Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 497 (2004) 41–47
www.elsevier.com/locate/ejphar

Melanin-concentrating hormone-1 receptor antagonism decreases feeding by reducing meal size

Timothy J. Kowalski[a,*], Constance Farley[a], Mary E. Cohen-Williams[b], Geoffrey Varty[b], Brian D. Spar[a]

[a]*Cardiovascular/Metabolic Disease, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[b]*CNS Research Division, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

Received 1 March 2004; received in revised form 3 June 2004; accepted 17 June 2004
Available online 21 July 2004

Abstract

Prior work has demonstrated that melanin-concentrating hormone-1 (MCH-1) receptor antagonism decreases food intake and body weight in obese rodents. The purpose of this study was to determine if the MCH-1 receptor antagonist-mediated hypophagia was due a decrease in meal size, meal frequency, or both. We performed a meal pattern analysis in free-feeding hyperphagic diet-induced obese (DIO) rats treated with 1, 3 or 10 mg/kg p.o. of the MCH-1 receptor antagonist T-226296 (a (−)enantiomer of *N*-[6-(dimethylamino)-methyl]-5,6,7,8-tetrahydro-2-naphthalenyl]-4′-fluoro[1,1′-biphenyl]-4 carboxamide). Food intake was continuously monitored for 24 h using a BioDAQ® food intake monitoring system. A total of 10 mg/kg T-226296 significantly decreased body weight and 24-h food intake, and had no effect on locomotor activity. The decrease in food intake was due to a reduction in meal size, not meal frequency. We conclude that MCH-1 receptor antagonism with T-226296 decreases food intake in DIO rats by selectively reducing meal size, and that the reduced food intake is not due to a generalized behavioral malaise.

Keywords: Spontaneous feeding; Chronic obesity; Diet-induced obese, rat; Locomotor activity

1. Introduction

Melanin-concentrating hormone (MCH) has been implicated in the control of feeding behavior and energy homeostasis. Within the central nervous system, MCH is expressed in the lateral hypothalamus and the zona incerta (Bittencourt et al., 1992). Hypothalamic MCH expression is regulated by the nutritional status of the animal, with starvation or deficient leptin signaling ($Lep^{ob/ob}$) increasing mRNA levels (Qu et al., 1996). Intracerebroventricular (i.c.v.) MCH administration produces hyperphagia (Qu et al., 1996) while obesity and insulin resistance result from chronic MCH administration (Della-Zuana et al., 2002; Ito et al., 2003) or MCH overexpression (Ludwig et al., 2001). Lastly, ablation of the prohormone precursor of MCH (which encodes MCH, neuropeptide EI and neuropeptide GE) results in hypophagia and leanness (Shimada et al., 1998).

In rodents, MCH acts through the MCH-1 receptor, which is a member of the G-protein coupled receptor family (Saito et al., 1999). Messenger RNA for MCH-1 receptor has been identified in several brain regions including those associated with olfaction, the hippocampus, amygdala, the shell of the nucleus accumbens, and the ventromedial, dorsomedial and arcuate nuclei of the hypothalamus (Chambers et al., 1999; Lembo et al., 1999; Saito et al., 1999, 2001). MCH-1 receptor expression has also been observed in several brainstem nuclei, including the locus coeruleus, hypoglossal, motor trigeminal and dorsal motor vagus (Saito et al., 2001). Immunohistochemical analysis has demonstrated that MCH-1 receptor protein is present in the dorsomedial and ventromedial nuclei of the hypothalamus (Chambers et al., 1999). The receptor distribution is

* Corresponding author.
E-mail address: timothy.kowalski@spcorp.com (T.J. Kowalski).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.06.027

consistent with the actions of MCH on feeding behavior and energy homeostasis. Genetic ablation of the MCH-1 receptor produced leanness and resistance to diet-induced obesity (Chen et al., 2002; Marsh et al., 2002), similar to the MCH deficient mice. Unlike the MCH deficient mice, however, MCH-1 receptor deficient mice are hyperphagic and the leanness is due to a hyperactive and hypermetabolic phenotype (Chen et al., 2002; Marsh et al., 2002). Recently, a second MCH receptor has been identified in non-rodent species (MCH-2 receptor) (An et al., 2001; Sailer et al., 2001; Wang et al., 2001); however, the physiological role of this receptor has not been determined.

Diet-induced obesity (DIO) in rodents has been used as an animal model to investigate the interaction between the environment and genetic background. One such model extensively used is the outbred Sprague–Dawley rat. When outbred Sprague–Dawley rats are placed on an energy dense, high fat diet, there is a wide distribution in body weight gain ranging from animals that becomes very obese (DIO) to others that remain as lean as animals fed a lower fat diet (diet-resistant (DR)) (Archer et al., 2003; Chang et al., 1990; Levin et al., 1983; Sclafani and Springer, 1976). The physiologic aspects of the diet-induced obesity in this model replicate many of the features observed with the human obesity syndrome: a polygenic mode of inheritance (Levin et al., 1997; Levin and Sullivan, 1987), a persistence of the phenotype once it is established (Levin et al., 1989), and dysregulated glucose homeostasis (Chang et al., 1990). These features have made the Sprague–Dawley DIO model an attractive tool for investigating human obesity.

Prior work in Sprague–Dawley DIO rats has shown that hyperphagia and increased energy efficiency often accompany the persistent obesity produced by long-term, high-fat feeding (Levin et al., 1985), and that the hyperphagia is due to an increase in meal size, not meal frequency (Farley et al., 2003). Recent reports have shown that pharmacological blockade of MCH-1 receptor in lean rodents abolishes MCH-induced feeding (Takekawa et al., 2002), and promotes hypophagia and weight loss in rodents (Borowsky et al., 2002; Haynes et al., 2001; Lewis et al., 2002), consistent with its role in energy homeostasis. Although the hypophagia produced in rodents is robust and has been replicated with three different chemical entities, it is not known if this action is mediated through a reduction in meal size, meal frequency, or both. This study aimed to use 24-h meal pattern analysis to determine how MCH-1 receptor blockade affects meal size and frequency in a model of rat diet-induced obesity.

2. Materials and methods

2.1. Animals

150 male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were weaned onto a semipurified diet containing 45% of calories as fat with an energy density of 4.7 kcal g^{-1} (D12451, Research Diets, New Brunswick, NJ). Animals had ad libitum access to food and water and were housed individually in hanging wire caging at 22 °C in a 12/12-h light/dark cycle. Weekly body weight measurements and animal husbandry were performed by Charles River Laboratories for 13 weeks. At approximately 16 weeks of age, animals were delivered to the Schering-Plough Research Institute animal facility. Animals were housed in Plexiglas caging with cob bedding in a 12/12-h light/dark cycle (10:00 h lights out) at 22 °C and continued to be fed the high fat diet ad libitum. Body weight was monitored for an additional 35 weeks. The 150 rats used displayed a normal distribution of body weight gain, similar to that observed by Archer et al. (2003) but different from that reported by Levin and Sullivan (1987). At 51 weeks of age, the highest weight gainers (DIO rats) were selected for meal pattern study (n=24). The next highest weight gainers were used in the locomotor activity assessment (n=40). All studies were conducted in an American Association for Laboratory Animal Care accredited facility following protocols approved by the Schering-Plough Research Institute Animal Care and Use Committee. The procedures were performed in accordance with the principles and guidelines established by the National Institutes of Health for the care and use of laboratory animals.

2.2. Meal pattern analysis

After 48 weeks of high fat feeding, DIO rats were transferred into wire bottomed caging equipped with a BioDAQ® food intake monitoring system (Research Diets). Rats were housed individually and provided access to the high fat diet and water ad libitum. Total food intake and meal pattern analysis were assessed using the BioDAQ® system as previously described (Farley et al., 2003). Briefly, the system uses a food hopper mounted on an electronic strain gauge-based load cell to measure food intake. The food hopper is weighed 50 times per second (accurate to 0.01 g) and a mean and standard deviation (S.D.) over approximately 1 s is calculated by a peripheral computer. Feeding is signaled by a fluctuation in the food hopper weight (defined as a S.D.>2000 mg) caused by the animal eating, at which time the date, time and hopper weight are recorded. The end of a feeding bout (but not necessarily a meal, see below) is signaled when the hopper is left undisturbed for 2 min (defined as a S.D.<2000 mg), at which time the duration of the feeding event and the amount eaten (initial-final hopper weight) is calculated. Each feeding event record (cage/animal number, start date and time, feeding duration, final hopper weight and the amount eaten) is exported to a central computer and a Microsoft Excel (Microsoft, Redmond, WA)-based program is used for calculating the desired parameters (see below) and for data summary. Two hours prior to lights out, access to the hoppers was denied and both the animals and food hoppers

were weighed. Food spillage was monitored during the study and no spillage was observed during the test periods. Twenty-four-hour intake was calculated from the difference in hopper weight. Routine maintenance was also performed during this time. Animals were given access to the hoppers 5 min prior to lights out.

A 14-day acclimation period was provided, after which time drug testing commenced (see below). The amount of food eaten, meal size and meal frequency were separately calculated for the 24-h period, as well as for the nocturnal and diurnal periods. A meal was defined as a difference in hopper weight of >0.3 g and separated from other feeding bouts by >15 min (Eckel et al., 1998; Surina-Baumgartner et al., 1995). Evaluating the data after varying the minimum meal size and IMI between 0.1 and 0.5 g, and 5 and 60 min, respectively, did not appreciably change the results (data not shown). When using this meal definition criterion, the daily food intake recorded by the BioDAQ® was 100.4±0.4% of the intake recorded manually as the 24-h hopper weight difference.

2.3. Drug administration

The selective MCH-1 receptor antagonist T-226296 (a (−)enantiomer of *N*-[6-(dimethylamino)-methyl]5,6,7,8-tetrahydro-2-naphthalenyl]-4′-fluoro[1,1′-biphenyl]-4 carboxamide) was used (Takekawa et al., 2002) (synthesized as described; Kato et al., 2001; Fig. 1). T-226296 was reported to have an IC_{50} of 5.5 nM in an MCH1-R binding assay and to lack any appreciable activity for other (unspecified) receptors involved in food intake (Takekawa et al., 2002). During acclimation to the BioDAQ® (see above), animals were orally dosed with a solution of 20% hydroxypropyl-β-cyclodextrin (HPβCD; Cerestar, Hammond, IN) in a volume of 800 μl using a 3-in. 16-g stainless steel dosing needle. The effects of MCH-1 receptor antagonism on food intake and body weight were assessed on two separate test days separated by 1 week. During week 1, animals were randomly assigned to receive an oral dose of vehicle (20% HPβCD), 1, 3 or 10 mg/kg T-226296 1 h prior to lights out in a volume of 800 μl (*n*=6/group). The following week, animals again were assigned to receive one of the four treatments, ensuring that each animal did not get the same treatment as week 1. Intake data and body weight change over the 24-h following dosing was analyzed.

One week after the final dose in the feeding studies, animals were randomly assigned to receive vehicle, 1, 3 or 10 mg/kg T-229296 1 h prior to lights out (*n*=6/group). One half of the rats within each treatment group were killed by CO_2 asphyxiation at 6 h post-dose (*n*=3/group), while the remaining animals within each group were killed 24 h post-dose (*n*=3/group). Blood was collected by cardiac puncture, and plasma was isolated and stored at −20 °C. Plasma concentrations of T-226296 were determined by LC-MS.

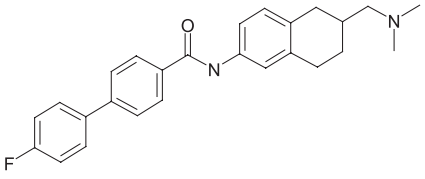

Fig. 1. Structure of T-226296.

2.4. Locomotor activity assessment

A separate cohort of DIO rats (*n*=40) was used for this experiment. The cohort was divided into four treatment groups of 10 rats. Rats were transferred from the colony room to the testing room in the late light cycle. Groups were treated with either vehicle (20% HPβCD) or T-229296 at doses of 1, 3 or 10 mg/kg p.o., and 2 h later tested in a locomotor activity assay. The locomotor activity assay was performed in the light, although the time it was performed corresponded to the early dark phase of the rats used. Briefly, rats were placed into one of 16 clear Plexiglas Digiscan locomotor activity assessment boxes (41×41×30 cm, L×W×H; Accuscan Instruments, Columbus, OH) and activity was recorded for 60 min. Two sets of 16 photobeams spaced 2.5 cm apart recorded horizontal activity and the total distance traveled (X–Y vectors). A separate series of 16 photobeams placed 15 cm above the floor recorded rearing behavior (Z vector). Activity was recorded automatically by Versamax software (Accuscan Instruments) and stored on a PC computer. The four treatment groups were balanced equally across the 16 locomotor activity assay boxes to allow for any differences in photobeam sensitivity.

2.5. Statistical analysis

All values are presented as means±S.E.M. Differences in intake, meal size and meal number between treatment groups over the 24-h period and the nocturnal and diurnal phases were assessed using one-way analysis of variance (ANOVA). Cumulative food intake was binned in hours post dose and significant differences between groups over the 24-h testing period were assessed using repeated measures ANOVA. ANOVAs were followed by Fishers PLSD where appropriate. LMA parameters were assessed using a one-way ANOVA. A *P*-value <0.05 was considered significant.

3. Results

3.1. Plasma concentrations of T-226296

The plasma concentrations of T-226296 6 h after oral dosing were 70.4±3.6, 369.4±25.1 and 1273.7±72.8 nM for the 1, 3 and 10 mg/kg treated groups, respectively. At 24 h after dosing, plasma concentrations were 0, 68.7±30.2 and 535.8±96.2 nM for the 1, 3 and 10 mg/kg treated groups, respectively. Brain penetrability of T-226296 has

been reported to be good (Takekawa et al., 2002); however, we did not measure brain concentrations in this study.

3.2. Food intake and body weight

The initial body weight of the rats used in this study was 1033±6 g. There was a significant overall effect of treatment on cumulative food intake over the 24-h test period ($F_{3,38}$=7.4, P<0.001; Fig. 2). Animals treated with 10 mg/kg T-226296 ate significantly less than vehicle-treated rats from the fourth hour on and less than the 1 and 3 mg/kg T-226296-treated rats from the seventh hour on. Animals treated with 3 mg/kg T-226296 ate significantly less than vehicle-treated rats from the 12th to the 15th hour post-dose (Fig. 2). Twenty-four-hour intake of the 10 mg/kg T-226296-treated group was significantly lower than the vehicle, 1 and 3 mg/kg T-226296 treatment groups (Table 1). The significant decrease in food intake produced by T-226926 was accompanied by a significant difference in 24-h body weight change ($F_{3,38}$=51.8, P<0.0001; Table 1), with the 10 mg/kg treated animals losing significantly more than vehicle, 1 and 3 mg/kg treated animals, and the 3 mg/kg treated animals losing significantly more than vehicle-treated animals.

There was a significant effect of treatment on the average meal size over the 24-h period ($F_{3,38}$=3.4, p<0.05; Table 1). Meal size in rats treated with 10 mg/kg T-226296 was significantly smaller than rats treated with vehicle or 1 mg/kg. There was, however, no significant difference in meal size between rats treated with vehicle, 1 or 3 mg/kg. There was no significant effect of treatment on meal number ($F_{3,38}$=0.8, n.s.).

Treatment with T-226296 significantly affected nocturnal ($F_{3,38}$=5.4, P<0.005; Table 1) and diurnal food intake ($F_{3,38}$=6.1, P<0.005; Table 1), with the 10 mg/kg treated rats eating less than those treated with vehicle, 1 or 3 mg/kg. During the nocturnal phase, average meal size in the 10 mg/kg treated rats was significantly smaller than that in the vehicle or 1 mg/kg treated rats. The average meal size of 10 mg/kg treated rats during the diurnal period, however, was significantly smaller than all of the other groups. There was no significant effect of treatment on meal number during both the nocturnal and diurnal phases (Table 1), and the percent of food eaten in the nocturnal phase was similar between groups (77±2%, 73±2, 71±3% and 78±4% for vehicle, 1, 3 and 10 mg/kg, respectively; $F_{3,38}$=1.3, n.s.).

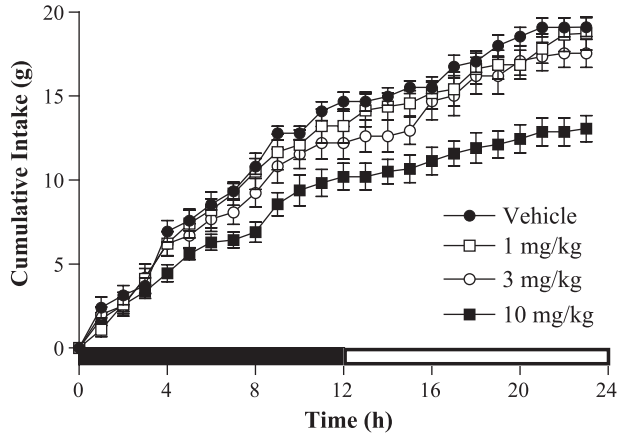

Fig. 2. Cumulative 24-h food intake of DIO rats treated with vehicle or T-226296 at a dose of 1, 3 or 10 mg/kg. Values displayed are means± S.E.M. The filled bar at the bottom denotes the nocturnal period and the open bar the diurnal period.

Table 1
Effect of vehicle, 1, 3 or 10 mg/kg T-226296 on 24-h body weight change, 24-h, nocturnal and diurnal food intake, and meal size and meal number in DIO rats

Measure	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg
24-h body weight change (g)	−0.4±1.0[a]	−0.9±1.3[a]	−3.7±0.6[b]	−15.5±1.1[c]
24-h intake (g)	19.1±0.6[a]	18.7±1.0[a]	17.5±0.8[a]	13.1±0.8[b]
Meal size (g)	3.2±0.1[a]	3.2±0.3[a]	3.0±0.2[a,b]	2.5±0.2[b]
Meal #	6.0±0.3[a]	6.1±0.5[a]	6.2±0.5[a]	5.4±0.3[a]
Nocturnal intake (g)	14.7±0.6[a]	13.8±1.1[a]	12.6±1.0[a]	10.2±0.8[b]
Nocturnal meal size (g)	3.3±0.1[a]	3.4±0.3[a]	2.8±0.3[a,b]	2.5±0.1[b]
Nocturnal meal #	4.4±0.3[a]	4.2±0.5[a]	4.5±0.4[a]	4.0±0.3[a]
Diurnal intake (g)	4.4±3.8[a]	4.9±0.3[a]	4.9±3.7[a]	2.9±0.5[b]
Diurnal meal size (g)	3.4±0.2[a]	3.4±0.5[a]	3.4±0.2[a]	2.1±0.4[b]
Diurnal meal #	1.5±0.2[a]	1.8±0.3[a]	1.5±0.2[a]	1.3±0.2[a]

Values are mean±S.E.M. Values with different superscripts within rows are significantly different (P<0.05).

3.3. Locomotor activity

The average body weight of the DIO rats used in this experiment was 833±15 g. Despite high body weight, vehicle-treated DIO rats displayed a pattern of locomotor activity similar to young lean rats when placed into a novel environment, i.e., initial high activity and exploration followed by a gradual habituation to the environment and reduced activity (data not shown). Administration of T-226296 to DIO rats, at doses of 1, 3 or 10 mg/kg had no significant effect on any activity measure (Table 2) when compared to the vehicle-treated control group.

Table 2
Effect of vehicle, 1, 3 or 10 mg/kg T-226296 on locomotor activity in diet-induced obese rats

Measure	Dose (mg/kg)			
	0	1	3	10
Horizontal activity (beam breaks)	3404±343	3384±569	4009±743	3669±606
Total distance (cm)	919±142	988±217	1064±257	1084±199
Vertical activity (beam breaks)	1162±196	928±166	1032±187	997±208

4. Discussion

We show that acute oral administration of the MCH-1 receptor antagonist T-226296 in free-feeding male DIO rats produces a dose-related decrease in 24 h food intake and body weight, consistent with prior reports evaluating MCH-1 receptor antagonists (Borowsky et al., 2002; Haynes et al., 2001; Lewis et al., 2002; Takekawa et al., 2002). The novel finding of this study was that the decrease in food intake observed with MCH-1 receptor antagonism is due to a reduction in meal size, not meal frequency.

The control of meal size is determined by food stimuli acting directly on pre-absorptive receptors during a meal; orosensory stimuli provide a positive feedback to maintain eating, while post-ingestive stimuli in the stomach and intestine provide a negative feedback to stop eating. The relative potencies of these peripheral feedback signals, termed "direct controls" (Smith, 1996), to central sites involved in the control of food intake determine the size of a meal. All other factors that modulate the potency of these direct controls, such as central MCH-1 receptor signaling, have been termed "indirect controls". The decrease in meal size observed with central MCH-1 receptor antagonism must be due to a decrease in the potency of positive feedback signals, an increase in the potency of negative feedback signals or both. Further studies are required to determine which feedback is regulated by MCH-1 receptor signaling.

The effect of MCH-1 receptor antagonism on food intake described in this experiment is consistent with the hypophagia and leanness observed in mice lacking the prohormone precursor of MCH (Shimada et al., 1998), but is not consistent with the hyperphagia resulting from MCH-1 receptor ablation (Ludwig et al., 2001; Marsh et al., 2002). One explanation for this is that the hyperactive and hypermetabolic phenotype of mice lacking the MCH-1 receptor may prevent the emergence of hypophagia. Another possibility is that the acute pharmacological actions of MCH-1 receptor antagonism on feeding and activity may be different from those that would be observed with chronic antagonist administration; however, studies to date provide no evidence for this (Borowsky et al., 2002).

Hypothalamic MCH is overexpressed in rodents that are hyperphagic due to null mutations in the leptin ($Lep^{ob/ob}$) or leptin receptor ($Lepr^{db/db}$, $Lepr^{fa/fa}$) genes (Qu et al., 1996; Stricker-Krongrad et al., 2001), and leptin administration to leptin-deficient animals reverses the hyperphagia (Pelleymounter et al., 1995). In rats of normal body weight, leptin administration has been shown to reduce food intake by selectively reducing meal size and not frequency in both males and females (Eckel et al., 1998; Kahler et al., 1998). Because MCH-1 receptor antagonism also selectively reduces meal size, the effects of leptin may, in part, be mediated by a reduction in MCH-1 receptor signaling. This may occur via changes in the expression, synthesis, or release of MCH or changes in the expression, density or second messenger signaling of MCH-1 receptor. Interestingly, Kokkotou et al. have shown that brain expression of MCH-1 receptor is higher in leptin deficient $Lep^{ob/ob}$ mice and in fasted hypoleptinemic mice, and that leptin replacement decreases MCH-1 receptor expression (Kokkotou et al., 2001). This increase in MCH-1 receptor expression occurs despite higher MCH expression under similar conditions (Qu et al., 1996). In contrast, studies in the leptin-signaling deficient $Lepr^{fa/fa}$ rat have shown that hypothalamic expression of MCH-1 receptor is down-regulated, while expression of the ligand is up-regulated (Stricker-Krongrad et al., 2001). It is not known if this represents a species difference or a difference in methodology. Future studies addressing whether the changes in expression of MCH-1 receptor are reflective of changes in MCH-1 receptor protein and signaling are required to better assess the role of leptin in modulating brain MCH tone.

Prior studies evaluating the action of MCH on motor activity in rats have produced variable responses, showing that central administration of MCH increases locomotion, grooming and rearing activity (Monzon and De Barioglio, 1999) or has no effect (Kela et al., 2003; Sanchez et al., 1997). Studies with MCH-1 receptor antagonists have failed to show a change in locomotor activity or induction of malaise at doses that reduce food intake (Borowsky et al., 2002; Lewis et al., 2002). Similar to these findings, we did not see an effect of T-226296 on locomotor activity at doses which produced a decrease in food intake and meal size. Therefore, the effect of MCH-1 receptor antagonism on feeding appears to be specific, and not due to a generalized behavioral malaise. This is reinforced by findings demonstrating that hypophagia produced by agents that elicit malaise and illness (lithium chloride) principally affect meal frequency, not meal size (West et al., 1987) and manipulations that render the diet unpalatable or aversive (quinine adulteration) impact the frequency of feeding bouts (Blundell et al., 1985; Ishii et al., 2003). It must be noted, however, that the results from the locomotor activity experiment using a 1 h test period in the light phase can not be extrapolated over a 24-h diurnal period. Further studies evaluating 24-h locomotor activity in response to a MCH-1 receptor antagonist are required to fully assess this aspect.

In summary, we have shown that acute MCH-1 receptor antagonism suppresses spontaneous food intake in DIO rats and that this is due to a selective reduction in meal size. T-226296 did not significantly affect meal frequency or the percent of calories consumed during the nocturnal period. These findings provide an important initial step in our understanding of MCH-1 receptor antagonist-mediated hypophagia, supporting a role for MCH in the regulation of meal size. These findings also suggest that MCH-1 receptor antagonists may provide an effective treatment for obesity or eating disorders.

Acknowledgements

We thank Cymbelene Nardo and Hong Mei for technical assistance in the measurement of plasma T-226296.

References

An, S., Cutler, G., Zhao, J.J., Huang, S.G., Tian, H., Li, W., Liang, L., Rich, M., Bakleh, A., Du, J., Chen, J.L., Dai, K., 2001. Identification and characterization of a melanin-concentrating hormone receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 7576–7581.

Archer, Z.A., Rayner, D.V., Rozman, J., Klingenspor, M., Mercer, J.G., 2003. Normal distribution of body weight gain in male Sprague–Dawley rats fed a high-energy diet. Obes. Res. 11, 1376–1383.

Bittencourt, J.C., Presse, F., Arias, C., Peto, C., Vaughan, J., Nahon, J.L., Vale, W., Sawchenko, P.E., 1992. The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J. Comp. Neurol. 319, 218–245.

Blundell, J.E., Rogers, P.J., Hill, A.J., 1985. Behavioural structure and mechanisms of anorexia: calibration of natural and abnormal inhibition of eating. Brain Res. Bull. 15, 371–376.

Borowsky, B., Durkin, M.M., Ogozalek, K., Marzabadi, M.R., DeLeon, J., Lagu, B., Heurich, R., Lichtblau, H., Shaposhnik, Z., Daniewska, I., Blackburn, T.P., Branchek, T.A., Gerald, C., Vaysse, P.J., Forray, C., 2002. Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. Nat. Med. 8, 825–830.

Chambers, J., Ames, R.S., Bergsma, D., Muir, A., Fitzgerald, L.R., Hervieu, G., Dytko, G.M., Foley, J.J., Martin, J., Liu, W.S., Park, J., Ellis, C., Ganguly, S., Konchar, S., Cluderay, J., Leslie, R., Wilson, S., Sarau, H.M., 1999. Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. Nature 400, 261–265.

Chang, S., Graham, B., Yakubu, F., Lin, D., Peters, J.C., Hill, J.O., 1990. Metabolic differences between obesity-prone and obesity-resistant rats. Am. J. Physiol. 259, R1103–R1110.

Chen, Y., Hu, C., Hsu, C.K., Zhang, Q., Bi, C., Asnicar, M., Hsiung, H.M., Fox, N., Slieker, L.J., Yang, D.D., Heiman, M.L., Shi, Y., 2002. Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. Endocrinology 143, 2469–2477.

Della-Zuana, O., Presse, F., Ortola, C., Duhault, J., Nahon, J.L., Levens, N., 2002. Acute and chronic administration of melanin-concentrating hormone enhances food intake and body weight in Wistar and Sprague–Dawley rats. Int. J. Obes. Relat. Metab. Disord. 26, 1289–1295.

Eckel, L.A., Langhans, W., Kahler, A., Campfield, L.A., Smith, F.J., Geary, N., 1998. Chronic administration of OB protein decreases food intake by selectively reducing meal size in female rats. Am. J. Physiol. 275, R186–R193.

Farley, C., Cook, J.A., Spar, B.D., Austin, T.M., Kowalski, T.J., 2003. Meal pattern analysis of diet-induced obesity in susceptible and resistant rats. Obes. Res. 11, 845–851.

Haynes, A.C., Stevens, A.J., Muir, A.I., Avenell, K.Y., Clapham, J.C., Tadayyon, M., Darker, J.G., Wroblowski, B., O'Toole, C., Witty, D.R., Arch, J.R.S., 2001. A melanin-concentrating hormone receptor (SLC-1) antagonist reduces food intake and body weight gain in rats and mice. Obes. Res. 9, 90S.

Ishii, Y., Blundell, J.E., Halford, J.C., Rodgers, R.J., 2003. Palatability, food intake and the behavioural satiety sequence in male rats. Physiol. Behav. 80, 37–47.

Ito, M., Gomori, A., Ishihara, A., Oda, Z., Mashiko, S., Matsushita, H., Yumoto, M., Sano, H., Tokita, S., Moriya, M., Iwaasa, H., Kanatani, A., 2003. Characterization of MCH-mediated obesity in mice. Am. J. Physiol., Endocrinol Metabol. 284, E940–E945.

Kahler, A., Geary, N., Eckel, L.A., Campfield, L.A., Smith, F.J., Langhans, W., 1998. Chronic administration of OB protein decreases food intake by selectively reducing meal size in male rats. Am. J. Physiol. 275, R180–R185.

Kato, K., Terauchi, J., Mori, M., Suzuki, N., Shimomura, Y., Takekawa, S., Ishihara, Y., 2001. WO-0121577 (Takeda Chemical Industries).

Kela, J., Salmi, P., Rimondini-Giorgini, R., Heilig, M., Wahlestedt, C., 2003. Behavioural analysis of melanin-concentrating hormone in rats: evidence for orexigenic and anxiolytic properties. Regul. Pept. 114, 109–114.

Kokkotou, E.G., Tritos, N.A., Mastaitis, J.W., Slieker, L., Maratos-Flier, E., 2001. Melanin-concentrating hormone receptor is a target of leptin action in the mouse brain. Endocrinology 142, 680–686.

Lembo, P.M., Grazzini, E., Cao, J., Hubatsch, D.A., Pelletier, M., Hoffert, C., St-Onge, S., Pou, C., Labrecque, J., Groblewski, T., O'Donnell, D., Payza, K., Ahmad, S., Walker, P., 1999. The receptor for the orexigenic peptide melanin-concentrating hormone is a G-protein-coupled receptor. Nat. Cell Biol. 1, 267–271.

Levin, B.E., Sullivan, A.C., 1987. Glucose-induced norepinephrine levels and obesity resistance. Am. J. Physiol. 253, R475–R481.

Levin, B.E., Triscari, J., Sullivan, A.C., 1983. Relationship between sympathetic activity and diet-induced obesity in two rat strains. Am. J. Physiol. 245, R364–R371.

Levin, B.E., Finnegan, M., Triscari, J., Sullivan, A.C., 1985. Brown adipose and metabolic features of chronic diet-induced obesity. Am. J. Physiol. 248, R717–R723.

Levin, B.E., Hogan, S., Sullivan, A.C., 1989. Initiation and perpetuation of obesity and obesity resistance in rats. Am. J. Physiol. 256, R766–R771.

Levin, B.E., Dunn-Meynell, A.A., Balkan, B., Keesey, R.E., 1997. Selective breeding for diet-induced obesity and resistance in Sprague–Dawley rats. Am. J. Physiol. 273, R725–R730.

Lewis, D.R., Joppa, M.A., Markison, S., Gogas, K.R., Schwarz, D.A., Zhu, F., Maki, R.A., Foster, A.C., 2002. Melanin-concentrating hormone receptor antagonists decrease food intake in rodents. Appetite 39, 89.

Ludwig, D.S., Tritos, N.A., Mastaitis, J.W., Kulkarni, R., Kokkotou, E., Elmquist, J., Lowell, B., Flier, J.S., Maratos-Flier, E., 2001. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. J. Clin. Invest. 107, 379–386.

Marsh, D.J., Weingarth, D.T., Novi, D.E., Chen, H.Y., Trumbauer, M.E., Chen, A.S., Guan, X.M., Jiang, M.M., Feng, Y., Camacho, R.E., Shen, Z., Frazier, E.G., Yu, H., Metzger, J.M., Kuca, S.J., Shearman, L.P., Gopal-Truter, S., MacNeil, D.J., Strack, A.M., MacIntyre, D.E., Van der Ploeg, L.H., Qian, S., 2002. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc. Natl. Acad. Sci. U. S. A. 99, 3240–3245.

Monzon, M.E., De Barioglio, S.R., 1999. Response to novelty after i.c.v. injection of melanin-concentrating hormone (MCH) in rats. Physiol. Behav. 67, 813–817.

Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., Collins, F., 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269, 540–543.

Qu, D., Ludwig, D.S., Gammeltoft, S., Piper, M., Pelleymounter, M.A., Cullen, M.J., Mathes, W.F., Przypek, R., Kanarek, R., Maratos-Flier, E., 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380, 243–247.

Sailer, A.W., Sano, H., Zeng, Z., McDonald, T.P., Pan, J., Pong, S.S., Feighner, S.D., Tan, C.P., Fukami, T., Iwaasa, H., Hreniuk, D.L., Morin, N.R., Sadowski, S.J., Ito, M., Bansal, A., Ky, B., Figueroa, D.J., Jiang, Q., Austin, C.P., MacNeil, D.J., Ishihara, A., Ihara, M., Kanatani, A., Van der Ploeg, L.H., Howard, A.D., Liu, Q., 2001. Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. Proc. Natl. Acad. Sci. U. S. A. 98, 7564–7569.

Saito, Y., Nothacker, H.P., Wang, Z., Lin, S.H., Leslie, F., Civelli, O., 1999. Molecular characterization of the melanin-concentrating-hormone receptor. Nature 400, 265–269.

Saito, Y., Cheng, M., Leslie, F.M., Civelli, O., 2001. Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. J. Comp. Neurol. 435, 26–40.

Sanchez, M., Baker, B.I., Celis, M., 1997. Melanin-concentrating hormone (MCH) antagonizes the effects of alpha-MSH and neuropeptide E-I on grooming and locomotor activities in the rat. Peptides 18, 393–396.

Sclafani, A., Springer, D., 1976. Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol. Behav. 17, 461–471.

Shimada, M., Tritos, N.A., Lowell, B.B., Flier, J.S., Maratos-Flier, E., 1998. Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature 396, 670–674.

Smith, G.P., 1996. The direct and indirect controls of meal size. Neurosci. Biobehav. Rev. 20, 41–46.

Stricker-Krongrad, A., Dimitrov, T., Beck, B., 2001. Central and peripheral dysregulation of melanin-concentrating hormone in obese Zucker rats. Brain Res. Mol. Brain Res. 92, 43–48.

Surina-Baumgartner, D.M., Langhans, W., Geary, N., 1995. Hepatic portal insulin antibody infusion increases, but insulin does not alter, spontaneous meal size in rats. Am. J. Physiol. 269, R978–R982.

Takekawa, S., Asami, A., Ishihara, Y., Terauchi, J., Kato, K., Shimomura, Y., Mori, M., Murakoshi, H., Suzuki, N., Nishimura, O., Fujino, M., 2002. T-226296: a novel, orally active and selective melanin-concentrating hormone receptor antagonist. Eur. J. Pharmacol. 438, 129–135.

Wang, S., Behan, J., O'Neill, K., Weig, B., Fried, S., Laz, T., Bayne, M., Gustafson, E., Hawes, B.E., 2001. Identification and pharmacological characterization of a novel human melanin-concentrating hormone receptor, mch-r2. J. Biol. Chem. 276, 34664–34670.

West, D.B., Greenwood, M.R., Marshall, K.A., Woods, S.C., 1987. Lithium chloride, cholecystokinin and meal patterns: evidence that cholecystokinin suppresses meal size in rats without causing malaise. Appetite 8, 221–227.