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# A cannabinoid receptor antagonist attenuates ghrelin-induced activation of the mesolimbic dopamine system in mice

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ABSTRACT

Ghrelin has been attributed various physiological processes including food intake and reward regulation, through activation of the mesolimbic dopamine system. Reward modulation involves the mesolimbic dopamine system, consisting of the ventral tegmental area (VTA) dopamine neurons targeting nucleus accumbens (NAc), a system that ghrelin activates through VTA-dependent mechanisms. In the first study, we found that systemic intraperitoneal (ip) administration of rimonabant attenuated intracerebroventricular (icv) ghrelin's ability to cause locomotor stimulation and NAc dopamine release in mice. Ghrelin-induced (icv) chow intake was not altered by rimonabant administration (ip). Finally, we showed that bilateral VTA administration of rimonabant blocks the ability of intra-VTA administered ghrelin to increase locomotor activity, but does not affect food intake in mice. Collectively, these data indicate clear dissociation between regulation of food intake and activation of the mesolimbic dopamine system.

## 1. Introduction

Regulation of energy homeostasis involves complex interactions between multiple signaling systems, among which ghrelin is the only orexigenic hormone identified to date (for review see [1,2]). Ghrelin was originally recognized for its ability to increase food intake as well as appetite [3–6] via circuits within the hypothalamus, amygdala and nucleus of the solitary tract [5,7,8]. Accordingly, elevated ghrelin levels are associated with meal initiation [9] and correlate with hunger scores in healthy subjects [10].

More recently, studies have identified ghrelin as a modulator of additional brain functions including reward, memory and mood [11–13]. Both intracerebroventricular (icv) and peripheral administration of ghrelin activate the mesolimbic dopamine system as shown by increased locomotor stimulation, increased nucleus accumbens (NAc) dopamine release, firing of dopamine neurons and increased dopamine turnover in rodents [14–18]. In agreement with those findings, a functional magnetic resonance imaging study showed that intravenous ghrelin administration to healthy volunteers increased neural response to food pictures in brain areas responsible for the motivational value of environmental stimuli, including the striatum [19].

Reward is at least in part mediated via the mesolimbic dopamine circuit, which consists of dopamine producing neurons projecting from the ventral tegmental area (VTA) to the NAc [20]. Nevertheless, reward processing is complex and involves other neural systems and neurotransmitters, like endogenous opioids [21]. Preclinical studies show that intra-VTA administration of ghrelin activates this dopaminergic projection in rodents [22,23] and increases motivation to obtain palatable food [6,24]. Ghrelin receptors (GHS-R1A) are expressed on dopaminergic neurons in the VTA [16], suggesting the possibility that ghrelin stimulates the mesolimbic dopamine system via ventral tegmental mechanisms. Previous reports have shown that this ghrelin-induced stimulation involves nicotinic acetylcholine receptors, NMDA as well as delta opioid receptors in the VTA, rather than orexin and μ opioid receptors [25–27]. Systems that regulate the activity of VTA dopamine neurons also include the endocannabinoids (for review see [28]) and the orexigenic properties of ghrelin are modulated by cannabinoid signaling as shown by the findings that 1) the ability of ghrelin to increase food intake is abolished in cannabinoid type 1 (CB1) receptor knockout mice, 2) pharmacological as well as genetic suppression of CB1 receptors abolishes the CB1 – AMP-activated protein kinase appetite signaling which is induced by ghrelin and 3) CB1 receptor blockade counteracts the orexigenic properties of icv ghrelin administration [29,30].

Together, these observations lead to the present hypothesis that CB1 receptors located in the VTA may regulate the ability of ghrelin to

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activate the mesolimbic dopamine system. The present study was therefore designed to investigate the effects of systemic administration (intraperitoneal, ip) of the CB1 antagonist rimonabant on the ability of icv-administered ghrelin to cause a locomotor stimulation and NAc dopamine release as well as its effect on chow and peanut butter in satiated mice. We also evaluated the ability of rimonabant (ip) to influence ghrelin mediated chow consumption (as an indicator of food intake) and palatable food (peanut butter) consumption (as an indicator of food reward) in satiated mice. In addition, the possibility that ventral tegmental CB1 receptors regulate ghrelin-induced locomotor stimulation, as well as food and palatable food intake, was evaluated. The present experiments will thus contribute to the identification of neurochemical mechanisms involved in ghrelin-induced activation of the mesolimbic dopamine system and the possible dissociation from ghrelin's food and palatable food intake regulation.

## 2. Materials and methods

### 2.1. Animals

Adult post-pubertal age-matched male NMRI mice (8–12 weeks old and 25–40 g body weight; Charles River; Susfeldt, Germany) were used. The mice were maintained at a 12/12 h light/dark cycle and at 20 °C with 50% humidity and were allowed to acclimatize at least one week before the start of the experiments. Tap water and food (normal chow; Harlan Teklad; Norfolk, England) were supplied ad libitum. The experiments were approved by the Swedish Ethical Committee on Animal Research in Gothenburg. All efforts were made to minimize animal suffering, and to reduce the number of animals used. Each experiment used an independent set of mice.

### 2.2. Drugs

Acylated rat ghrelin (Bionuclear; Bromma, Sweden) was diluted in Ringer solution (NaCl 140 mM, Ca $Cl_2$ 1.2 mM, KCl 3.0 mM and $MgCl_2$ 1.0 mM; Merck KGaA; Darmstadt, Germany) for intracerebroventricular (icv, third ventricle) as well as VTA administration. The selected doses for icv (1 μg in 0.5 μl) as well as intra-VTA (1 μg in 0.5 μl per side) administration of ghrelin previously been shown to increase locomotor activity and NAc dopamine release [15,22] as well as palatable food intake [31] in mice. Local intracranial, as opposed to peripheral injections were used since the effects of ghrelin on food intake as well as activation of the mesolimbic dopamine system is more pronounced and reliable following icv administration. In addition, icv ghrelin injections are more commonly used allowing comparison to previous studies. Rimonabant, a CB1 receptor antagonist (NIDA Drug Supply Program), was diluted in 5.5% D-glucose and acetic acid (HAc), 10 ml/kg for peripheral administration (ip) and in 75% DMSO + 25% Ringer for bilateral intra VTA-administration. A dose of 1 mg/kg (ip) was used, since our first dose-response study indicated it as the highest without an effect on baseline locomotor activity. For bilateral administration into the VTA, a dose of 2 μg in 0.5 μl per side was selected from our second dose-response study, since this was the highest without an effect per se. For icv ghrelin administrations, Ringer was used as the dilution solution. For the control groups, the respective dilution solutions were used as vehicle solutions, allowing comparison of behavior to appropriate vehicle treatment rather than e.g. DMSO free vehicle solutions.

### 2.3. Guide cannula implantation

For administration of ghrelin, rimonabant or vehicle solution into the brain, guide cannulas (one targeting the third ventricle (icv) or two for bilateral VTA administrations) were surgically implanted four days prior to the experiments. Mice were anesthetized with isoflurane (Isoflurane Baxter; Univentor 400 Anaesthesia Unit, Univentor Ldt., Zejtun, Malta), placed in a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA) and kept on a heating pad to prevent hypothermia. The skull bone was exposed and two drops of a 5 μg/ml solution of xylocaine adrenaline (Pfizer Inc.; New York, USA) was used as local anesthetic. Subsequently, one or two holes for the guide cannulas (stainless steel, length 10 mm, with an o.d./i.d. of 0.6/0.45 mm) and one for the anchoring screw were drilled. The guide cannulas were placed according to the following coordinates from Bregma; VTA: 3.4 mm AP, ± 0.5 mm ML and 1.0 mm below the brain surface and third ventricle: 0.9 mm AP, ± 0.0 mm ML and 1.0 mm below the brain surface [32]. Guide cannulas and screws were stabilized with dental cement (DENTALON plus; AgnTho's AB; Lidingö, Sweden). Carprofen (5 mg/kg subcutaneously, Rimadyl®; Astra Zeneca, Gothenburg, Sweden) was used to relieve pain following surgery. The mice were thereafter kept in individual cages (Macrolon III) for four days prior to the experiment. At the day of the experiment, a dummy cannula targeting the infusion area (third ventricle or VTA) was inserted and then retracted to remove clotted blood and hamper spreading depression. The dummy as well as injection cannula was inserted and extended another 3.8 mm or 1.1. mm ventrally beyond the tip of the guide cannula implanted during surgery, aiming at VTA or third ventricle (icv) respectively. One hour later, a cannula for drug administration was inserted and the drug was infused. For all local injections a 5 μl syringe (Kloehn microsyringe; Skandinaviska Genetec AB, V. Frölunda, Sweden) was used, which was connected to a graded thin tubing. The drugs were infused over a period of 1 min and the flow was remarked with the help of a bubble in the tubing, thus monitoring the volume infused. This allows injection of 0.5 μl into either the third ventricle or per side into the VTA. The cannula was left in place for another minute and was then retracted. The cannula placement was verified following the termination of the experiment and only animals with the correct placement were included in the statistical analysis.

### 2.4. Locomotor activity experiments

Locomotor activity experiments were performed to assess dopamine system, providing that this behavior involves dopamine and may in all probability involve several other neurotransmitters [33–35].

#### 2.4.1. Apparatus

Locomotor activity was registered in six sound attenuated, ventilated and dim lit locomotor boxes (Open Field Activity System; Med Associates Inc., Fairfax, Vermont, USA; 420 × 420 × 200 mm). In this system, 15 × 15 infrared beams at the bottom of the floor allow a computer-based system to register the distance travelled (cm per 5 min) of each mouse during 60 min. Prior to drug administrations the mice were allowed to habituate to the locomotor activity box for 60 min.

#### 2.4.2. Procedures

*Experiment 1.1* was conducted to establish the highest dose of systemic administration of rimonabant, which has no effect on baseline locomotor activity. Vehicle (ip) or rimonabant (1, 3 or 6 mg/kg, ip) were injected and locomotor activity of the mice was recorded.

In *experiment 1.2*, the effects of rimonabant (1 mg/kg, ip) on ghrelin-induced (1 μg, icv) locomotor stimulation were investigated. Rimonabant or vehicle was administered 30 min prior to ghrelin or vehicle, as the dose response study showed that this time frame was necessary for the expression of the drug's effect. Each mouse received one treatment combination in a 2 × 2 factorial between subjects design (vehicle/vehicle, vehicle/ghrelin, rimonabant/vehicle or rimonabant/ghrelin) and was only subjected to one experimental trial.

*Experiment 1.3* was conducted to establish the highest dose of rimonabant into the VTA, which does not affect baseline locomotor activity. Thus, the effects of bilateral intra-VTA administration of rimonabant (1, 2 or 4 μg in 0.5 μl per side) or an equal volume of vehicle on locomotor activity were investigated in mice.

In *experiment 1.4*, the effects of bilaterally administered intra-VTA