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Effects of 18-methoxycoronaridine on ghrelin-induced increases in sucrose intake and accumbal dopamine overflow in female rats

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Abstract

Rationale—18-Methoxycoronaridine (18-MC), a selective antagonist of $\alpha 4 \beta 2$ nicotinic receptors, has been previously shown, in rats, to reduce the self-administration of several drugs of abuse, reduce operant responding for sucrose, and prevent the development of sucrose-induced obesity. It has become increasingly apparent that there is a significant overlap between the systems regulating drug reward and food intake, therefore, we investigated whether 18-MC might modulate the effects of ghrelin, one of several orexigenic peptides recently implicated in both feeding and drug reward.

Objectives—In female Sprague–Dawley rats, we determined whether acute 18-MC treatment would reduce both ghrelin-induced increases in sucrose intake and ghrelin-elicited increases in accumbal dopamine levels.

Results—Pretreatment with 18-MC (20 mg/kg, i.p.), given prior to the administration of ghrelin (1 μ g, lateral ventricle), blocked ghrelin-induced increases in sucrose (5%) intake in a two-bottle open access paradigm. Using in vivo microdialysis, 18-MC (both 20 and 40 mg/kg) prevented ghrelin (2 μ g, intraventricular tegmental area)-induced increases in extracellular dopamine in the nucleus accumbens. 18-MC had no effect on deposition of fat or on serum levels of glucose, triglycerides, and cholesterol in ghrelin-treated rats.

Conclusions—The present results suggest that one potential mechanism by which 18-MC exerts its effects on palatable food consumption is via modulation of ghrelin's effects.

Keywords

Sucrose; Obesity; Nicotinic receptors; Ghrelin; 18-Methoxycoronaridine

Introduction

Obesity is a source of significant morbidity and increased mortality in the US population. Despite the demand for safe and effective agents, there are only a few agents currently approved by the FDA and available for clinical use in obese patients. 18-Methoxycoronaridine (18-MC), a potential anti-addictive agent and a selective antagonist of

$\alpha 4 \beta 2$ nicotinic receptors (Pace et al. 2004), has been previously shown to attenuate sensitized morphine-induced dopamine release in the nucleus accumbens of morphine-experienced rats (Taraschenko et al. 2007). Furthermore, systemic pretreatment with 18-MC has been shown to reduce the intravenous self-administration of morphine and other drugs and alleviate several signs of opioid withdrawal in rats (Glick et al. 2002; Maisonneuve and Glick 2003). In recent studies, 18-MC reduced consumption of sucrose in an operant self-administration paradigm and attenuated intake of sucrose, saccharin, and saline solutions by female rats in ad libitum access models (Taraschenko et al. 2008). Chronic treatment with 18-MC has also been shown to attenuate the development of sucrose-induced and high-fat-induced obesity in rats by reducing their intake of sucrose and dietary fat pellets, respectively (Taraschenko et al. 2008). Specifically, 18-MC appears to increase satiety in animals consuming palatable food while having no effect on consumption of normal chow and water (Taraschenko et al. 2008). Furthermore, 18-MC has a safe side effect profile (Maisonneuve and Glick 2003) and does not produce a conditioned taste aversion in rats (Taraschenko et al. 2010a).

The mechanism of 18-MC's effects on palatable food consumption could involve the drug's interaction with accumbal dopamine release in rats consuming sweet liquids. It does not appear that 18-MC acts directly within the mesolimbic dopamine pathway, as 18-MC administered directly into the ventral tegmental area (VTA) has no effect on either drug or sucrose self-administration (Glick et al. 2008). However, an interaction could be indirectly mediated and one such possibility involves the orexigenic peptide, ghrelin.

Ghrelin, a 28-amino acid peptide synthesized in the stomach and hypothalamus, has been shown to potently enhance appetite and induce deposition of fat in rodents (Gil-Campos et al. 2006; Tschöp et al. 2000). Although there are both central and peripheral sources of ghrelin in the body, the hyperphagic properties of this hormone are thought to be primarily mediated by its action in the brain (for review, see Olszewski et al. 2008). Furthermore, receptors for ghrelin (the growth hormone secretagogue receptor) are found in several discrete brain regions, including the hypothalamus, hippocampus, raphe nuclei, substantia nigra, and ventral tegmental area (Guan et al. 1997). Central administration of ghrelin (i.e., in the dorsolateral tegmentum, ventral tegmental area, and the third ventricle) increases the release of dopamine in the nucleus accumbens (Jernagel et al. 2006). Such increases appear to be mediated by nicotinic acetylcholine receptors since the response is blocked by systemic or central injections of nicotinic receptor antagonists (Jernagel et al. 2007, 2008). As dopamine is involved in the expression of compulsive eating behavior (Berridge 1996; Berthoud 2002), 18-MC, a selective $\alpha 4 \beta 2$ nicotinic receptor antagonist, could interact with ghrelin-induced accumbal dopamine release to affect sucrose intake in rats. Given such evidence, the present experiments were undertaken to examine the role of 18-MC in the mechanism of ghrelin's effects on sucrose intake and accumbal dopamine levels.

Methods

Animals

Naïve female Sprague–Dawley rats (250–275 g; Taconic, Germantown, NY) were housed individually and maintained on a normal 12-h light cycle (light on/off at 7 a.m./7 p.m.) in the colony room. For all experiments, normal chow and water were provided ad libitum. The experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

Drugs

18-MC (Albany Molecular Research, Albany, NY) was dissolved in 0.01 M NaH₂PO₄ (vehicle) and administered intraperitoneally (20 or 40 mg/kg). Sucrose (5% w/v, MP Biomedicals, Inc., Solon, OH) was dissolved in water. Acylated and desacylated rat ghrelin (Pi Proteonomics, LLC, Huntsville, AL) were dissolved in phosphate-buffered saline (PBS, vehicle) and administered intracerebrally into the VTA (2 µg in 1 µl) or into the lateral ventricle (1 µg in 5 µl).

Stereotaxic surgery

The surgery was performed according to the previously described protocol (Maisonneuve and Glick 1999). Specifically, for a two-bottle drinking experiment, the rats were implanted with one 22-gauge microinjection guide cannula (Plastics One, Roanoke, VA, USA) over the lateral ventricle. Left and right sides for placement of the microinjection guide were alternated from rat to rat. Coordinates in millimeter were chosen according to Paxinos and Watson (1986) such that when an injector was lowered, the tip was located in the lateral ventricle (in millimeter; anterior/posterior (AP)=−0.8; medial/lateral (ML)=±1.5; dorsal/ventral (DV)=−5.3).

For microdialysis experiments, the rats were implanted with one microdialysis guide cannula (CMA Microdialysis, North Chelmsford, MA) over the nucleus accumbens (NAc) and one injector cannula over the ipsilateral VTA. The sides for the microdialysis/microinjection guides were alternated from rat to rat. Coordinates in millimeter were chosen such that when a dialysis probe was inserted, the tip was located in the NAc (in millimeter, AP=+1.6; ML=±3.0; DV=−8.3 using a 14° angle) and when an injector was lowered the tip was located in the VTA (in millimeter, AP=−6.0; ML=±2.6; DV=−8.5 using a 14° angle). Rats were monitored daily for proper recovery and allowed to recover for 3–5 days before any testing was performed.

Two-bottle sucrose drinking paradigm

Baseline (three sessions; Monday–Wednesday) and treatment (four sessions; Monday–Thursday) were administered in two consecutive weeks. On the first day of the experiment (i.e., 3–4 days after surgery), the regular cage tops were replaced with custom-made cage tops containing metal holders for two 100-ml graduated glass bottles (Lab Products Inc., Seaford, DE). Animals received an additional bottle containing 5% sucrose solution; the bottles containing water and sucrose solution were placed approximately 50 mm apart. To control for possible side preferences, the left–right positions of the bottles were switched daily. Animals with an obvious side preference, not consuming sucrose, or those who lost weight or appeared to be in poor health were excluded from the analysis (*n*=5).

The baseline consumption levels of fluids were established during the initial three 1-h sessions conducted 24 h apart, Monday–Wednesday of the first week. The following Monday, rats were given a single injection of 18-MC (20 mg/kg, i.p.) or vehicle, immediately followed by a single treatment with ghrelin or PBS (vehicle). Desacylated ghrelin (1 µg) or PBS was infused into the lateral ventricle (ivt) in a volume of 5 µl over 1 min by means of a microsyringe (Hamilton, Reno, NV). The consumption of sucrose and water in the 1 h immediately following the treatments was recorded. The same treatments were repeated 24 h apart for the following 3 days such that each group of animals received a total of four injections (i.e., two injections of ghrelin and two injections of vehicle) during the same week. The orders of treatments were either ghrelin–vehicle–vehicle–ghrelin or vehicle–ghrelin–ghrelin–vehicle. The rats remained on sucrose for an additional 3 days, then were euthanized in CO₂ chambers and decapitated. Trunk blood was collected and analyzed for serum glucose, cholesterol, and triglycerides and brains were removed for

cannulae placement verification. Four animals lost weight and/or appeared sick during week 3 and were excluded from the study due to poor health, leaving a total of ten rats in the study.

In vivo microdialysis

A different group of naïve female, Sprague–Dawley rats was used for the microdialysis experiments. Of a total of 37 rats, four had to be discarded due to misplacement of injectors and/or probes and two were excluded due to defective probes, resulting in a total of 31 rats used in the study. On the afternoon before microdialysis, the rats were placed in the dialysis chamber and a dialysis probe was inserted through the guide cannula on one side of the brain. The probe was continuously perfused at a flow rate of 1 μ l/min with artificial cerebrospinal fluid (146 mM NaCl, 2.7 mM KCL, 1.2 mM CaCl_2 , 1.0 mM MgCl_2). At this time (19 h prior to ghrelin administration), rats were injected with either vehicle or 18-MC (40 mg/kg) as higher doses of 18-MC have been shown to lower basal extracellular levels of dopamine (Glick et al. 1998). The collection of brain perfusates began the following morning. The samples were collected in tubes containing 2 μ l of 1.1 N perchloric acid solution (containing 50 mg/l Na_2EDTA and 50 mg/l sodium metabisulfite). After collection of six 20-min baseline samples, rats received an injection of ghrelin (2 μ g) or vehicle into the VTA. Acetylated ghrelin (2 μ g/ μ l) was infused into the VTA in a volume of 1 μ l over 1 min by means of a microsyringe. To prevent backflow through the microinjection guide, the injection cannula (26 gauge) was kept in place for an additional minute after the solution was administered. To examine whether the same regimen of 18-MC (acutely administered, 20 mg/kg) would affect both sucrose consumption and increases in extracellular dopamine induced by ghrelin, in a separate group of animals, 20 mg/kg 18-MC was injected 20 min prior to an intrategmental infusion of ghrelin (2 μ g) or vehicle.

Following the delivery of ghrelin into the VTA, the collection of dialysate samples was then continued for 3 h. The samples were analyzed for dopamine, 3,4-dihydroxy-phenylacetic acid (DOPAC), and homovanillic acid (HVA) with high-performance liquid chromatography (HPLC). Upon completion of a microdialysis experiment, the animals were sacrificed and their brains were processed for verification of probe and injector placements.

In vitro recovery of dopamine—The afternoon of the microdialysis experiment, probes were placed in a standard solution of dopamine (14 nM) and flushed with ACSF at 1 μ L/min for 20 min. The in vitro recovery of dopamine was calculated as a percentage of the concentration in the standard. The mean recovery of dopamine obtained from the 2-mm probes was $23.1 \pm 1.87\%$.

HPLC—The HPLC system with electrochemical detection was comprised of an ESA 540 autosampler (ESA, North Chelmsford, MA), an ESA solvent delivery unit, an ESA column (MD-150/RP-C18; diameter=3.0 μ m), and an ESA Coulochem II electrochemical detector with an ESA 5020 guard cell and an ESA 5014B analytical cell. The glassy carbon working electrode was set at a potential of 300 mV with respect to the reference electrode. The MD-TM mobile phase (ESA, North Chelmsford, MA) was comprised of 75 mM sodium dihydrogen phosphate monohydrate, 1.7 mM 1-octanesulfonic acid sodium salt, 100 μ l/l triethylamine, 25 μ M EDTA in 10% acetonitrile (pH=3.0); it was pumped at a flow rate of 0.530 ml/min. Chromatograms were analyzed using an Agilent Technologies Chem Station Plus software (Agilent Technologies, Wilmington, DE).

Assessment of trunk blood composition and fat depot mass

Trunk blood was collected and analyzed for serum glucose, cholesterol, and triglyceride levels at the clinical biochemistry laboratory of Albany Medical Center; the tests were run in

duplicates. Necropsies were performed to remove periovarian, perirenal, and inguinal fat pads, and the combined fat tissue was weighed. The experimenter was blinded as to the treatment status of the animals.

Verification of cannula and/or probe placement

Brains were rapidly removed and frozen at -80°C until thinly sectioned ($40\text{ }\mu\text{m}$) using a cryostat. Only the data from animals with injectors located within the boundaries of the lateral ventricle or the VTA and probes located within the boundaries of the NAc were included in the analysis of data.

Statistical analysis

One hour of sucrose consumption by rats during a baseline period and after treatment with vehicle+ghrelin or 18-MC+ ghrelin was compared using paired t tests. The fat depot data and metabolic data for the same animals were analyzed separately using unpaired t tests. For microdialysis studies, the basal levels of dopamine and metabolites expressed as picomolar per $10\text{ }\mu\text{l}$ were analyzed separately for each dose of 18-MC (20 or 40 mg/kg) with repeated measures analysis of variance (ANOVA) with treatment group as an independent variable and time as the repeated measures variable, followed by planned comparisons of samples collected post-ghrelin injection. Levels of dopamine and metabolites were expressed as percents of the appropriate baseline means and were used as such for all subsequent analyses.

Results

Effects of intracerebroventricular ghrelin and systemic 18-MC on sucrose intake

The data for 1 h intake of 5% sucrose solution and water in the vehicle- and 18-MC-treated groups receiving intracerebroventricular (ivt) infusions of ghrelin are shown in Fig. 1. Prior to the initiation of treatments (week 1), the average 1-h levels of sucrose intake in the vehicle- and 18-MC-assigned groups were 5.80 ± 2.11 and $5.53\pm 1.29\text{ ml}$, respectively (mean $\pm$ SEM); there was no significant difference between the two groups ($t(8)=0.11$; $p>0.05$). The average baseline period water intake (1 h measurement) in the vehicle and 18-MC-assigned groups was 1.50 ± 0.3 and $1.50\pm 0.2\text{ ml}$, respectively with no significant difference between groups ($t(8)=0.05$; $p>0.05$). Ghrelin significantly increased 1 h of sucrose consumption in the vehicle-pretreated group (Fig. 1a; $t(4)=2.7$; $p=0.05$). However, in rats treated with 18-MC followed by ghrelin, sucrose intake was not different from consumption in PBS-treated rats, suggesting that 18-MC blocked ghrelin-induced increases in sucrose intake (Fig. 1b; $t(4)=1.4$; $p>0.23$). Ghrelin did not appear to affect 1 h of water consumption compared to baseline ($t(4)=0.22$; $p>0.83$; data not shown); nor did pretreatment with 18-MC affect water consumption ($t(4)=1.40$; $p>0.22$; data not shown).

Effects of systemic 18-MC on fat depot weights and serum markers of obesity in rats treated with ghrelin

The total weights of periovarian, perirenal, and inguinal fat pads fat tissue from the rat carcasses examined upon completion of the experiment are shown in Table 1. An unpaired t test revealed no significant effects of 18-MC on total fat depot weight ($t(8)=0.88$; $p>0.41$).

The average levels of glucose, cholesterol, and triglycerides in the serum of trunk blood of the same animals are shown in Table 1. An unpaired t test for each serum marker revealed no significant effects of 18-MC (glucose: $t(8)=-1.05$; $p>0.32$; cholesterol: $t(8)=-0.28$; $p>0.78$; triglycerides $t(8)=-0.70$; $p>0.50$).

Effect of systemic 18-MC on ghrelin-induced increases in accumbal DA

In order to assess whether the observed ghrelin-induced increases in sucrose intake might involve changes in mesolimbic dopamine, we chose to examine the effects of intrategmental ghrelin on extracellular levels in the NAc. Figure 2 shows the injector and probe cannulae placement in the regions of the VTA and NAc, respectively. Rats having injector cannulae not within the boundaries of the VTA or probe tips in the NAc were excluded from analysis ($n=4$).

The average basal levels of extracellular dopamine, DOPAC, and HVA of vehicle–ghrelin, 18-MC–ghrelin, 18-MC–PBS, and vehicle–PBS groups, expressed as picomoles per 10 μ l are found in Table 2. Consequently, extracellular concentrations of dopamine, DOPAC, and HVA revealed no significant difference among the four groups of rats (for dopamine: group effect, $F_{3, 15}=0.58$, $p>0.64$; group $\times$ time interaction, $F_{15, 75}=1.00$, $p>0.46$; for DOPAC: group effect, $F_{3, 15}=0.11$, $p>0.95$; group $\times$ time interaction, $F_{15, 75}=0.72$, $p>0.76$; for HVA: group effect, $F_{3, 15}=0.60$; group $\times$ time interaction, $F_{15, 75}=0.34$, $p>0.99$).

Consistent with previous reports (Jerlhag et al. 2007, 2008), administration of ghrelin into the VTA of vehicle-pretreated rats increased extracellular levels of dopamine in the NAc, a maximum of $152.19\pm 26.31\%$ of baseline was achieved at 20 min after treatment (time effect: $F_{14, 70}=2.24$, $p<0.014$; Fig. 3). In contrast, the infusion of PBS into the same brain area of vehicle-pretreated rats had no effect on basal extracellular levels of accumbal dopamine (time effect: $F_{14, 42}=0.80$, $p>0.66$). As shown in Fig. 3, intraperitoneal injection of 18-MC (40 mg/kg) 19 h prior to ghrelin treatment abolished the ghrelin-induced increase in accumbal dopamine (group effect: $F_{3, 15}=4.74$, $p<0.02$; group $\times$ time interaction: $F_{42, 210}=1.54$, $p<0.03$). Further analysis between the vehicle+ghrelin and 18-MC+ghrelin groups revealed that this effect was significant at 20–80 min after ghrelin injection (Bonferroni post hoc test). Consistent with this analysis, the ANOVA of the 18-MC effects in PBS-treated group alone revealed no significant effects (time effect: $F_{14, 42}=1.00$, $p>0.47$).

In order to confirm that the same regimen of 18-MC used in the sucrose studies would also have an effect on extracellular dopamine levels, rats were injected with 20 mg/kg 18-MC (i.p.), just prior to a local infusion of ghrelin into the VTA. Figure 3 (inset) shows that, similar to the higher dose of 18-MC tested, 20 mg/kg 18-MC administered just prior to ghrelin prevented ghrelin-induced increases in extracellular dopamine; no differences were observed between rats treated with 18-MC+PBS compared to rats treated with 18-MC+ghrelin (group effect: $F_{1, 8}=0.24$; $p>0.63$).

As shown in Fig. 4, administration of ghrelin into the VTA of vehicle-pretreated rats significantly increased extracellular DOPAC levels in the NAc (time effect: $F_{14, 70}=2.89$, $p<0.002$). On the other hand, administration of PBS into the VTA of these control rats produced no change in basal levels of extracellular DOPAC in the NAc (Time effect: $F_{14, 42}=1.08$, $p>0.41$). Administration of 18-MC 19 h prior to ghrelin treatment prevented the ghrelin-induced increase in DOPAC levels (group effect: $F_{3, 15}=1.86$, $p>0.18$, group $\times$ time interaction: $F_{42, 210}=1.44$, $p<0.05$). As apparent from Fig. 4, this effect was significant at 40 and 80–180 min after ghrelin injection. The ANOVA of the 18-MC effects in the PBS-injected rats showed no significant effects (time effect: $F_{14, 42}=0.62$, $p>0.83$). When acute 18-MC (20 mg/kg; Fig. 4, inset) was examined, there were no differences between 18-MC+PBS and 18-MC+ghrelin groups (group effect: $F_{1, 8}=0.06$; $p>0.81$), again, suggesting that 18-MC pretreatment blocked ghrelin-induced increases in extracellular DOPAC.

As indicated in Fig. 5, administration of PBS into the VTA of vehicle-pretreated rats did not alter basal extracellular levels of HVA in the NAc ($F_{14, 42}=1.11$, $p>0.38$), but the infusion of ghrelin into the same brain area as well as systemic 18-MC increased accumbal HVA levels (time effect: $F_{14, 70}=2.18$, $p<0.02$; for 18-MC: time effect, $F_{14, 70}=2.34$, $p<0.02$). However, further analysis revealed no significant difference among the three treatment groups (group effect: $F_{2, 10}=1.66$, $p>0.24$; group $\times$ time interaction: $F_{28, 140}=1.35$, $p>0.13$). Furthermore, the ANOVA with inclusion of all four treatment groups failed to reveal any significant differences among the groups (group effect: $F_{3, 15}=0.88$, $p>0.47$; group $\times$ time interaction, $F_{42, 210}=0.67$, $p>0.93$). These data suggest that administration of either ghrelin (into the VTA) or 18-MC (intraperitoneally) failed to alter extracellular levels of HVA in the NAc. The inset to Fig. 5 shows the effect of acute (20 mg/kg) 18-MC on extracellular HVA levels. As expected, no differences between rats treated with 18-MC+PBS or 18-MC+ghrelin were observed, (group effect: $F_{1, 8}=0.34$; $p>0.57$).

Discussion

In this study, we examined the mechanism of the previously demonstrated 18-MC-induced suppression of sucrose intake in female rats (Taraschenko et al. 2008). This is the first demonstration that pretreatment with systemic 18-MC can abolish the increase in 1 h sucrose intake produced by infusion of ghrelin into the lateral ventricles. Many factors have been shown to influence ingestive behavior and taste in female rats, particularly the sex hormone estradiol (Clarke and Ossenkopp 1998; Atchley et al. 2005). However, females consume sucrose more readily than males (Taraschenko et al. 2010b); therefore, in order to assess sucrose drinking behavior we used female rats. In this study, day of estrous was not controlled for, rather, we used random cycling female rats. Nevertheless, we were able to observe a pronounced effect of ghrelin on sucrose intake.

The orexigenic peptide ghrelin has been characterized as a potent inducer of food searching and food intake behaviors in animals and humans (for review cf. Korbonits and Grossman 2004). Ghrelin increases intake of fatty and carbohydrate-rich foods (Egecioglu et al. 2010) and increases sucrose intake (Bomberg et al. 2007). Consistent with these reports, in the present study, ghrelin increased sucrose drinking after administration into the lateral ventricle. In other reports, the same dose of ghrelin administered repeatedly into the lateral ventricle has been shown to also increase water intake and promote adiposity in rats (Nesic et al. 2008; Tschop et al. 2000). The lack of the effects of ghrelin on both water intake and fat depot weight in the present study could be attributed to the lower number of treatments administered repeatedly herein.

In order to assess whether 18-MC might affect ghrelin's effects in the mesolimbic dopamine pathway, we measured ghrelin-induced changes in extracellular dopamine using in vivo microdialysis. Here, we report that infusion of ghrelin into the VTA of rats increased extracellular levels of dopamine and DOPAC in the NAc, while pretreatment with 18-MC prevented these ghrelin-induced increases. It has previously been shown that ghrelin increases extracellular levels of dopamine in the nucleus accumbens by acting directly in the brain (Jerlhag et al. 2006, 2007). The increase in extracellular dopamine levels in the NAc produced by intrategmental injection of ghrelin in the present study is in agreement with those reports (Fig. 3). Ghrelin also increased extracellular DOPAC levels (Fig. 4), suggesting that the effects of ghrelin could be prolonged and also possibly affect dopamine synthesis. This finding is in agreement with previously reported ghrelin-induced-increases in accumbal dopamine turnover (Abizaid et al. 2006) and expression of tyrosine hydroxylase mRNA in midbrain dopamine neurons (Andrews et al. 2009).

A selective antagonist of the $\alpha 4 \beta 2$ nicotinic acetylcholine receptor, 18-MC, alters a number of motivated behaviors, including drug self-administration and food intake (Taraschenko et al. 2008; for review cf. Maisonneuve and Glick 2003). Specifically, previous studies in this laboratory demonstrated that 18-MC (20 mg/kg, i.p.) reduces consumption of palatable fluids (i.e., saccharin, sucrose, and saline) when given either acutely or repeatedly, and reduces sucrose reward (Taraschenko et al. 2008). Furthermore, the same dose of 18-MC administered chronically for 2 weeks prevents the development of obesity in rats consuming a high sucrose diet (Taraschenko et al. 2008). Consistent with this report, in the present study, 18-MC (20 mg/kg, i.p.) reduced consumption of sucrose in PBS-treated rats. Furthermore, 18-MC also prevented the ghrelin-induced increase in sucrose intake without having an effect on water consumption in the same rats. These findings indicate that 18-MC could reduce sucrose reward by modulating central effects of ghrelin.

In these experiments, the systemic administration of 18-MC prevented increases in extracellular dopamine in the nucleus accumbens evoked by intrategmental ghrelin. It is unlikely that 18-MC acts in the VTA to block $\alpha 4 \beta 2$ nicotinic receptors; for one, there is little expression of $\alpha 3$ or $\alpha 4$ subunit mRNA found in this region (Azam et al. 2002; Klink et al. 2001). One indirect mechanism by which 18-MC might affect mesolimbic dopamine release is via its effects on ghrelin or other orexigenic hormones. 18-MC could also possibly attenuate the release of ghrelin at a downstream location. One such location is the nucleus of the solitary tract, a brainstem structure responsible for the recognition of basic taste qualities in rats known to express $\alpha 4 \beta 2$ nicotinic receptors (Dhar et al. 2000; Roussin et al. 2007). Receptors for ghrelin, the growth hormone secretagogue receptors appear to be expressed on cholinergic neurons in the laterodorsal tegmentum, another brainstem region that may potentially mediate effects of 18-MC and other nicotinic receptor antagonists on ghrelin-induced sucrose intake (Dickson et al. 2010). It is noteworthy that ghrelin-induced increases in accumbal dopamine are sensitive to blockade by other nicotinic receptor antagonists, including mecamylamine and α -conotoxin MII, but not the $\alpha 4 \beta 2$ -specific antagonist dihydro-beta-erythroidine, suggesting that a non- $\alpha 4 \beta 2$ nicotinic receptor mediates this response (Jerlhag et al. 2006, 2008).

In summary, 18-MC attenuates intraventricular ghrelin-induced increases in sucrose intake, a mechanism of which might be inhibition of ghrelin-induced increases in mesolimbic dopamine overflow. While 18-MC has been shown to reduce self-administration of multiple drugs of abuse via the habenulo-interpeduncular pathway (e.g., Glick et al. 2008), 18-MC, like other nicotinic receptor antagonists, may modulate the effects of ghrelin via a distinct mechanism. Considered together with our previous data showing attenuated sucrose reward following 18-MC treatment (Taraschenko et al. 2010a, b) and recent studies implicating ghrelin in both food and drug reward (Jerlhag et al. 2010), these findings indicate that $\alpha 4 \beta 2$ nicotinic receptors and ghrelin could be jointly involved in the regulation of food reward and drug addiction.

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Fig. 1.

Intraventricular ghrelin increases sucrose consumption, a response that is blocked by pretreatment with systemic 18-MC. **a** One hour of sucrose consumption before (baseline) and after ivt ghrelin treatment. Mean 1-h sucrose consumption was determined during three consecutive days prior to drug treatment (baseline). Then, for 2 days, rats were injected with vehicle (i.p.) and infused with ghrelin (1 μ g, ivt); shown is mean sucrose consumption from the 2 days of ghrelin treatment. An *asterisk* indicates a significant difference following a paired *t* test ($n=5$). **b** Pretreatment with 18-MC prevents ghrelin-induced increases in sucrose consumption. In an identical paradigm to that described in a, following measurement of

baseline sucrose consumption, rats were injected with 20 mg/kg 18-MC (i.p.) immediately followed by an infusion of ghrelin (1 µg; ivt) and mean 1-h sucrose consumption was recorded for the 2 days of treatment. There was no difference between baseline sucrose consumption and consumption following treatment with 18-MC+ghrelin (paired *t* test; *n*=5)

Fig. 2. Schematic representations of injector cannulae in the VTA (**a**) and microdialysis probes in the NAc (**b**). Cannulae and probes were inserted unilaterally. Microdialysis probe tips (2 mm) were primarily located in the NAc shell. Diagrams were taken from Paxinos and Watson (1986), and numbers represent the anterior-posterior position in millimeters posterior from the bregma. *Gray squares* indicate misplaced injector cannulae

Fig. 3.

Time course of extracellular dopamine (mean±S.E.M.) in the NAc of rats infused with ghrelin or PBS into the VTA and pretreated with 18-MC or vehicle (40 mg/kg, i.p., 19 h prior). Asterisk, $p < 0.05$, Fisher's least significant difference (LSD) tests, vehicle-ghrelin vs. 18-MC-ghrelin. Arrows indicate injections of ghrelin (2 µg; or PBS, VTA). Inset, time course of extracellular dopamine (mean±S.E.M.) in the NAc of rats administered with either 20 mg/kg 18-MC+ghrelin (2 µg) or 18-MC+PBS into the VTA. The dose of 20 mg/kg 18-MC was delivered i.p., 20 min prior to the intrategmental injection of ghrelin or PBS. No differences in extracellular dopamine were observed between the two groups ($p > 0.05$ following repeated measures ANOVA)

Fig. 4.

Time course of extracellular DOPAC (mean ± S.E.M.) in the NAc of rats infused with ghrelin or PBS into the VTA and pretreated with 18-MC or vehicle (40 mg/kg, i.p., 19 h prior).

Asterisk $p < 0.05$, Fisher's LSD tests, vehicle-ghrelin vs. 18-MC-ghrelin. Arrows indicate injections of ghrelin (2 µg; or PBS, VTA). *Inset*, time course of extracellular DOPAC (mean ± S.E.M.) in the NAc of rats administered either 20 mg/kg 18-MC+ghrelin (2 µg) or 18-MC+PBS into the VTA. The dose of 20 mg/kg 18-MC was delivered i.p., 20 min prior to the intrategmental injection of ghrelin or PBS. No differences in extracellular DOPAC were observed between the two groups ($p > 0.05$ following repeated measures ANOVA)

Fig. 5.

Time course of extracellular HVA (mean $\pm$ S.E.M.) in the NAc of rats infused with ghrelin or PBS into the VTA and pretreated with 18-MC or vehicle (40 mg/kg, i.p., 19 h prior). Arrows indicate injections of ghrelin (2 μ g; or PBS, VTA). Ghrelin alone (*open circles*) increased accumbal HVA levels overall (time effect: $F_{14, 70} = 2.18$, $p < 0.02$) but post hoc analyses revealed no differences among the groups. *Inset*, time course of extracellular HVA (mean $\pm$ S.E.M.) in the NAc of rats administered either 20 mg/kg 18-MC+ghrelin (2 μ g) or 18-MC +PBS into the VTA. The dose of 20 mg/kg 18-MC was delivered i.p., 20 min prior to the intrategmental injection of ghrelin or PBS. No differences in extracellular HVA were observed between the two groups ($p > 0.05$ following repeated measures ANOVA)

Table 1

Mean serum levels of glucose, cholesterol, and triglycerides (milligrams per deciliter) and total weight (grams) of fat depots in rats treated with systemic 18-MC or vehicle

	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Fat depots (g)
Treatment				
18-MC (<i>n</i> =5)	165.9±3.0	63.4±3.0	101.3±3.0	8.4±1.3
Vehicle (<i>n</i> =5)	154.9±10.0	60.6±5.3	87.3±15.0	10.1±1.5

Rats received 18-MC (20 mg/kg; i.p.) or vehicle followed by an intraventricular infusion of ghrelin (1 µg) for 2 days, then PBS for 2 days (once-daily treatments; order counterbalanced). Student's unpaired *t* tests were performed for all measures; there were no differences between 18-MC and vehicle-treated groups (*p*>0.05)

Table 2

Mean basal levels of accumbal dopamine, DOPAC, and HVA in baseline samples collected during in vivo microdialysis

	Dopamine	DOPAC	HVA
Treatment			
18-MC+PBS	0.052±0.018	19.45±0.90	7.99±1.46
18-MC+ghrelin	0.052±0.015	21.22±2.69	9.63±1.52
Vehicle+ghrelin	0.054±0.010	21.49±1.68	8.14±0.77
Vehicle+PBS	0.077±0.020	20.52±4.45	10.73±2.38

Values are expressed as picomole per 10 µl. Two-way ANOVA revealed no differences between basal extracellular levels of dopamine, DOPAC, or HVA among treatment groups ($p>0.05$ for all comparisons)