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Bromocriptine Administration Reduces Hyperphagia and Adiposity and Differentially Affects Dopamine D2 Receptor and Transporter Binding in Leptin-Receptor-Deficient Zucker Rats and Rats with Diet-Induced Obesity

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Abstract

Background—The dopamine (DA) D₂ receptor (D2R) agonist bromocriptine (BC) decreases body fat in animal and human models and increases lean muscle mass, improves glucose intolerance and insulin resistance, and reduces triglycerides and free fatty acids. We have previously shown a negative correlation between D2R and body weight in obese individuals and in rodents, and that chronic food restriction increases D2R binding in genetically obese rats. The purpose of this study was to assess whether the antiobesity and metabolic effects of BC are related to changes in midbrain DA and D2R activity by measuring D2R and DA transporter (DAT) binding in a genetic (leptin-receptor-deficient) and environmental (diet-induced) rodent obesity model.

Methods—Obese (fa/fa) (leptin-receptor-deficient), lean (FA/FA) Zucker rats and rats with diet-induced obesity (DIO) were treated with 10 mg/kg BC for 4 weeks. Body weight, food intake, locomotor activity and blood glucose levels were measured along with D2R- and DAT-binding levels using in vitro receptor autoradiography.

Results—BC decreased food intake and body fat and increased locomotor activity in both the (fa/fa) and DIO rats. Furthermore, BC increased D2R binding in (fa/fa) but not in DIO rats. Finally, BC increased DAT binding in DIO rats but not in the (fa/fa) rats.

Conclusion—These observations are all consistent with the existence of unique leptin-DA interactions and the hypothesis that there is hyposensitivity of the DA system in obesity.

Keywords

Bromocriptine; Dopamine; Dopamine D₂ receptor; Dopamine transporter; Leptin

Introduction

Numerous studies have demonstrated that the dopamine (DA) D₂ receptor (D2R) agonist, bromocriptine (BC), produces significant reductions in body fat in both animals [1] and humans [2], increases lean muscle mass [3], improves glucose intolerance and insulin resistance [2–6], and reduces triglycerides and free fatty acids [3]. Defective DA neurotransmission has been described in both obese humans and animals. Imaging studies have shown that severely obese individuals have decreased striatal D2R availability [7]. Genetic studies have also found that individuals carrying the Taq 1 A1 allele of the D2R gene have decreased D2R binding in striatum (ST) [8], though one study did not find this association [9]. Some studies have also found an association between this allele and vulnerability to addictive behaviors such as compulsive food intake including propensity to obesity [for reviews, see 10,11]. What remains to be elucidated is whether such individuals are born with inherently lower numbers of D2R, or whether the obesity results from pathological eating behaviors which increase peptides such as leptin and insulin that alter DA signaling [12,13], eventually leading to changes in D2R. The present study examined whether a D2R agonist can ameliorate the behavioral, physiological and neurochemical sequelae associated with obesity and whether these effects were related to changes in midbrain D2R and DA transporter (DAT) binding.

DA is an important regulator of energy expenditure [14–16], food intake [17] and goal-oriented behaviors [18]. Reduced DA signal transduction may give rise to overeating and decreased energy expenditure, both of which contribute to the positive energy balance seen in obesity. The above observations are all consistent with the construct that there is a hyposensitivity of the DA system involving the D2R in obesity and obesity-related metabolic disturbances. To our knowledge, there have been no studies evaluating the effect of BC on both the central and peripheral indicators of obesity that could shed light on whether reductions in D2R density are a part of the cause or the effect in obesity.

In order to examine the effectiveness of BC in treating obesity and determine a potential mechanism of action, we evaluated two rodent models of obesity: (1) fatty (fa/fa) Zucker rats, characterized by a leptin receptor defect [5,19] and lower striatal and hypothalamic D2R [20, 21,22], resulting in a phenotype of hyperphagia, morbid obesity [23], and inactivity [24], and (2) rats with diet-induced obesity (DIO), characterized by a polygenetic predisposition to weight gain [25] and hyperleptinemia [26], similar to that of most human forms of obesity.

Experimental Procedures

Subjects

The genetically obese subjects were 9-week-old, male (fa/fa) Zucker rats weighing 491.1 ± 11.4 g, and their lean (Fa/Fa) Zucker controls weighing 318.5 ± 4.9 g. The DIO subjects were 9-week-old, male, Sprague-Dawley rats weighing 198.3 ± 6.7 g. DIO rats have been shown to become obese when fed a high-energy diet (8% corn oil, 44% sweetened condensed milk, 48%

Purina 5001) over a 2-week period [27]. After 26 days of receiving the high-energy diet, the DIO rats weighed 402 ± 6.3 g. Mortality was at 0% as all rats made it to the end of the experiment.

Experimental Design

Three groups of 6 obese (fa/fa) Zucker rats were assigned to receive 4 weeks of: BC + ad libitum food intake (BC-OZ); placebo (PL) + ad libitum food intake (PL-OZ) to control for effects of BC; or placebo, but pair-feeding to amounts consumed by the BC-OZ group (PL/PF-OZ) to control for effects of feeding. A group of lean (Fa/Fa) Zucker rats received no treatment + ad libitum food intake (NTx-LZ) as a broad control for effects of obesity associated with leptin receptor deficiency and high-fat diet. Two groups of 6 DIO rats were randomized to receive 4 weeks of: BC + ad libitum food intake (BC-D) or placebo + pair-feeding to the treated DIO group (PL/PF-D) to control for effects of feeding. Group assignment was performed at baseline in such a way as to ensure that initial mean body weights did not differ between treatment and control groups. Table 1 summarizes the composition and characteristics of the groups.

All Zucker rats were fed standard pellet rat chow (RMH 1000) and water throughout the study. DIO rats consumed the high-energy diet throughout the study. Rodents randomized to the treatment groups received intraperitoneal injections of 10 mg/kg BC (Sigma-Aldrich, St. Louis, Mo., USA) 1 h after light onset daily for 4 weeks. BC was dissolved in 80% peanut oil and 20% ethanol for intraperitoneal administration [1]. Animals fed ad libitum and pair-fed control animals received injections of vehicle, except for NTx-LZ rats, which did not receive injections. All animal procedures were in strict accordance with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals [28] and approved by the Johns Hopkins University Animal Care and Use Committee.

Measurements

Body weight (grams) and food intake (grams) were measured daily in animals receiving BC, and 1–2 times weekly in those fed ad libitum. In treated rodents, food was weighed daily. Spillage was collected, weighed and subtracted from the gross food intake measured in order to calculate the net daily food intake of each animal.

Locomotor activity (total horizontal distance traveled, centimeters) was assessed by placing individual subjects into open-field boxes equipped with infrared beams to monitor activity for ~48 h on 2 separate occasions: prior to treatment, and during the last week (week 4) of treatment. Rats acclimated to the activity cages for 24 h before monitoring began, and continued to receive water, food and treatment according to their assigned group. Thus, analysis was based on the measurement from day 2 of each 48-hour monitoring period.

At the end of 4 weeks, rats were euthanized by decapitation 1–3 h after light onset on the day following the final drug treatment, and 24–26 h after the last injection. Blood glucose was obtained from core blood upon sacrifice using the Glucometer Elite with Ascensia test strips (Bayer Healthcare AG, Leverkusen, Germany).

After decapitation, brains were removed and cut into right and left hemispheres. The brains were immediately placed on dry ice and stored at -80°C ; later they were cut into sagittal sections (20 μm thickness) in a microtome cryostat (Bright model OTF 5040; Hacker Instruments Inc., Fairfield, N.J., USA) at -22°C . Sections were thaw-mounted on Ultrastick adhesion slides (Tedpella Inc., Redding, Calif., USA) and stored at -80°C until used. Brain regions of interest (ROIs) included the: ST (caudate-putamen), nucleus accumbens shell (NA

shell), nucleus accumbens (NA core), ventral tegmental area (VTA), and lateral hypothalamus (LH) 1.55–1.4 mm from the bregma, according to the rat brain atlas [29].

D2R Binding

D2R binding was measured by quantitative autoradiography in all rats according to well-established procedures [30,31]. To quantify D2R binding, brain sections were preincubated at 4 ° C for 30 min in 50 mM Tris-HCl buffer (pH 7.4) containing 60 mM NaCl and 5 mM KCl, and then incubated for 120 min in the same buffer with (250 µCi) 2 nM [³H]raclopride (Perkin Elmer Life Sciences, Mass., USA) at room temperature. Nonspecific binding was determined in adjacent sections incubated with 300 µM (±) sulpiride HCl (Tocris, St. Louis, Mo., USA). Slides were dried at room temperature and apposed to [³H]-Hyperfilm (Amersham Biosciences, Piscataway, N.J., USA) for 9 weeks with microscale-calibrated [³H]-impregnated standards [31]. Standard curves from [³H]-microscales were used to convert density levels into femtomoles/milligram of tissue equivalent. Autoradiograms were digitized using a Umax Powerlook 3000 Phosphoimager (Umax Data Systems Inc., Taipei, Taiwan) and Silver Fast Ai (Laser Soft Imaging AG, Kiel, Germany) software. Optical-density measurements were taken from the ST, NA (shell and core), VTA and LH. Densities were quantified by comparing autoradiographic signals to standards containing known concentrations of [³H]raclopride. The data were analyzed by Quantity One (Bio-Rad Laboratories Inc., Hercules, Calif., USA), and quantified with the use of computer-generated curves derived from the standards. The effect of treatment on regional densities of the labeled receptors was compared to controls.

DAT Binding

To measure DAT densities, sections were preincubated at 22 ° C for 30 min in PBS, pH 7.4, and then incubated at 22 ° C for 1 h in the presence of 26 pM [¹²⁵I]RTI-121 (2,200 Ci/mmol; Amersham Biosciences). Nonspecific binding was assessed by incubating adjacent sections with 10 mM GBR 12909 (Tocris). Slides were apposed to Bmax Hyperfilm (Amersham Biosciences) for 16 h. Standard curves from [¹²⁵I]RTI-121-microscales were used to convert density levels into femtomoles/milligram of tissue equivalent. Autoradiograms were digitized using a Umax Powerlook 3000 Phosphoimager (Umax Data Systems) and Silver Fast Ai (Laser Soft Imaging AG) software. Densities were quantified by comparing autoradiographic signals to standards containing known concentrations of [¹²⁵I]RTI-121. The data were analyzed by the analysis program Quantity One (Bio-Rad Laboratories Inc.) and quantified with the use of computer-generated curves derived from the standards. The effect of treatment on regional densities of the labeled receptors was compared to controls.

Statistical Analysis

All data were collected using a standardized format, entered into Microsoft Excel, and then exported to the Stata (Stata, College Station, Tex., USA) statistical software package for analysis. The Wilcoxon signed-rank test was used to test for equality of matched pairs, and Wilcoxon rank-sum (Mann-Whitney 2-sample statistic) was performed to test equality of unmatched data. The Kruskal-Wallis test was used to test equality of multiple samples. To account for multiple comparisons in Zucker rats, a Bonferroni corrected p value was used: $p = 0.05/3 = 0.017$ in all instances except for food intake in which a Bonferroni-corrected p value of $0.05/4 = 0.0125$ was used. Linear regression analyses were performed and Pearson correlation coefficients determined to examine associations between outcome variables and treatment with BC. Results in each group were analyzed for survivors only: BC-OZ (n = 6), PL-OZ (n = 5), PL/PF-OZ (n = 6), NTx-LZ (n = 6); and BC-D (n = 6), PL/PF-D (n = 5). In neither case did nonsurvivors significantly differ in initial characteristics and both served as control animals. Unless otherwise specified, data were considered statistically significant if $p < 0.05$.

Results

Food Intake

Food intake was significantly reduced (fig. 1) in BC-OZ rats compared to pretreatment (37.7 ± 0.8 g): 28.6 ± 0.98 g, $p = 0.001$ (week 1); 28.0 ± 0.7 g, $p = 0.004$ (week 3), and 29.6 ± 1.4 g, $p = 0.001$ (week 4); and in BC-D rats compared to pretreatment (24.3 ± 1.3 g): 19.25 ± 0.25 g, $p = 0.009$ (week 1); 19 ± 0.9 g, $p = 0.03$ (week 2); 18.2 ± 1.2 g, $p = 0.003$ (week 3), and 16.5 ± 1.0 g, $p = 0.001$ (week 4). There was no significant difference in mean food intake (average intake over 4 weeks) between BC-OZ and PL-OZ groups ($p = 0.3$).

Body Composition

Using mean percent body weight change, we found no significant effect of BC treatment in the obese Zucker rats (fig. 2). However, significant reductions in body fat with BC were demonstrated among DIO rats; BC significantly reduced epididymal fat pads (3.8 ± 0.5 vs. 6.2 ± 0.5 g, $p = 0.02$) and showed a trend in retroperitoneal fat pads (6.1 ± 0.84 vs. 8.4 ± 0.95 g, $p = 0.07$) (table 2).

Locomotor Activity

Compared to pretreatment measures, the BC-OZ group became more active ($+421.8 \pm 221$ cm, $p = 0.03$) whereas the PL-OZ (-706.6 ± 240 cm, $p = 0.04$) and PL/PF-OZ (-377.3 ± 149 cm, $p = 0.03$) became less active [$\chi^2_{(2 \text{ d.f.})} = 11.9$, $p = 0.003$] during the 12-hour dark phase (fig. 3). There was no significant difference in dark phase locomotor activity between BC-OZ and NTx-LZ rats ($1,425 \pm 229.4$ vs. $2,226 \pm 372$ cm) after treatment. Among DIO rats, the BC-D group became significantly more active during the 12-hour dark period ($1,433.97 \pm 1,490$, $p = 0.04$) compared to pretreatment, while PL/PF-D rats became less active during the 12-hour period ($-2,168 \pm 416$ cm, $p = 0.04$). No significant difference was found before treatment between the BC-D and PL/PF-D groups during the 12-hour period ($z = -1.46$, $p = 0.14$), but after treatment BC-D rats showed significantly greater locomotor activity than PL/PF-D rats ($z = 1,766.4$, $p = 0.06$).

Blood Glucose

Among Zucker rats, BC significantly lowered nonfasting glucose levels (mg/dl) (108.2 ± 2.16) compared to levels in the PL-OZ (162.4 ± 25.4) and PL/PF-OZ groups (122 ± 5.0) [$\chi^2_{(2 \text{ d.f.})} = 6.65$, $p = 0.035$]. The BC-OZ group also had lower blood glucose levels than lean Zucker rats (127 ± 17.0), but the mean difference was not significant ($z = 1.45$, $p = 0.15$). We found no significant differences among BC-D (162.7 ± 9.1) and PL/PF-D (141.6 ± 5.6) rats in nonfasting blood glucose values ($z = 1.7$, $p = 0.08$).

Binding Data

D2R-binding data for each group are presented as percent of lean Zucker rat (NTx-LZ) binding in figure 4 a and as femtomoles per milligram in table 3. In instances when we were unable to accurately quantify specific brain regions in an animal, analyses were performed without these individual animals. Significant mean differences in D2R binding were found among obese Zucker rats (BC-OZ, PL-OZ, PL/PF-OZ) in all brain regions studied: ST [$\chi^2 = 10.75$ (2 d.f.), $p = 0.005$], NA shell [$\chi^2 = 8.6$ (2 d.f.), $p = 0.014$], NA core [$\chi^2 = 7.35$ (2 d.f.), $p = 0.025$], VTA [$\chi^2 = 10.6$ (2 d.f.), $p = 0.005$] and LH [$\chi^2 = 11.25$ (2 d.f.), $p = 0.004$]. Lean Zucker rats had significantly higher binding in ST ($p = 0.02$) and NA core ($p = 0.039$) than treated obese Zucker rats, but not in NA shell ($p = 0.07$), VTA ($p = 0.24$) or LH ($p = 0.61$). No significant differences were found among obese Zucker rats in [^{125} I]RTI-121 binding in any brain region evaluated (table 3).

There were no significant differences between BC-D and PL/PF-D rats in [³H]raclopride binding (table 3). There were, however, significant increases in [¹²⁵I]RTI-121 binding in BC-D rats in the ST ($z = 2.0$, $p = 0.04$) and NA shell ($z = 2.0$, $p = 0.04$) compared with PL/PF-D controls (fig. 4b). DAT binding among DIO rats is presented as percent of control.

Correlations

Across groups, D2R binding significantly correlated with obesity-related behavioral, physiological and neurochemical measures in Zucker rats. Inverse correlations between D2R binding and food intake (weeks 2 and 4) were found in each of the following ROI: ST ($p \leq 0.002$); NA shell ($p < 0.016$); NA core ($p < 0.003$); VTA ($p < 0.05$). Positive correlations between D2R binding and locomotor activity (12 and 22 h) were found in each ROI: ST ($p = 0.000$); NA shell ($p = 0.000$); NA core ($p = 0.000$); VTA ($p = 0.000$) and LH ($p = 0.002$). Among DIO rats, significant inverse correlations were found between food intake (weeks 1 and 2) and DAT binding in the NA shell ($p = 0.05$), and between body fat (epididymal and retroperitoneal) and DAT binding in the NA shell ($p = 0.05$) and body fat (epididymal) and DAT binding in the ST ($p = 0.03$). Significant positive correlations were found between locomotor activity (12 and 22 h) in the NA shell ($p = 0.05$) and ST ($p < 0.02$).

Discussion

The present study examined the effect of a D2R agonist on brain concentrations of D2R in (leptin-receptor-deficient) genetically obese and DIO rodents. The salient findings of this study were that BC significantly increased D2R binding in the leptin-receptor-deficient obese Zucker rats, whereas it increased DAT binding in the DIO rats. Furthermore, the increases in D2R in the BC-OZ rats were significantly correlated with the marked increases in locomotor activity and inversely with the reductions in food intake, whereas in DIO rats the increases in DAT correlated with reductions in food intake, body fat composition and increases in locomotor activity.

Bromocriptine and Behavior

Food Intake, Body Weight and Adiposity—BC reduced food intake in both rodent models of obesity (fig. 1); this finding supports previous findings of reduced hyperphagia in leptin-deficient obese mice [17]. We also found significant reductions in body fat (table 2) among DIO rats with BC treatment in epididymal fat and a trend in retroperitoneal fat. With regard to the lipolytic effects of BC, our results agreed with previous studies that BC significantly reduced body fat in laboratory animals [1] and in human subjects [2], including reductions in triglycerides and free fatty acids [3]. In contrast to the DIO rats, there was no significant reduction in body fat in Zucker rats. This is in contrast to previous findings of a 29% reduction in body fat in obese Zucker rats treated with BC [1]. However, the lack of an effect of BC on body fat in our experiment compared to previous findings may be due to the fact that rats in the previous study were exposed to BC treatment for 8 weeks while in our study BC treatment was limited to 4 weeks. Our finding supports the notion that BC may be more potent in causing body fat reductions in DIO than in genetic obesity associated with leptin receptor deficiency. This may be due to both differences in food intake (obese Zucker rats treated with BC consumed on average 28% more food than BC-treated DIO rats), as well as differences in concentrations of other regulatory peptides associated with leptin receptor deficiency [32]. We did not observe significant reductions in body weight in response to BC treatment in either group (fig. 2), although BC-treated rats showed marginally less weight gain than animals fed ad libitum. The lack of a significant reduction in body weight may be secondary to a protein-sparing effect of BC as increases in lean muscle mass have been demonstrated with its administration in other studies. It may also be due to the duration of treatment (only 4 weeks) as well as to the fact that the rats studied were young (9 weeks of

age) and thus still in development. Future studies are planned to investigate higher BC doses and longer treatment durations.

Blood Glucose—A number of studies have demonstrated that BC improves glucose tolerance and insulin resistance in both humans [2,5,6] and laboratory animals [3,4]. We found significant reductions in nonfasting levels of blood glucose in Zucker rats treated with BC compared to PL-OZ (33%) and NTx-LZ (15%) controls. Pair-feeding of PL-treated obese Zucker rats to match the food consumption of BC-treated obese Zucker rats did not significantly reduce glucose levels (11.4%), indicating a blood-glucose-lowering effect of the drug itself rather than a weight/diet effect. There were no significant differences in nonfasting blood glucose in DIO rats treated with BC.

Locomotor Activity—We found that BC significantly increased locomotor activity in both obese Zucker (~35%) and DIO rats (~30%), whereas all other groups showed generalized decreases in locomotor activity during the treatment period (fig. 3). This finding supports the reductions we observed in body fat for the DIO since increased locomotor activity would translate into increased energy expenditure. Failure to see a decrease in body fat in the obese Zucker rats could reflect the fact that even though BC increased their locomotor activity, their activity was markedly lower than that of the DIO rats (treated or untreated) or the lean Zucker rats. The decreased locomotor activity in obese Zucker rats is likely the result of leptin receptor deficiency since leptin-deficient mice also show a marked reduction in locomotor activity [33]. This indicates the involvement of the leptin receptor in regulating locomotor activity, which is likely to reflect in part DA [34] and DA neuron regulation [12,34,35] by leptin. Increases in locomotor activity with BC correlated significantly with increased D2R binding in BC-OZ rats in all regions. DA is an important regulator of energy expenditure [14–16] and locomotion [18] (for a complete review, see [36]). Specifically, DA agonists increase, while antagonists decrease locomotor activity [16,37]. Also, increased locomotor activity leads to increases in DA [38]. Furthermore, mice lacking the D2R are characterized by decreased locomotor activity and hypothermia, and therefore lower energy expenditure [39]. Similarly, humans characterized by impaired D2R function also show lower energy expenditure [40]. Our findings are consistent with others that have demonstrated that the NA core and CPu are striatal regions particularly involved in voluntary motor functions [41] possibly mediated via D2R. Among DIO rats, we found increases in both locomotor activity and DAT binding in the ST and NA core, but not in D2R, suggesting that increases in locomotor activity with BC appear to be occurring via a different DA-related mechanism between Zucker and DIO. The lack of an effect of BC on DAT binding in the obese Zucker rats may reflect the involvement of leptin in upregulation of DAT by BC [42].

BC Increased D2R Binding in Leptin-Receptor-Deficient Obese Zucker Rats

Genetically obese Zucker rats had significantly lower D2R-binding levels compared to their lean controls, and BC treatment decreased this difference (table 4; fig. 4a). Specifically, whereas PL-OZ and PL/PF-OZ rats exhibited a 9-fold decrease in D2R binding when compared to NTx-LZ rats, BC-OZ rats exhibited a 1.5- to 3-fold decrease in D2R binding in all striatal regions examined (table 4). We did not find any significant differences in striatal D2R binding between PL/PF-OZ and PL-OZ rats. This is different from our previous findings of increases in D2R binding in response to food restriction [21]. However, the increases in D2R binding in our previous study were observed after a much longer period of food restriction (3 months) and used a different radioligand ([³H]spiperone) for D2R binding measurements. Furthermore our previous study used much greater food restriction (30%) compared to the decrease in food intake observed in both PL-OZ and PL/PF-OZ rats in the present study. Nevertheless, we did find that the PL/PF-OZ rats showed significantly greater D2R binding in the LH compared to PL-OZ rats. Previous findings have shown that obese Zucker rats are characterized by greater

LH DA levels compared to lean rats during periods of acute food deprivation [43] and show increased DA release during feeding [43]. The conclusion of this study was that the obese Zucker rat may be characterized by a higher DA ‘threshold’ than the lean rat, which may be a reason contributing to the increased feeding behavior observed in these rats (obese rats need to eat more in order to reach the higher threshold than lean rats) [43]. Subsequent experiments showed that DA infusion into the LH significantly reduced food intake in obese Zucker rats [44] and that these rats were also characterized by reduced expression of D2R in the LH [45], a finding that supports our results of lower D2R binding in the LH in obese compared to lean Zucker rats. However, if the obese rats never meet this ‘threshold’ due to the limited and ‘inadequate’ amount of food consumed, as is the case in the PL/PF-OZ group, then it may be possible for the ‘threshold’ to decrease in order for the rat to adapt to this new state or environment. The increase that we observe in D2R binding in the LH in the PL/PF-OZ group may serve as an adaptation to decrease this ‘threshold’. Finally, a lack of a difference in striatal D2R binding and the increased LH D2R binding in PF-Z rats may indicate that mesolimbic (VTA/NA core) DA is less sensitive than the LH to short-term decreases in food intake. Among obese Zucker rats, we found an approximate 3-fold increase in D2R binding in striatal and hypothalamic brain regions of BC-OZ compared with PL-OZ and PL/PF-OZ rats. Specifically, BC treatment led to increases in D2R binding in obese Zucker rats that were independent of any differences in food intake between the three groups. This was surprising since BC, as a DA agonist, could have presumably resulted in D2R downregulation as recently reported in male mice treated with BC [46]. The opposite effect in the Zucker rats suggests that leptin may modulate D2R expression.

Overall, these results support previous findings of decreased D2R binding in obese rats fed ad libitum compared to lean Zucker rats [20,21] and reinforce recent views on interactions between leptin, insulin and DA in regulating physiological functions associated with food consumption and energy regulation (for a review, see Palmiter [47]). Furthermore, these results reinforce the notion of unique leptin-DA interactions in genetic obesity, and suggest that selective D2R agonists that target striatal and hypothalamic D2R may prove valuable tools in further studying both physiological conditions such as hepatic steatosis [48] and possibly even behavioral (i.e. craving, food-related novelty seeking) symptoms associated with overeating and obesity. Improvement of metabolic parameters with BC, a DA agonist, is consistent with the deterioration in metabolic parameters (metabolic syndrome) reported with D2R antagonists (neuroleptics used in the treatment of schizophrenia) [49].

Increases in DAT Binding in DIO Rats

DAT is responsible for clearing DA from the extracellular space [50]. A role for D2R in DAT regulation is demonstrated by in vivo electrochemistry showing that D2R-deficient mice (–/–) clear only 50% of the synaptic DA that (+/+) mice clear [51]. Although it has been shown that obese Zucker rats exhibit higher concentrations of DAT mRNA in the CNS, and specifically the VTA compared to lean controls [42], we did not detect significant differences in DAT binding between any of the four Zucker rat groups (even though DAT-binding levels in the VTA were 30% higher in obese compared to lean Zucker rats) (table 4). The lack of significance may be attributed to the intrinsic variability within the obese group, which may have originated from differences in insulin profile (since differences in insulin are thought to be the main cause for the increased DAT mRNA in obese Zucker rats) [42].

While BC did not significantly affect DAT binding in the obese Zucker rats, it significantly increased DAT binding among DIO rats, in both the ST and NA (table 3; fig. 4b). A likely explanation is that the increased expression of DAT in the ST and NA of DIO rats reflects an accelerated clearance of synaptic DA in response to BC, since D2R agonists have been shown to increase the half-life of DAT in NA [52]. The increased half-life of DAT in the NA could

translate into a higher expression of functional DAT on the cell membrane surface. This higher level of functional DAT available on the cell surface could potentially translate into greater DA clearance from the extracellular space [53]. In support of this result are previous findings which showed that chronic restricted eating led to decreases in extracellular DA in the NA [54,55]. Taken together with our results, the decreases in extra-cellular DA previously reported in response to restricted feeding (and consequently weight loss) may be a consequence of increased DAT in the ST and NA. Furthermore, these findings suggest that BC may be mediating this increase in DAT binding and consequently decreases in DA in the ST and NA as part of its anti-obesity effects. Our results further support a role for DA (and specifically increased DA clearance) in regulating food intake in DIO rats, and also supports the use of D2R-specific agonists (that would regulate DAT binding) as potential pharmacotherapeutic agents for DIO. Though stimulant medications such as methylphenidate, which block DAT, decrease food intake, their clinical usefulness is limited by tolerance to this effect [56].

Limitations

Our interpretation of whether the antiobesity effects of BC treatment are actually dependent on D2R and DAT is limited by the fact that we only tested a single (10 mg/kg) dose of BC in our design. The main reason we decided to limit ourselves to this specific dose of BC is based on our previous findings which showed that this specific dose and treatment regimen (4 weeks daily intraperitoneal injections) caused marked reductions in obesigenic behaviors and markers of nonalcoholic fatty liver disease (NAFLD) in obese Zucker rats [48]. Based on these findings, in this study we wanted to see whether these improvements in obesity and NAFLD markers may coincide with changes in D2R and DAT binding. Although we cannot directly argue for the results that we report as being D2R and DAT dependent, the fact that we do report changes in D2R and DAT binding which coincide with a reduction in obesity and NAFLD markers using this specific dose and treatment regimen is suggestive of the potential involvement of D2R and DAT in these effects. This view is further supported by a recent study that examined BC-induced hyperglycemia and blood corticosterone increases in nonobese diabetic mice [57]. This study also utilized a 10 mg/kg dose of BC and concluded that the BC-induced hyperglycemia and blood corticosterone increases involved D2R since concurrent administration of 10 mg/kg of the D2R antagonist metoclopramide blocked these effects [57]. Another recent study also selectively utilized a 10 mg/kg dosing regimen in combination with *Hypericum perforatum* (a natural antioxidant) to show reduced 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-induced neurotoxicity in mice [58]. MPTP is a neurotoxin that selectively destroys dopaminergic neurons in the ST of rodents and primates [59] and therefore, these findings suggest that at this specific dose (10 mg/kg), BC is acting on striatal DA receptors.

Our interpretation is also limited by the fact that we did not utilize a BC-treated lean Zucker and non-DIO rat group in our design. As a result, we are limited in addressing how BC would affect a rat that eats a normal amount of food. This is particularly relevant to our finding of increased D2R binding in the LH in obese Zucker rats pair-fed the equivalent food consumed by BC-treated obese Zucker rats. We believe that while this is a very important and viable point to address, it goes beyond the scope of this study. Moreover, there is no experimentally valid reason of why a lean Zucker rat would differ in D2R or DAT binding compared to a lean non-DIO rat. Nevertheless, the main reason not to include these groups was because this study represents a continuation of our previous study which did not include BC-treated lean Zucker or non-DIO rat groups [48]. We believe that this study offers a valid interpretation of a relationship between obesity and central DA circuits even without the use of BC-treated lean rats. In support of this view, previous studies have utilized a similar design which did not focus on looking at effects of BC in normal (lean) mice [16,17,60,61] or humans [6,62], but rather focused on assessing a potential improvement in obesity-related markers in obese rodents and

humans in response to BC treatment. In terms of how BC may affect normal rats, previous studies have demonstrated that BC leads to decreases in body weight in normal Sprague-Dawley rats [1]. In terms of how the neurochemistry of these rats is affected by BC treatment is still unknown, however.

Conclusion

BC has previously been shown to reduce hyperphagia, particularly when administered in conjunction with the D1R agonist, SKF38393 [15,16]. We therefore postulated that D2R agonists would decrease food consumption. Our results demonstrate significant reductions in food intake in response to BC in both models of obesity studied. While both genetic and DIO models of obesity had significant reductions in food intake, only the genetic model had significant increases in D2R binding (in all striatal ROIs) that correlated with reductions in food intake. BC increased D2R binding in genetically obese rodents characterized by central leptin receptor defects, and it increased DAT binding in DIO rodents with comparatively normal levels of central leptin, but characterized by peripheral leptin resistance. The absence of an association between D2R binding, yet similarly robust reductions in food intake observed in the DIO rats may suggest that, while BC yielded similar satiety-producing effects in DIO animals to those observed in genetically obese rodents, the underlying mechanism of action is different.

Overall, we found that the D2R agonist BC has activities that give it the potential to treat several behavioral as well as pathophysiologic components of obesity. Behaviorally, reductions in food intake coupled with increases in locomotor activity demonstrate the potential of BC as an antiobesity agent. Furthermore, BC or other D2R agonists may protect against certain obesity-related complications by improving body composition and blood glucose levels. The positive effects of BC in both genetic and diet-induced rodent models of obesity are consistent with clinical studies showing beneficial effects of BC in type 2 diabetes and obesity [2,5,62,63].

In the context of our current obesity epidemic, our research suggests that two subsets of obese humans could potentially benefit from D2R agonist treatment: (1) those with relatively normal levels of D2R, but leptin resistance in the periphery, and (2) those who are genetically predisposed to a D2R deficiency (i.e. carriers of the Taq 1 A1 allele of the D2R gene) or who have documented D2R signaling deficiencies (i.e. as shown in a PET scan). Since nearly 20% of the population carries the Taq 1 A1 allele [64], and 30/65% of the population is categorized as obese/overweight, it is possible that 6–13% of the population may benefit from D2R agonists [65].

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Fig. 1. Mean + SEM food intake levels before (week 1) and after (week 4) BC treatment. Zucker and DIO rats treated with BC showed significant reductions in food intake at week 4 compared to week 1 (* $p < 0.05$).

Fig. 2. Mean + SEM body weight levels before and after BC treatment. There were no significant differences in body weight in response to BC treatment.

Fig. 3.

Mean + SEM dark-phase locomotor activity levels before (week 1) and after (week 4) BC treatment. BC significantly increased locomotor activity in both Zucker and DIO rats (* $p < 0.05$). All other experimental groups except lean Zucker rats showed significant reductions in locomotor activity between weeks 1 and 4 (* $p < 0.05$).

Fig. 4.

a Mean + SEM D2R binding in Zucker rats. BC treatment caused significant increases in D2R binding in obese rats in all brain regions (* $p < 0.05$). Obese nontreated rats showed significantly lower D2R binding in all brain regions compared to lean controls († $p < 0.05$). Lean Zucker rats showed greater D2R binding in the ST and NA core compared to BC-treated rats (^ $p < 0.05$). **b** Mean + SEM DAT binding in DIO rats. Results are presented as a percent from control. BC caused significant decreases in DAT binding in the ST and NA shell (* $p < 0.05$). Results are presented as a percent from control.

Table 1

Treatments and abbreviations for the 6 groups of animals tested

Strain	Treatment	Abbreviation
Obese Zucker	BC + ad libitum food	BC-OZ
Obese Zucker	PL + ad libitum food	PL-OZ
Obese Zucker	PL + pair-feeding to BC-Z	PL/PF-OZ
Lean Zucker	No tx + ad libitum food	NTx-LZ
DIO	BC + ad libitum food	BC-D
DIO	PL + pair-feeding to BC-D	PL/PF-D

Each group comprised 6 animals and treatments were given over a 4-week period.

Table 2
Mean ($\pm$ SEM) epididymal and retroperitoneal body fat (grams) in Zucker and DIO rats

	n	Epididymal	Retroperitoneal
Zucker rats			
BC-OZ	6	10.4 $\pm$ 0.9	17.4 $\pm$ 1.46
PL-OZ	5	10.3 $\pm$ 1.0	18.6 $\pm$ 2.8
PL/PF-OZ	6	9.9 $\pm$ 0.7	16.5 $\pm$ 2.77
NTx-LZ	6	2.8 $\pm$ 0.23	2.4 $\pm$ 0.31
DIO rats			
BC-D	6	3.8 $\pm$ 0.5 ^{<i>I</i>}	6.1 $\pm$ 0.84 ^{<i>I</i>}
PL/PF-D	5	6.2 $\pm$ 0.5	8.4 $\pm$ 0.95

^{*I*}DIO rats showed significant reductions in epididymal ($p = 0.05$) and retroperitoneal ($p = 0.07$) fat pads. There were no significant reductions in body fat in Zucker rats.

Table 3
Mean ($\pm$ SEM) absolute D2R and DAT binding in DIO rats (fmol/mg tissue equivalent)

	n	ST	NA shell	NA core	VTA	LH
^[125I] JRTI-121 DAT binding, fmol/mg						
BC-D	6	22.6 $\pm$ 4.4	14.9 $\pm$ 2.0	25.0 $\pm$ 4.0	11.3 $\pm$ 2.7	
PL/PF-D	5	16.1 $\pm$ 2.8	7.4 $\pm$ 3.6	14.2 $\pm$ 4.4	7.1 $\pm$ 1.65	
^[3H] raclopride D2R binding, fmol/mg						
BC-D	4	77.3 $\pm$ 28.9	63.8 $\pm$ 19.9	65.6 $\pm$ 26.4	33.5 $\pm$ 15.3	19.9 $\pm$ 9.6
PL/PF-D	5	64 $\pm$ 15	58.1 $\pm$ 13.6	86.0 $\pm$ 21.0	50.7 $\pm$ 3.8	38.1 $\pm$ 13.5

DIO rats showed no significant difference in D2R binding.

Table 4
Mean ($\pm$ SEM) absolute D2R and DAT binding in Zucker rats (fmol/mg tissue equivalent)

	n	Striatum	NA shell	NA core	VTA	LH
^[125I] JRTI-121 DAT binding, fmol/mg						
BC-OZ	6	14.6 $\pm$ 3.2	16.4 $\pm$ 4.1	10.3 $\pm$ 2.7	11.4 $\pm$ 1.7	
PL-OZ	5	15.3 $\pm$ 3.8	10.6 $\pm$ 1.0	13.6 $\pm$ 3.7	12 $\pm$ 2.6	
PL/PF-OZ	6	20.1 $\pm$ 3.0	14.7 $\pm$ 2.1	14.0 $\pm$ 1.4	14.5 $\pm$ 0.9	
NTx-LZ	6	19.7 $\pm$ 2.4	15.2 $\pm$ 2.5	10.9 $\pm$ 2.0	9.6 $\pm$ 1.0	
^[3H] raclopride D2R binding, fmol/mg						
BC-OZ	6	78.4 $\pm$ 12.5	79.8 $\pm$ 10.5	74.2 $\pm$ 11.5	64.1 $\pm$ 10.0	62.5 $\pm$ 8.4
PL-OZ	5	22.7 $\pm$ 5.8	28.7 $\pm$ 13.3	31 $\pm$ 12.7	13.0 $\pm$ 5	6.9 $\pm$ 3.1
PL/PF-OZ	6	25.2 $\pm$ 4.3	27.2 $\pm$ 7.1	24.6 $\pm$ 2.5	14.3 $\pm$ 2.6	11.6 $\pm$ 2.7
NTx-LZ	6	237 $\pm$ 50.4	144.3 $\pm$ 27.1	176 $\pm$ 32.1	96.1 $\pm$ 24.2	64.7 $\pm$ 6.2

There were no significant DAT-binding differences in Zucker rats.