Activation of mesolimbic dopamine neurons during novel and daily limited access to palatable food is blocked by the opioid antagonist LY255582

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Sahr AE, Sindelar DK, Alexander-Checko JT, Eastwood BJ, Mitch CH, Statnick MA. Activation of mesolimbic dopamine neurons during novel and daily limited access to palatable food is blocked by the opioid antagonist LY255582. Am J Physiol Regul Integr Comp Physiol 295: R463–R471, 2008. First published June 4, 2008; doi:10.1152/ajpregu.00390.2007.—An analog of the trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine series (LY255582) exhibits high in vitro binding affinity and antagonist potency for the μ-, δ-, and κ-opioid receptors. In vivo, LY255582 inhibits high in vitro binding affinity and antagonist potency for the μ-, δ-, and κ-opioid receptors blocking morphine- and U50,488-mediated antinociception, and bremazocine-induced diuresis (43, 64). Importantly, LY255582 has also been shown to potentiate increase food intake after central or peripheral administration and produces a sustained weight loss in several rodent models of obesity (38, 52, 53, 55).

Our laboratory has developed a hedonic feeding paradigm for investigating compound effects on HP food consumption. In this model, rats are fed regular chow ad libitum, and are given daily limited access (1 h) to an HP diet during the light portion of the light-dark cycle when food intake is normally low. Under these conditions, rats progressively increase their intake of the HP diet following several days of HP diet access resulting in a large bout of feeding during the 1-h access period. The objectives of the present studies were to determine 1) the effect of LY255582 on consumption of the HP diet in animals given daily limited HP diet access, 2) whether the effect of LY255582 on HP diet induced increases in NAc shell DA levels in animals consuming the HP diet for the first time, and 3) the effect of LY255582 on the progressive increase in HP diet intake and the accompanying alterations in NAc shell DA levels that occur during the escalation phase of HP diet intake. The NAc shell subregion was targeted, as several studies have demonstrated, a preferential increase in DA in the NAc shell vs. core in response to the consumption of palatable diets (9, 58).

MATERIALS AND METHODS

General Methods

Subjects. Male Sprague-Dawley rats (250–270 g; purchased from Harlan Sprague Dawley, Indianapolis, IN) were single housed in plastic shoe box cages in a temperature- and humidity-controlled colony maintained on a 12:12-h light-dark cycle [lights on at 0400 h for experiment 1 (effect of LY255582 on consumption of an HP diet) and lights on at 0600 h for experiments 2a, 2b and 2c (microdialysis experiments)]. In their home cage, animals were maintained on

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normal rat chow (LabDiet 5001; Purina Mills, Richmond, IN) and water ad libitum. The normal rat chow consisted of 59.4% carbohydrate, 28.4% protein, and 12.3% fat (3.3 kcal/g). The HP diet that was used in all experiments was Teklad 95217 (Harlan Teklad, Madison, WI), consisting of 41.3% carbohydrate (primarily as sucrose: 157 g/100 g food), 18.7% protein, and 40% fat (4.25 kcal/g). All experiments were conducted during the light portion of the light cycle between 0700 and 1500 h, when baseline feeding is normally low. HP diet access occurred an average of 6 h into the light cycle. All animal protocols used in the study were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 86–23, 1985) and approved by the Lilly Research Labs Institutional Animal Care and Use Committee. All efforts were made to minimize animal discomfort and to use the minimum number of animals required to obtain appropriate statistical power.

Compounds. LY255582 was synthesized at Lilly Research Laboratories according to Mitch et al., (43). An in vivo formulation of LY255582 was prepared in water acidified with 1% lactic acid (vol/vol) and administered subcutaneously in a 1 ml/kg dose volume.

**Experiment 1: Effect of LY255582 on Consumption of an HP Diet**

Experiment 1 examined the effect of acute LY255582 treatment on food intake in animals given daily limited access to the HP diet. On days 1–3, all rats (n = 7/group) were dosed (sc) with vehicle (1 ml/kg) and returned to their home cage. One hour after dosing, rats were given 1-h access to the HP diet (at ~1130) in addition to having their regular chow in their home cage. On day 4, rats received either vehicle or 0.1–3.0 mg/kg sc LY255582. One hour following compound treatment, rats were given access to the HP diet in the same manner. Daily 1-h HP diet intake and 24-h chow intake were measured throughout the study.

**Experiments 2a, 2b, and 2c: Microdialysis Experiments**

Common methods. STEREOTAXIC SURGERY. Rats were anesthetized with 2–3% isoflurane anesthesia and placed in a stereotaxic apparatus with the incisor bar set at ~3.3 mm (David Kopf Instruments, Tujunga, CA). Briefly, the skull was exposed by removing a small piece of scalp and the tissue was retracted. Two to three pilot holes were drilled into the skull for anchor screws, and a separate hole was made to allow the insertion of the guide cannula over the NAc shell using the following coordinates: AP = +1.7 mm, ML = +0.8 mm, DV = −6.0 mm, relative to bregma (46). The guide cannula was fixed into place with Duralar dental adhesive [Bioanalytical Systems (BASI), West Lafayette, IN]. Following surgery, the animals were allowed 2–5 days to recover before any experimentation. Twenty-four hours prior to testing, microdialysis probes (BR-2 probes, 30,000 Daltons; BASI) were inserted into the guide cannulae. The active membrane portion of the probe was 2 mm in length.

GENERAL MICRODIALYSIS PROCEDURE. On the morning of the experiment, animals were moved to the testing room at ~0700 and placed in round-bottom cages and tethered to a counterbalance arm. The probe inlet was connected to a syringe pump that delivered Ringers solution (in mM: 145 NaCl, 2.7 KCl, 1.0 MgCl₂, 2.5 CaCl₂, 2.0 Na₂HPO₄, pH 7.2–7.4) at a flow rate of 1.5 μl/min. Animals were perfused for 2.5 h before sample collection began. Following this equilibration, samples were collected every 10 min. All microdialysates were collected into vials containing 5 μl of 0.2 N perchloric acid and maintained at 4°C in a fraction collector. Water was not available during microdialysis testing. Food was not available in the microdialysis cages until the presentation of the HP diet (~4 h after rats were placed in the cages and 5 h into the light cycle).

**Specific Methods**

**Experiment 2a: microdialysis following novel access to an HP diet.** This study examined the effect of LY255582 on the mesolimbic DA response to the HP diet in animals that had no previous history of HP diet consumption (i.e., when the HP diet was novel). Following setup and equilibration, three baseline samples were collected, and animals were injected subcutaneously with either vehicle or 0.3 mg/kg LY255582 (n = 6–7/group). The dose of 0.3 mg/kg was chosen based on ex vivo binding studies that showed 0.3 mg/kg of LY255582 produced > 80% occupancy of opioid receptors in the central nervous system, ensuring that the dose of LY255582 was sufficient to fully antagonize opioid receptors throughout the testing period (data not shown). One hour after treatment, animals were given a preweighed amount of the HP diet on the floor of the testing bowl. After 1 h, the food remaining was removed and weighed so that the amount eaten could be calculated. A pair-fed, vehicle-treated control group was given the amount of HP diet consumed by LY255582-treated rats (0.7 g). The pair-fed animals consumed all 0.7 g of the HP diet during the 1-h access period.

**Experiment 2b: microdialysis following repeated access to an HP diet.** This study examined the effect of LY255582 on the intake of the HP diet and the changes in NAc shell DA levels in response to the HP diet during daily 1-h limited HP diet access sessions. The study employed a between-subjects design with groups run concurrently (n = 5–9/group), as probe integrity can begin to deteriorate 24 h after implantation. Each day, rats were taken to the testing room at ~0700, placed in the testing bowls, and tethered to the counterbalance arm. On day 1 all animals were given vehicle injections (1 ml/kg sc) and presented with HP diet 1 h later. On days 2–4 animals were given either vehicle or LY255582 (0.3 mg/kg sc) and presented with HP diet 1 h later. On day 5, all animals were given vehicle injections 1 h prior to HP diet presentation. Microdialysis sampling occurred on either day 3 (during the period when HP diet intake is increasing) or day 5 (to determine whether HP diet intake and changes in DA levels occur following cessation of LY255582 treatment) of the 1-h HP diet access. The procedure of the microdialysis testing was identical in both experiments 2a and 2b with a 2.5-h equilibration period, a 30-min baseline sampling period occurring before drug treatment, and HP diet presentation occurring ~4 h after the animals were placed in the cages (~5 h into the light cycle). For clarity, Fig. 1 graphically illustrates the experimental time line.

**Experiment 2c: microdialysis following access to a regular chow diet.** To test whether the changes in consumption of the HP diet and subsequent increase in NAc shell DA levels were the result of the animals being in a mild calorie restricted state resulting in a deprivation-induced feeding response (since they were placed in the microdialysis bowls for 4 h prior to food availability) we examined whether access to regular chow under similar conditions elicited similar increases in feeding and NAc DA levels. Experimental conditions were identical to experiment 2a and 2b except that a preweighed amount of regular rodent Chow diet (LabDiet 5001) was provided to the rats for 1 h during the microdialysis sampling (occurring ~4 h after the animals were placed in the cages and ~5 h into the light cycle).

**Histology.** At the end of microdialysis experiments, animals were euthanized by CO₂ inhalation and tetrazolium red dye (5 mg/ml; Sigma, St. Louis, MO) was perfused through the probe to mark the probe track. The brain was removed and frozen at −20°C and sliced on a cryostat at 40 μm to verify probe location. Only animals with ~80% of the dialysis membrane in the shell of the NAc were included in the data analyses (data not shown).

HPLC. An ESA 542 pump delivered MD-TM mobile phase (ESA, Chelmwood, MA; pH adjusted to 5.0) through a reverse-phase column (3.2 × 150 mm; C-18, 3 μM particle size) at 0.6 ml/min. Samples (15 μl) were injected onto the column with the aid of an autosampler and DA was quantified using a Coulachem III detector equipped with a
guard cell and a 5014B analytical cell. The potential of the guard cell was set at +350 mV. The potential of the first electrode in the analytical cell was set at +220 mV, and the second electrode was set at −290 mV. DA was detected on electrode 2 set at a sensitivity of 5 nA. DA was quantified based on peak area using a standard curve generated from DA standards run the day samples were analyzed. The software used to run the instrumentation and acquire data was EZChrom Elite version 2.8.3.

**Data Analysis**

**Food intake data.** For experiment 1, to analyze the change in food intake across the 4 days, 1-h HP and 24-h chow intakes from the vehicle group were analyzed separately using one-way repeated-measures ANOVAs. To analyze the effect of LY255582 treatment, 1-h HP and 24-h chow intakes on day 4 were analyzed separately using one-way ANOVAs with Dunnett’s post hoc tests. In the microdialysis studies, food intake data were compared using a two-tailed t-test (experiment 2a) or a two-way (time × group) repeated-measures ANOVA with a compound symmetry matrix (experiment 2b). Significant interactions were followed up using contrast effects and Fisher’s least significant difference test. Significance was set at *P < 0.05.*

**Microdialysis data.** All microdialysis data were normalized to percent of baseline (baseline DA = average of the three samples prior to injection). Baseline DA levels were analyzed using an one-way ANOVA (experiment 2a) or a two-tailed t-test (experiment 2b) on the untransformed data. Normalized DA levels were analyzed with a two-way mixed ANOVA (time × group) with either a heterogeneous Toeplitz (experiment 2a) or a Toeplitz matrix (experiment 2b). In experiment 2c normalized DA levels were analyzed with a one-way ANOVA using a heterogeneous compound symmetry matrix. To determine the effect of LY255582 treatment on NAc shell DA levels, the first portion of the data were analyzed from −20 min through 60 min of data collection. To determine the effects of HP diet access on NAc shell DA levels and the effect of LY255582 treatment on this measure, a separate analysis was performed on the latter part of the data by analyzing −20 min to 140 min, excluding time points from 10 to 60 min. Statistically significant interactions were followed up using contrast effects and a post hoc Fisher’s test. Statistical significance was set at *P < 0.05.*

**RESULTS**

**Experiment 1: Effect of LY255582 on Consumption of an HP Diet**

In vehicle-treated rats, daily 1-h access to the HP diet produced a progressive increase in the amount of the HP diet consumed over the 4 days of the study (Fig. 2A). A one-way repeated-measures ANOVA revealed a significant main effect of day [F(3,18) = 23.68; *P = 0.0001] on HP diet intake. Treatment on day 4 with LY255582 produced a significant decrease in HP diet consumption [F(4,30) = 9.17; *P = 0.001]. Post hoc analysis revealed that all doses of LY255582 decreased HP diet intake (*P < 0.01). Interestingly, as HP diet intake increased 24-h regular chow intake decreased [F(3,18) = 6.14; *P = 0.005] over the 4-day access period (Fig. 2B). A main effect of treatment was observed with administration of LY255582 [F(4,30) = 6.31; *P = 0.0008]. Compared with HP diet intake, LY255582 had a reduced effect on 24-h chow intake on *day 4* in that only the highest dose (3 mg/kg) of LY255582 decreased 24-h chow intake (*P < 0.01* vehicle vs. 3 mg/kg).

**Experiment 2a: Microdialysis Following Novel Access to an HP Diet**

In the group of animals tested during their first exposure to the HP diet, LY255582 (0.3 mg/kg sc) significantly suppressed 1-h HP diet intake during the access period [*t*(11) = −7.69; *P = 0.014] (Fig. 3, *inset*). Vehicle-treated animals consumed 5.9 ± 0.6 grams of HP diet during the 1-h access, whereas LY255582-treated animals ate only 0.7 ± 0.2 g of the HP diet.

Raw baseline DA, which was defined as the three time points prior to injection, did not differ between any of the three groups [F(2, 17) = 0.78; *P = 0.47; overall mean = 1.01 ± 0.17 nM]. Furthermore, normalized DA levels were stable during the hour immediately following injection of vehicle or LY255582 (Fig. 3). This was confirmed by a mixed ANOVA run on the data from time −20 through 60 min which demonstrated no significant main effects or interactions: group [F(2,13) = 0.75; *P = 0.49], time [F(8,23) = 0.88; *P = 0.55], or group × time [F(16, 32) = 0.96; *P = 0.52].

Analysis of DA levels during the hour of HP diet availability indicated main effects of group [F(2,31) = 8.04; *P = 0.002], time [F(11,30) = 3.22; *P = 0.005], and a significant group × time interaction [F(22,42) = 2.85; *P = 0.002] (Fig. 3). Individual comparisons demonstrated that vehicle-treated rats exhibited a significant increase in DA levels during the HP diet access period at 80, 90, 100, and 110 min compared with *time 0*. The group labeled pair fed had the amount of HP diet yoked to that consumed by rats treated with LY255582 and was given only 0.7 g of HP diet. The pair-fed group consumed all 0.7 g of the HP diet during the 1-h access period. Moreover, the increase in DA levels in the pair-fed group demonstrated a significant increase in DA at all time points during the hour of HP diet availability and was similar to the vehicle control group. Interestingly, the group of animals treated with 0.3 mg/kg LY255582, which consumed 0.7 g of the HP diet, failed
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Day 3 HP diet intake and microdialysis. As shown in Fig. 4, inset, following 3 days of HP diet access, rats showed a progressive increase in HP diet intake. Interestingly, the progressive intake in HP diet consumption was prevented in rats treated with LY255582. A two-way mixed ANOVA performed on the HP food intake data demonstrated a main effect of treatment \([F(1,13) = 20.25; P = 0.0006]\), day \([F(2,26) = 7.39; P = 0.003]\), and a significant treatment \(\times\) day interaction \([F(2,26) = 12.83; P = 0.0001]\). Individual comparisons supported that LY255582-treated animals showed a reduction in HP diet intake relative to vehicle-treated animals on days 2 and 3. Moreover, intake did not increase with repeated HP diet access in the LY255582-treated group, while intake was significantly higher on days 2 and 3 compared with day 1 in vehicle-treated animals.

Analysis of the raw baseline NAc shell DA levels on day 3 demonstrated no difference between the vehicle and LY255582-treated groups \([t(13) = 1.623; P = 0.129]\); overall mean: 0.92 \pm 0.13 nM. During the 1st hour postinjection (Fig. 4) there was a slight but significant decrease in DA levels over time \([F(8,53) = 4.46; P = 0.0003]\); however, there was neither a main effect of treatment \([F(1,126) = 0.0007; P = 0.98]\) nor a significant treatment \(\times\) time interaction \([F(8,53) = 1.89; P = 0.08]\). During the HP diet access period, NAc DA levels increased significantly in vehicle-treated animals, but did not increase in animals treated with LY255582. The statistical analysis revealed no main effect of treatment \([F(1,20) = 0.73; P = 0.4]\), but significant main effects of time \([F(11, 53) = 3.12; P = 0.003]\), and a treatment \(\times\) time interaction \([F(11, 53) = 3.79; P < 0.0005]\). Detailed analysis of each time point during the HP diet access period revealed an increase in NAc shell extracellular DA levels in vehicle-treated animals at time 70, 80, and 90 min (relative to time 0). LY255582-treated animals did not show a change in DA levels at any time point during the HP diet access period on day 3.

Day 5 HP diet intake and microdialysis. Analysis of food intake in the group of animals undergoing microdialysis testing on day 5 (Fig. 5, inset) revealed main effects of treatment \([F(1,12) = 35.52; P < 0.0001]\), day \([F(4,48) = 18.49; P < 0.0001]\), and a treatment \(\times\) day interaction \([F(4,48) = 6.69; P = 0.0002]\). Following up on the significant interaction revealed that animals treated with LY255582 had significantly lower food intakes compared with vehicle-treated animals on days 2–5, suggesting that animals treated with LY255582 did not progressively increase intake of the HP diet over the days of limited access. On day 5 when all animals were given vehicle injections, those animals previously treated with LY255582 consumed 6.8 \pm 1.5 g of HP diet. The amount of HP diet intake in animals with LY255582 treatment history was significantly more than this group consumed on previous days (during treatment with LY255582); however, it was still significantly less than the amount consumed on day 5 (11.7 \pm 0.7 g) by animals treated with vehicle over the entire testing period. Interestingly, on day 5 animals previously treated with LY255582 ate a similar amount to vehicle-treated animals on day 2. Therefore, the effects of LY255582 on HP diet intake were reversible following cessation of drug treatment.

On day 5, raw baseline DA levels did not differ between the two groups with different treatment histories, but there was a

Fig. 2. The effect of trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine series (LY255582) on highly palatable (HP) diet intake (A) and concomitant 24-h normal chow intakes (B). Data are expressed as means \(\pm\) SE \((n = 7\) group\). HP diet increased over the first 4 days when only vehicle (Veh) injections were administered. On the day 4 of HP diet access, animals were treated (Trt) with LY255582, and all doses \((0.1–3.0\ mg/kg\ sc)\) caused a significant decrease in HP diet intake compared with vehicle \((P < 0.01,\ Dunnett’s\ multiple\ comparison\ test)\). Only the highest dose tested \((3.0\ mg/kg)\) caused a reduction in 24-h chow intake, suggesting that the effect of LY255582 was selective for the HP diet \((P < 0.01,\ Dunnett’s\ multiple\ comparison\ test)\).

Experiment 2b: Microdialysis Following Repeated Access to HP Diet

Experiment 2b was designed to examine the effect of LY255582 on the progressive increase in HP diet intake and the accompanying alterations in NAc shell DA levels that occur during the escalation phase of HP diet intake. Microdialysis sampling occurred on either day 3 (Fig. 4) or day 5 (Fig. 5) of HP diet access. In the group of animals tested on day 5, LY255582 administration was terminated on day 4 and groups differed only in their treatment histories (See Fig. 1 for time line).

Day 3 HP diet intake and microdialysis. As shown in Fig. 4, inset, following 3 days of HP diet access, rats showed a progressive increase in HP diet intake. Interestingly, the progressive intake in HP diet consumption was prevented in rats treated with LY255582. A two-way mixed ANOVA performed on the HP food intake data demonstrated a main effect of treatment \([F(1,13) = 20.25; P = 0.0006]\), day \([F(2,26) = 7.39; P = 0.003]\), and a significant treatment \(\times\) day interaction \([F(2,26) = 12.83; P = 0.0001]\). Individual comparisons supported that LY255582-treated animals showed a reduction in HP diet intake relative to vehicle-treated animals on days 2 and 3. Moreover, intake did not increase with repeated HP diet access in the LY255582-treated group, while intake was significantly higher on days 2 and 3 compared with day 1 in vehicle-treated animals.

Analysis of the raw baseline NAc shell DA levels on day 3 demonstrated no difference between the vehicle and LY255582-treated groups \([t(13) = 1.623; P = 0.129]\); overall mean: 0.92 \pm 0.13 nM. During the 1st hour postinjection (Fig. 4) there was a slight but significant decrease in DA levels over time \([F(8,53) = 4.46; P = 0.0003]\); however, there was neither a main effect of treatment \([F(1,126) = 0.0007; P = 0.98]\) nor a significant treatment \(\times\) time interaction \([F(8,53) = 1.89; P = 0.08]\). During the HP diet access period, NAc DA levels increased significantly in vehicle-treated animals, but did not increase in animals treated with LY255582. The statistical analysis revealed no main effect of treatment \([F(1,20) = 0.73; P = 0.4]\), but significant main effects of time \([F(11, 53) = 3.12; P = 0.003]\), and a treatment \(\times\) time interaction \([F(11, 53) = 3.79; P < 0.0005]\). Detailed analysis of each time point during the HP diet access period revealed an increase in NAc shell extracellular DA levels in vehicle-treated animals at time 70, 80, and 90 min (relative to time 0). LY255582-treated animals did not show a change in DA levels at any time point during the HP diet access period on day 3.

Day 5 HP diet intake and microdialysis. Analysis of food intake in the group of animals undergoing microdialysis testing on day 5 (Fig. 5, inset) revealed main effects of treatment \([F(1,12) = 35.52; P < 0.0001]\), day \([F(4,48) = 18.49; P < 0.0001]\), and a treatment \(\times\) day interaction \([F(4,48) = 6.69; P = 0.0002]\). Following up on the significant interaction revealed that animals treated with LY255582 had significantly lower food intakes compared with vehicle-treated animals on days 2–5, suggesting that animals treated with LY255582 did not progressively increase intake of the HP diet over the days of limited access. On day 5 when all animals were given vehicle injections, those animals previously treated with LY255582 consumed 6.8 \pm 1.5 g of HP diet. The amount of HP diet intake in animals with LY255582 treatment history was significantly more than this group consumed on previous days (during treatment with LY255582); however, it was still significantly less than the amount consumed on day 5 (11.7 \pm 0.7 g) by animals treated with vehicle over the entire testing period. Interestingly, on day 5 animals previously treated with LY255582 ate a similar amount to vehicle-treated animals on day 2. Therefore, the effects of LY255582 on HP diet intake were reversible following cessation of drug treatment.

On day 5, raw baseline DA levels did not differ between the two groups with different treatment histories, but there was a
trend for the baseline values in rats with a LY255582 treatment history to be higher compared with vehicle treatment. However, normalized DA values were stable following the injection of vehicle as analysis of the first part of the microdialysis data (20 to 60 min; Fig. 5) revealed no main effects of group, time, nor an interaction between the two variables: treatment \([F(1,12) = 0.067; \ P = 0.42]\), time \([F(8,28) = 0.49; \ P = 0.85]\). During the period of HP diet access, both groups of animals exhibited an increase in NAc shell DA levels (Fig. 5), regardless of their prior treatment history. This conclusion was supported by a finding of only a main effect of time \([F(11,30) = 2.11; \ P = 0.05]\) during HP diet access (i.e., excluding 10 to 60 min). There was neither a significant main effect of treatment \([F(1,23) = 0.66; \ P = 0.42]\) nor a treatment \(\times\) time interaction \([F(11,30) = 0.39; \ P = 0.95]\). These data suggest that following cessation of LY255582 treatment, both HP diet consumption and extracellular DA levels in the NAc shell increase when animals are given access to an HP diet.

**Experiment 2c: Microdialysis Following Access to a Regular Chow Diet**

To understand if changes in consumption of the HP diet and subsequent increases in NAc shell DA levels were the result of...
the animals being in a mild calorie restricted state (since they were placed in the microdialysis bowls for 4 h prior to food availability), we examined whether access to regular chow under similar conditions elicited similar increases in feeding and NAc DA levels. In this separate group of animals treated identically to previous HP diet groups, 1-h access to regular rodent chow resulted in very little food consumption. Rats ate an average of 0.05 ± 0.02 g of chow (Fig. 6, inset), with several animals consuming none of the chow diet. Normalized baseline NAc shell DA levels (~20 to 60 min; Fig. 6) revealed no effect of time [F(8,12.2) = 1.81; P = 0.169] in the 1st hour following vehicle injection. Moreover, following presentation of regular chow DA levels remained unchanged as indicated by no effect of time [F(10,10.9) = 0.81; P = 0.629] during the chow access period (i.e., excluding 10 to 60 min; Fig. 6).

**DISCUSSION**

The present study demonstrated that with daily limited access rats increased HP diet intake while concurrently decreasing their regular chow consumption. Additionally, extracellular DA levels in the NAc shell increased during access to the HP diet. Acute administration of LY255582 inhibited both the HP diet intake and the corresponding increase in NAc shell DA levels. In animals with repeated access to the HP diet, LY255582 prevented the progressive increase in HP diet consumption and prevented the increase in extracellular NAc DA levels that occurred during HP diet access. However, when LY255582 treatment was stopped, rats increased their consumption of HP diet and there was an associated increase in DA levels in the NAc shell. Moreover, animals given access to a regular chow diet under identical conditions ate very little and exhibited no change in NAc shell DA levels.

Rats given daily limited access to the HP diet over several days demonstrated an increase in HP diet consumption resulting in a large bout of feeding during the 1-h access period. This bout of feeding represented more than one-third of the animal’s total daily caloric intake. Additionally, 24-h regular chow intake decreased over this time period. Administration of LY255582 significantly reduced HP diet consumption under limited access conditions at all doses tested (0.1–3.0 mg/kg).

**Fig. 5.** The effect of cessation of LY255582 (0.3 mg/kg sc) treatment on HP diet intake (inset; n = 5–9/group) and NAc shell DA levels on day 5 of the limited HP diet access schedule. On day 5 both groups of animals were treated with vehicle and differ only in their treatment histories. Data are expressed as means ± SE (food intake data) or are expressed as %baseline defined using the 3 time points prior to injection (microdialysis data; means ± SE). Vehicle-treated animals exhibited an increase in HP diet intake across days 1–4 (\(P < 0.001\)) compared with day 1; post hoc Fisher’s test), whereas in LY255582-treated animals HP diet intake remained low. On day 5 when LY255582 treatment was stopped, HP intake increased significantly (\(P < 0.001\) compared with day 1; post hoc Fisher’s test). NAc shell DA levels increased significantly during the HP diet access period on day 5 in both groups regardless of prior treatment history (\(P < 0.05\); 2-way repeated-measures ANOVA).

**Fig. 6.** Food intake and NAc shell DA levels following 1-h access to a normal chow diet (n = 6 animals per group). Data are expressed as means ± SE (food intake data) or are expressed as %baseline defined using the 3 time points prior to injection (microdialysis data; means ± SE). Rats consumed an average of 0.05 ± 0.02 g of chow during the 1-h access period (inset). No changes in NAc DA levels were observed over the experimental protocol period indicated by no observed effect of time following vehicle injection (\(P = 0.169\); one-way ANOVA) or during 1-h access to a chow diet (\(P = 0.629\); 1-way ANOVA).
Interestingly, it appeared to be somewhat selective for reducing HP diet intake since only the highest dose (3.0 mg/kg) decreased 24-h chow consumption. Although LY255582 administration only occurred once per day, this finding is not likely due to insufficient levels of LY255582 during the 24-h chow feeding period. Previous studies have demonstrated that 18 h after oral administration of LY255582, opioid receptor occupancy in the central nervous system (measured by ex vivo receptor binding) is still significantly high. Furthermore, the level of opioid receptor occupancy at 18 h is significantly correlated with the reduction in 24-h food intake (55). Moreover, our finding of a preferential effect on palatable food intake is consistent with previous studies suggesting that opioid receptor antagonists primarily alter the intake of palatable food or a preferred macronutrient (23, 25, 34, 37).

Rats that were given access to the HP diet for the first time demonstrated an increase in DA levels in the NAc shell during the HP diet access period. This finding is consistent with other reports using in vivo microdialysis, demonstrating an increase in NAc DA levels in response to palatable food (4, 7, 9, 10, 21, 27, 40–42, 47, 58). The response is somewhat selective for the HP diet since we found that rats given access to a regular Chow diet under identical conditions ate very little and exhibited no alterate in NAc DA levels. In animals treated with LY255582, HP diet intake was decreased significantly and there was no corresponding rise in mesolimbic DA levels. Interestingly, animals that had their HP diet intake yoked to the group treated with LY255582 (i.e., pair-fed group in Fig. 3) exhibited a robust increase in NAc shell extracellular DA levels. The increase in extracellular DA levels in the NAc shell of pair-fed animals was similar to that seen in the vehicle-treated animals, even though these rats consumed only 0.7 g of the HP diet compared with 5.9 ± 0.6 g in the vehicle group. This finding suggests that the lack of increase in DA levels seen in the LY255582-treated group was a pharmacological one and not merely a consequence of the decreased HP diet consumption. The DA response to the small quantity of food given to the pair-fed animals was somewhat unexpected. A few reports indicate that the DA response to palatable food is correlated with the amount of food consumed (28, 39, 51, 61). Methodological differences in the cited studies, including the use of within-subject microdialysis, different types of food (nutritionally complete vs. macronutrient-specific diets), sham feeding, and/or food deprivation may have contributed to these differences. Within-subject microdialysis studies are generally ill advised due to damage induced by the probe over time (12, 13, 50). Additionally, food deprivation and restricted, scheduled feeding are known to influence the “amount-dependent” rise in NAc DA, in that food-deprived animals appear to exhibit this phenomenon, whereas ad libitum-fed animals do not (4, 47, 61).

When animals underwent microdialysis testing during the escalation phase of the HP diet access, vehicle-treated animals exhibited an increase in NAc shell DA levels in response to the HP diet. Rats treated with LY255582 on days 2–4 consumed less HP diet compared with vehicle-treated animals and did not progressively increase their intake of HP diet from days 2–4. Moreover, LY255582-treated rats showed no change in NAc shell DA levels during this time. When LY255582 treatment was lifted on day 5 of the testing period, rats consumed significant quantities of HP diet and demonstrated a significant rise in NAc shell DA levels during HP diet access. Although the animals that had been treated on days 2–4 with LY255582 consumed less than those that had been treated with vehicle (6.8 ± 1.5 vs. 11.7 ± 0.7), the increase in the extracellular NAc DA level was not statistically different from 5-day vehicle-treated animals. These results are consistent with our findings in experiment 2a with the pair-fed animals suggesting that consumption of the HP diet stimulated mesolimbic DA neurons independent of the quantity of the HP diet consumed.

Collectively, the results from our microdialysis studies suggest that LY255582 decreased HP diet intake via an inhibition of the mesolimbic DA pathway. In support of this hypothesis, it has been demonstrated that μ- and δ-opioid receptors are located on inhibitory GABAergic neurons within the ventral tegmental area (18, 30). Activation of μ- and δ-opioid receptors through exogenous administration of opioid agonists results in increased DA release within the NAc shell (16, 29, 31, 54). Moreover, consumption of a high-fat diet increases expression of endogenous opioid peptides (14), providing a mechanism whereby consumption of the HP diet, which contains 40% of calories/g from fat, may stimulate DA release in the NAc shell. Therefore, blockade of opioid receptors by LY255582 would prevent the increase in DA produced by HP diet access, a hypothesis that is supported by microinjection studies of other opioid antagonists (57, 58). The opioid receptor subtype responsible for the effects seen in the present study is unknown since LY255582 binds to all three major opioid receptors with high affinity. A likely candidate is the μ-opioid receptor since germ-line deletion of the μ-opioid receptor gene was found to reduce body weight gain in mice fed a high-fat diet (56). Moreover, it is doubtful that these results arise from nonopioid receptor-mediated activity of LY255582, such as activity at DA receptors, since autoradiographic binding studies with [3H]-labeled LY255582 revealed a nearly identical opioid receptor-like binding pattern in wild-type mice compared with naltrexone, and no specific binding in triple opioid receptor (μ, δ, κ) knockout mice throughout the entire neural axis (20).

It is interesting that our animals did not exhibit habituation of the NAc shell DA response to the HP diet, since we saw a robust increase in DA on day 3 and day 5 of HP diet access. Bassareo and Di Chiara (8) have shown that NAc shell DA response to palatable foods decreases following repeated exposure. However, this does not appear to be the case in food-deprived animals (10, 61) nor in rats on a schedule of intermittent sucrose access (4, 47). An important point is that our rats did not have access to any food once they were placed in the microdialysis bowls until HP diet was presented (~4 h), which may have contributed to a semirestricted and/or intermittent schedule. Therefore, it is possible that our rats are in a mild negative energy balance before the HP diet access period in the microdialysis sessions contributing to the lack of habituation seen in our studies. However, the HP diet access and microdialysis studies were conducted during the lights-on period of the light-dark cycle, a time when chow intake is normally low. Moreover, we found that in animals given access to a regular chow diet under identical conditions, food intake was very low suggesting that the period when the animals are in the microdialysis bowls is not inducing a generalized deprivation-induced feeding response. Thus, it is unlikely that the increase in NAc shell DA found in the present study arises by

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an energy deficit that lends salient properties to any ingestant, but rather encodes salient properties of the HP diet itself. Taken together, our data suggest that an increase in DA release in the NAc shell during the HP diet access period may be involved in the progressive increase in HP diet intake. This finding is consistent with other studies demonstrating that DA transmission in the NAc shell is important in the acquisition of motivated behaviors and motivational learning in particular (8, 17, 21, 41, 42). Our findings demonstrated that animals treated with the opioid antagonist LY255582 did not show a rise in NAc shell DA levels in response to the same amount of HP diet that elicited an increase in DA levels in the pair-fed animals. These findings suggest that LY255582 may be preventing the DA response to the HP diet and thus altering the incentive salience of the food. Moreover, the fact that the increase in DA is prevented by an opioid antagonist argues that an increase in endogenous opioid peptides are at least partially involved in regulating HP diet intake. Finally, our data suggest that the pan opioid antagonist LY255582 may be a useful tool in understanding the neural mechanisms involved in processing the saliency of palatable food and the reinforcement mechanisms regulating food intake.

**Perspectives and Significance**

The activation of the mesolimbic DA system comprises a critical part of the neural network regulating reward and reinforcement mechanisms for substances of abuse. Indeed, many drug classes with addictive properties (including ethanol, nicotine, opiates, cannabinoids, and psychostimulants) activate the mesolimbic DA pathway, increasing DA levels within the NAc (35, 62). However, the neural circuitry encoding salience and hedonic properties of addictive drugs likely did not evolve from the substances described above, but rather from natural reinforcers, such as palatable, calorically-dense food (which primitive animals would require to maintain energy balance in an environment where food availability was limited), and engaging in sexual behavior (essential for continuation of the species). Given the results of the present study with the opioid receptor antagonist LY255582 it appears that, similar to ethanol intake (45, 48), activation of the mesolimbic DA system by consumption of palatable food is, in part, mediated by endogenous opioids. Indeed, microinjection of μ-opioid receptor agonists into the NAc increases the preferential intake of palatable food (63). This raises the question: does chronic and excessive consumption of palatable foods, which often occurs for reasons other than maintaining energy balance, produce neurochemical and behavioral effects resembling addiction to drugs of abuse? Evidence supporting this hypothesis is mounting. Colantuoni et al., (15) reported that rats given an intermittent access schedule to glucose develop a binge-like feeding pattern, and exhibit somatic signs of withdrawal and anxiety following administration of the opioid antagonist naloxone. Moreover, rats bingeing on sugar exhibit increases in μ-opioid and DA D1 receptor binding and decreases in DA D2 receptor binding in the NAc (15). These findings are particularly relevant since similar changes in striatal D2 receptor binding have been reported in positron emission tomography (PET) studies of both human substance abusers and patients with chronic obesity (60). Similar to other drugs of abuse, sugar bingeing rats exhibit locomotor cross-sensitization to amphetamine (3) and cocaine (24) and when forced to abstain from sugar access show an enhanced intake of unsweetened alcohol (2). Interestingly, patients with alcohol dependence exhibit an enhanced sucrose preference compared with nonalcoholic controls (32). Given these findings it is reasonable to speculate that following repeated, overconsumption of palatable food alterations in brain DA and opioid systems may support mechanisms and behaviors associated with addiction similar to the development of addiction to drugs of abuse. Indeed, many people exhibit cravings for and an uncontrollable urge to eat high-fat, high-sugar foods much in the same way that addicts crave and seek drugs of abuse. Together, these observations offer an interesting corollary to drug addiction. Whether food addiction exists in humans and contributes to the growing epidemic of obesity will be an important topic for future study.

**REFERENCES**


