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Research report

A role for dopamine in feeding responses produced by orexigenic agents

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Abstract

Dopamine-deficient (DD) mice become hypophagic and die of starvation by 3 to 4 weeks of age unless dopamine is restored by daily treatment with L-3-4-dihydroxyphenylalanine (L-dopa). We demonstrate here that DD mice mount qualitatively normal counter-regulatory blood glucose responses to insulin and 2-deoxy-D-glucose (2-DG). However, unlike control mice, DD mice fail to eat in response to acute glucoprivation induced by insulin or 2-DG. They also have a severely blunted response to central administration of peptide YY (PYY). Viral-mediated restoration of dopamine synthesis to the central caudate putamen (CPu) of DD mice rescues feeding and survival. However, this treatment fails to restore insulin- and 2-DG-induced feeding despite normalizing feeding in response to food deprivation and PYY. Since dopamine signaling in the CPu is not sufficient for glucoprivation-induced feeding, we propose that this feeding behavior may be mediated by dopamine in an anatomically distinct brain region.

Theme: Neural basis of behavior
Topic: Ingestive behaviors

Keywords: Dopamine; Dopamine-deficient; Tyrosine hydroxylase; L-3-4-dihydroxyphenylalanine; Feeding; Cholecystokinin; Insulin; 2-Deoxy-D-glucose; Peptide YY; Adeno-associated virus; Caudate putamen

1. Introduction

Multiple factors converge in the central nervous system (CNS) to coordinate behaviors that modify food consumption and energy expenditure. Peripheral hormones such as leptin (from adipose tissue) and insulin (from the pancreas) activate receptors on neurons in the arcuate nucleus of the hypothalamus. Activation of these receptors alters the firing properties of pro-opiomelanocortin (POMC)-expressing neurons in the arcuate resulting in a net decrease in food intake (for review, see Refs. [24,32,33]). Similarly, the stomach secretes ghrelin when it is empty, which acts in the hypothalamus to stimulate feeding [9]. Conversely, in response to a meal, the intestine secretes cholecystokinin octapeptide (CCK-8) which suppresses feeding via vagal inputs to the brainstem [21].

The brain also responds to changes in blood glucose, its primary energy source. When blood glucose levels fall, neurons in the brainstem and arcuate are activated, which initiates the physiological and behavioral counter-regulatory responses including glycogenoloysis, gluconeogenesis, and increased food consumption [13,14]. Hypoglycemia and cytoglucopenia can be experimentally induced by peripheral administration of insulin or 2-deoxy-D-glucose (2-DG), respectively. Insulin, which is normally released by the pancreas after a meal, lowers blood glucose by stimulating its uptake into muscle, liver and other tissues. 2-DG is a glucose analog that is taken up by cells whereupon it acts as

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a competitive glycolytic inhibitor, resulting in cytoglucopenia [26]. Lesioning glucose-sensitive, noradrenergic neurons in the hindbrain that project to the arcuate has been shown to reduce hypoglycemia-induced feeding [27,28]. However, lesioning a different population of glucose-sensitive, hindbrain noradrenergic neurons that activate the sympathetic nervous system abrogates the physiological responses to glucoprivation [28].

The role of dopamine in the control of food intake has been studied using both pharmacological and lesioning approaches. Perturbations of the dopaminergic system generally result in a reduction in feeding [7,42]. Indeed, severe reductions in striatal dopamine levels can eliminate feeding to the point that the animal will starve without intervention [46,47]. However, because these lesions lead to akinesia and the near elimination of all motivated behaviors, it is likely that striatal dopamine is required more generally to highlight a variety of salient sensory stimuli that require a voluntary behavioral response [36].

Here we explore the role of dopamine in consummatory behavior using a genetic mouse model in which the ability to synthesize dopamine has been selectively eliminated [45]. The CNS of dopamine-deficient (DD) mice appears to develop normally and the mice suckle and grow normally for the first week. The mice then begin to display symptoms of bradykinesia and hypophagia and they will die of starvation by 3 to 4 weeks without intervention [45]. DD mice can be maintained by daily injection of L-dopa, which produces a bout of hyperlocomotion and hyperphagia that lasts 8–10 h [39,45]. In the absence of L-dopa, DD mice are hypoactive but can move about in their cages and manifest infrequent consummatory responses. We have demonstrated the efficacy of viral-mediated gene transfer as a tool to locally restore dopamine synthesis in DD mice [38,41]. When this technique is used to restore dopamine to the central caudate putamen (CPu), but not when restored to the nucleus accumbens (NAc), DD mice are able to feed adequately and maintain body weight for many months in the absence of L-dopa treatment [41]. These virally treated or 'rescued' DD mice (RDD) are a useful model for studying the role of dopamine in brain regions outside of the CPu. In this study, we use the DD and RDD mice to determine if dopamine is required for the physiological and/or behavioral responses to acute treatment with CCK-8, peptide YY (PYY), insulin and 2-DG, agents that modify feeding behavior of control mice.

2. Materials and methods

2.1. Generation and maintenance of DD and virally rescued (RDD) mice

DD ($Th^{-/-}$; $Dbh^{Th/+}$) mice carrying two inactive tyrosine hydroxylase (*Th*) alleles, one intact dopamine β-hydroxylase allele (Dbh^{+}), and one *Dbh* allele with a targeted insertion of the *Th* gene (Dbh^{Th}) were created as described [45]. Controls included animals that carry at least one intact *Th* allele and one intact *Dbh* allele, because these mice synthesize nearly normal levels of catecholamines. Mice were maintained on a mixed C57BL/6×129/SvEv genetic background. All mice were housed under a 12:12 light/dark cycle and temperature controlled environment with food and water available ad libitum unless noted. All mice were treated in accordance with guidelines established by the National Institute of Health and the University of Washington Animal Care Committee.

Recombinant adeno-associated viruses (rAAV) used for these experiments were generated as described [8]. The vector preparations had titers of 3.6×10^{12} (TH) and 5.0×10^{12} (GTPCH1) genomic particles/ml. The two rAAV vectors were mixed 1:1 and bilateral injections were performed on anesthetized (ketamine/xylazine/acepromazine) mice as follows. Mice were placed into a stereotaxic frame (Cartesian Instruments, Sandy, OR) and the head was leveled in the *x*, *y*, and *z* planes using the sagittal suture, lambda, and bregma as landmarks. Coordinates (in mm) used for injection into the central CPu were 0.80 anterior–posterior, 2.00 and −2.00 medial–lateral, and 3.60 dorsal–ventral, using bregma as a reference for *x* and *y* coordinates and the skull surface as a reference for the *z* coordinate. Virus (0.65–0.80 μl) was injected through a 5-μl Hamilton syringe fitted with a 24-gauge needle at a rate of 0.25 μl/min. After each injection, the needle remained stationary for an additional 2 min, was then raised 0.1 mm and maintained in this position for an additional 2 min before being withdrawn. Bilaterally injected mice were removed from L-dopa treatment 1 week after viral injection and assayed for restoration of feeding behavior. Mice that maintained at least 80% of their body weight after 1 week without L-dopa treatment were designated as rescued (RDD).

2.2. Food deprivation and CCK-8 administration

Individually housed mice were moved into new cages without food and fasted overnight (18 h). The following morning the mice were injected with either saline or CCK-8 (8 μg/kg BW, Sigma, St. Louis, MO) and returned to the cage with one or two pre-weighed chow pellets (Purina 5LJ5) on the floor. The food was weighed 30 min, 60 min and 5 h after injection. The experimental paradigm was a repeated-measure, Latin-square design such that each animal received both CCK-8 and saline. Treatments were separated by 1 week. CCK-8 was dissolved in PBS and administered intraperitoneally at 10 μl/g.

2.3. Insulin- and 2-DG-induced feeding

Non-rescued DD and control mice were individually housed for 7 days prior to the start of feeding experiments.

DD mice received daily injections of 50 mg/kg L-dopa and control mice received an equivalent volume of PBS vehicle at 10:00 each day. Pre-weighed amounts of chow (Purina 5015) were placed on the bottom of the cage each day. On day 8, both groups of mice were injected with PBS (10 μl/g) at 10:00 and food was weighed hourly. At 14:00, DD mice were injected with L-dopa and food was left in the cage. On day 9, both groups received their normal daily treatment of either L-dopa or PBS at 10:00 and allowed access to food. On day 10, the same control and DD mice were injected at 10:00 with insulin (1 U/kg, HumulinR, Eli Lilly, Indianapolis, IN) or 2-DG (500 mg/kg, Sigma) and food consumption was measured. A new cohort of mice was used for each drug treatment.

Singly housed RDD and littermate control mice were injected with 2-DG (250 mg/kg) or saline and returned to their home cage. Four-hour food intake (Purina 5LJ5) was recorded. The experimental paradigm was a repeated measure, Latin-square design such that each animal received both 2-DG and saline. Treatments were separated by 1 week. To acclimate the mice to being handled and injected, singly housed RDD and controls were injected with saline on four consecutive mornings and 4-h food intake was recorded. The fifth day the mice were injected with insulin (2 U/kg) and the amount of food consumed was measured 4 h later. Insulin and 2-DG were dissolved in PBS and administered intraperitoneally at 10 μl/g.

2.4. Blood glucose measurements

Blood was collected after removing 1 mm from the tip of the tail with a scalpel blade. Blood was drawn into a cuvette containing glucose oxidase and the concentration was measured directly using a portable blood-glucose analyzer (HemoCue, Ängelholm, Sweden).

2.5. PYY experiments

Anesthetized mice (ketamine/xylazine) were placed in a stereotaxic frame and a stainless steel guide cannula (24 or 26 gauge) was implanted into the lateral ventricle (LV) of DD and control mice and into the dorsal third ventricle (D3V) of RDD and control mice [LV coordinates (in mm); 0.5 posterior to bregma, 1.8 lateral to the midline, and 1.9 below the skull surface; D3V coordinates; 0.3 posterior to bregma on the midline suture and 3.0 below the skull surface]. The cannula was secured in place by dental acrylic cement (Jet, Lang Dental Manufacturing, Wheeling, IL) and the mice were returned to their cages and allowed to recover for at least 5 days.

In the first experiment involving DD mice, access to chow (Purina 5015) was restricted to an 8-h period for 8 consecutive days so that the feeding patterns of control and DD mice were synchronized. Chow was placed on the bottom of the cage each day at 08:00 and removed at 16:00. DD mice were injected daily with L-dopa at 08:00 each day. All mice were injected with L-dopa on the 9th day at 08:00, fresh chow was placed on the bottom of the cage, and food intake was measured at 1-h intervals. The next day at 08:00, mice were injected with L-dopa, and 6 h later, four mice of each genotype were infused with 1 μl of PYY (1 μg/μl, Bachem) and the remaining four were infused with saline into the lateral ventricle via a 30-gauge injector needle lowered 0.5 mm below the tip of a guide cannula. Food intake was measured 2 h after injection of PYY or vehicle. The experimental paradigm was a repeated-measure, Latin-square design such that each animal received both PYY and saline. Treatments were separated by 2 days.

In another experiment involving non-rescued DD mice, 24 h after the last L-dopa injection (08:00), eight control and eight DD mice were given an i.p. injection of saline and placed in transparent Plexiglas cages (40×20×20 cm) that contained a feeding and drinking station at both ends of the cage. A small dish of highly palatable food (F1850, Bioserve) was available at one station and a matching dish with sham food was present at the other station. Sham food was appropriately colored, non-toxic modeling clay (FIMO®) that was shaped to look like the highly palatable food. At 11:00, PYY or vehicle was infused, and food intake was measured 2 h later. The amount of time spent at food and sham stations was recorded and quantified using a video camera and a computer running activity-monitoring software (Poly-track, San Diego Instruments, San Diego, CA). To determine cannula placement, 2 μl of 1% cresyl violet was bilaterally injected into the implanted cannulae of each mouse prior to sacrifice. Mice were considered to have proper cannula placement if the cannula tract terminated within the ventricle and if cresyl violet-stained cells lining the ventricles were detected.

For the experiments with virally rescued mice, RDD and control mice were placed into a new cage with fresh food (Purina 5LJ5). After 4 h of acclimation, the mice were injected with 1 μl of saline or PYY (5 μg/μl, Bachem) through the cannula. The amount of food consumed was measured 4 h later. Each mouse received both treatments.

3. Results

3.1. Viral-mediated restoration of feeding behavior

Bilateral injection of adeno-associated viruses engineered to express tyrosine hydroxylase, GTP cyclohydrolase I and DsRed2 (rAAV-CBA-TH-CMV-DsRed2 and rAAV-CBA-GTPCH1-CMV-DsRed2) into the central region of the CPu of a DD mouse rescues their feeding deficit and makes daily L-dopa treatment unnecessary for survival [41]. The approximate injection sites are shown in Fig. 1A. Histological analysis of brain tissue was performed to confirm rAAV protein expression. Fig. 1B and C show DsRed2 fluorescence in a representative unstained coronal section

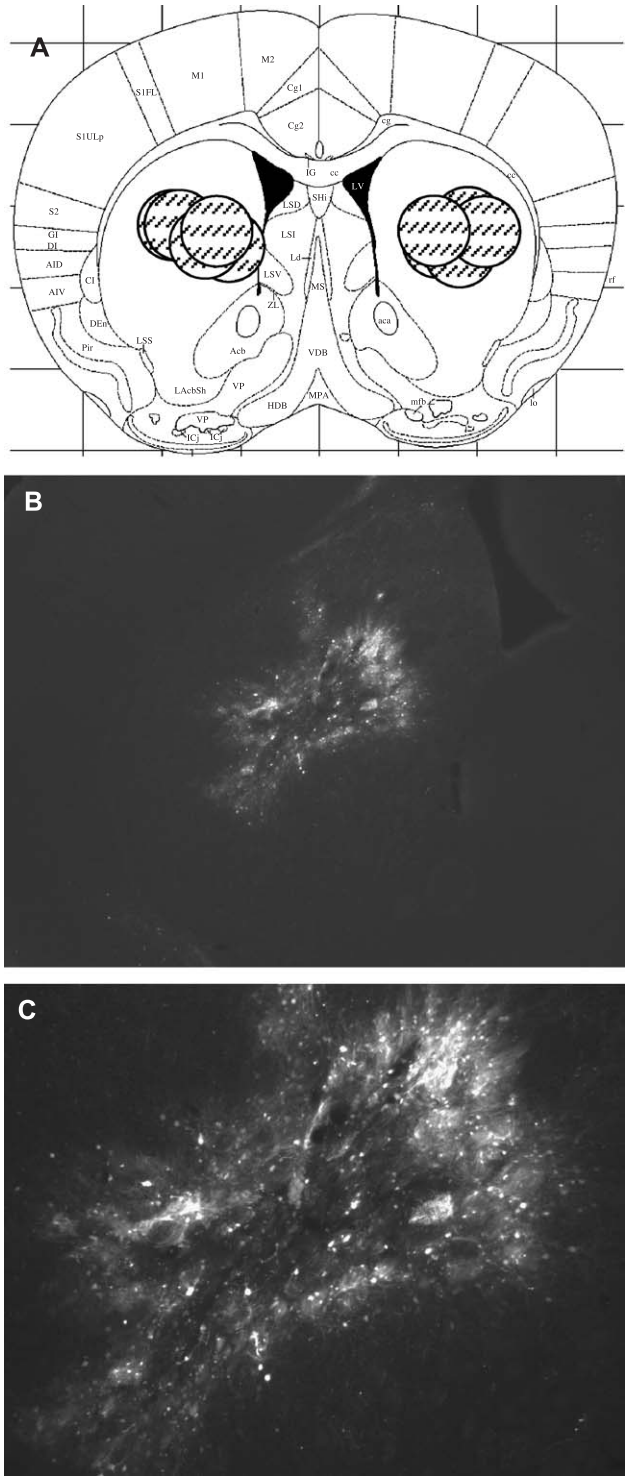

Fig. 1. Histological analysis near the site of viral transduction. (A) Schematic illustrating approximate bilateral injection sites of rAAV injection in the central CPu of five RDD mice used in these studies (adapted from Paxinos and Franklin [23]). (B) Virally transduced neurons in the central CPu express the fluorescent marker protein, DsRed2 (C) higher magnification.

through the central CPu. We have shown previously that DsRed2 expression co-localizes with expression of tyrosine hydroxylase [8].

3.2. RDD mice respond normally to food deprivation and display normal CCK-induced appetite suppression

RDD mice consumed enough food to survive in the absence of L-dopa, but their body weights remained approximately one-third less than littermate controls. To determine if dopamine is required outside of the central CPu for RDD mice to respond to endogenous hunger signals we measured food intake after an 18-h fast. There were no significant differences between the rates at which the RDD and control mice consumed food after food deprivation (Fig. 2A,B).

Additionally, to determine if dopamine outside of the central CPu plays a role in CCK-8-induced inhibition of feeding we administered CCK-8 (8 μg/kg) to fasted mice immediately prior to returning chow to the cage. CCK-8 significantly inhibited cumulative food intake in both groups of animals at the 30-min and 1-h time points but

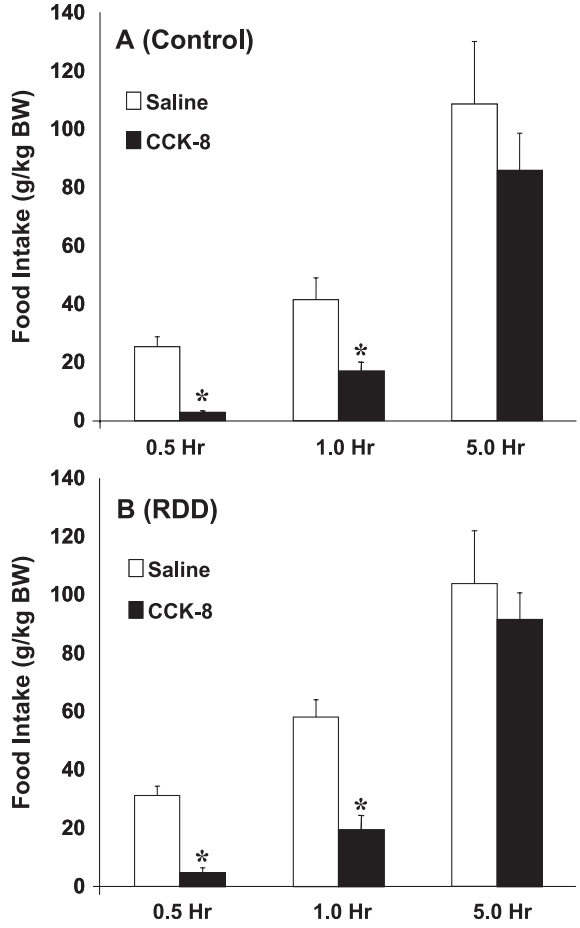

Fig. 2. Effect of CCK-8 on food intake by RDD mice after a fast. Cumulative food intake was measured at three different time points after 18 h of food deprivation. CCK-8 (8 μg/kg) attenuated short-term food intake in both the control (A) and RDD (B) groups. There was no difference between the control and RDD mice in their response to a fast or to CCK-8. Data represent means ± S.E.M. control (n=8), RDD (n=8). Data were analyzed by separate repeated measures ANOVA for each time point and no significant interactions were found between genotype and drug; combined drug effect *p<0.05.

the effect was no longer significant by 5 h (Fig. 2A,B). There was no significant difference in the effect of CCK-8 on food consumption between the two groups of mice.

3.3. DD mice do not increase food intake in response to insulin- or 2-DG-induced glucoprivation

To determine if dopamine is required to mount an appropriate behavioral response to glycemic challenge, DD mice and controls were treated peripherally with insulin or 2-DG. Control mice treated with insulin (1 U/kg) ate significantly more food over the course of 4 h compared to saline treatment (Fig. 3A). However, administration of the same dose of insulin to DD mice 24 h after their last L-dopa injection did not increase food intake significantly compared to saline treatment (Fig. 3A). An insulin dose of 10 U/kg produced a larger increase in food consumption in control mice (data not shown) but was lethal to DD mice within 2 h, presumably because they did not consume enough food to maintain adequate blood glucose levels. Consistent with this interpretation, control mice treated with insulin at 10 U/kg also perished if food was not available. Administration of 2-DG (500 mg/kg) also increased 4-h food intake in control, but not in DD mice, relative to saline treatment (Fig. 3A).

Average blood glucose concentrations in control mice under ad libitum conditions and after a 16-h fast were 161 and 82 mg/dl, respectively. Average blood glucose concentrations of DD mice 1 h after L-dopa treatment were similar to ad libitum-fed control mice and remained at 144 mg/dl 8 h after L-dopa treatment; however, by 24 h after L-dopa administration (roughly equivalent to a 16-h fast because of their hypophagia in the absence of dopamine) blood glucose fell to 102 mg/dl. Thus, blood glucose of DD mice was maintained in a range comparable to that of similarly treated control mice. Two hours after injection of control mice with insulin (1 U/kg), blood glucose fell by only 11% when there was ad libitum access to food, whereas in the DD mice, which did not eat in response to this treatment, blood glucose fell by 45% (Fig. 3B). Treatment of control mice with 2-DG elevated blood glucose concentration at 2 h compared to saline-injected controls (Fig. 3B), reflecting the action of counter-regulatory physiological responses. A significant increase in blood glucose concentration was also detected in DD mice 2 h after 2-DG treatment (Fig. 3B), indicating that they mount the appropriate physiological response to 2-DG-induced cytoglucopenia even though they do not eat.

3.4. RDD mice do not increase food intake in response to insulin- or 2-DG-induced glucoprivation

To determine if restoration of dopamine to the CPu is sufficient to achieve an appropriate behavioral response to drug-induced glycemic challenge, food intake by RDD mice was compared to that of control mice. Unlike their controls, RDD mice do not increase their food intake over the course

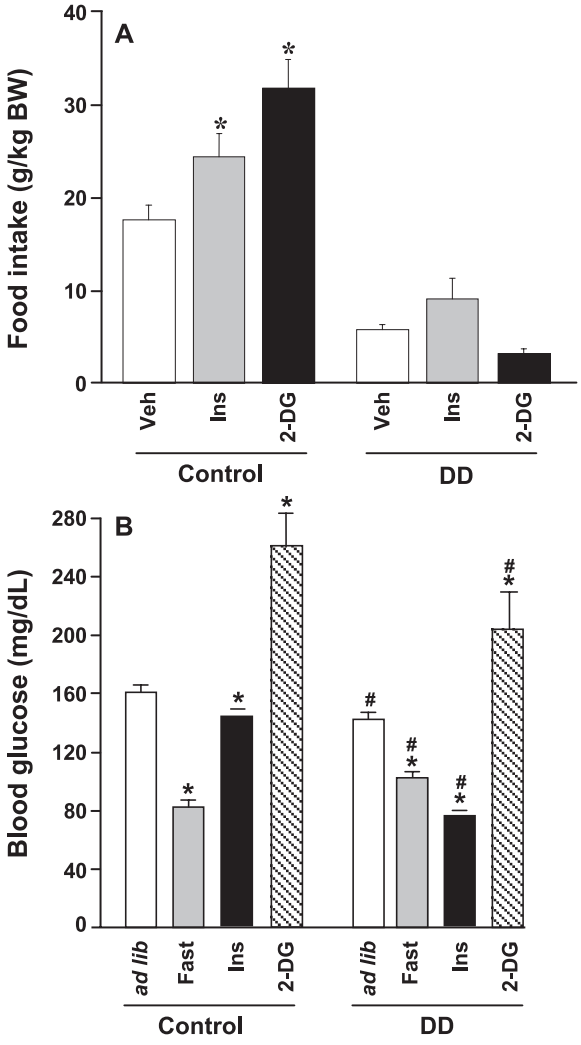

Fig. 3. Response of DD mice to a glycemic challenge. (A) Food consumed by control and DD mice during the 4 h following indicated treatments. Separate groups of control and DD mice were treated with either insulin (n=18 of each genotype) or 2-DG (n=16 of each genotype). (B) Blood glucose concentrations were determined from 20 control and 12 DD mice under ad libitum conditions (8 h after L-dopa and food presentation); 3 control and 21 DD mice after a 16-h fast (24 h after L-dopa); 15 control and 16 DD mice 3 h after administration of insulin (1 U/kg); five control and five DD mice 3 h after treatment with 2-DG (500 mg/kg). Data represent means±S.E.M. Data were analyzed by ANOVA followed by Tukey post-hoc analysis for comparison between vehicle and drug treatment within group; *p<0.05. Data were analyzed by two-way ANOVA followed by Tukey post-hoc analysis for comparison between ad libitum-fed control mice and DD mice; #p<0.05.

of 4 h following treatment with 2-DG at 250 mg/kg (Fig. 4A) or insulin at 2 U/kg (Fig. 4B). We repeated the 2-DG results three successive times producing equivalent results. In the case of insulin treatment, repeated measures ANOVA did not show a significant overall drug effect [p=0.073], but it appeared that an effect of insulin on control mice was being masked by the RDD values. We therefore performed two-tailed paired t-tests which revealed that the effect of insulin on control mice was highly significant [p=0.009] but not on RDD mice [p=0.582].

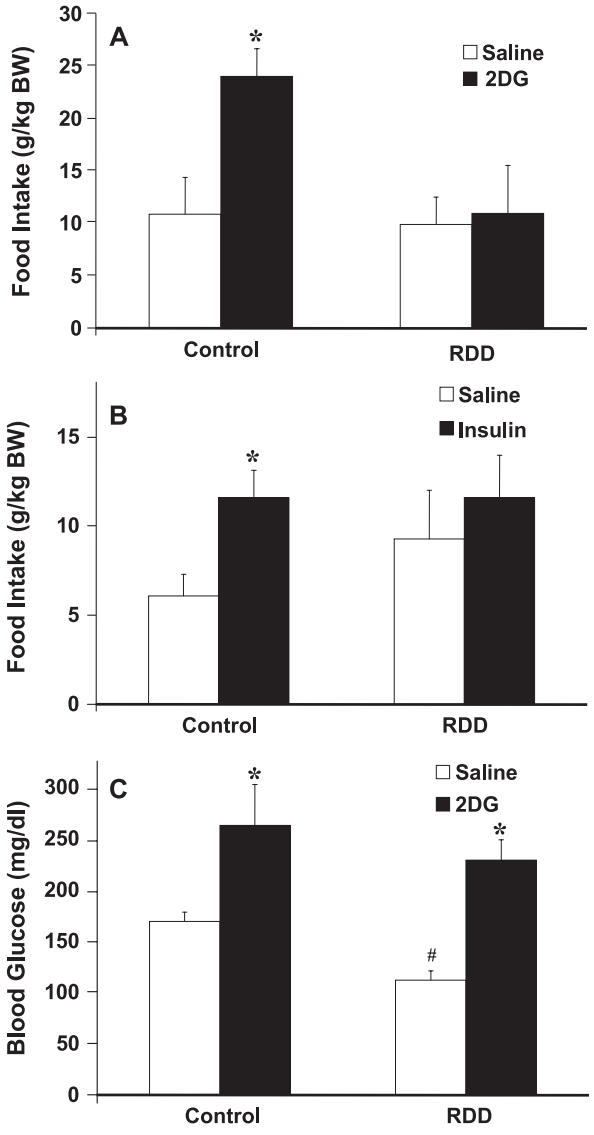

Fig. 4. Response of RDD mice to glycemic challenge. (A) Food intake by control and RDD mice during the 4 h following treatment with 2-DG (250 mg/kg); n=8 both groups. (B) Four-hour food intake in response to insulin (2 U/kg); control n=7; RDD, n=6. (C) Blood glucose concentrations of the same two groups of eight animals reported in (A) were measured 1 h after treatment with 2-DG (250 mg/kg). Data represent means±S.E.M. Data in each graph were analyzed by separate repeated measures ANOVA (followed by a two-tailed paired t-tests for WT mice treated with insulin; see results); *p<0.05. A two-tailed t-test was also performed to compare the saline-treated groups across genotypes; #p<0.05.

Blood glucose levels in response to 2-DG were measured to make certain that the lack of behavioral effect found in the RDD mice was not simply due to a failure of 2-DG to elicit cytoglucopenia in the RDD mice. Mice were fasted for 4 h, administered 2-DG (250 mg/kg), and blood was collected 1 h later. Blood glucose levels increased significantly in both control (56%) and RDD (105%) mice (Fig. 4C). RDD mice also had a significantly lower basal blood glucose concentration than control mice when measured after saline treatment [control=169.0±9.1, RDD=111.8±9.3; t-test p=0.0006].

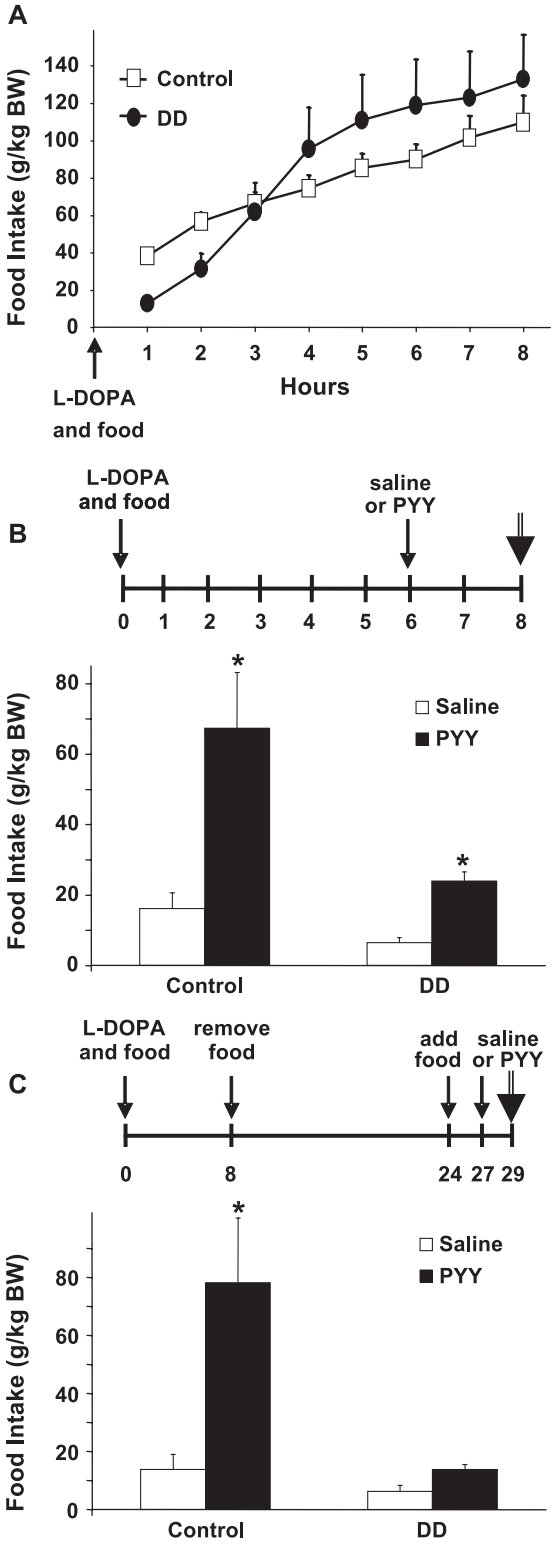

Fig. 5. Feeding response of control and DD mice to PYY treatment. (A) The cumulative food intake by control and DD mice that were trained to eat between 08:00 and 16:00 was measured; eight mice per group. (B) Mice were injected with L-dopa, 6 h later PYY (1 μg/μl) or saline was infused intraventricularly, and the amount of food consumed over the next 2 h was measured (double arrow); controls (n=8; DD, n=7). (C) Same as (B) except 27 h after L-dopa injection. The mice were videotaped during the 2 h after PYY infusion. Data represent means±S.E.M. Data were analyzed by ANOVA followed by Tukey post-hoc analysis for comparison between saline and PYY treatment within group; *p<0.05.

3.5. DD mice have an attenuated response to PYY that is partially restored in RDD mice

PYY was administered centrally to determine if DD mice are capable of responding to this orexigenic peptide. PYY was used because it is slightly more efficacious than NPY, which is naturally expressed in the brain and activates the same receptors [17]. To synchronize feeding in mice used in the PYY experiments, DD and control mice were injected with L-dopa at 08:00 and food was only available for the subsequent 8 h. This regimen was repeated for 8 days. Although control mice were injected with L-dopa, this treatment has no effect on brain dopamine content or behavior of control mice [45]. The amount of food consumed during 1-h epochs by these mice was measured on the 9th day. Control mice ate ~1 g of chow during the 1st h of access to food, whereas DD mice only ate 0.3 g of chow; however, by 6 h, control and DD mice had eaten similar amounts of food (Fig. 5A). Control mice injected centrally with 1 μg PYY either 6 or 27 h after their last L-dopa injection ate significantly more food during the subsequent 2-h test period compared to those injected with saline vehicle (Fig. 5B,C). They also spent a higher percentage of time in the target area around the feeding station [41.5%±12% PYY vs. 12%±4% vehicle]. Injection of PYY had a much smaller effect on food consumption when administered to DD mice 27 h after their last L-dopa injection (Fig. 5C), but it increased the time they spent in the target area containing food [20.5%±3% PYY vs. 7.8%±2% vehicle]. PYY stimulated more feeding by DD mice 6 h after L-dopa injection (when dopamine was still present), but consumption was still less than that of control mice (Fig. 5B).

To determine if PYY stimulates feeding by RDD mice, they were allowed to feed ad libitum for 4 h and then injected in the 3rd ventricle with 5 μg of PYY. Both the RDD and control mice increased food intake significantly in response to PYY [RDD, 2.2-fold increase; Control, 3.4-fold increase] (Fig. 6).

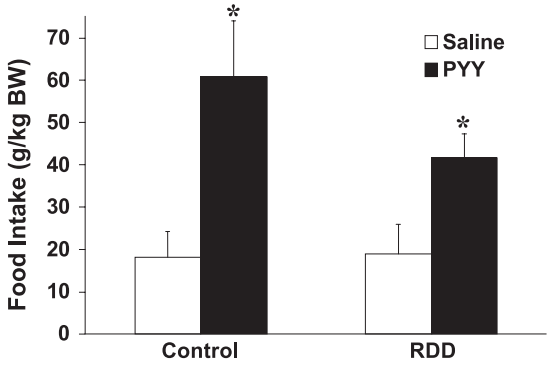

Fig. 6. Food intake by control and RDD mice during the 4 h following intraventricular administration of PYY (5 μg/mouse); control, n=7; RDD, n=7. Data represent means±S.E.M. Data were analyzed by repeated measures ANOVA; combined drug effect; *p<0.05.

4. Discussion

4.1. Hypothalamic dopamine is not required for inhibition of food intake by CCK-8

CCK-8 is released from the gut during a meal and activates CCK-A receptors on vagal afferents to inhibit food intake [21,34]. Dopamine release in the nucleus of the solitary tract is one of the downstream consequences of feeding or intraperitoneal injection of CCK-8 [2] and it has been shown that dopamine receptor antagonists can potentiate [3] the actions of CCK-8 on feeding. Furthermore, intra-hypothalamic 6-OHDA lesions completely blocked the effects of CCK-8 on food consumption suggesting that hypothalamic dopamine may be necessary for CCK-8-induced inhibition of feeding (Ref. [43], but see also Ref. [25]). Our results with the RDD mice suggest that this result does not extend to mice. In this model, dopamine is entirely absent, except from the central CPu. We found that RDD mice mount a quantitatively normal response to food deprivation and that this feeding response can be delayed by exogenous CCK-8 in a manner similar to control mice. These results indicate that neither hypothalamic nor brainstem dopamine is required for CCK-8-induced appetite suppression in mice.

4.2. Dopamine is not required for physiological responses to glucoprivation

Dopamine may play an important role in the physiological regulation of blood glucose. For instance, dopaminergic agonists have been shown to increase blood glucose [29] and insulin administration increases dopamine turnover and release in the hypothalamus [30]. Furthermore, central administration of apomorphine, a dopamine receptor agonist, increases blood glucose levels, likely through descending activation of the adrenal gland resulting in an increase in epinephrine secretion [1]. Together, these data are consistent with the hypothesis that central dopamine may be involved in regulating the physiological responses to glucoprivation. Our observation that DD mice show the anticipated physiological changes in blood glucose concentration in response to both 2-DG and insulin indicate that glucose-sensing and effector mechanisms are intact. The hyperglycemia in response to 2-DG is perhaps the best evidence for an intact counter-regulatory response, as this requires autonomic nervous system function. Signals of perceived hypoglycemia as a result of cytoglucopenia are thought to originate in glucose-sensitive, neurons in the hypothalamus and brainstem that activate descending pathways to release glucagon from the pancreas and epinephrine from the adrenal medulla [4,5,26,27]. Infusion of 2-DG directly into the ventromedial hypothalamus triggers the counterregulatory response [4]. On the other hand, lesioning brainstem noradrenergic neurons with a DBH-specific immunotoxin abrogates the physiological

response to insulin or 2-DG [28]. In DD mice, the increase in blood glucose in response to treatment with 2-DG is most likely due to gluconeogenesis (rather than glycogenolysis) because they are effectively fasted 24 h after their last L-dopa injection.

4.3. Dopamine is required outside of the CPu for glucoprivation-induced food intake

Despite observations that the DD mice mount appropriate physiological changes in response to 2-DG, the behavioral response appears to depend on dopamine. Our results are similar to those obtained in lateral hypothalamic and 6-OHDA lesion models [6,15,37], and suggest that dopamine is needed to facilitate feeding in response to glucoprivation. However, because DD mice and 6-OHDA-treated rats are deficient in many goal-directed behaviors, the lack of effect of glucoprivation on feeding may be nonspecific. We therefore explored these questions with the RDD mice that are able to maintain sufficient feeding in the absence of L-dopa. We found that they also manifest the appropriate changes in blood glucose, but lack the behavioral response to 2-DG and insulin. This result underscores a differential requirement for dopamine in the CPu to facilitate sufficient feeding for survival or in response to a fast and suggests that there is a separate requirement for dopamine outside of the CPu for glucoprivation-induced feeding. There are several dopaminergic cell groups in the hypothalamus that might serve this function. Indeed, dopamine release decreases in the ventral medial nucleus (VMN) [19,20] and increases in the lateral hypothalamic area (LHA) [18,20] during feeding. In addition, dopaminergic cell grafts into the VMN increase food intake [20,44] and specific 6-OHDA lesions of the preoptic area, that do not alter dopamine levels in the CPu, can blunt the feeding response produced by 2-DG [12]. Hindbrain neurons that express norepinephrine and NPY and project to the arcuate and periventricular areas of the hypothalamus are implicated in the feeding response to glucoprivation [27,31]; thus, we suggest that a local hypothalamic dopaminergic input may also be necessary for a feeding response to glucoprivation.

Another possibility is that the extent of dopamine signaling achieved in the striatum by viral transduction is too low or too restricted. We have shown that this approach restores dopamine levels in the CPu to ~25% of that of a normal mouse [39]. Hence, there could be a threshold effect such that increasing the distribution and/or concentration of dopamine in the CPu would restore further aspects of feeding behavior. It is also possible that dopamine signaling in the nucleus accumbens could be important because disruptions of neuronal signaling there can modify feeding [11,16,35]. The experiments with the RDD mice were performed with half the dose of 2-DG, but twice the dose of insulin, that were used in earlier experiments with DD mice. It is unlikely, that these differences affect our interpretation, because both doses produce significant physiological responses and both dosages are used by other investigators to explore this phenomenon.

4.4. Dopamine action in the CPu restores feeding responses to PYY

Substantial evidence indicates that leptin derived from adipose tissue can affect appetite and metabolism by acting on hypothalamic neurons. The POMC and NPY neurons in the arcuate region of the hypothalamus appear to be prime targets of leptin action [24]. Melanocortin release from the POMC neurons inhibits feeding and stimulates metabolism. Disruption of melanocortin signaling, either pharmacologically or genetically leads to obesity [32,33]. Although the physiological role of NPY in long-term regulation of energy balance is not resolved [10,22], acute intracranial delivery of NPY or agouti-related protein stimulates robust feeding, at least in part by antagonism of melanocortin release [22,32,33]. We demonstrated previously that DD mice fail to eat in response to leptin deficiency, despite the fact that they are hyperphagic and become obese when treated with L-dopa [40]. Here, we extend those observations by showing that the feeding response to central administration of PYY, an NPY analog, is also severely blunted. Central injection of PYY increased the amount of time that both DD and control mice spent in the vicinity of the food, but DD mice did not eat much when dopamine levels were low (27 h after their last L-dopa treatment). To determine if the PYY response is dependent on dopamine outside of the CPu, we tested the effects of intracerebroventricular PYY administration on RDD mice. We found that PYY led to a significant increase in food intake in the RDD mice, although still less than controls, and thus we conclude that the ability of PYY to stimulate feeding is at least partially restored by dopamine signaling in the central region of the CPu. The blunted feeding response by RDD mice was similar in magnitude to the blunted response of DD mice when dopamine was present (6 h after L-dopa injection). Perhaps compensatory changes have occurred as a consequence of development in the absence of dopamine that attenuates the response to PYY. We suggest that dopamine action in the central region of the CPu allows many goal-directed behaviors, including the initiation of bouts of feeding [39]. We recognize that the amount of PYY used and its site of injection for the RDD experiments were different than for the experiments performed with DD mice; however, because the feeding responses of the control mice were similar in the two experiments this is unlikely to affect our conclusions.

4.5. Conclusions

The aphagia of adult DD mice demonstrates an essential role for dopamine in sustained feeding behavior [39,45], but this phenotype can be rescued through viral restoration of dopamine synthesis in the CPu [41]. In these studies we

established a role for dopamine in the feeding that occurs after either a hypoglycemic challenge or infusion of PYY. However, dopamine signaling in separate brain regions appears to be required for these two feeding responses. Dopamine action in CPu restores the response to PYY, which is thought to activate NPY receptors in the medial hypothalamus to suppress melanocortin signaling [22,32,33]. In contrast, dopamine signaling somewhere outside the CPu, perhaps the hypothalamus, appears to be required for the feeding response to glucoprivation. It is noteworthy that RDD mice lack a feeding response to acute glucoprivation, but they manifest a normal feeding response to an overnight fast. There are many redundant mechanisms to ensure adequate food consumption any of which may be sufficient to stimulate food intake after a fast. Indeed, glucose concentrations change very little during the course of normal feeding [33]; therefore, it may not be one of the primary mediators of meal initiation. In fact, the feeding response triggered by experimentally induced glucoprivation is generally considered to be an emergency response mechanism that is used as a last resort to counter an acute energy deficit [33].

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