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5-HT1A receptor antagonists reduce food intake and body weight by reducing total meals with no conditioned taste aversion

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ABSTRACT

Serotonin acts through receptors controlling several physiological functions, including energy homeostasis regulation and food intake. Recent experiments demonstrated that 5-HT1A receptor antagonists reduce food intake. We sought to examine the microstructure of feeding with 5-HT1A receptor antagonists using a food intake monitoring system. We also examined the relationship between food intake, inhibition of binding and pharmacokinetic (PK) profiles of the antagonists. Ex vivo binding revealed that, at doses used in this study to reduce food intake, inhibition of binding of a 5-HT1A agonist by ~40% was reached in diet-induced obese (DIO) mice with a trend for higher binding in DIO vs. lean animals. Additionally, PK analysis detected levels from 2 to 24 h post-compound administration. Male DIO mice were administered 5-HT1A receptor antagonists LY439934 (10 or 30 mg/kg, p.o.), WAY100635 (3 or 10 mg/kg, s.c.), SRA-333 (10 or 30 mg/kg, p.o.), or NAD-299 (3 or 10 mg/kg, s.c.) for 3 days and meal patterns were measured. Analyses revealed that for each antagonist, 24-h food intake was reduced through a specific decrease in the total number of meals. Compared to controls, meal number was decreased 14–35% in the high dose. Average meal size was not changed by any of the compounds. The reduction in food intake reduced body weight 1–4% compared to Vehicle controls. Subsequently, a conditioned taste aversion (CTA) assay was used to determine whether the feeding decrease might be an indicator of aversion, nausea, or visceral illness caused by the antagonists. Using a two bottle preference test, it was found that none of the compounds produced a CTA. The decrease in food intake does not appear to be a response to nausea or malaise. These results indicate that 5-HT1A receptor antagonist suppresses feeding, specifically by decreasing the number of meals, and induce weight loss without an aversive side effect.

1. Introduction

Serotonin control of feeding behavior has been studied since the 1960s (Joyce and Mrosovsky, 1964; Myers and Yaksh, 1968). Multiple lines of evidence indicate the 5-HT1A receptor to be one of the several serotonergic receptor subtypes involved in physiological functions including control of energy balance. The 5-HT1A receptors are extensively distributed in key feeding centers throughout the brain including the PVN, arcuate and ventromedial nuclei along with the lateral hypothalamic area (Leibowitz and Alexander, 1998; Collin et al., 2000; Voigt et al., 2000). Early evidence demonstrated that both the 5-HT1A receptor selective partial agonist ipsapirone and the full agonist 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin), could promote food intake in rodents (Dourish et al., 1985; Gilbert et al., 1988; De Vry and Schreiber, 2000; Ebenezer and Surujbally, 2007). Moreover, the hyperphagia induced by the 5-HT1A receptor agonist in mice could be abolished by simultaneous administration of the selective 5-HT1A receptor antagonist WAY100635.

Early experiments testing the ability of 5-HT1A receptor antagonists to regulate energy balance demonstrated a reduction in the intake of palatable food (Moreau et al., 1992) when the antagonists were administered alone or in combination with SSRIs (Li et al., 1998). Recently, a selective 5-HT1A receptor antagonist has been shown to reduce 24-h food intake in leptin deficient ob/ob mice or chow fed WT mice when administered by itself. Moreover, the hypophagia was absent in mice in which the 5-HT1A receptors had been specifically knocked out of POMC neurons in the arcuate nucleus indicating the regulation of appetite was being directly modulated by the 5-HT1A expression in these hypothalamic neurons (Yadav et al., 2011).

While these recent data are suggestive that the 5-HT1A receptor system can play a role in the regulation of energy balance, previous data with WAY100635, a 5-HT1A receptor antagonist, demonstrated that, in normal weight rats, low doses of the compound did not affect food intake (Arkle and Ebenezer, 2000). Moreover, some 5-HT1A compounds have been shown to invoke conditioned taste aversions in rodents and nausea in humans (Feighner and Boyer, 1989; Wegener et al., 1997). Since drug-induced malaise or nausea in rodents can reduce food intake

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due to their lack of an emetic response, it remains that the hypophagia after dosing with 5-HT1A receptor antagonists at the higher doses could be due to nausea or general malaise.

In the present study, we investigated four different 5-HT1A receptor antagonists in both lean and DIO mice to inhibit binding at the 5-HT1A receptor. We then investigated if the four different 5-HT1A receptor antagonists could reduce food intake by either specifically altering the size of meals or the number of meals consumed by diet-induced obese (DIO) mice by analyzing feeding microstructure utilizing a food intake monitor system. We further investigated whether the four 5-HT1A receptor antagonists produced a conditioned taste aversion in mice indicating the reduction in food intake was due to aversive or nauseating properties of the compounds and not the proposed centrally regulated pathways. Pharmacokinetic (PK) analysis was also completed with each compound at the highest dose to examine the PK relationships with the food intake effects.

2. Methods

2.1. Animals

All animals were housed under a 12 h light/dark cycle and controlled temperature (72–74 °F) with water and food provided ad libitum unless otherwise noted. Chow fed "lean" animals were used for the ex vivo and CTA studies. Male, C57Bl/6 mice from Harlan Laboratories (Indianapolis, IN) or Taconic Labs (Germantown, NY) were maintained on Teklad 2014 chow. Adult male diet-induced obese (DIO) C57Bl/6 mice (20–22 weeks of age, ~30–45 g body weight, Taconic or Harlan Laboratories) were maintained on Teklad Adjusted Fat Diet TD95217 (~40% fat, ~40% sucrose) for the ex vivo, pharmacokinetic and food intake monitor studies. All experimentation was performed with approval from the Eli Lilly Institutional Animal Care and Use Committee in accordance with the NIH Guide for Care and Use of Laboratory Animals.

2.2. Compounds

The 5-HT1A receptor antagonists LY439934 (LY426595.HCl), WAY100635, and SRA-333 (also known as Lecozotan) were synthesized at Eli Lilly and Company. NAD-299 (also known as Robalzotan) was purchased from Tocris Bioscience, Minneapolis, MN. LY439934 and SRA-333 were dissolved in sterile water (Hospira, Inc., Lake Forest, IL) and orally dosed at 10 or 30 mg/kg. A 1% Lactic acid (L-(+)-lactic acid solution, 30% w/w, Sigma-Aldrich, St. Louis, MO) was added to SRA-333 to facilitate compound dissolution. WAY100635 and NAD-299 were dissolved in 0.9% Saline (Hospira, Inc., Lake Forest, IL) and administered at 3 or 10 mg/kg subcutaneously. All solutions were prepared on the day of dosing and kept at room temperature until administration. LiCl for the condition taste aversion studies was dosed IP at 0.30 M, 2% of body weight. Drug dose and route of administration was selected based on published reports outlining preparation, route and experimental results along with internal data examining ex vivo binding results.

2.3. Ex vivo binding

Chow fed "lean" and DIO mice (n = 5/group) were dosed either with vehicle or compound and sacrificed by decapitation 90 min later for dose response evaluation. The cerebral cortex was dissected, homogenized in 10 volumes of 50 mM TRIS buffer and frozen overnight at −80 °C. Homogenates were incubated at 37 °C. Binding of each compound to the 5-HT1A receptors was evaluated by incubating aliquots of homogenate with [^{3}H]-8-OH-DPAT (1.6 nM) in 50 mM TRIS buffer for 30 min at room temperature. To assess non-specific binding, a set of homogenates from each group were incubated with 50 μM WAY100635. The reaction mixtures were filtered under vacuum with a cell harvester fitted with a Whatman GF/B glass fiber filter. Radioactivity was measured by a liquid scintillation spectrophotometer. Percent of vehicle control inhibition was calculated for each dose response study.

2.4. BioDAQ food intake monitors

Animals were individually housed and acclimated to the system for a minimum of 2 weeks and after sufficient acclimation to the system caging, studies were initiated. The ex vivo studies indicated a higher level of 5-HT1A receptor binding in the DIO, high fat-fed mouse compared to the chow-fed mouse, as also reported in the literature (Li et al., 2011; Peleg-Raibstein et al., 2012), so DIO mice were used in the feeding and pharmacokinetic studies. Additionally, the TD95217 high fat diet the DIO mice were maintained on is a solid pellet which does not crumble easily and aids in the cleanliness of the food monitor to help reduce the possibility of monitor malfunctioning from food interference in the system. Feeding behavior was monitored using the BioDAQ Food Intake Monitor system (Research Diets Inc., New Brunswick, NJ) for mice allowing constant recording of food acquisition in the home cage with limited disturbance from outside manipulation. The system consisted of a food hopper affixed on a peripheral sensory controller attached to the home cage. Water bottles were placed on the wire lid and offered ad libitum. Cages had a wire floor with Alpha-dri® bedding on cage bottom. This minimized the inadvertent deposition of bedding material in the monitor or hopper that could result in erroneous feeding activity as well as minimizing coprophagia. Animals were also provided nesting material for enrichment.

On Day −1, mice were dosed with vehicle and the microstructure of feeding was monitored for 24 h. On Day 1 animals were randomized into 3 groups (n = 8/group) based on 24-h food intake following vehicle dosing. On Days 1 through 3 animals were dosed either with compound or vehicle. Manual body weights and food hopper weights were measured daily as well. The experiment and data collection ended 24 h after the last dose.

2.4.1. BioDAQ analysis

Microstructure analysis of feeding activity was performed for each 24-h post-dose period. Following compound administration, feeding was measured from the time the gates to the feed hoppers were opened until gates were closed for measurements the following day. Dosing was performed approximately 30 min prior to "lights out". Gates were opened only after all animals were dosed.

The BioDAQ food monitoring system records the weight of the food hopper once per second. A bout is initiated when the hopper is disturbed by the animal and the weight becomes unstable. A bout is defined as change in weight of hopper at time unstable weight is initiated until a stable weight can be re-established. A meal is defined as the number of bouts following a predetermined amount of stable hopper weight time (inter-meal interval (IMI)) and a minimal amount of food intake. All of the studies outlined had an IMI defined as 10 min and a minimum meal amount of 0.02 g. Meal patterns measured by the BioDAQ system and analyzed included number of meals, meal size, meal onset, post-meal interval, total meal intake and time spent in meals. Data was exported from BioDAQ software, BioDAQ Data Viewer 2.2.02, into Excel spreadsheet for analysis. Although manual food intake was measured in all experiments, all food intake analysis reported in the manuscript is from the BioDAQ data.

Food intake analysis was broken down into varying timeframes to determine when onset of effect occurred. Effects on food intake were seen in the LY439934 as early as 2 h post dose, however, with other compounds the effect varied with longer times (~6 h post dose in WAY100635 and 12 h post dose in SRA-333) to find significant changes from the Vehicle dosed group. The pattern and rate of food intake did not vary greatly between the earlier timepoints where significance started to be measured and 24-h data; therefore, 24-h parameters are reported for final results and analysis.