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Serotonin 5-HT_{2C} Receptor Agonist Promotes Hypophagia via Downstream Activation of Melanocortin 4 Receptors

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The neurotransmitter serotonin (5-hydroxytryptamine) is a well-established modulator of energy balance. Both pharmacological and genetic evidence implicate the serotonin 2C receptor (5-HT_{2C}R) as a critical receptor mediator of serotonin's effects on ingestive behavior. Here we characterized the effect of the novel and selective 5-HT_{2C}R agonist BVT.X on energy balance in obese and lean mice and report that BVT.X significantly reduces acute food intake without altering locomotor activity or oxygen consumption. In an effort to elucidate the mechanism of this effect, we examined the chemical phenotype of 5-HT_{2C}R-expressing neurons in a critical brain region affecting feeding behavior, the arcuate nucleus of the hypothalamus. We show that 5-HT_{2C}Rs are coexpressed with neurons containing proopiomelanocortin, known to potently affect appetite, in the arcuate nucleus of the hypothalamus of the mouse. We then demonstrate that prolonged infusion with BVT.X in obese mice significantly increases *Pomc* mRNA and reduces body weight, percent body fat, and initial food intake. To evaluate the functional importance of melanocortin circuitry in the effect of BVT.X on ingestive behavior, we assessed mice with disrupted melanocortin pathways. We report that mice lacking the melanocortin 4 receptor are not responsive to BVT.X-induced hypophagia, demonstrating that melanocortins acting on melanocortin 4 receptor are a requisite downstream pathway for 5-HT_{2C}R agonists to exert effects on food intake. The data presented here not only indicate that the novel 5-HT_{2C}R agonist BVT.X warrants further investigation as a treatment for obesity but also elucidate specific neuronal pathways potently affecting energy balance through which 5-HT_{2C}R agonists regulate ingestive behavior. (*Endocrinology* 149: 1323–1328, 2008)

DEMONSTRATING THE IMPORTANCE of brain serotonin in energy balance, depletion of central serotonin using selective neurotoxins results in hyperphagia and obesity (1, 2), whereas pharmacological compounds increasing serotonin bioavailability, such as D-fenfluramine, potently inhibit feeding and reduce body weight (3–5). However, such compounds producing a general enhancement of brain serotonin levels may promote effects unrelated to food intake, given the involvement of this neurotransmitter in a diverse array of behavioral, psychological, and physiological processes.

Efforts to identify the specific mechanism through which serotonin regulates feeding behavior have indicated a critical role for the serotonin 2C receptor (5-HT_{2C}R) subtype. Indeed, mice lacking 5-HT_{2C}Rs are hyperphagic and obese (6), unlike mice lacking other serotonin receptors. These findings illustrate that functional 5-HT_{2C}Rs are required to promote normal energy balance. Complementing these genetic data, administration of nonselective 5-HT_{2C}R agonists such as *m*-chlorophenylpiperazine reduces food intake in a manner consistent with the advancement of satiety (7–12). The anorectic effects of nonselective 5-HT_{2C}R agonists and fenfluramine are attenuated by 5-HT_{2C}R antagonists (9–11). However, the high degree of sequence homology between receptors within the 5-HT_2R family has made it exceedingly difficult to pharmacologically distinguish between them. Here we report effects of a novel and highly selective 5-HT_{2C}R agonist, BVT.X, on energy balance in murine models of obesity. We further attempt to elucidate the mechanism through which serotonin, via action at 5-HT_{2C}R, promotes hypophagia.

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Abbreviations: ARC, Arcuate nucleus of the hypothalamus; BVT.X, 5-HT_{2C}R agonist; CLAMS, Comprehensive Lab Animal Monitoring System; DEXA, dual-energy x-ray absorptiometry; DIO, diet-induced obesity; HFD, high-fat diet; 5-HT_{2C}R, serotonin 2C receptor; IHC, immunohistochemistry; IR, immunoreactivity; ISHH, *in situ* hybridization histochemistry; MC4R, melanocortin 4 receptor; NDS, normal donkey serum; PBT, Triton X-100 in PBS; POMC, proopiomelanocortin; VO_2, oxygen consumption; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

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Materials and Methods

Animals

Mice used were adult male *Lep*^{ob} (*ob/ob*; Jackson Laboratories, Bar Harbor, ME; age on receipt 4–6 wk, average body weight 30 g) mice maintained on a chow pellet diet; lox-TB *Mc4r* null and wild-type littermate [breeding pairs kindly provided by Drs. Joel Elmquist and Bradford Lowell (Beth Israel Deaconess Medical Center, Boston, MA) (13, 14)] mice maintained on a powdered chow diet until 4–5 wk of age at which time they were switched to the same diet in pellet form; *Pomc tau-lacZ*$^{+/-}$ and wild-type mouse brain tissue [kindly provided by Drs. Stephen O'Rahilly and Anthony Coll (University of Cambridge, Cambridge, UK) (15)]; and C57BL/6 (Jackson Laboratories, age on receipt 3

wk, average body weight 16 g) mice maintained on a high-fat diet (HFD; 58.4 kcal percent fat; Research Diets, Inc., New Brunswick, NJ) for 4.5 months to produce diet-induced obesity (DIO) or maintained on a chow pellet diet. All mice were on a C57BL/6 background except the lox-TB *Mc4r* strain, which were on a mixed 129Sv and C57BL/6 background and the *Pomc tau-lacZ* strain, which were generated on a 129/SvEv background.

Mice were individually housed with *ad libitum* access to food and water in a light- (12 h on, 12 h off) and temperature (21.5–22.5 C)-controlled environment. All studies were conducted in the home cage, unless otherwise specified. All procedures used were in accordance with the guidelines for the care and use of animals established by the U.S. National Institutes of Health or the U.K. Animals (Scientific Procedures) Act 1986.

Tissue preparation

Male *Pomc tau-lacZ*$^{+/-}$, *ob/ob*, and wild-type mice were terminally anesthetized with pentobarbitone (50 mg/kg of body weight, ip) and perfused transcardially with heparinized saline, followed by 4% paraformaldehyde or 10% neutral buffered formalin (Sigma, St. Louis, MO). Brains were removed, placed in 15% sucrose/fixative solution, and immersed in 30% sucrose/PBS at 4 C. Twenty-five-micrometer coronal sections were cut using a freezing sliding microtome.

Dual-label ^{35}S-POMC and X-gal

Pomc tau-lacZ$^{+/-}$ mice are expected to express the β-galactosidase gene under the *Pomc* promoter in sites of endogenous POMC expression (15). Histochemical validation of exclusive expression of β-galactosidase activity in endogenous POMC-containing neurons was performed in *Pomc tau-lacZ*$^{+/-}$ mice and control$^{+/+}$ littermates. First, free-floating sections were incubated with X-gal staining buffer (15) in darkness at 37 C for 12 h. Sections were then mounted onto slides and air dried before processing for ^{35}S-POMC *in situ* hybridization histochemistry (ISHH).

The protocol for ISHH used was a modification of that previously reported (16, 17). Briefly, an antisense POMC ^{35}S-labeled riboprobe was generated from cDNA template. The linearized plasmid was subjected to *in vitro* transcription with SP6 polymerase according to the manufacturer's protocol (Promega, Madison, WI). The ^{35}S-POMC probe was diluted to 2×10^7 cpm/ml. Sections were incubated in the hybridization solution for 12–16 h at 56 C. Sections were next immersed in 0.002% RNase A (Roche Molecular Biochemicals, Indianapolis, IN) for 30 min. After stringency washes, slides were dipped in 3% parlodion (Fisher Scientific, Fair Lawn, NJ), air dried, dipped in photographic emulsion (NTB2; Kodak, Rochester, NY), and stored in light-tight boxes at 4 C for 1 wk. Sections were developed (D-19, Kodak) and fixed (Fixer, Kodak) and analyzed with an Axioskop 2 mot plus microscope (Zeiss, Thornwood, NY).

5-HT_{2C}R and POMC colocalization

To assess colocalization of 5-HT_{2C}R and POMC, dual-label immunohistochemistry (IHC) for β-galactosidase immunoreactivity (IR) and 5-HT_{2C}R-IR was performed in *Pomc tau-lacZ*$^{+/-}$ and wild-type mice. Modified standard IHC procedures, as previously reported (18, 19), were used. Briefly, tissue was washed with PBS and then blocked in 3% normal donkey serum (NDS) in 0.25% Triton X-100 in PBS (PBT) for 1 h. Tissue was then incubated with goat anti-5-HT_{2C}R (1:200; Santa Cruz Biotechnology, Santa Cruz, CA) and mouse anti-β-galactosidase (1:5000; Promega) antibodies in 3% NDS and PBT-azide (0.02% sodium azide in PBT) overnight at room temperature. The anti-5-HT_{2C}R antibody has previously been shown to be specific, with no background staining in 5-HT_{2C}R-deficient mice (20). After PBS washes, sections were incubated for 1 h with biotinylated donkey antimouse serum (1:1000; Jackson Laboratories) in 3% NDS and PBT. Sections were washed and then incubated for 1 h with appropriate fluorophores, Alexa Fluor 594 conjugated to streptavidin (1:1000; Molecular Probes, Eugene, OR) and Alexa Fluor 488 conjugated to donkey-antigoat IgG (1:500; Molecular Probes) in 3% NDS and PBT. Finally, after PBS washes, sections were mounted onto slides, air dried, and coverslipped with mounting medium (Vectashield; Vector Laboratories, Burlingame, CA).

Effect of BVT.X on acute food intake, locomotor activity, and oxygen consumption (VO_2)

The effect of saline and BVT.X on 6 h dark-cycle HFD paste (58.4 kcal percent fat) intake in murine models of obesity, *ob/ob* and DIO mice, was assessed. HFD paste was provided in glass jars in the home cage. DIO mice were maintained on HFD after weaning, whereas *ob/ob* mice were maintained on a chow diet until 3 d before the initiation of the study, at which time they were switched to the HFD. Forty-five minutes before the onset of the dark cycle, the HFD was removed from the home cage and saline or BVT.X (20 or 60 mg/kg) was administered by ip injection. At the onset of the dark cycle, fresh preweighed HFD was returned to the home cage, and intake was recorded for the next 6 h. These studies were performed using a within-subject experimental design (*i.e.* each animal was assessed with all treatments), such that animals received one of the three treatments on alternating days, with a minimum of 3 d elapsing between experimental treatments.

The effect of saline and BVT.X on 6-h, dark-cycle, powdered laboratory rodent chow (Purina) intake in young *Mc4r* null and wild-type littermates was also examined. Mice were recently weaned and maintained on the chow diet provided in the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH). Mice were housed in the CLAMS chambers 3 d before the initiation of the study. CLAMS chambers are rectangular 1-liter containers with a two-level photobeam array for measuring locomotor activity. Powdered chow diet is provided under a spring-loaded grate that is fixed to a balance. VO_2 is measured using indirect calorimetry. All data are collected and stored electronically in a time-stamped data file. While mice were acclimating to the chambers, food intake and body weight were measured and compared with home cage measurements to ensure stable feeding patterns. To assess the effect of BVT.X on ingestive behavior, food was removed 45 min before the onset of the dark cycle, and mice were injected with saline or BVT.X (60 mg/kg, ip). At the onset of the dark cycle, food was returned, and 6-h food intake, locomotor activity, and VO_2 were measured. This study was performed using a within-subject experimental design, such that each animal received saline or BVT.X on different days, with 2 d elapsing between the experimental treatments.

Effect of prolonged BVT.X infusion on POMC mRNA, body weight, percent body fat, and food intake

To assess the prolonged effect of a selective 5-HT_{2C}R agonist on POMC mRNA, body weight, percent body fat, and food intake, *ob/ob* mice were treated with saline or BVT.X (60 mg/kg·d) via sc osmotic minipump for 7 d. Body weight and chow pellet intake were recorded daily. Immediately before pump implantation (d 0) and after 7 d of treatment (d 7), percent body fat was assessed with dual-energy x-ray absorptiometry (DEXA; Lunar PIXImus, MEC Lunar Corp., Minster, OH). After the DEXA scan on d 7, mice were perfused with fixative, and brain tissue was collected and prepared as described above. Arcuate nucleus of the hypothalamus (ARC) POMC mRNA was determined using densitometric quantification of ^{35}S-labeled POMC with ISHH. ISHH was performed as outlined above, and then slides were placed in x-ray film cassettes with BMR-2 film (Kodak). The film was developed 24 h later. Comparisons of POMC mRNA after saline or BVT.X treatment were made by assessing the autoradiographic ARC (coordinates from Bregma, −1.34 to −2.70 mm) ^{35}S-labled POMC signal as measured with a light box, a digital camera interface, and Scion Image software (Frederick, MD). Determination of the level of bregma for each section of brain tissue on the film was made by examining the sections on the slides counterstained with thionin on a Zeiss Axioskop 2 mot plus microscope. At comparable levels of the rostral to caudal ARC for each mouse, the ^{35}S-POMC signal within each section was analyzed by computing the mean density minus background using Scion Image software.

Drug

The selective 5-HT_{2C}R agonist used was BVT.X [K_i (nM): 5-HT_{2C}R, 9; 5-HT_{2A}R, > 1000; 5-HT_{2B}R, > 1000; 5-HT_{1A}R, > 800; 5-HT_{1B}R, > 1000; other 5-HT receptors, not active] which was kindly provided by Biovitrum (Stockholm, Sweden). BVT.X was dissolved in 0.9% pyrogen-free saline.

Data analysis

All data were normally distributed as determined by Shapiro-Wilk's test. Data were analyzed with either a dependent *t* test or repeated-measures ANOVA followed by Tukey's *post hoc* tests for saline and multiple drug-dose or time-point comparisons or with independent-samples *t* test for comparisons of effects of drug treatment on POMC mRNA. Data were analyzed using SPSS PC Advanced Statistics (version 11.5; SPSS Inc., Chicago, IL) software. For all analyses, statistical significance was assigned at the $P \leq 0.05$ level.

Results

Food intake

In addition to modulating feeding, serotonin also affects other processes that may interfere with ingestive behavior (*e.g.* arousal). The induction of behaviors that compete with feeding using serotonergic agonists is likely associated with a lack of serotonin receptor selectivity, which confounds the interpretation of the specific pathways modulating serotonin's effects on satiety. We therefore investigated the mechanism of serotonin-induced satiety via action at the 5-HT_{2C}Rs using the selective agonist BVT.X.

BVT.X (60 mg/kg, ip) significantly reduced palatable HFD intake in a mouse model of obesity, DIO mice (n = 11, average body weight = 53 g; Fig. 1A). We next replicated this study using another mouse model of obesity, *ob/ob* mice (n = 6, average body weight = 51 g; Fig. 1B). As observed with DIO mice, BVT.X (60 mg/kg, ip) significantly reduced acute HFD intake in obese and hyperphagic *ob/ob* mice. These data illustrate that BVT.X is effective in decreasing intake of a palatable diet in rodent models of obesity.

Coexpression of 5-HT_{2C}R and POMC

Whereas the general expression of 5-HT_{2C}R mRNA in the rodent has been reported (21), the chemical phenotype of 5-HT_{2C}R expressing neurons in satiety centers within the mouse brain has not been described. We hypothesized that 5-HT_{2C}Rs are coexpressed with POMC neurons synthesizing the anorectic endogenous melanocortin agonist α-MSH in the ARC, as is observed in the rat (17). To investigate this hypothesis, we used *Pomc tau-lacZ*$^{+/-}$ mice to facilitate the identification of POMC-expressing cells. We first confirmed the exclusive expression of β-galactosidase activity in endogenous POMC-containing neurons in these mice but not in their wild-type littermates using dual labeling with X-gal and ^{35}S-POMC mRNA (n = 10). As expected, no β-galactosidase activity was found in wild-type mice, but β-galactosidase activity was evident in *Pomc tau-lacZ*$^{+/-}$ mice (Fig. 2, A–D). Dual-label analyses revealed that approximately 100% of X-gal-stained neurons contained ^{35}S-POMC mRNA. These data illustrate that *Pomc tau-lacZ*$^{+/-}$ mice may be used as a tool to visualize cells expressing endogenous POMC mRNA.

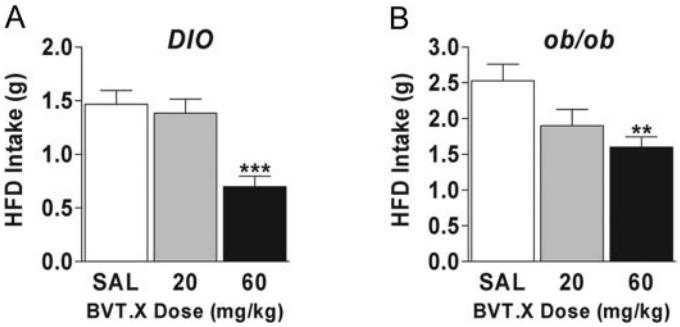

FIG. 1. BVT.X significantly reduces food intake. Effects of BVT.X on acute intake of HFD in DIO (n = 11, average body weight 53 g; A) and *ob/ob* (n = 6, average body weight 51 g; B) mice. Saline (SAL) or BVT.X (20 or 60 mg/kg) were administered ip 45 min before the onset of the dark cycle. Total food intake was measured 6 h after injection (mean ± SEM). Significant differences are indicated as follows: **, $P < 0.01$; ***, $P < 0.001$.

Consistent with earlier reports in rats using ISHH (17, 21), we observed that 5-HT_{2C}R protein is widely expressed in the wild-type mouse hypothalamus using IHC (n = 4). We next investigated the hypothesis that 5-HT_{2C}Rs are positioned to affect melanocortin action in the mouse by performing dual-label IHC using antibodies directed against the 5-HT_{2C}R and β-galactosidase in *Pomc tau-lacZ*$^{+/-}$ mice. 5-HT_{2C}R-IR neurons were abundantly coexpressed with β-galactosidase-IR cells in the ARC (Fig. 2, E–G). Specifically, approximately 75% of β-galactosidase-IR neurons were 5-HT_{2C}R-IR positive, and 28% of 5-HT_{2C}R-IR neurons were β-galactosidase-IR positive. These findings indicate that 5-HT_{2C}Rs are positioned to affect the activity of the majority of ARC POMC neurons in the mouse. Furthermore, they suggest that 5-HT_{2C}Rs are also expressed in non-POMC cells in the ARC.

Effect of prolonged BVT.X infusion on POMC mRNA, body weight, percent body fat, and food intake

Obese *ob/ob* mice (n = 8, mean body weight 46 g) received saline or BVT.X (60 mg/kg·d) via sc osmotic minipump for 7 d. Using densitometric quantification of ARC POMC mRNA, we observed that BVT.X treatment significantly elevated POMC expression throughout the extent of the ARC (Fig. 3A). This BVT.X-induced increase in POMC mRNA was associated with a significant reduction in percent body fat (Fig. 3B) and body weight (Fig. 3C) on d 7, compared with pretreatment levels. Food intake was significantly reduced during the first 2 d of treatment but then normalized after that (Fig. 3D).

Effects of BVT.X in Mc4r null mice

We have thus far presented evidence demonstrating that 5-HT_{2C}Rs are anatomically positioned to affect POMC neuronal activity and that 5-HT_{2C}R agonist application modulates POMC mRNA expression. POMC is the gene precursor to the endogenous melanocortin agonist, α-MSH, which acts at melanocortin 4 receptor (MC4Rs) to reduce food intake (22). To determine whether 5-HT_{2C}R-mediated regulation of α-MSH availability at MC4Rs is a necessary mechanism through which 5-HT_{2C}R agonists affect feeding behavior, we assessed the efficacy of BVT.X in mice with a genetic disruption of this receptor, *Mc4r* null mice.

Responses of young wild-type and *Mc4r* null littermates (n = 9, average body weight 20 g) to BVT.X were compared in the CLAMS system. BVT.X reduced 6-h food intake in wild-type mice by approximately 30% (Fig. 4A) but had no significant effect on ingestive behavior in *Mc4r* null mice (Fig. 4D). BVT.X did not significantly affect locomotor activity

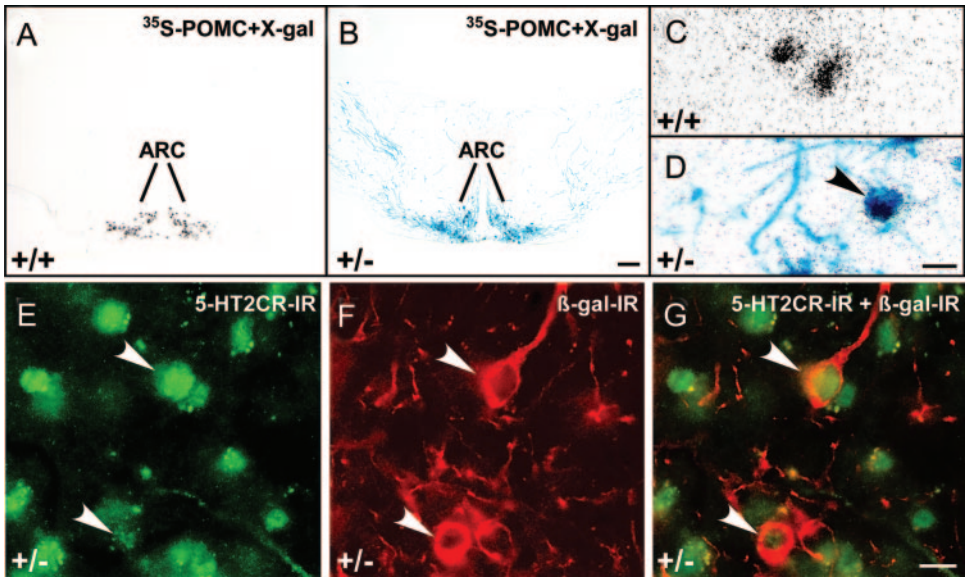

FIG. 2. 5-HT$_{2C}$Rs are coexpressed with POMC in the ARC. A, Wild-type (+/+) mice exhibit endogenous POMC mRNA using ISHH with ^{35}S-POMC (identified by clusters of *black grains*) but no β-galactosidase activity (X-gal stain of *blue cytoplasm*) in the ARC. B, *Pomc tau-lacZ*$^{+/-}$ mice display ^{35}S-POMC in every neuron positive for X-gal. C, Higher level magnification of wild-type$^{+/+}$ mouse ARC, showing ^{35}S-POMC grains but no X-gal stain. D, Higher-level magnification of *Pomc tau-lacZ*$^{+/-}$ mouse ARC, showing a typical cell containing both ^{35}S-POMC grains and X-gal stain (*arrow*). E–F, Fluorescent IHC for 5-HT$_{2C}$R (E) and β-galactosidase (F) in *Pomc tau-lacZ*$^{+/-}$ mouse ARC. G, Merge of E and F, showing cells coexpressing 5-HT$_{2C}$R and β-galactosidase (*arrows*). *Scale bar* (B), 200 μm and applies to A; (D), 20 μm and applies to C; (G), 20 μm and applies to E–G.

(Fig. 4, B and E) or VO_2 (Fig. 4, C and F) in either wild-type or *Mc4r* null mice. These data support a specific role for the MC4R in 5-HT$_{2C}$R agonist-induced hypophagia.

Discussion

Numerous studies have demonstrated that nonselective 5-HT$_{2C}$R agonists reduce ingestive behavior in lean rodents (reviewed in Ref. 23). Whether 5-HT$_{2C}$R agonists affect feeding in hyperphagic and obese animals is of great clinical relevance. We therefore investigated the mechanism of serotonin-induced satiety via action at the 5-HT$_{2C}$Rs using the selective agonist BVT.X in models of obesity. We demonstrate that this novel and highly selective 5-HT$_{2C}$R agonist significantly reduced acute food intake in an obese mouse, the DIO, and in a hyperphagic and obese mouse, the *ob/ob*. Tolerance to the anorectic effect, but not rebound hyperphagia, was observed after 2 d of continuous infusion of BVT.X via minipump to *ob/ob* mice. Despite this, 1 wk of treatment with this concentration of BVT.X was sufficient to significantly reduce body weight and percent body fat. These data indicate that further characterization of the effect of BVT.X on energy balance, body weight, and body composition is necessary. Taken together with previous 5-HT$_{2C}$R pharmacological and genetic studies, a critical role of the 5-HT$_{2C}$R in the modulation of energy homeostasis is indicated, and selective agonists warrant consideration for obesity treatment.

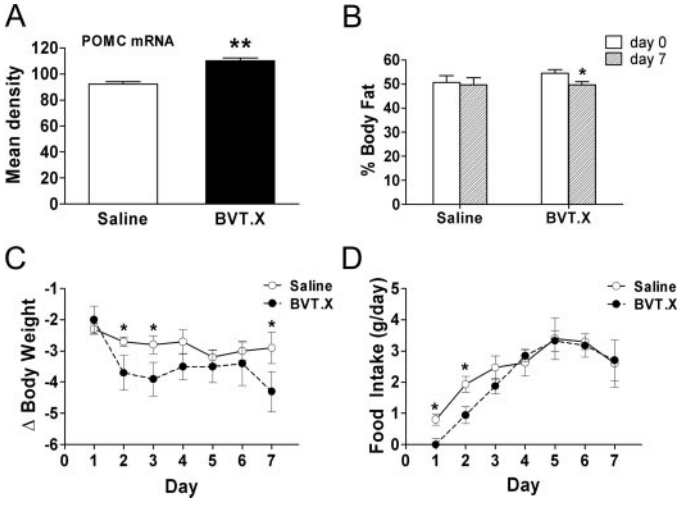

FIG. 3. Prolonged 5-HT$_{2C}$R agonist infusion increases POMC mRNA expression and reduces percent body fat, body weight, and initial food intake. Obese *ob/ob* mice (n = 8, mean body weight 46 g) were treated with saline or BVT.X (60 mg/kg·d for 7 d, sc minipump). A, Prolonged BVT.X treatment increased POMC mRNA in the ARC, compared with saline treatment, as determined by densitometry analysis after ISHH with a ^{35}S-labeled antisense POMC riboprobe. Prolonged BVT.X treatment significantly decreased percent body fat as determined by DEXA (B) and body weight (grams) (C), compared with pretreatment levels. D, BVT.X treatment decreased chow pellet intake on d 1 and 2, but no further significant differences were observed. Data are presented as mean ± SEM. Significant differences are indicated as follows: *, $P < 0.05$; **, $P < 0.01$.

We investigated a possible mechanism through which 5-HT$_{2C}$R agonists regulate energy balance. Previously we reported that POMC/α-MSH neurons in the ARC express 5-HT$_{2C}$R mRNA and are activated by the nonselective 5-HT$_{2C}$R agonist *m*-chlorophenylpiperazine in the rat (17). More recently the activation of POMC neurons by 5-HT$_{2C}$R has been shown to involve desensitization to prominent inhibitory inputs (24). Here we extend these findings by demonstrating ARC 5-HT$_{2C}$R and POMC coexpression in mice. Whereas the majority of POMC neurons coexpressed 5-HT$_{2C}$Rs, 5-HT$_{2C}$Rs were also identified in cells that were not POMC positive. The full chemical phenotype of ARC 5-HT$_{2C}$R-expressing cells and their role in physiology and behavior remain to be determined.

We investigated the functional relevance of ARC 5-HT$_{2C}$R and POMC coexpression by demonstrating that prolonged infusion with a 5-HT$_{2C}$R agonist increases POMC mRNA in the ARC. A limitation of this study is that it was performed in only one line of obese mice. However, the data are consistent with those reported by Nonogaki *et al.* (25), who demonstrated that treatment with drugs increasing serotonin bioavailability or with high binding affinity for the 5-HT$_{2C}$Rs significantly increase POMC mRNA in food-deprived wild-

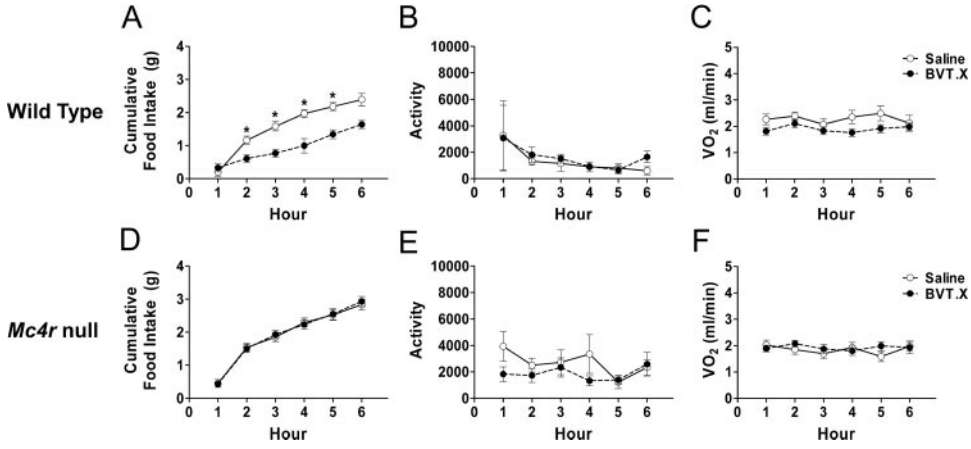

FIG. 4. BVT.X significantly decreases food intake in wild-type but not *Mc4r* null mice. Wild-type (A–C) and *Mc4r* null littermates (n = 9, average body weight 20 g) (D–F) received an acute injection of either saline or BVT.X (60 mg/kg, ip) and were assessed for 6 h in the CLAMS apparatus. BVT.X significantly reduced 6-h food intake (powdered chow) in wild-type mice (A) but not *Mc4r* null mice (D). BVT.X did not significantly affect average hourly locomotor activity (*horizontal counts*) (B and E) or VO_2 (C and F) in either genotype. Data are presented as mean ± SEM and significant differences are indicated: *, $P < 0.05$.

type mice. Together these findings suggest that 5-HT_{2C}R activation affects POMC mRNA expression, and by extrapolation, α-MSH synthesis and release.

Of the five melanocortin receptors, the MC4R is most closely associated with the regulation of food intake. Indeed, mutations of the *Mc4r* gene produce hyperphagia and obesity in both rodents and humans (26, 27). Selective MC4R agonists decrease food intake (28, 29), whereas selective MC4R antagonists increase food intake (28, 30). We show that, unlike in other obese and lean mice, BVT.X does not affect feeding in *Mc4r*-deficient mice, demonstrating that 5-HT_{2C}R agonists exert most of their hypophagic effect through downstream melanocortin signaling, dependent on MC4R. The MC4R population mediating this effect may be located in the paraventricular nucleus of the hypothalamus because restoration of MC4R expression in the paraventricular hypothalamus and amygdala reverses the hyperphagia of *Mc4r*-deficient mice (13).

In summary, here we characterize for the first time the effect of a novel and selective 5-HT_{2C}R agonist, BVT.X, on energy balance. We demonstrate that BVT.X significantly reduces acute ingestive behavior to a similar extent in lean and obese mice. Furthermore, we report that the anorectic effect is achieved without altering locomotor activity or VO_2. We show that prolonged treatment with BVT.X promotes weight loss and reductions in body fat. We determined that a specific neuronal pathway highly relevant to obesity, the melanocortin system, is a necessary mechanism through which this selective 5-HT_{2C}R agonist reduces food intake. These findings provide additional insight into the neural circuitry underlying the serotonergic modulation of ingestive behavior and suggest that BVT.X warrants consideration as a treatment for obesity.

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