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Acute methylphenidate treatments reduce sucrose intake in restricted-fed bingeing rats

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Abstract

Recent evidence suggests that methylphenidate HCl may be effective at limiting the frequency and the amount of binge eating. The present study investigated if daily treatments with methylphenidate reduced the bingeing-like behavior observed in restricted-fed adult male rats. Three groups ($n = 6$) received peripheral injections of methylphenidate in doses of 1.5 or 0.75 mg/kg/day, or saline, 3 days prior and 7 days during a previously characterized intermittent feeding regimen that results in a gradual increase of sucrose and food intake. The higher, but not the lower, dose of methylphenidate reduced sucrose intake to an asymptotic level starting after 3 days of the feeding protocol and concurrently led to an increase in the intake of chow. The high dose methylphenidate group also had two-fold lower plasma insulin levels compared with the saline-treated animals at the time of sacrifice on the last day of the feeding regimen. Further histological assays revealed that the methylphenidate treatments, irrespective of the dose used, resulted in selectively higher dopamine transporter and D2-like receptor labeled bindings in the shell region of the nucleus accumbens. These results suggest that relatively low-dose methylphenidate treatments may be effective for the management of binge eating by reducing the intake of palatable foods and may not interfere with short-term regulation of energy balance. These findings further support the notion that the mesoaccumbens dopamine system plays an important role in restricted access-induced sucrose bingeing in this rat model.

Keywords: Bingeing; Ritalin; Nucleus accumbens; Eating disorders; Food restriction

1. Introduction

Intermittent episodes of excessive food consumption (i.e., bingeing) are likely contributory to the increase in the prevalence rate of obesity and related eating disorders in the United States and other affluent countries [19,39]. In fact, bingeing without purging has been reported to occur in ∼25% of obese patients seeking medical intervention [59] and imposes a greater difficulty upon the treatment [5]. The bingeing episodes are perceived as uncontrollable [52] with the most likely food of choice being highly palatable, i.e. high in fats or carbohydrates or both ("forbidden foods") [13,31]. This suggests that binge eating represents a maladaptive non-homeostatic behavior insofar as the energy homeostasis is overridden by altered motivational mechanisms [15]. Specific binge eating disorders also have a strong temporal component as exemplified by nighttime eating disorders [51]. Along these lines, scheduled access to hydrogenated vegetable fat (Crisco) has been proposed as an animal model for binge eating [14]. Bingeing in this model has been observed with or without food restriction [12,17]. A similar effect has been illustrated with the phenomenon of scheduled-induced polydipsia [38] and scheduled-induced drug taking [47,55].

Additional evidence indicates that the rewarding properties of palatable foods can be augmented by food restriction with long-term effects on feeding behavior. For example, a persistent hyperphagia for palatable foods is expressed in ad libitum-fed rats that were previously exposed to a cycle of scheduled food restriction with intermittent access to the same palatable food [23,24]. Long-term exposure (≥21 days) to intermittent access to palatable sugar solutions (25% glucose or 10% sucrose) under scheduled feeding conditions results in binge-like eating and dopaminergic alterations reminiscent of drug dependence in rats [10,11,42]. Previously, it has been demonstrated that a 7-day scheduled-feeding regimen with brief (20 min) access to 0.3 M

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sucrose solution in restricted-fed rats resulted in eating more chow than the control groups [3]. Importantly, it was also found that these bingeing rats had higher dopamine (DA) transporter (DAT) and lower D2-like DA receptor binding selectively in the mesoaccumbens DA system [2,3]. Similar alterations have been observed in human studies, which have characterized polymorphisms in D2-like receptors and the DAT1 genes associated with bingeing eating disorders [46,48], and may be suggestive of a reduction in basal (i.e., tonic) DA levels [7,16,37].

Methylphenidate HCl (MPH; e.g., Ritalin© and Concerta©) treatments have been found to decrease bingeing in bulimia nervosa patients with attention deficit disorder (ADD) symptoms [18,44,49]. Recently, it has been reported that acute low doses of MPH reduced the intake of a palatable meal (i.e., pizza) in obese patients without ADD symptoms [35]. MPH inhibits the monoamine uptake transporters for DA and norepinephrine (NE) [32] and it has been suggested that MPH and other ADD medications are effective as a treatment for ADD because they raise tonic DA levels and thereby attenuate the relative rise in phasic (i.e., pulsatile) DA [45]. MPH and other ADD medications, in particular *d*-amphetamine, have been shown to dose dependently increase the concentration of forebrain catecholamines and reduce food intake [20,27,28,60]. In fact, anorexia with weight loss is a commonly reported side effect in patients chronically treated with MPH [43]. This anorectic property in combination with its relatively lower abuse potential makes MPH a prospective drug for the treatment of eating disorders.

On this basis, the present experiment was designed to test the hypothesis that daily, low doses of MPH would be effective at reducing the bingeing observed in scheduled-fed food restricted rats. In addition, assuming that this escalating feeding response is causal to the effects of acute MPH treatments on the mesoaccumbens DA system, we assessed D2-like DA receptor and DAT binding as markers of phasic and tonic DA regulation, respectively.

2. Materials and methods

2.1. Animals

Eighteen male Sprague–Dawley rats (Charles River, Wilmington, MA) with an initial body weight of 425–475 g were housed and maintained on a 12/12 h light–dark schedule (lights on 07:00 h) throughout the experiment. During the handling period (1 wk before the feeding regimen), all rats had ad libitum access to regular laboratory chow pellets (Rodent Diet-W 8604, Harlan Teklad, Madison, WI) and filtered tap water. Throughout the entire experiment all animals remained in their home cages except when daily body weights (BW) were measured between 08:00 and 08:30 h. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were in accordance with NIH Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals and their suffering throughout the experiment.

2.2. Experimental groups and design

Three days prior to the feeding regimen the rats were divided into three groups ($n = 6$ for each) with respect to receiving either 1.5 or 0.75 mg/kg MPH-HCl (Sigma St. Louis, MO) or 0.9% saline (i.e., vehicle) treatments. Accordingly, each group received a single daily injection (intraperitoneal; IP) at 08:30 h for 10 days. All groups were maintained on a food and water restriction schedule consisting of a daily 20 min access to a 0.3 M sucrose solution at 10:00 h, 20 min access to chow at 12:20 h, and a 2 h access to chow and water at 14:00 h. This procedure continued for 7 days and was similar to previously reported scheduled feeding paradigms [3,25,30]. The MPH treatments began 3 days prior to the feeding regimen not only to ensure appropriate pharmacological effects but also to avoid the initial association between the administration of the drug and the presentation of the palatable sucrose solution.

2.3. Behavioral measurements

Chow and water consumption were recorded daily at 08:30 h during the 3-day MPH pretreatment period and sucrose, chow and water intakes were recorded accordingly throughout the 7-day feeding paradigm. Daily cumulative total caloric intake was calculated from the intakes of chow + sucrose and was expressed in kilocalories (Kcal).

2.4. Blood parameters

All groups were decapitated in a staggered fashion (i.e., to ensure consistency with respect to the feeding regimen) on day 7 at ∼11:20 h. Trunk blood from each rat was collected into individual EDTA (K_3, 15%) vacutainer tubes. After removing 20 μl for blood glucose assay (Elite Glucometer, Bayer, Elkhart, IN), the remainder of blood sample was gently agitated and maintained on ice until centrifugation at 3000 rpm for 10 min. Plasma was then aliquoted into individual microcentrifuge tubes and stored at −80 °C. Standard radioimmunoassay kits were used to determine plasma levels of corticosterone (Cort; sensitivity; 25 ng/ml; ICN Biomedical, Redding, CA), insulin (sensitivity; 0.02 ng/ml; Linco Research, St. Charles, MO) and leptin (sensitivity; 0.5 ng/ml; Linco Research, Inc., St. Louis, MO).

2.5. Histology

Immediately following the decapitations, brains were removed and stored at −80° C. Tissue was sectioned at 20 μm and thaw-mounted on poly-L-lysine subbed slides. The regions of interest were the dorsal and ventral striatum (1.7–1.1 mm from Bregma; inclusive of the nucleus accumbens) and from the mesencephalon (−5.6 to −6.1 mm from Bregma, inclusive of the ventromedial tegmental area; VTA and substania nigra pars compacta; SNpc) [41]. Four sections from each brain region were serially mounted on a slide and each section on the slide represented an approximate distance of 160 μm. One slide from each brain region was stained with cresyl echt violet to accurately determine anterior–posterior anatomical coordinates for proper analytical comparisons.

2.6. D2-like autoradiography

Slide mounted sections were air dried for 3 min after removal from −80 °C storage. Then the dried slides were pre-incubated for 15 min in 50 mM Tris–HCl (pH 7.4), 120 mM NaCl at room temperature; followed by an incubation for 30 min at room temperature in 50 mM Tris–HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM CaCl, 1 mM $MgCl_2$ and a 0.6 nM concentration of [^{125}I] iodosulpride (Amersham, specific activity 2000 Ci/mmol) [4]. Iodosulpride has a K_d of 0.6 and 1.2 nM for D_2 and D_3, respectively [50]. The concentration of iodosulpride used in this assay was determined by previously running a series of test sections at stepped concentrations bracketing a suggested concentration of 0.3 nM. Slides were rinsed two times for 5 min each time in ice-cold 50 mM Tris–HCl (pH 7.4) buffer and placed before a stream of air at room temperature to dry for several hours. Non-specific binding was determined with the addition of a 50 μM concentration of s (+)-apomorphine to the [^{125}I]-iodosulpride incubation solution [2,36].

2.7. DAT autoradiography

Slide mounted sections were thawed for at least 30 min at room temperature. Slides were then incubated for 90 min at 4 °C in a buffer solution which