.3g, during the first session, to 7.5g during the twelfth session.
Figure 1. The acquisition of binge eating behavior (Mean ± SEM chocolate intake in grams; n = 22) over the course of 12 one-hour sessions of exposure to chocolate. There was an overall significant effect due to session intake of chocolate \([F(11,143) = 28.269, p < 0.001]\).

3.2. Effect of LDX on Chocolate Consumption

Analysis of the effect of LDX on chocolate consumption was performed using a repeated measures ANOVA followed by a planned comparisons test comparing the effect of the vehicle to each dose of LDX. There was an overall significant effect due to LDX on chocolate consumption \([F(4,24) = 11.593, p < 0.001]\) Fig. 2]. Doses of 0.1875, 0.75, and 1.5mg/kg LDX showed a significant reduction in the amount of chocolate consumed.

Figure 2. Administration of varying doses of LDX and its effects on overall chocolate intake (Mean ± SEM chocolate intake in grams) during a one-hour chocolate exposure session. There was an overall effect of LDX on chocolate consumption \([F(4,24) = 11.593, p < 0.001]\). *Dose of
0.1875 mg/kg demonstrated a significant effect of p < 0.05. **Doses of 0.75, and 1.5 mg/kg demonstrated a significant effect of p < 0.001.

3.3. Effect of Haloperidol on Chocolate Consumption

Analysis of the effect of haloperidol on chocolate consumption was performed using a repeated measures ANOVA. There was no significant overall effect of haloperidol on chocolate consumption [F(2,12) = 0.577, p = 0.576) Fig. 3].

**Figure 3.** Administration of varying doses of haloperidol and its effects on overall chocolate intake (Mean ± SEM chocolate intake in grams) during a one-hour chocolate exposure session. There was no overall effect of haloperidol on chocolate consumption [F(2,12) = 0.577, p = 0.576)].
3.4. Effects of Haloperidol on Reversing LDX Reduction of Chocolate Consumption

Analysis of the effect of haloperidol on the reversal of LDX reduction of chocolate consumption was performed using a regression analysis. This showed a significant effect of haloperidol on the partial reversal of the effects of LDX on chocolate consumption \( F(1,23) = 4.647, p < 0.05 \) Fig. 4b] with an \( R^2 \) value of 0.174.

**Figure 4a.** Coadministration of LDX and varying doses of haloperidol (Mean ± SEM chocolate intake in grams; incomplete data set).
Figure 4b. Regression analysis of the coadministration of LDX and varying doses of haloperidol. There was a partial reversal of LDX reduction of chocolate consumption by haloperidol as marked by the linear dose-related effect [$F(1,23) = 4.647$, $p < 0.05$].

3.5. Microdialysis & HPLC

Overall, LDX substantially increased the concentration of DA in the PFH region, with DA concentrations reaching nearly 450% of the mean baseline.
Figure 5. Effect of LDX on the extracellular levels of DA in CSF samples taken from the PFH during microdialysis in an individual rat.

4. Discussion

The behavioral acquisition data of this experiment (Figure 1) support the idea that this animal model can successfully induce binge-like eating behavior in rats through the use of chocolate exposure sessions, consistent with Vickers et al. (2015) and Presby et al. (2020). LDX was shown to significantly decrease chocolate consumption during the one-hour exposure sessions, particularly at the two highest doses. LDX, being a d-amphetamine prodrug, increases the release of DA and decreases its uptake, two effects that lead to an increase in extracellular DA. As previously discussed, the existing literature by Leibowitz (1975a&b) and Leibowitz and Rossakis (1978, 1979) reported the anorexigenic effects of d-amphetamine administration. Existing literature by Leibowitz and Rossakis (1978) theorized that the reduced binge eating behavior due to increased DA transmission induced by amphetamines like LDX is localized
within the PFH. The initial microdialysis data collected in this thesis further supplements the validity of this theory, indicating that the concentration of DA in the PFH substantially increases from baseline after administration of the highest dose of LDX. Furthermore, the DA D2 antagonist haloperidol was shown to partially reverse the effects of LDX on chocolate consumption, consistent with the data produced by Prada et al. (1988), and alluding to the presence of a DAergic mechanism underlying BED. It should be noted that, due to the ongoing COVID-19 pandemic, this study’s LDX/haloperidol reversal dataset was incomplete. Therefore, a regression analysis was performed to analyze and draw from the existing data. However, data from the experiment show only a partial reversal of LDX effects on chocolate consumption from haloperidol administration. This may be due to the specific binding mechanism of haloperidol, and the specific actions of LDX in enhancing DA transmission by binding to a different protein (DAT). Haloperidol is a relatively selective DA D2 competitive antagonist and while LDX increases extracellular DA, haloperidol would only limit binding of DA to D2 receptors. However, it is likely that the increased DA transmission induced by LDX is stimulating both DA D1 and D2 receptors, thereby making haloperidol’s inhibition of the effects of LDX only partial.

Previous research indicates that amphetamines, such as LDX, affect the transmission of other catecholamines apart from DA, such as norepinephrine and epinephrine. Previous work by Grossman (1962) and Leibowitz (1970) has investigated and identified adrenergic effects in the hypothalamus on food intake and satiation. α- and β-adrenergic receptors were found to have opposing functions within the hypothalamus. Leibowitz (1970) found that administration of an α-adrenergic agonist in the hypothalamus increased food intake in rats, whereas this behavior could then be suppressed using a β-adrenergic agonist. Furthermore, administration of an α-adrenergic antagonist decreased food intake, and this behavior was reversed using a β-adrenergic antagonist.
(Leibowitz, 1970). It can be reasoned from these findings that the hypothalamus has α-adrenergic and β-adrenergic pathways that regulate hunger and satiation, respectively (Leibowitz 1970). Considering NE transmission can have such implications on appetite regulation, future research should focus on the possibilities of using selective NE uptake inhibitors, such as atomoxetine. This drug has been looked into as a possibly viable treatment for BED, and it has been found to successfully reduce the quantity of binging sessions as well as lessen the psychological symptoms associated with the disorder (Brownley et al., 2014).

Apart from catecholamines, serotonin (5-HT) has also been shown to play a role in the regulation of appetite. Increased 5-HT transmission has been shown to have anorexigenic effects, as illustrated in a study conducted by Wurtman & Wurtman (1977). Fenfluramine, a drug which increases the release and blocks the uptake of 5-HT, was shown to have effects similar to what is seen upon administration of amphetamine (Wurtman & Wurtman, 1977). Interestingly, fenfluramine administration seems to directly target and decrease the consumption of foods with high carbohydrate content. A similar effect was seen in the data of this experiment, in which LDX demonstrably decreased the consumption of chocolate, which is similarly high in sugar content. This research, in combination with the findings of this study, suggests the possibility that appetite for different macromolecules, such as carbohydrates and proteins, are regulated through transmission of different neurotransmitters.

The microdialysis data shows DA transmission in the PFH to have implications in binge eating behavior. Specifically, the data show that enhanced DAmnergic transmission in the PFH may be linked to the reduction of food intake as induced by the administration of LDX. However, more recent studies have focused their investigation on the arcuate nucleus (ARC), a region in the hypothalamus that has overarching connections to the PFH, paraventricular nucleus,
and other hypothalamic nuclei. The widespread connections between the ARC and the rest of the hypothalamus have led researchers to believe it may be the main drive in the regulation of appetite by regulating secondary structures, such as the PFH (Ramos et al., 2005). The ARC contains a dense population of neuropeptide-Y (NPY) releasing neurons which influence the downstream regions of the PFH (Sohn et al., 2013; Varela & Horvath, 2012). Pharmaceutical manipulations have evidenced the antagonistic relationship between DA and NPY in the PFH. AMPH administration into the PFH resulted in decreased NPY-induced feeding behavior, which was then completely reversed through administration of HAL (Gillard et al., 1993).

The overall goals of this study were to observe the effects of pharmaceutical manipulations of DA, through the administration of LDX and HAL, on the binge-like eating behavior of a rat model. LDX, which increases overall DA transmission, was shown to reduce the binge-like eating behavior resulting in a decrease in chocolate consumption. HAL, a DA D2 competitive antagonist, did not have an effect on overall chocolate consumption when administered alone but partially reversed the decrease in chocolate consumption induced by LDX when co-administered. The partial nature of the reversal may be due to the relative D2 receptor selectivity of haloperidol, and future studies need to explore the role of D1 DA receptors in the actions of LDX. With the preliminary microdialysis study, LDX administration substantially increased DA concentrations in the PFH, supporting the theory that there may be a DAergic mechanism in the PFH underlying appetite regulation. These findings have important implications for future studies of BED and its viable treatments. Understanding the mechanisms behind the disorder are an important first step in developing specific and neurochemically targeted medications.
References


Corwin, R.L., Wojnicki, F.H., 2006. Binge eating in rats with limited access to vegetable


