DAYTIME MELATONIN TREATMENT INFLUENCES FOOD-CARRYING (HOARDING) BEHAVIOR IN RATS

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Objective. Three experiments were performed to verify whether melatonin (MEL) may influence hoarding behavior in rats. This hypothesis was supported by the consideration that leptin treatment decreases food hoarding in hamsters and that an inverse relation exists between plasma leptin concentration and MEL treatment.

Methods. Male Sprague-Dawley rats housed individually and kept on 12h:12h light-dark cycle were used. First experiment was performed to check whether a bimodal distribution of food hoarding scores exists in rats, and to select two groups of high (HH-rats) or low (LH-rats) hoarding. Second experiment was designed to verify whether MEL treatment modifies food-hoarding, while the third one was performed to investigate whether MEL treatment was able to modify the reciprocal relation between leptin and MEL plasma concentration.

Results. In rats the hoarding tendency fell into a bimodal distribution and the plasma leptin concentration was significantly higher in HH-rats than LH-rats. When MEL was injected, circulating concentration of leptin was decreased in both HH-rats and LH-rats and such MEL treatment significantly increased the number of pellets hoarded by LH-rats but not that hoarded by HH-rats.

Conclusions. MEL influences the food-hoarding in rats either directly, or indirectly by the MEL and leptin reciprocal interaction. Our results support the hypothesis that the endocrine system either directly, by the action of one or more combined hormones (MEL, leptin), or indirectly via its actions on neural substrates determines, at least in part, food-hoarding of rats.

Key words: Low and high hoarding tendency – Melatonin-leptin interaction – Psychosocial behavior – Security hypothesis – Object value hypothesis

The term food hoarding covers a variety of behaviors that are united by two common criteria: post-ponement of food consumption that may vary greatly among food-hoarding species ranging from a few minutes to as much as two years (VANDER WALL 1990); and food carrying, conservation and storage through special handling. In rodents, a prominent aspect of foraging behavior is carrying or hoarding food to a new location for subsequent consumption or storage (WHISHAW et al. 1990). Since the transport of food is a conspicous and important component of food-hoarding behavior, the terms food-carrying, caching and storing may be considered synonymous with food-hoarding (VANDER WALL 1990). Thus, in this paper carrying-food and hoarding-food are synonym.

Some animals lineages differ markedly in their propensity to hoard food. For example, food-deprived black-hooded rats begin to hoard pellets sooner, hoard more, and continue to hoard longer after *ad libitum* feeding resumes than do Irish rats (STAMM 1954). Hoarding behavior may be correlated with the mechanisms that regulate food intake, body fat as well as body mass (CABANAC and GOSSELIN 1996). However, different neurological and/or endocrine states underlie specific food

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intake and hoarding behavior (BARTNESS and CLEIN 1994).

Leptin is produced by adipocytes in response to nutrient cycling (WANG et al. 1998). BUCKLEY and SCHNEIDER (2003) demonstrated that Syrian hamsters respond to energetic challenges by changing their hoarding behavior and that leptin is one factor that mediates this response. They found a significant increase of food hoarding in food-deprived hamsters and a significant attenuation of the hoarding response by systemic treatment with leptin. Moreover, circulating leptin has a diurnal pattern, and the pattern of leptin levels in the adult male Syrian hamster is MEL and/or photoperiod dependent (GUNDUZ 2002).

Daily MEL administration suppresses or significantly decreases plasma leptin levels in rats (RASMUSSEN et al. 1999; MASTRONARDI et al. 2000; WOLDEN-HANSON et al. 2000; BAYDAS et al. 2001; CANPOLAT et al. 2001; RASMUSSEN et al. 2001) and raccoon (NIEMINEN et al. 2002). Moreover, MEL suppresses not only plasma leptin levels, but also leptin production in anterior pituitary cells (KUS et al. 2004). A photoperiodic and seasonal regulation of leptin release seems related to MEL secretion in rodents (HORTON et al. 2000; GUNDUZ 2002; NIEMINEN et al. 2002).

Since MEL has been implicated in food intake, body mass gain and adiposity (BUBENIK and PANG 1994; RASMUSSEN et al. 1999; BRYDON et al. 2001; JASNOW et al. 2002; GENIN et al. 2003), taken together these data suggest a potential role for MEL in both consummatory and appetitive ingestive behavior, such as food -hoarding, considered an appetitive aspect of ingestive behavior (DIBATTISTA and BEDARD 1987; DAY and BARTNESS 2004; RHINEHART and BARTNESS 2005; for review see VANDER WALL 1990). Moreover, MEL significantly influences plasma leptin level which is involved in food hoarding (BUCKLEY and SCHNEIDER 2003) thus, it seems reasonable that MEL treatment might also influence food hoarding behavior in rats.

First aim of the present experiments was to check for a bimodal distribution of food hoarding scores exists in rats, as known for hamsters (BUCKLEY and SCHNEIDER 2003). The second main purpose was to test whether peripherally administered MEL influences food hoarding in rats. Third aim was to clarify whether MEL treatment was capable of modifing the reciprocal relation between leptin and MEL plasma concentration. It was investigated whether manipulation of MEL signals, by its daytime administration, influences levels of circulating leptin.

Methods and Results

Experiment 1: Hoarding tendency

A bimodal distribution of hoarding scores (high and low hoarding) among male Syrian hamsters has been documented by BUCKLEY and SCHNEIDER (2003). The present experiment was performed: a) to ascertain whether a bimodal hoarding tendency also exists in rats; b) if that is so, to select two groups of rats with high or low tendency to hoard food.

Methods

Animals. Experiments were carried out in conformity with the Guiding Principles of AMERICAN PHYSIOLOGICAL SOCIETY (2002) and were approved by Local Ethical Committe and the Institutional Review Board of the University of Modena and Reggio Emilia (Italy). Special care was taken to minimize animal suffering and to reduce the number of animals used. Subjects were 16 naive, male Sprague-Dawley rats weighing 290-300 g at the beginning of the experiments. Since food was available *ad libitum*, it is reasonable to presume that rats were sated at the beginning of tests, for this they hoard food (carry-to-leave). Rats were housed individually in stainless steel cages and maintained under controlled conditions of temperature (22 ± 1 °C) and humidity (65 ± 5 %). Animals were kept on a 12h:12h light/dark cycle (light on at 7.00 h). A dim red light of about 1 lux was always on. Normal pelleted food and tap water were available *ad libitum* during all phases of experiments except during the hoarding period.

Apparatus and procedures. Food-hoarding apparatus consisted of a home cage, connected through a 50 cm long opaque Plexiglas alley (15 L x 15 H, cm) to a hoarding box, a wire mesh cage (25 x 20 x 25 cm) resembling the rat's home cage. After the hoarding period, the food hoarded in the rat's home was removed; then, the rat was returned to the home cage.

The experiment was conducted in three phases: 1. adaptation; 2. pre-training; 3. baseline food hoarding (hoarding tendency). During the adaptation period (days 1-5), each rat was placed in the apparatus for 30 min a day, and access to the alley and hoarding box was closed. This period permitted rats to explore and to familiarize with the new environment. During the pre-training phase (days 6-11), 20 pellets were scattered on the floor of hoarding box. The animal was placed in the hoarding apparatus for 30 min a day and hoarding behavior monitored. Actual

food hoarding testing (days 12-22) took place immediately after the pre-training phase. To avoid a ceiling level during baseline that will obscure any potential modulation on MEL treatment, 40 pellets were scattered on the floor of hoarding box. The pre-training phase was necessary because not all rats started to hoard on the same day, and the individual scores during the first days of hoarding were usually variabile. The mean daily hoarding scores for each rat remained fairly constant during the hoarding tests that followed the pre-training phase. The number of pellets collected and left in the hoarding box and/or alley were monitored. The weight and dimensions of each pellet were constant (10 mm diameter and 15 to 20 mm long; about 2.5 g each). The amount of food in the hoarding box was weighed before and after the hoarding session. The food remaining in the hoarding box and alley at the end of the session and the food hoarded by the rat were also weighed. It is important to note that rats never ate pellets during the hoarding tests. Hoarding behavior was monitored in the morning (9.00 – 12.00 h).

Statistical evaluation. Data were analyzed using ANOVA, and significant interactions were followed by pair wise comparisons (Student-Newman-Keul and/or Bonferroni "t" test). Differences were considered significant if $p< 0.05$.

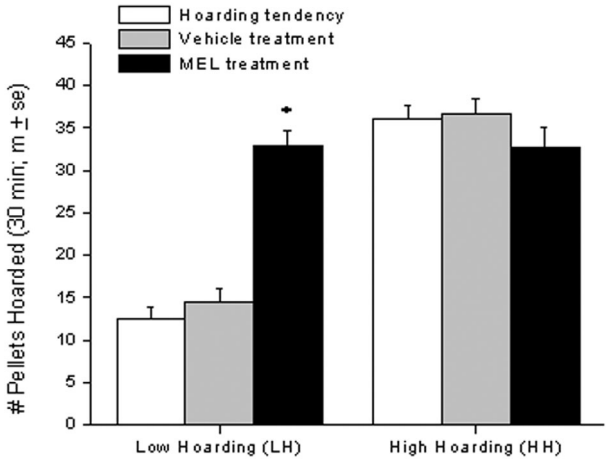

Fig. 1 Hoarding tendency test (30 min; 40 pellets scattered on the hoarding apparatus) showed a bimodal hoarding behavior in rats, which were divided into two groups, Low Hoarding (LH) and High Hoarding (HH). MEL treatment significantly increased the number of pellets hoarded by LH-rats as compared to that hoarded during the tendency period (*p< 0.0001) and vehicle treatment (*p< 0.0001). MEL treatment did not significantly modify the number of pellets hoarded by HH-rats.

Results

The actual food hoarding period showed a bimodal hoarding behavior and permitted to select rats having high or low hoarding scores. Rats hoarding 70 % or more of pellets scattered in the hoarding apparatus during the 30 min test period were designed as "high hoarding rats" (HH-rats), whereas rats that hoarded 30 % or less of the pellets were designed as "low hoarding rats" (LH-rats). Hoarding tendency was bimodal, so that 8 rats hoarded significantly more pellets than the other 8 rats. On the average, the HH-rats hoarded 28 pellets and the LH-rats hoarded 12 pellets (Fig. 1). There were no significant differences in mean body weight, daily (24 h) food intake and water intake between HH-rats and LH-rats at the beginning and at the end of the hoarding period.

Experiment 2: MLT treatments

Methods

This experiment was performed to verify whether MEL treatment modifies the food-hoarding in the groups with high hoarding (HH) and low hoarding (LH). Manipulations of MEL levels, by its daytime administration, have revealed that MEL modifies several biological functions toward a typical nightime pattern (Cagnacci et al. 2002). The precise timing of MEL injections at light out, has been shown to extend the normal long-day pattern of endogenous MEL secretion; the resulting extended pattern of MEL is interpreted by animals as a short-day (Demas et al. 2004). Thus the pattern of MEL injections generated in experimental animals, rather than being artificial or supraphysiological, accurately reflects typical short-day patterns (Demas et al. 2004). Moreover, exogenous MEL treatment in the daytime would cause a relatively high concentration of the hormone during the light phase to mimic that of the dark period reflecting a typical short-day patterns of MEL secretion (Jasnow et al. 2002). In line with observations that the period of the day when tests are conducted might be a critical factor in the action of the MEL since a significant diurnal variations in the amplitude of MT1 and MT2 receptors agonist action may occur (Golombek et al. 1993), the effects of MEL were assessed by two pilot experiments at two circadian times (dark/light).

The first pilot hoarding experiment, MEL or vehicle injections were performed in the dark after 2h light out of the light/dark cycle; the second one, MEL or vehicle injections were performed in the light part of the cycle after 2 h of light on. Each rat used in pilot experiments was tested on two occasions: MEL treatment and control sessions. The order of the two pilot experimets was randomized, with at lest 1 week between them. Pilot experiments showed that hoarding behavior was not significantly influenced by the period of the day when MEL treatment was performed. Thus, it was decided to perform hoarding experiments during the light phase (9.00 – 12.00 h). Animals were treated with either exogenous MEL or vehicle after 2h light was on (9.00 h) to mimic short-day patterns of MEL secretion.

MEL (11.6 mg) was dissolved in 0.5 ml 70 % ethanol and then diluted to 50 ml with saline. A portion of this solution was diluted 1:1 in saline to obtain a working solution that contained 1 mmol MEL (and 0.012 mmol ethanol). Two dosages of MEL solution were used: a relatively low dosage (0.5 mg/kg b.w./day; 1.55 ml/kg b.w. of the working solution injected s.c.) and high dosage (1.0 mg/kg b.w./day; 3.10 ml/kg b.w. of the working solution injected s.c.). To reduce the number of animals used in the experiment, each rat served as control of itself. According to a randomized cross-over design, all rats received both high and low dosages of MEL (low: 0.5 mg/kg b.w./day; high: 1.0 mg/kg b.w./day) for 10 consecutive days, and both high and low dosage of control vehicle solution (0.012 mmol ethanol in saline; low dosage, 1.55 ml/kg b.w.; high dosage, 3.10 ml/kg b.w./day) for another 10 days. In particular, the treatment was started with MEL for four HH-rats (0.5 mg/kg b.w./day; HH-L), with vehicle solution for four HH-rats (HH-VL, 1.55 ml/kg b.w.), with MEL for four LH-rats (LH-L, 0.5 mg/kgb.w./day), and with vehicle solution for four LH-rats (LH-VL, 1.55 ml/kg b.w./day). All rats recovered from both vehicle and MEL injections for two weeks, then either a high dosage of MEL solution (1mg/kg b.w./day) or a high dosage of vehicle solution (3.10 ml/kgb.w./day) were injected according to the previous treatment scheme. Injection of either MEL or vehicle solutions were performed s.c. in the neck region, each morning at 10:00 am.

Results

Daily mean body weight, food and water intake at the start and end of treatment with vehicle or MEL were not significantly different in both HH-rats and LH-rats groups. Since results obtained by low and high dosage of either MEL or vehicle used for HH-rats and LH-rats groups did not vary significantly, they were pooled and considered as one dosage for all further statistical analyses. MEL treatment significantly increased the number of pellets hoarded by LH-rats but not that hoarded by HH-rats (Fig. 1). In LH-rats, ANOVA [$F(2, 21) = 45.89$, $p = 0.0001$] and post-hoc analyses revealed that MEL treatments significantly increased the number of pellets hoarded, as compared to baseline ($t = 12.304$, $p<0.0001$) and vehicle treatment ($t = 11.063$, $p< 0.0001$). On the contrary, no significant difference was found in the HH-rats group [$F(2, 21) = 2.28$, $p = 0.079$]. ANOVA comparisons between HH-rats and LH-rats [$F(5, 42) = 45.83$, $p = 0.0001$] and *post-hoc* analyses showed significant differences between baseline (baseline HH-rats vs baseline LH-rats, $t = 14.664$, $p< 0.0001$) and vehicle treatment (vehicle HH-rats vs vehicle LH-rats, $t = 13.660$, $p<0.0001$) but not between MEL treated rats (MEL-HH-rats vs MEL-LH-rats, n.s.).

Experiment 3: Plasma leptin and MLT concentrations

Methods

Blood samples to determine plasma leptin and MEL concentrations were collected from HH-rats and LH-rats at the end of the hoarding tendency experiment and at the end of either MEL or vehicle treatment period. Since the light/dark cycle influences the concentration of plasma leptin and MEL, blood samples were always collected at the same time of the day during the light phase and soon after hoarding tests.

Blood samples were taken by puncture of the tail vein using a 1 ml syringe connected to a 26-g needle. Rats were placed in a retention plastic cylinder, and blood was collected on EDTA (1 %) anticoagulant solution. Plasma was then separated by centrifugation (1300 *g* ; 15 min) and stored at -70 °C until assay. Serum leptin concentrations (ng/ml) were measured using a rat leptin radio-immunoassay (RIA) kit manufactured by Linco Research (St. Charles, 63304, MO, USA) in 100 µl of plasma. Plasma MEL concentrations (pg/ml) were measured with an MEL radio-immunoassay (RIA) kit developed by DLD Diagnostika GmbH (Hamburg, Germany).

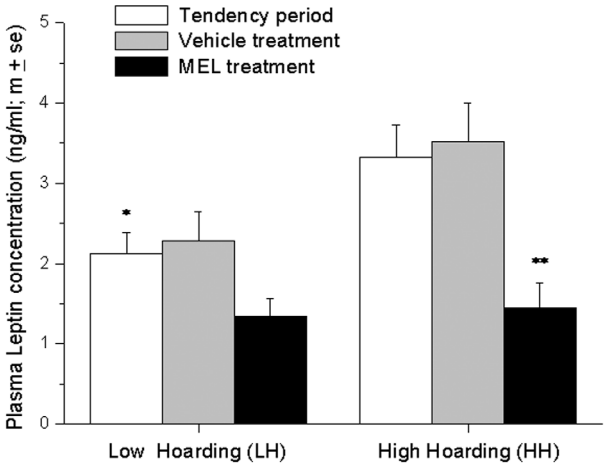

Fig. 2 At the end of tendency period, the plasma leptin concentration of LH-rats was inferior to that of HH-rats, and the difference just met the statistical criterion of significance (*p = 0.0505). MEL treatment decreased plasma leptin levels compared to those of vehicle treatment and those before treatment in HH-rats and LH-rats, respectively, but the differences reached statistical significance only in HH-rats (HH-rats: vehicle vs MLT, t = 3.926, p<0.001; Baseline vs MEL, t = 3.547, p< 0.01). No significant differences were noted between HH and LH rats after MEL treatment.**

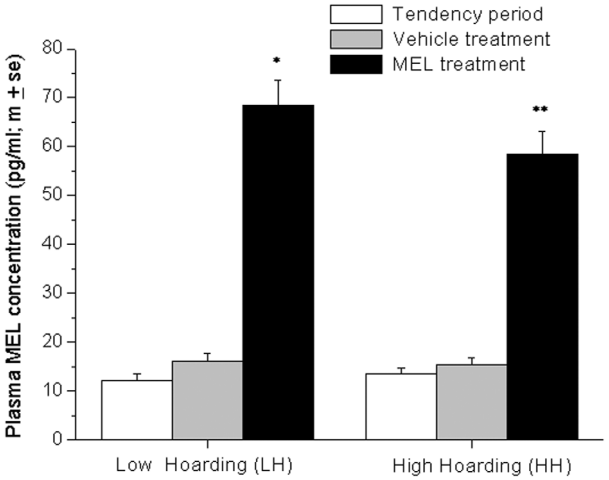

Fig. 3 MEL concentrations at the end of tendency period were not significtly different between HH-rats and LH-rats. Vehicle administration had no effect on MEL concentration. On the contrary, MEL concentration increased in both LH and HH rats after treatment with MEL, as compared to the concentration of the tendency period and of vehicle treatment (*LH-rats: MEL vs Tendency; MEL vs Vehicle; **HH-rats: MEL vs Tendency; MEL vs Vehicle; in all comparisons t < 0.0001). After MEL treatment, no significant difference were found between MEL concentration of LH and HH rats.

Results

Plasma leptin concentration. *A. Hoarding Tendency period.* Leptin plasma concentrations at the end of hoarding tendency experiment were determined to verify whether food hoarding behavior might depend on plasma leptin levels. The plasma leptin concentration of LH-rats (2.12 + 0.38 ng/ml) was lower than that of HH-rats (3.32 + 0.43 ng/ml), and the difference just met the statistical criterion of significance [F(1, 14) = 4.37, p = 0.0505)] (Fig. 2).

B. MEL treatment. Leptin concentrations measured during the tendency experiment were used as baseline in the comparisons between leptin concentrations determined at the end of either MEL or vehicle treatment period. Leptin concentrations at the end of either MEL or vehicle treatments were independent of dosages used in both HH-rats and LH-rats. Thus, respective data were pooled for all further statistical analyses. Pooled results are shown in Fig. 2. MEL treatment significantly decreased plasma leptin levels as compared to those of the vehicle group (control) and those before treatments in both HH-rats and LH-rats [F(5, 42) = 6.01, p = 0.0001] (Fig. 2). In particular, *post-hoc* results showed that the administration of MEL in HH-rats significantly reduced leptin concentration (HH-rats: vehicle vs MEL, t = 3.926, p<0.001; Baseline vs MEL, t = 3.547, p< 0.01). No significant differences were noted between HH and LH rats after MEL treatment.

Plasma MEL concentration. *A. Hoarding Tendency period.* The MEL plasma concentration at the end of the hoarding tendency experiment was not significantly different between HH-rats and LH-rats, and ANOVA [F(1, 14) = 0.525; n. s.] did not reveal significant interactions (Fig. 3).

B. MEL treatment. ANOVA [F(5, 42) = 68.98, p<0.0001] showed that vehicle administration had no effect on MEL concentration in both HH-rats and LH-rats at the end of the treatment period. In contrast, a marked increase in plasma MEL concentration was observed in both HH-rats and LH-rats treated with this hormone (Fig. 3). In particular, *post-hoc* results showed that MEL concentration increased in both LH and HH rats, as compared with hording tendency and vehicle data (LH-rats: MEL vs Tendency, t = 12.890; MEL vs Vehicle, t = 9.879; HH-rats: MEL vs Tendency, t = 10.319; MEL vs Vehicle, t = 9.879; in all comparisons t<0.0001). On the contrary, after MEL treatment, no

significant differences were found between plasma MEL concentration of LH and HH rats.

Discussion

We hypothesized that MEL might be involved in the induction of food-hoarding behavior and we based our hypothesis on the evidence that MEL is involved in the regulation of body weight, fat deposition, metabolism, and several seasonal processes (Morgan and Mercer 2001; Puchalski et al. 2003a; Puchalski et al. 2003b). We tested MEL since this hormone is correlated with leptin secretion (Baydas et al. 2001; Kus et al. 2004), and thus it might be a factor influencing food-hoarding. Moreover, Buckley and Schneider (2003) discussing their data on the influences of leptin treatments on food-hoarding in hamsters state that ".... leptin is not the only factor that influences hoarding, and thus hoarding behavior might be sensitive to changes in other hormones... (pg. R1027)".

The primary findings of the present study are: 1) rats show a bimodal distribution of the hoarding score; 2) MEL treatment influence food-hoarding in rats; 3) MEL treatment reduces plasma leptin concentration in high and low hoarding rats.

The result that about one-half of the rats showed a low tendency to hoard (LH-rats) whereas the other half showed an high tendency (HH-rats) is consistent with data on hamsters (Buckley and Schneider 2003). Our data demonstrated that rats present a bimodal distribution of food hoarding scores and that this behaviour may be a general behaviour in low rodents. The differences in hoarding tendency are not related to innate differences in the food intake; indeed, no significant difference was observed in food intake in home cages between LH and HH rats. The bimodal hoarding distribution prompts two questions. The first is whether hoarding is an innate or acquired behavior, or rather, whether differences in hoarding tendency stem from genetic or environmental factors (Buckley and Schneider 2003). Vander Wall (1990) suggests that the hoarding behavior in rodents might be a product of genetic endowment and environmental influences. Indeed, Manosevitz (1967) estimated that the heritability of hoarding in mice ranged from 0.25% to 0.55% and that the rest of phenotypic variability was due to environmental factors. Thus, the genetic component is large enough to be of considerable importance in understanding hoarding behavior, but it is not completely exhaustive. In other words, hoarding behavior may be the result of interactions between developmental processes corresponding to maturation of the endocrine and nervous system and learning from individual experience (Vander Wall 1990).

Within the context of genetic and neuro-hormonal regulation of hoarding, hoarding intensity may be further modulated by stimuli impinging on the organism from the environment. There is a trend towards an additive effect of isolation-reared and sawdust floors on increasing both the number and weight of pellets hoarded by rats (Heidbreder et al. 2000). Social experiences such as dominant-subordinate relationships that develops when the animals are group housed before weaning or during shipping may influence food-hoarding (Buckley and Schneider 2003). Since the endocrine system is able to transduce events of the social environment into an endocrine message, some endocrine glands (e.g., pineal) may play an important role in central processing of social stress (Heinzeller et al. 1988).

In accordance with the literature (Baydas et al. 2001; Canpolat et al. 2001; Rasmussen et al. 1999; Rasmussen et al. 2001; Wolden-Hanson et al. 2000; Kus et al. 2004; Puchalski et al. 2003a), our second experiment showed that MEL treatment decreased plasma leptin levels in both LH and HH rats, and this effect was independent of total food consumption and body weight. Since the body weight does not change, it is possible argue that the decrease of circulating leptin may be explained by a direct effect of MEL on leptin production rather by modification in body composition.

Our results confirmed the inverse correlation between MEL and leptin plasma concentration in rats, too. In particular, at the end of MEL treatment leptin concentration becomes almost equal in both LH and HH rats (Fig. 2). Obviously, MEL concentration increased significantly in both LH and HH rats after MEL treatment (Fig.3). After MEL treatment, the number of pellets hoarded by LH-rats increased significantly; on the contrary, the pellets hoarded by HH-rats decreased but not significantly (Fig. 1).

An important question arising from these results is why, after MEL treatment, LH-rats increased hoarding behavior in spite of the fall in leptin concentration, whereas the reduction of leptin concentration in HH-rats did not significantly influence hoarding behavior. One explanation may be that MEL treatment directly influences hoarding behavior, independent of its inverse correlation with leptin concentration. Moreover, the inability of MEL to increase food hoarding in rats that

have natural proclivities to hoard high amounts of food likely may be a ceiling effect.

It is known that MEL treatment may influence psychosocial behavior (Heinzeller et al. 1988; Fuchs and Schumacher 1990) and that factors leading to hoarding are by no means entirely alimentary. Food hoarding has been explained by two main hypothesis. The "*object value*" hypothesis, which suggests that rats hoard objects that they perceive as valuable as related to some state or need (Fantino and Cabanac 1980), and the "*security hypothesis*" (Bindra 1948) which postulates that rats hoard food to escape exposure while eating in an open environment. According to the last hypothesis, eating in an open field induces fear or anxiety, which a rat can avoid by carrying the food to a covered and familiar environment.

The "*security hypothesis*" has been validated in rats by the use of the anxiolytic drug diazepam (McNamara and Whishaw 1990). MEL treatment reduces neophobia and exerts anxioloytic-like properties without sedative effects (Golombek et al. 1993; Delagrange et al. 2003). Exogenous MEL treatment raises aggression in male and female hamsters (Jasnow et al. 2002; Demas et al. 2004; Fleming et al. 1988). In rodents, the MEL mediates aggression, indeed male house mice treated daily with MEL for 5 days displayed significant increase in territorial aggression (Paterson and Vickers 1981). In addition, pinealectomy suppresses aggression in female hamsters (Fleming et al. 1988) and in mice (Paterson and Vickers 1981). Melatonin-induced increase in aggression may be due to the direct action of this hormone on neural substrates mediating aggression (e.g., hypothalamus, amygdala, limbic system) or mediated by adrenocortical rather that adrenomedullary hormones (Demas et al. 2004). Moreover, increased aggression is not due to changes in body mass or gonadal regression, as both these parameters are unaffected by exogenous MEL treatment (Demas et al. 2004). MEL treatment might increase aggressiveness and daring, might reduce anxiety and neophobia in LH-rats and, in turn, in accordance with the "*security hypothesis*", it might be responsible for the increase of number of pellets hoarded. In HH-rats, MEL treatment reduced leptin concentration, which reached the same level of LH-rats. Our results showed that in HH-rats the number of pellets hoarded decreased but not significantly (Fig. 1). Two explainations may be advanced: 1) the concentration of MEL used during the treatment probably did not allow leptin concentration to reach the critical level essential for significantly decreasing food hoarding; 2) MEL treatment increased MEL concentration (Fig. 3), which might nullify the effects of the reduced plasma leptin levels.

In conclusion, our results do not clarify whether food-hoarding is an innate or an acquired behavior, but clearly confirm that the endocrine system either directly, by the action of one or more combined hormones (MEL, leptin), or indirectly via its actions on neural substrates (e.g., hypothalamus, amygdala, limbic system, hippocampus, thalamus) determines, at least in part, hoarding behavior of rats. Moreover, our data show that hoarding behavior is controlled to some extent not only by sex hormones (Vander Wall 1990), but also by MEL and leptin and provide new insight into whole body integration and endocrine functions.

References

American Physiological Society: Guiding principles for research involving Animals and Human Beings. Am J Physiol (Regul Integr Comp Physiol) **283**, R281-R283, 2002

Bartness TJ, Clein MR. Effects of food deprivation and restriction, and metabolic blockers on food hoarding in Siberian hamsters. Am J Physiol (Regul Integr Comp Physiol) **266**, R1111-R1117, 1994

Baydas G, Gursu F, Canpolat S, Konar V, Yasar A, Canatan H, Kelestimur H. Effects of pinealectomy on the circadian release pattern of leptin in male rat. Neuroendocrinology Letters **22**, 449-452, 2001

Bindra, D. What makes rats hoard? J Comp Physiol Psychol **41**, 397-402, 1948

Brydon L, Petit L, Delagrange P, Strosberg AD, Jockers R. Functional expression of MT2 (Mel1b) melatonin receptors in human PAZ6 adipocytes. Endocrinology **142**, 4264-4271, 2001

Bubenik GA, Pang SF. The role of serotonin and melatonin in gastrointestinal physiology: ontogeny, regulation of food intake, and mutual serotonin-melatonin feedback. J Pineal Res **16**, 91-99, 1994

Buckley CA, Schneider JE. Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters. Am J Physiol (Regul Integr Comp Physiol) **285**, R1021-R1029, 2003

CABANAC M, GOSSELIN C. Ponderostat: hoarding behavior satisfies the condition for a lipostat in the rat. Appetite **27**, 251-261, 1996

CAGNACCI A, MALMUSI S, ZANNI A, ARANGINO S, CAGNACCI P, VOLPE A. Acute modifications in the levels of daytime melatonin do not influence leptin in postmenopausal women. J Pineal Res **33**, 57-60, 2002

CANPOLAT S, SANDAL S, YILMAZ B, YASAR A, KUTLU S, BAYDAS G, KELESTIMUR H. Effects of pinealectomy and exogenous melatonin on serum leptin levels in male rat. Europ J Pharmacol **428**, 145-148, 2001

DAY DE, BARTNESS TJ. Agouti-related protein increases food hoarding more than food intake in Siberian hamsters. Am J Physiol (Regul Integr Comp Physiol) **286**, R38-R45, 2004

DELAGRANGE P, ATKINSON J, BOUTIN JA, CASTEILLA L, LESIEUR D, MISSLIN R, PELLISIER S, PENICAUD L, RENARD P. Therapeutic perspectives for Melatonin agonists and antagonists. J Neuroendocrinology **15**, 442-448, 2003

DEMAS GE, POLACEK KM, DURAZZO A, JASNOW A. Adrenal hormones mediate melatonin-induced increases in aggression in male Siberian hamsters (*Phodopus sungorus*). Horm Behav **46**, 582-591, 2004

DIBATTISTA D, BEDARD M. Effects of food deprivation on hunger motivation in gold hamsters (*Mesocricetus auratus*). J Comp Psychol **101**, 183-189, 1987

FANTINO M, CABANAC M. Body weight regulation with a proportional hoarding response in the rat. Physiol Behav **24**, 939-942, 1980

FLEMING AS, PHILLIPS A, RYDALL A, LEVESQUE L. Effects of photoperiod, the pineal gland and the gonades on agonistic behavior in female golden hamsters (*Mesocricetus aureatus*). Physiol Behav **44**, 227-234, 1988

FUCHS E, SCHUMACHER M. Psychosocial stress affects pineal function in the tree shrew (*Tupaia belangeri*). Physiol Behav **47**, 713-717, 1990

GENIN F, SCHILLING A, CLAUSTRAT B. Melatonin and methimazole mimic short-day-induced fattening in gray mouse lemurs. Physiol Behav **79**, 553-559, 2003

GOLOMBEK DA, MARTINI M, CARDINALI DP. Melatonin as an anxiolytic in rats: time dependence and interaction with the central GABAergic system. Eur J Pharmacol **237**, 231-236, 1993

GUNDUZ B. Daily rhythm in serum melatonin and leptin levels in the Syrian hamster (Mesocricetus auratus). Comp Biochem Physiol Part A **132**, 393-401, 2002

HEIDBREDER CA, WEISS IC, DOMENEY AM, PRYCE C, HOMBERG J, HEDOU G, FELDON J, MORAN MC, NELSON P. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience **100**, 749-768, 2000

HEINZELLER T, JOSHI BN, NURNBERGER F, REITER RJ. Effects of aggressive encounters on pineal melatonin formation in male gerbils (*Meriones unguiculatus, Cricetide*). J Comp Physiol (A) **164**, 91-94, 1988

HORTON TH, BUXTON OM, LOSEE-OLSON S, TUREK FW. Twenty-four-hour profiles of serum leptin in Siberian and Golden hamsters: photoperiodic and diurnal variations. Horm Behav **37**, 388-398, 2000

JASNOW AM, HUHMAN KL, BARTNESS TJ, DEMAS GE. Short days and exogenous melatonin increase aggression of male Syrian hamsters (*Mesocricetus auratus*). Horm Behav **42**, 13-20, 2002

KUS I, SARSILMAZ M, COLAKOGLU N, KUKNER A, OZEN OA, YILMAZ B, KELESTIMUR H. Pinealectomy increases and exogenous melatonin decreases leptin production in rat anterior pituitary cells: an immunohistochemical study. Physiol Res **53**, 403-408, 2004

MANOSEVITZ M. Hoarding and inbred strains of mice. J Comp Physiol Psychol **63**, 148-150, 1967

MASTRONARDI CA, WALCZEWSKA A, YU WH, KARANTH S, PARLOW A F, MCCANN SM. The possible role of prolactin in the circadian rhythm of leptin secretion in male rats. Proc Soc Exp Biol Med **224**, 152-158, 2000

MCNAMARA RK, WHISHAW IQ. Blockade of hoarding in rats by diazepam: an analysis of the anxiety and object value hypotheses of hoarding. Psychopharmacology **101**, 214-221, 1990

MORGAN PJ, MERCER JG. The regulation of body weight: lessons from the seasonal animal. Proc Nutr Soc **60**, 127-134, 2001

NIEMINEN P, MUSTONEN AM, ASIKAINEN J, HYVARINEN H. Seasonal weight regulation of the raccoon dog (*Nyctereutes procyonoides*): interactions between melatonin, leptin, ghrelin, and growth hormone. J Biol Rhythms **17**, 155-163, 2002

PATERSON AT, VICKERS C. Melatonin and the adrenal cortex: relationship to territorial aggression in mice. Physiol Behav **27**, 983-987, 1981.

PUCHALSKI SS, GREEN JN, RASMUSSEN DD. Melatonin effect on rat body weight regulation in response to high-fat diet at middle age. Endocrine **21**, 63-167, 2003a

PUCHALSKI SS, GREEN JN, RASMUSSEN DD. Melatonin effects on metabolism independent of gonad function. Endocrine **21**, 169-173, 2003b

RASMUSSEN DD, BOLDT BM, WILKINSON CW, YELLON SM, MATSUMOTO AM. Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. Endocrinology **140**, 1009-1012, 1999

RASMUSSEN DD, MITTON DR, LARSEN SA, YELLON SM. Aging-dependent changes in the effect of daily melatonin supplementation on rat metabolic and behavioral responses. J Pineal Res **31**, 89-94, 2001

RHINEHART K, BARTNESS TJ. Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. Am J Physiol (Regul Integr Comp Physiol) **288**, R716-R722, 2005

STAMM JS. Genetics of hoarding. I. Hoarding differences between homozygous strains of rats. J Comp Physiol Psychol **47**, 157-161, 1954

VANDER WALL SB. Food hoarding in animals. The University of Chicago Press, Chicago 1990

WANG J, LIU R, HAWKINS M. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature **393**, 805-808, 1998

WHISHAW IQ, ODDIE SD, MCNAMARA RK, HARRIS TL, PERRY BS. Psychophysical methods for study of sensory-motor behavior using a food-carrying (hoarding) task in rodents. J Neurosci Methods **32**,123–133, 1990

WOLDEN-HANSON T, MITTON DR, MCCANTS RL. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology **141**, 487-497, 2000.