Appetite Suppression and Weight Reduction by a Centrally Active Aminosterol

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The rise in obesity and its complications has generated enormous interest in the regulation of feeding and body weight. We show that a spermine metabolite of cholesterol (MSI-1436) decreases body weight, specifically fat, by suppressing feeding and preventing the reduction in energy expenditure, hormonal changes, and patterns of neuropeptide expression normally associated with weight loss. MSI-1436 enters the brain after peripheral injection and is more potent when injected into the cerebral ventricle (intracerebroventricular [ICV]). Systemic or ICV MSI-1436 administration induced similar patterns of Fos immunoreactivity in the brain, especially the paraventricular hypothalamic nucleus (PVN). This brain region integrates neural signals from hypothalamic and brain stem nuclei and regulates feeding behavior, autonomic function, and neuroendocrine function. Microinjection of MSI-1436 into the PVN potently suppressed feeding and reduced body weight for several days. Unlike calorie restriction, MSI-1436 decreased mRNA levels of agouti-related peptide and neuropeptide Y in the hypothalamus. These findings indicate that MSI-1436 acts in the brain to regulate food intake and energy expenditure, likely through suppression of orexigenic hypothalamic pathways. Diabetes 51: 2099–2104, 2002

Obesity is highly prevalent in the U.S. and other developed countries and is increasing worldwide (1,2). This epidemic has serious public health consequences because obesity is associated with excess mortality and morbidity from type 2 diabetes, cardiovascular disease, and other complications (1,2). Diet and exercise remain the cornerstone of obesity management; however, it is likely that many patients will require drug treatment to reduce body weight and prevent complications (3). Here, we describe the anti-obesity action of a novel aminosterol. MSI-1436 is a spermine metabolite of cholesterol that was originally isolated from the dogfish shark (Squalus acanthias) liver during a search for naturally occurring antimicrobial compounds (4,5). MSI-1436 is structurally similar to squalamine (MSI-1256) except for a spermine side-chain at C-3 on the cholesterol A-ring (4,5). The bioactivity of MSI-1436 is also dependent on a seven α-OH and sulfated moiety at C-25 (5). Unexpectedly, MSI-1436 was shown to inhibit feeding and decrease body weight in a highly specific manner in normal and obese rodents (5).

MSI-1436 is distributed to the brain and several peripheral tissues (5). A single or intermittent treatment with MSI-1436 results in a prolonged reduction in food intake and body weight and has been partly attributed to its long half-life (~7 days in rodents) (5). However, it is unclear whether MSI-1436-induced weight loss is due to appetite suppression alone. Moreover, although it had been suggested that MSI-1436 is more potent when administered into the cerebral ventricle, its targets in the central nervous system are unknown. The objective of this study was to investigate the contributions of energy intake and expenditure to the sustained effect of MSI-1436 on body weight and determine whether the biological activity of MSI-1436 in the brain is mediated by well-known hypothalamic neuronal pathways that mediate feeding behavior and energy balance.

RESEARCH DESIGN AND METHODS

Experiments were performed in accordance with guidelines and regulations of the National Institutes of Health and Institutional Animal Care and Use Committee of the University of Pennsylvania.

Determine the effect of MSI-1436 on food intake and energy expenditure. Male 12-week-old C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) were housed individually in a 12:12 h light-dark cycle (lights on at 0600; temperature 22°C) and allowed normal laboratory diet and water ad libitum. Synthetic MSI-1436 and squalamine (MSI-1256) were provided by Genaera (Plymouth Meeting, PA). Our preliminary studies confirmed that squalamine did not affect food intake or body weight (5). In contrast, intraperitoneal (IP) injection of MSI-1436 during the light or dark cycle reduced food intake and body weight in a dose-dependent manner (data not shown). We selected a lower dose of MSI-1436 that did not suppress drinking as previously described (5). MSI-1436 (5 mg/kg IP × three doses) was administered at 0900–1000 at 3-day intervals to a group of mice (n = 6). Control mice (n = 6) were treated with vehicle (100 μl endotoxin-free H2O IP). A third group of mice (n = 6) was pair-fed to the daily intake of MSI-1436 mice. Indirect calorimetry was performed after the final treatment. The mice were acclimatized to the metabolism cage for 2 days, and energy expenditure was measured at 15-min intervals for 24 h on the third day (Oxymax Equalflow System; Columbus Instruments, Columbus, OH) (6). The following settings were used per cycle: air flow 500 ml/min, sample flow 400 ml/min, settle time 120 s, measuring time 50 s, temperature 22°C, respiratory exchange ratio (RER) = volume of carbon dioxide generated (VCO2) divided by oxygen consumption (VO2). Heat (kcal/h) = 3.815 + 1.232 × RER. Total and ambulatory activity were measured simultaneously with photodetectors (Optovarimex System; Columbus Instru-
The mice were killed by CO₂ inhalation, and blood was obtained by cardiac puncture. Glucose and triglycerides (Sigma, St. Louis, MO) and nonesterified fatty acids (NEFAs) (Wako Chemicals, Richmond, VA) were measured with enzyme assays. Plasma insulin, leptin, corticosterone, and thyroxine were measured by radioimmunoassay as previously described (7,8). Uncoupling protein (UCP)-1 mRNA was measured in brown adipose tissue (BAT) by Northern blot analysis using a cDNA probe (provided by Mitch Lazar, University of Pennsylvania). The rest of the carcass was dried to a constant weight at 60°C to determine water content and digested in ethanol–KOH, and fat (triglyceride) content was measured with a colorimetric assay (Sigma) (8).

We determined whether MSI-1436 could prevent the hyperphagia normally associated with fasting. Male 12-week-old C57Bl/6J mice were fasted for 48 h and received a single injection of MSI-1436 (5 mg/kg IP) or vehicle (n = 6/group) after the fast. They were housed in individual cages and allowed ad libitum access to normal diet and water. Food intake and body weight were measured daily. Feeding frequency and duration were monitored using infrared detectors (Vitalview System; Mini Mitter, Bend, OR). The data on feeding frequency and duration were collated in 1-h bins and analyzed using Actiview software (Mini Mitter).

**Determine the dose response to intracerebroventricular versus systemic MSI-1436 treatment.** Male Sprague-Dawley rats (250–300 g) were obtained from Harlan Industries (Indianapolis, IN), housed under a 12-h light–dark cycle (lights on at 0600; temperature 22°C), and allowed normal laboratory food and water ad libitum. The animals were anesthetized with sodium pentobarbital, and a 22-gauge stainless steel guide cannula with obturator (Plastics One, Roanoke, VA) was implanted unilaterally in the lateral cerebral ventricle using the following coordinates: 0.8 mm posterior to lambda (Plastics One), 1.0 mm lateral to the midline, and 4 mm below the skull. The intracerebroventricular (ICV) cannula was attached to the cranial with stainless steel screws and dental cement. Cannula placement was verified with the drinking response to ICV angiotensin II injection and histologically after the experiment. Only data from rats with correctly positioned cannulas were included in the analysis.

The animals were housed individually after surgery and handled daily to habituate, and two sham injections were performed at 3-day intervals. Experiments were performed after restoration of body weight (~1 week). MSI-1436 (10 or 30 µg) or vehicle (5 µl endotoxin-free H₂O) was administered ICV over 1 min to unanesthetized rats (n = 6/group) with a preweighed amount of pellet food, and food consumption and body weight at 60 min were measured. Feeding frequency and duration were monitored using infrared detectors (Vitalview System; Mini Mitter, Bend, OR). The data on feeding frequency and duration were collated in 1-h bins and analyzed using Actiview software (Mini Mitter).

**Results**

MSI-1436 decreases body weight by inhibiting food intake and increasing energy expenditure. To ascertain whether weight reduction by MSI-1436 was mediated entirely by inhibition of feeding, the daily intake of a group of pair-fed mice was matched with that of MSI-1436–treated mice. Cumulative food intake was decreased by ~20% in MSI-1436–treated and pair-fed mice (Fig. 1A); however, body weight (Fig. 1B) and body fat (Fig. 1C) were significantly lower in MSI-1436–treated mice than in pair-fed mice, indicating that inhibition of food intake alone could not account for the weight loss in MSI-1436–treated mice. Oxygen consumption (VO₂) (Fig. 1D) and heat (data not shown) were maintained at high levels in MSI-1436–treated mice despite weight reduction. RER, an index of metabolic fuel use, was not different between MSI-1436–treated (0.8 ± 0.01) and vehicle-treated (0.84 ± 0.02) mice. In contrast, VO₂ was lower in pair-fed mice (Fig. 1D). RER was significantly lower in pair-fed mice (0.77 ± 0.02) than in vehicle-treated mice (0.84 ± 0.02) (P < 0.05). Body temperature, UCP-1 mRNA expression in BAT, and locomotor activity (beam breaks) were not affected by MSI-1436 (data not shown).

Serum triglycerides, insulin, and leptin were reduced in both MSI-1436–treated and pair-fed mice (Table 1). NEFAs and the NEFA-to-triglyceride ratio were twofold greater in pair-fed mice than in MSI-1436–treated mice, consistent with increased lipolysis (Table 1). Thyroxine was decreased and corticosterone was increased in pair-fed mice but remained normal after MSI-1436 treatment (Table 1). Feeding frequency and food consumption increased rapidly in vehicle-treated fasted mice, leading to restoration of body weight within 48 h (Fig. 2A–C). In contrast, MSI-1436 blunted the postfast hyperphagia and weight gain (Fig. 2A–C). The duration of feeding bouts was not significantly different between MSI-1436 treatment and pair-feeding (data not shown).

**The potency of MSI-1436 is greater after central administration than after peripheral treatment.** Cerebroventricular injection of MSI-1436 resulted in a dose-related decrease in food intake and body weight (Fig. 3A and B). Water intake was reduced slightly by the highest ICV dose (30 µg MSI-1436) but was not statistically significant (33.7 ± 2.6 vs. 30 ± 4.6 ml/24 h; P = 0.21). The effective ICV dose of MSI-1436 was <1,000 of the periph-
eral dose (Fig. 3A and B). In all cases, body weight was restored several days after MSI-1436 treatment (Fig. 3B). MSI-1436 treatment did not cause taste aversion, as evidenced by a normal saccharin preference ratio of 0.8 in vehicle, ICV, and IP MSI-1436–treated rats. In contrast, saccharin preference ratio was markedly suppressed to 0.38 ($P < 0.001$) after LiCl administration, consistent with aversion behavior.

**MSI-1436 induces Fos immunoreactivity in the PVN and reduces food intake and body weight after direct injection into the PVN.** The distribution of potential MSI-1436 targets in the brain was evaluated using Fos immunohistochemistry. A robust induction of Fos-immunoreactive cells was detected in the PVN after central or peripheral MSI-1436 administration (Fig. 4A and B). Fewer numbers of Fos-positive cells were observed in other forebrain regions involved in ingestive behavior, energy balance, and glucose homeostasis, i.e., arcuate nucleus, perifornical region, zona incerta, lateral hypothalamic area, dorsolateral and ventromedial hypothalamic nuclei, and central amygdala (Fig. 4B). Injection of MSI-1436 into the PVN caused a dose-dependent reduction in food intake (Fig. 4C) and body weight (Fig. 4D). As with ICV treatment, the response to a single PVN injection persisted for several days (Fig. 4C and D).

**DISCUSSION**

MSI-1436 and other aminosterols that affect feeding were discovered serendipitously during a search for antimicrobial compounds in the dogfish shark (4,5). Initial studies showed that MSI-1436 decreased body weight over long periods. However, there was a lack of understanding as to whether the sustained reduction in body weight was due solely to appetite suppression. Our studies provide evidence in support of a specific suppression of feeding as well as increased energy expenditure after MSI-1436 treatment. Importantly, MSI-1436 reduced body weight (specifically fat) without provoking the well-known response to caloric depletion (16,17). In the physiological model of energy homeostasis, the amount of energy stored in adipose tissue reflects the balance between energy intake and expenditure (16). A decrease in energy stores from fasting triggers various responses, e.g., reduced energy expenditure and overfeeding in an attempt to restore body weight (16). Other typical responses to food deprivation include decreased thyroid hormone, increased glucocorticoids, and increased lipolysis (evidenced by an increased NEFA-
These responses, which are mediated at least in part by reduced leptin level, are designed to defend body weight and lean mass by reducing energy expenditure and stimulating feeding (16,17). The counter-regulatory mechanisms may be responsible for the failure to sustain weight loss during forced caloric restriction (16). MSI-1436 was effective in reducing food intake and body weight in ad libitum–fed animals for several days and also prevented the hyperphagia and rapid weight gain normally associated with fasting, indicating that its pharmacological effects can override the physiological tendency to gain weight. The ability of MSI-1436 to maintain high-energy expenditure despite weight loss is not likely to be mediated through UCP-1 or locomotor activity because these factors were not altered.

Based on a previous study (5) and our own study (data not shown) showing that MSI-1436 reaches the brain after peripheral injection, we compared the dose-effect of ICV treatment versus systemic MSI-1436 treatment. Administration of MSI-1436 ICV was >1,000 times more potent than intraperitoneal treatment. Central or peripheral MSI-1436 treatment did not cause taste aversion, thus providing evidence against visceral illness as a cause of the reduced food intake and body weight. We mapped poten-
tial targets of MSI-1436 in the brain using Fos immunostaining. The induction of Fos immunoreactivity in the PVN is consistent with neuronal activation and might explain the effects of MSI-1436 on feeding and energy expenditure because this hypothalamic region mediates feeding behavior and autonomic function (10,16,17). Fos immunoreactivity in the PVN has been shown to be regulated by a variety of factors, such as leptin, urocortin, corticotropin-releasing factor, and AGRP (10–13). We observed fewer Fos-positive cells in other hypothalamic and forebrain regions implicated in energy homeostasis. However, activation of Fos within the PVN does not necessarily prove that MSI-1436 acts at this site. Thus, we determined whether direct administration of MSI-1436 into the PVN would produce an effect similar to that of ICV or peripheral MSI-1436 injection. Administration of one-thirtieth the ICV dose of MSI-1436 into the PVN resulted in a sustained reduction in food intake and body weight, suggesting that the PVN is a specific target of MSI-1436.

The PVN receives neuronal input from the arcuate nucleus, other hypothalamic regions, and the brainstem (16,17). The orexigenic neuropeptides NPY and AGRP are produced by the same neurons in the arcuate nucleus and increase body weight by stimulating appetite and increasing energy expenditure (16,17). Conversely, α-melanocyte-stimulating hormone (α-MSH) is produced by POMC neurons in the arcuate nucleus and decreases body weight by inhibiting appetite and increasing expenditure (16,17). The cellular action of α-MSH is mediated through antagonism of AGRP at melanocortin receptor-3 (MCR3) and MCR4. Hypothalamic neuronal circuits expressing these neuropeptides respond to leptin, insulin, glucocorticoids, and other endogenous factors (16,17) and can be pharmacologically influenced by other molecules such as ciliary neurotropic factor (CNTF) and the fatty acid synthase inhibitor C75 (18–21). MSI-1436 significantly inhibited AGRP mRNA and, to a lesser extent, NPY mRNA expression in the hypothalamus. This response was distinct from caloric deprivation, in which AGRP and NPY mRNA expression increased, consistent with their roles as stimulators of feeding (16,17).

We speculate that the central action of MSI-1436 is mediated at least in part through inhibition of AGRP/AGRP projection to the PVN (16,17). The suppression of AGRP may explain the prolonged inhibition of feeding by MSI-1436 because AGRP has the unique ability to regulate food intake and body weight for several days after a single central injection (21). The ability of MSI-1436 to profoundly inhibit appetite while increasing energy expendi-
turer for long periods distinguishes it from other weight-reducing compounds. In case the pharmacology of MSI-1436 is shown to be similar in humans, these properties could prove to be advantageous by counteracting the homeostatic metabolic responses that resist weight loss.

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