Am J Physiol Regul Integr Comp Physiol 287: R1071–R1079, 2004;
doi:10.1152/ajpregu.00294.2004.

Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation

B. Bodosi,[1] J. Gardi,[2] I. Hajdu,[1] E. Szentirmai,[1] F. Obal, Jr.,[1] and J. M. Krueger[3]

[1]*Department of Physiology and* [2]*Endocrine Unit, University of Szeged, A. Szent-Györgyi Medical Center, 6720 Szeged, Hungary; and* [3]*Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, Washington 99164-6520*

Submitted 4 May 2004; accepted in final form 21 June 2004

Bodosi, B., J. Gardi, I. Hajdu, E. Szentirmai, F. Obal, Jr., and J. M. Krueger. Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. *Am J Physiol Regul Integr Comp Physiol* 287: R1071–R1079, 2004; doi:10.1152/ajpregu.00294.2004.—To determine the relationships among plasma ghrelin and leptin concentrations and hypothalamic ghrelin contents, and sleep, cortical brain temperature (T_{crt}), and feeding, we determined these parameters in rats in three experimental conditions: in free-feeding rats with normal diurnal rhythms, in rats with feeding restricted to the 12-h light period (RF), and in rats subjected to 5-h of sleep deprivation (SD) at the beginning of the light cycle. Plasma ghrelin and leptin displayed diurnal rhythms with the ghrelin peak preceding and the leptin peak following the major daily feeding peak in *hour 1* after dark onset. RF reversed the diurnal rhythm of these hormones and the rhythm of rapid-eye-movement sleep (REMS) and significantly altered the rhythm of T_{crt}. In contrast, the duration and intensity of non-REMS (NREMS) were hardly responsive to RF. SD failed to change leptin concentrations, but it promptly stimulated plasma ghrelin and induced eating. SD elicited biphasic variations in the hypothalamic ghrelin contents. SD increased plasma corticosterone, but corticosterone did not seem to influence either leptin or ghrelin. The results suggest a strong relationship between feeding and the diurnal rhythm of leptin and that feeding also fundamentally modulates the diurnal rhythm of ghrelin. The variations in hypothalamic ghrelin contents might be associated with sleep-wake activity in rats, but, unlike the previous observations in humans, obvious links could not be detected between sleep and the diurnal rhythms of plasma concentrations of either ghrelin or leptin in the rat.

hypothalamic ghrelin; corticosterone; brain temperature; electroencephalogram delta power; sleep duration

GHRELIN AND LEPTIN are humoral feedback signals from the periphery to the hypothalamic neuronal network regulating energy homeostasis. Neuropeptide Y (NPY)-containing neurons in the arcuate nucleus are major targets of both hormones (reviewed in Ref. 24). Ghrelin and growth hormone (GH) secretagogues (GHSs), which are synthetic ligands of the GHS receptor that bind ghrelin, stimulate these neurons (14, 27), promote NPY expression (45, 47) and release (59), and elicit food intake (47, 60). Leptin inhibits NPY neurons (27) and NPY expression (2, 47, 56) and suppresses feeding (47, 56). Ghrelin promotes deposition of fat (55), whereas leptin stimulates energy expenditure (22) mediated by sympathetic activity-induced stimulation of uncoupling proteins in brown fat tissue and perhaps elsewhere (reviewed in Ref. 50). Leptin is released from white adipose tissue as a function of insulin-dependent glucose metabolism in fat cells (reviewed in Ref. 20). Ghrelin is produced predominantly by endocrine cells in the oxyntic gastric mucosa (12, 28). Ghrelin is also a neurotransmitter in the hypothalamic arcuate nucleus (28, 31).

Both leptin (53, 57) and ghrelin (and GHSs) (13, 46, 60) stimulate the somatotropic system. This ghrelin or GHS action requires intact functioning of the hypothalamic growth hormone (GH)-releasing hormone (GHRH) system (32, 38, 40, 52). Ghrelin/GHSs stimulate GHRH neurons (14) and elicit GHRH release (59). GHRH is also involved in the mediation of leptin's action on GH secretion. Leptin enhances GHRH mRNA expression (9) and elicits GHRH release (57). Several lines of evidence demonstrate that GHRH is part of a hypothalamic network promoting physiological non-rapid eye movement sleep (NREMS) (39). Through GHRH, ghrelin and leptin may modulate sleep. In fact, both leptin and ghrelin have been linked to sleep regulation. For example, systemic leptin decreases rapid eye movement sleep (REMS) and stimulates deep NREMS in rats (49). Two GHSs (GHRP-6 and MK-677) are reported to promote sleep (10, 18) and one (GHRP-2) was inactive (33) in human subjects. Ghrelin itself also enhances NREMS in humans (58) and mice (38) although it increased wakefulness in rats due to stimulation of feeding (54). Another line of experiments implicating ghrelin and leptin in sleep regulation suggests that secretions of ghrelin and leptin are in part related to sleep because sleep deprivation (SD) alters plasma concentrations of these hormones (16, 35), or shifts in the sleep period are followed by shifts in hormone secretions (48). These observations were all obtained in human subjects.

The aim of our experiments was to study whether variations in plasma leptin and ghrelin concentrations and hypothalamic ghrelin contents are related to sleep-wake activity in the rat. First, diurnal rhythms of ghrelin and leptin were determined in rats with normal diurnal feeding (free feeding) and sleep-wake rhythms. Then, hormone rhythms and sleep-wake rhythms were compared in rats on a restricted feeding (RF) schedule when food was available only during the 12-h light period. It was hypothesized that the normal relationship between sleep and hormone secretions is maintained in the RF condition if there are links between these events. Finally, SD was used to disturb sleep, and the changes in plasma hormone concentra-

Address for reprint requests and other correspondence: J. M. Krueger, Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520 (E-mail: krueger@vetmed.wsu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

 0363-6119/04 $5.00 Copyright © 2004 the American Physiological Society

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

tions and hypothalamic ghrelin were studied during SD and during recovery after SD.

METHODS

Animals. Male Sprague-Dawley rats weighing 300–330 g were used. There were 47–55 rats in each of the three experiments. Institutional guidelines for the care and use of research animals were followed and approved by the institutional committees. The experiments are consistent with the "Guiding Principles for Research Involving Animals and Human Beings" issued by the American Physiological Society (4).

Surgery. The surgeries were carried out under ketamine-xylazine (87 and 13 mg/kg, respectively) anesthesia. Each animal was implanted with stainless steel screws as electrodes for electroencephalographic (EEG) recording over the frontal and parietal cortexes and the cerebellum. A thermistor placed over the parietal cortex was used to record changes in cortical brain temperature (T_{crt}).

Sleep-wake recording. The rats were housed in individual Plexiglas cages. The cages were placed in recording chambers with a 12:12-h light-dark cycle and with an ambient temperature regulated at 26°C. Water was continuously available. The rats were kept in conditions identical to those in the recording chambers for at least 1 mo before the operation. After surgery, the rats were provided at least 10 days of recovery during which they were connected to the recording cable.

The recording cables were attached to commutators. The motor activity was assessed by means of recording potentials generated in electromagnetic transducers activated by movements of the cables. The digitized (64-Hz sampling rate) signals of the EEG, T_{crt}, and motor activity were collected by computers in an adjacent room. The states of vigilance were scored in 8-s epochs from signals restored on the computer screen. Power density values were calculated by fast-Fourier transformation for consecutive 8-s epochs in the frequency range of 0.25–20.0 Hz for 0.25-Hz bands and were integrated for 0.5-Hz bins. The spectra were also displayed on the screen. The states of vigilance were determined over 8-s epochs by the usual criteria as NREMS [high-amplitude slow waves in the EEG, lack of body movements, declining cortical temperature after entry, predominant power in the delta range (0.5–4.0 Hz)]; REMS [highly regular theta activity in the EEG with corresponding high theta power in the spectrum, general lack of body movements with occasional twitches, and a rapid rise in brain temperature at onset]; and wakefulness (less regular theta activity and significant theta power, higher delta power than during REMS, frequent body movements, and a gradual increase in brain temperature after arousal). The percentage of the time spent in each state of vigilance in consecutive 1-h periods and for the 12-h light and 12-h dark periods was determined. The power values for the 0.25- to 4-Hz delta range during NREMS were integrated and used to characterize sleep intensity (1) in each recording hour. T_{crt} was averaged for 1-h periods.

Recording of feeding activity. Food pellets were placed in vertical aluminum tubes hanging in one corner of the cage; stainless steel bars kept the pellet in the opening of the tube. Movements of the tube generated potentials in electromagnetic transducers, and these potentials were recorded together with the EEG signals, T_{crt}, and motor activity. The records were subjected to spectral analysis, and the total power in 8-s epochs was regarded as feeding activity. Feeding activity was integrated for 1-h periods. To standardize the different values obtained in the various feeders, the total feeding activity in 24 h was taken as 100%, and the percent fraction of feeding activity was calculated for each recording hour. It is noted that this method of determination of feeding activity does not measure the quantity of food consumed, and it also misses food spillage and eating from the cage floor. However, the reproducibility of feeding activity was high in the three experiments, indicating that the method was suitable for the aims of these studies.

Experimental protocol. Sleep-wake activity, T_{crt}, and feeding activity were recorded for 3 days in each rat. On *day 4*, groups of rats ($n = 7$–12) were killed by means of a guillotine at 4-h intervals at the following time points: light period at 1, 5, and 9 h after light onset; dark period at 13, 17, and 21 h after light onset, i.e., 1, 5, and 9 h after dark onset. The trunk blood was collected and immediately centrifuged, and the plasma was stored at −80°C until the hormone assays. The hypothalamus was harvested by using the following landmarks, i.e., frontal edge of the optic chiasm, lateral sulci, caudal edge of the mammary bodies, and a depth of 2 mm, and was also stored at −80° until used. The mean (±SE) mass of the hypothalamus was 42.0 ± 0.81 mg without significant differences among the groups.

In *experiment 1* with free-feeding rats, the access to the feeding tubes was continuous. In *experiment 2* with RF, the feeders were removed at dark onset and returned at light onset. These rats were on RF for 3 wk before recording. Rats in an identical experiment learned to adapt in 1 wk to RF so that the daily amount of food and their body weight remained normal (43). The amounts of daily-consumed food were determined in eight free-feeding rats (26.4 ± 0.73 g) and in eight RF rats (24.6 ± 0.59 g) without significant differences between them (Student's *t*-test) in our experiments. In *experiment 3*, the rats were sleep deprived by means of gentle handling. The rats stayed in their home cage, and whenever behavioral or EEG signs of sleep were observed, they were aroused by knocking on the cage or lightly touching them. The feeding tubes were continuously available. The SD started at light onset and lasted for 5 h. Because the groups of rats were killed at 4-h intervals, feeding activity could not be standardized as described above for 24-h on *day 4*. Feeding values were obtained on *day 4* in all rats until the time of their death. To make the percent distribution feeding calculation, a 24-h value is needed. To obtain those values for *day 4* in those rats killed before the end of *day 4*, the mean hourly feeding activity for the previous 3 recording days for each rat were used for the missing hours (after death). The calculation provided the fraction of feeding activity during and after SD assuming that the total 24-h feeding activity remained unaltered. The last group of rats was killed in *hour 21*, and the pattern of feeding activity in this group supported that this calculation was correct.

Hormone measurements. Commercially available radioimmunoassay kits were used to determine ghrelin (Phoenix Pharmaceuticals, Belmont, CA) and leptin (LINCO Research, St. Charles, MO), whereas corticosterone was measured by means of enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX). According to the supplier, the antiserum to rat ghrelin displayed 100% cross-reactivity with human and canine ghrelin, human and rat ghrelin-(des-octanoyl-Ser3), and ghrelin-(17–28) (human, rat). There was a 3% cross-reaction between the antiserum and the COOH-terminal ghrelin hexapeptide (human, rat). The antiserum to ghrelin did not recognize some other ghrelin fragments, human secretin, vasoactive intestinal peptide (human, rat, porcine), human and rat prolactin-releasing peptide-31, human and rat galanin, human and rat GHRH, NPY (human, rat), orexin A (human, rat, mouse), and human orexin B. The antiserum to rat leptin does not recognize glucagon, somatostatin, and insulin. The antiserum to rat corticosterone displayed 5.4% cross-reactivity with dehydroxycorticosterone. The hormones were measured in duplicate or triplicate with a sensitivity of 4 pg/tube for ghrelin, 0.5 ng/ml for leptin, and 4 ng/ml for corticosterone. The intra- and interassay coefficients of variation were less than 8% and 14%, respectively.

For extraction of hypothalamic samples, the frozen samples were weighed and placed in tubes containing 0.5 ml 2 M acetic acid and then boiled for 5 min. The tissues were individually homogenized by means of ultrasound. The homogenates were centrifuged at 15,000 *g* for 20 min at 4°C. The supernatant was lyophilized for the ghrelin radioimmunoassay.

Statistics. Two-way ANOVA for repeated measures was used to compare sleep-wake activity, T_{crt}, and feeding among groups/experiments. Hormone concentrations were analyzed by means of one-way

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

ANOVA. When ANOVA indicated significant variations, the different group or hour was identified by means of the Student-Newman-Keuls test. Although each experiment included 47–55 rats, the sample sizes varied slightly in the individual tests because a few records and samples were lost or omitted for technical reasons. In particular, the sample sizes dropped significantly in the SD experiment because the repeated-measures ANOVA on *day 4* accepted only the last survivor group of rats. Student *t*-test was used to compare mean values in the light and dark periods. An α-level of $P < 0.05$ was considered to be significant in all tests.

RESULTS

Experiment 1: normal rhythm in free-feeding rats. Free-feeding rats ate mostly at night; only a small fraction of the total daily feeding activity was observed in the light period (Table 1, Fig. 1). Dark onset was rapidly followed by vigorous eating, and 13.9 ± 1.24% of the daily feeding activity occurred during the first hour of the dark period. Feeding activity bouts recurred throughout the night.

The diurnal rhythm of sleep-wake activity displayed the normal patterns of rats (Fig. 1, Table 1). NREMS peaked at the beginning of the light cycle. Thereafter it declined steadily toward lights off. NREMS time dropped markedly at dark onset and stayed low during the dark period. As characterized by EEG delta power during NREMS, maximum NREMS intensity occurred at the beginning of the rest period. Delta power then decreased throughout the day. In the dark period, the depth of consecutive sleep bouts increased and delta power during NREMS gradually increased to reach the subsequent morning peak. REMS also displayed a diurnal rhythm with high and low amounts during the light and the dark period, respectively. REMS time was maximum in the second portion of the light period.

T_{crt} displayed 0.9°C difference between day and night [$F(23,1027) = 143.652$, $P < 0.001$] with the mean T_{crt} at night being significantly ($P < 0.001$) higher than during the light period (Fig. 1, Table 1).

Plasma leptin [$F(5,45) = 12.3$, $P < 0.0001$] and ghrelin [$F(5,44) = 5.05$, $P = 0.001$] displayed distinct diurnal rhythms with their peak values occurring at opposite times of the day (Fig. 1, Table 1). The leptin maximum followed the dark onset-elicited eating peak and occurred at 17 h (5 h after dark onset) at night. The ghrelin peak preceded the eating peak and occurred 5 h after light onset when leptin dropped to its diurnal trough value. Ghrelin was still relatively high in *hour 9* of the light period.

Table 1. *Mean (± SE) values of non-REM and REM sleep time, cortical brain temperature, feeding activity, plasma leptin and ghrelin concentrations, and hypothalamic ghrelin contents in 24-h, in the 12-h light period, and in the 12-h dark period in rats free-feeding on normal diurnal rhythm and in rats with food restricted to the 12-h light period*

	Free Feeding	Restricted Feeding
NREMS, %Rec time		
Total	43.5±0.42	43.0±0.44
Light	61.4±0.54	50.3±0.62*
Dark	25.7±0.78	35.6±0.63*
REMS, %Rec time		
Total	8.4±0.19	8.9±0.15
Light	12.4±0.37	6.2±0.25*
Dark	4.5±0.27	11.5±0.31*
T_{crt}, °C		
24 h	37.6±0.08	37.7±0.08
Light	37.2±0.09	37.6±0.09*
Dark	38.1±0.08	37.7±0.09*
Feeding Activity, %24 h		
Light	13.5±1.67	100
Dark	86.5±1.62	0
Leptin, ng/ml		
24 h	3.5±0.24	5.3±0.47*
Light	2.6±0.15	6.6±0.71*
Dark	4.6±0.38	3.7±0.33
Peak	6.2±0.91	7.8±0.72
Plasma ghrelin, pg/ml		
24 h	1,333.3±77.58	1,735.4±142.23*
Light	1,549.0±123.54	1,247.7±91.96*
Dark	1,099.6±64.60	2,365.3±252.96*
Peak	1,857.7±257.53	3,649.8±401.12*
Hypothalamic ghrelin, pg/mg		
24 h	1.04±0.027	1.10±0.018*
Light	1.01±0.038	1.07±0.020
Dark	1.07±0.038	1.15±0.029

Values are means ± SE. REM, rapid eye movement; REMS, REM sleep; NREMS, non-REM sleep; T_{crt}, cortical brain temperature; %Rec time, % recording time. * Significant difference (Student *t*-test) between free-feeding rats and rats on restricted feeding.

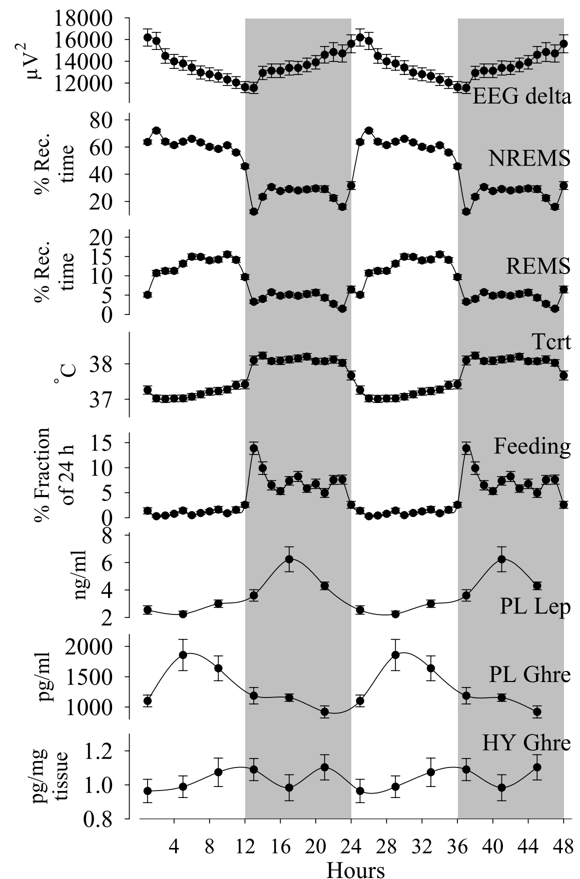

Fig. 1. Diurnal rhythms (hourly mean ± SE) of EEG delta power during non-rapid eye movement sleep (NREMS), time spent in NREMS and rapid eye movement sleep (REMS), cortical brain temperature (T_{crt}), and feeding activity were determined on 3 days and averaged for 24 h in 51 rats. Diurnal rhythms of plasma (PL) leptin (Lep) and ghrelin (Ghre) concentrations, and hypothalamic (HY) ghrelin contents were determined on *day 4* of recording in groups of rats ($n = 7$–10) killed at 4-h intervals. The 24-h curves are double-plotted to promote visualization of the rhythms. *Time 0*, light onset. Gray columns, dark period. %Rec time, %recording time; %Distrib/24 h, %distribution/24 h.

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

The ghrelin content of the hypothalamus was low (Table 1). Hypothalamic ghrelin showed modest oscillations with a rise after the plasma ghrelin peak and a second increase toward the end of the dark period (Fig. 1). However, these changes in hypothalamic ghrelin did not reach the level of statistical significance.

Plasma corticosterone was close or below detection limit in most rats in *hours 1* and *5* of the light cycle and started to rise toward the end of the light period [$F(2,22) = 25.099$, $P < 0.001$] (see Fig. 4). The value in *hour 9* differed significantly from corticosterone in *hour 1* or *5*.

Experiment 2: restricted feeding. When food was available only in the light cycle, the rats produced vigorous eating in *hour 1* of the light period (Fig. 2). The fraction of the daily feeding activity in *hour 1* of the light period was 31.8 ± 2.24%, and this value was significantly higher (*t*-test, $P < 0.001$) than the feeding activity of rats on the normal cycle in *hour 1* of the dark period. After *hour 1*, feeding initially dropped to low levels and then tended to increase toward the end of the light period in the RF rats.

RF altered the various sleep parameters differentially. Total NREMS did not change (Table 1).

NREMS time decreased by 11% in the light period [treatment factor: $F(1,100) = 179.012$, $P < 0.001$] and increased by 10% in the dark period [treatment factor: $F(1,100) = 102.147$, $P < 0.001$]. However, the diurnal rhythm with more NREMS during the day was clearly maintained (Table 1, Fig. 2). Reductions in NREMS during the day occurred only in *hour 1* and in the last hours when the rats spent more time with eating [group × time interaction: $F(11,1100) = 35.984$, $P < 0.001$]. In between, NREMS stayed at baseline values. At night, sleep bouts consisted of more NREMS epochs than in free-feeding rats [time × treatment interaction, $F(11,1100) = 11.333$, $P < 0.001$]. The daily rhythm EEG delta power during NREMS was particularly resistant to restricted feeding (Fig. 2). There were only three hourly values that differed from baseline [time × treatment interaction for 24-h recording: $F(23,2263) = 22.584$, $P < 0.001$]: *hour 1* of the light period when the rats slept little, and the first and the last hours of the dark period. In contrast to NREMS, RF fundamentally altered the diurnal rhythm of REMS (Fig. 2, Table 1). REMS decreased throughout the light period [treatment factor: $F(1,100) = 203.910$, $P < 0.001$] and increased in the dark period [treatment factor: $F(1,100) = 291.540$, $P < 0.001$], resulting in more REMS at night than during the day. These changes in REMS occurred without alterations of the total REMS time in 24 h.

T_{crt} was also responsive to RF. Mean T_{crt} increased in the light cycle and decreased in the dark cycle, and thereby the mean 24-h T_{crt} did not change although the diurnal difference disappeared between the means calculated for the light and the dark periods (Table 1). However, T_{crt} did vary [treatment × time interaction: $F(23,1191) = 30.207$, $P < 0.001$]: it peaked at the beginning of the dark period, and then T_{crt} dropped rapidly to low levels during the dark period followed by a slow increase in the subsequent light cycle (Fig. 2). The courses of T_{crt} differed between free-feeding and RF rats [time × treatment interaction: $F(23,2254) = 48.690$, $P < 0.001$].

RF had a significant impact on plasma ghrelin [comparison with free-feeding, group factor: $F(1,93) = 22.497$, $P < 0.001$; time factor: $F(5,93) = 7.360$, $P < 0.001$; time × group interaction: $F(5,93) = 21.896$, $P = 0.001$] and leptin [group factor: $F(1,94) = 9.722$, $P = 0.002$; time factor: $F(5,94) = 2.896$, $P = 0.02$; time × group interaction: $F(5,94) = 8.986$, $P < 0.001$]. The diurnal rhythm of these hormones reversed in such a way that they maintained their relationship with respect to one another and to feeding activity. The ghrelin peak continued to precede the major feeding peak, which was now in *hour 1* after lights-on, and thus the ghrelin maximum was observed at 21 h toward the end of the dark period. The leptin peak followed the major feeding activity peak, and it occurred

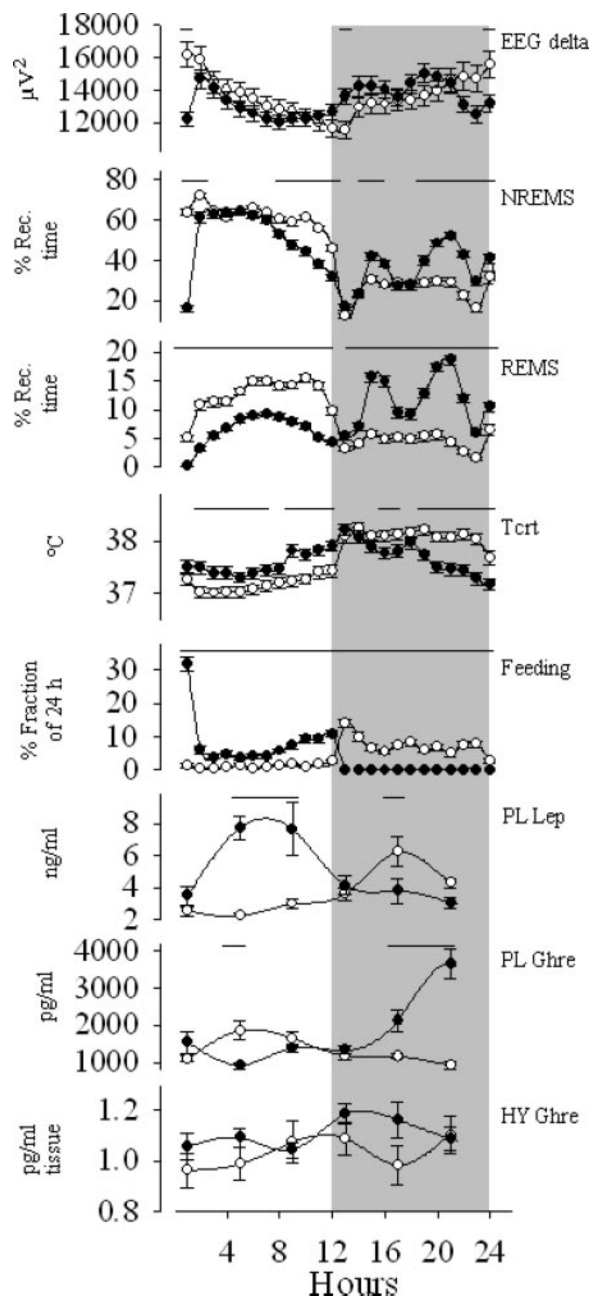

Fig. 2. Effects of feeding restricted to the light cycle on the diurnal rhythms in 55 rats (RF rats). Filled symbols, RF rats; open symbols, values obtained in the free-feeding rats shown in Fig. 1, plotted over the 24-h values of the RF rats. See legends of Fig. 1 for abbreviations. Horizontal lines above the curves: significant differences between free-feeding rats and RF rats (Student-Newman-Keuls test after ANOVA). Gray column, dark period.

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

during the day sometime between *hours 5* and *9* after light onset. The concentrations calculated for 24 h increased significantly for both leptin and ghrelin (Table 1). Although the leptin peak during RF did not differ significantly from the leptin peak on the normal cycle (Table 1), the diurnal rise in leptin obviously lasted longer than the nocturnal increase (Fig. 2). The nocturnal ghrelin peak in the rats on RF was almost double that of, and significantly higher than, the diurnal ghrelin peak in the rats on normal feeding rhythm (Table 1, Fig. 2).

Ghrelin content of the hypothalamus displayed slight oscillations (Fig. 2), which did not reach the level of statistical significance. However, comparisons of hypothalamic ghrelin contents revealed significant differences between free-feeding and RF rats [group factor: $F(1,85) = 5.149$, $P = <0.03$], and calculated for 24 h, the mean hypothalamic ghrelin content was significantly higher ($P = 0.03$) in the rats on RF than in the rats on the normal feeding schedule (Table 1).

Mean concentrations of corticosterone in the plasma of rats on RF slightly exceeded corticosterone in rats on the normal feeding at each time point during the light cycle, but significant differences were not detected (Fig. 4). Like in free-feeding rats, corticosterone concentration increased as the day progressed [$F(2,25) = 17.8$, $P < 0.001$] with the value in *hour 9* being significantly higher than corticosterone in the morning or in *hour 5*.

Experiment 3: sleep deprivation. Baseline values of sleep, T_{crt}, and feeding activity in the SD group did not differ from the values in the rats on the normal feeding cycle (Fig. 3). SD for 5 h was followed by significant albeit modest increases in the duration of both NREMS [treatment factor: $F(1,21) = 94.126$, $P < 0.001$; treatment $\times$ time interaction: $F(6,126) = 2.363$, $P = 0.03$] and REMS [treatment $\times$ time interaction: $F(6,126) = 6.570$, $P < 0.001$]. NREMS time increased immediately after SD and in the dark period [treatment factor: $F(1,6) = 29.842$, $P < 0.002$]. Enhancements of REMS were delayed to the end of the light period and into the dark period [$F(1,6) = 21.873$, $P < 0.005$]. Delta power during NREMS was the parameter most affected by SD [calculated for 21 h, treatment factor: $F(1,6) = 12.107$, $P = 0.013$; treatment $\times$ time interaction: $F(15,90) = 11.723$, $P < 0.001$]. The depth of NREMS increased promptly after SD. Thereafter, delta power declined and tended to decrease below baseline in the dark period.

T_{crt} rose significantly during SD and tended to decrease below baseline during recovery sleep [treatment $\times$ time interaction: $F(15,90) = 3.665$, $P < 0.001$] (Fig. 3).

Feeding activity was slightly but significantly stimulated during SD [treatment $\times$ time interaction: $F(20,452) = 3.291$, $P < 0.001$] (Fig. 3). When calculated for individual hours, feeding activity increased only in *hour 5*, but determined for the entire SD period, 11.1 $\pm$ 2.87% of the 24-h feeding activity occurred during SD, which was significantly higher (*t*-test, $P < 0.01$) than the corresponding value without SD (2.8 $\pm$ 1.22%). The rats tended to display less feeding activity during the recovery period subsequent to SD (*hours 6–12* of the light period: 5.8 $\pm$ 1.98% after SD and 11.8 $\pm$ 1.98% on the baseline day, *t*-test, $P < 0.05$), and the feeding activity peak after dark onset also decreased significantly.

SD failed to alter plasma concentration of leptin (Fig. 3). In contrast, plasma ghrelin increased significantly in *hour 1* of SD (*t*-test, $P < 0.001$). Thereafter, significant differences were not observed in plasma ghrelin between the SD and the baseline days.

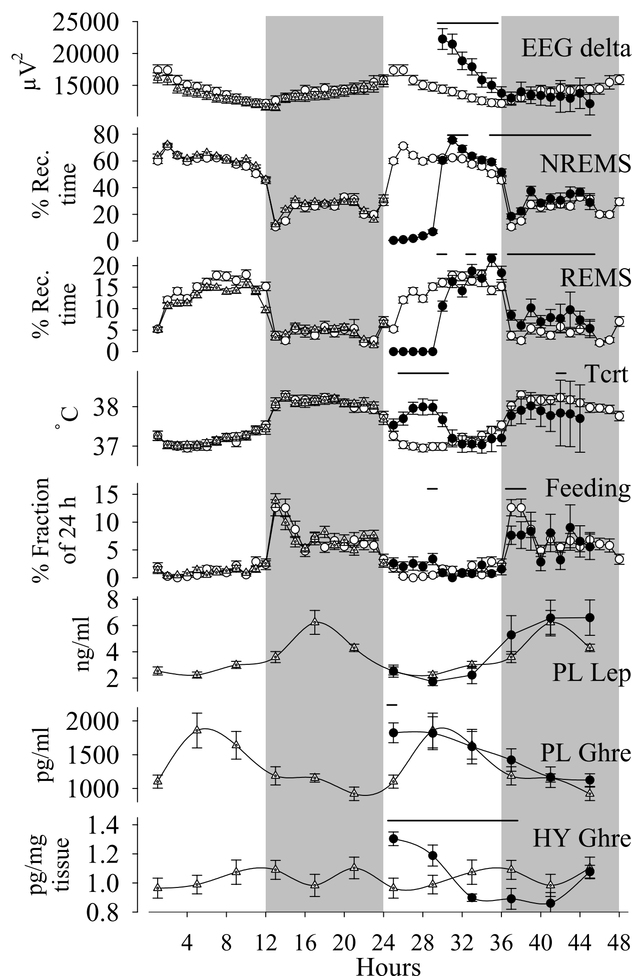

Fig. 3. Sleep, T_{crt}, feeding, and hormone responses to sleep deprivation (SD) in 47 rats. Open circle, baseline values (means of 3 days of recording) in the SD group double-plotted for 48 h. Triangle with dot, free-feeding rats depicted in Fig. 1 shown superimposed over the first 24 h. Filled circle, values during and after SD. SD started at light onset and lasted for 5 h. See legends of Fig. 1 for abbreviations. Horizontal lines above the curves: significant differences between free-feeding rats and RF rats (Student-Newman-Keuls test following ANOVA). Gray columns, dark period.

Hypothalamic ghrelin was highly responsive to SD, displaying statistically significant biphasic variations [$F(5,39) = 11.073$, $P < 0.001$]. Ghrelin contents of the hypothalamus increased significantly during SD and dropped below baseline after SD. These variations in the courses of ghrelin were significant between the free-feeding rats and sleep-deprived rats [time $\times$ treatment interaction: $F(5,75) = 6.411$, $P < 0.001$].

Plasma corticosterone concentration (Fig. 4) in the SD rats was significantly higher than in the other groups [treatment factor: $F(2,66) = 15.670$, $P < 0.001$, time factor: $F(2,4) = 8.792$, $P < 0.001$; treatment $\times$ time interaction: $F(4,66) = 15.012$, $P < 0.001$]. The difference was significant in *hour 1*. Corticosterone declined thereafter, it did not differ from corticosterone in the other groups in *hour 5*, and it tended to decrease below baseline during post-SD recovery, in *hour 9*.

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

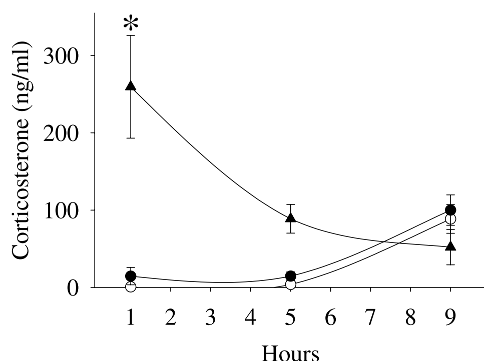

Fig. 4. Plasma corticosterone concentrations (mean ± SE) 1, 5, and 9 h after light onset in free-feeding rats (○), in RF rats (●), and in SD rats (▲; sleep deprivation: *hours 1–5*). *Significant difference between the SD rats and the other groups (Student-Newman-Keuls test after ANOVA).

DISCUSSION

The major current findings are that strong links between sleep and plasma ghrelin or leptin are not detected, whereas the intimate relationships between feeding and these hormones are confirmed in the rat. Rats do not eat or they consume little food during the light cycle (reviewed in Ref. 50). The onset of the dark phase is a potent stimulus for ingestive behavior, and rats generally have one large meal soon after lights go off. The last feeding bout before light onset is also special because it is based on learning the time of light onset. Our free-feeding rats displayed normal diurnal feeding patterns. Marked diurnal rhythms in plasma leptin and ghrelin were observed. The single nocturnal peak of plasma leptin after feeding corresponds to the dark phase-associated leptin peak described previously in free-feeding rats (61). Mice also exhibit a nocturnal rise in plasma leptin (3). The diurnal rhythm of ghrelin was opposite to that of leptin with its peak occurring in the light period. We found no signs of a previously reported second rise in plasma ghrelin before light onset in the rat (36). The highest values of ghrelin and the lowest values of leptin occurred at the same time, and this is in agreement with the suggestion that withdrawal of leptin tone may promote secretion of ghrelin (24). Concentrations of both leptin and ghrelin exhibit ultradian variations in the plasma (6, 54). These changes could not be detected with the 4-h sampling rate used in our experiments. The ultradian pulse variations of ghrelin correlate with feeding episodes (54); fasting increases the frequency and amplitude of ghrelin pulses and diminishes leptin pulse amplitudes (6). Ghrelin secretion is usually observed immediately before feeding, and plasma ghrelin levels fall within 1 h after a meal in humans (11) and sheep (51). It may seem, therefore, that the hormonal rhythms observed in our experiments are more consistent with a circadian regulation than a feeding-induced rhythm.

Although we are not aware of reports demonstrating that a circadian oscillator controls variations in plasma ghrelin, there is evidence showing that the suprachiasmatic nucleus is capable of regulating the diurnal rhythm of leptin. Lesion of the suprachiasmatic nucleus eliminates the diurnal rhythm of plasma leptin in the rat (25), and the nocturnal rise in plasma leptin is maintained in human subjects on continuous enteral nutrition, i.e., without specific feeding signals (48). Nevertheless, several observations suggest that meal timing might be more important than the circadian regulation for the diurnal rhythm of leptin. Plasma leptin increases regularly within 5 h after each meal in humans (17). More importantly, leptin secretion shifts with feeding. Restricted food availability to 4 h of the light period resulted in the abolishment of the nocturnal leptin peak, and the appearance of a diurnal leptin secretion 4 h after the initiation of feeding in the rat in a previous study (61), which is a finding fully confirmed in our experiments. Day/night reversal also reversed the timing of the peaks and minimum values of plasma leptin in humans (44). Our results with RF suggest that the diurnal rhythm of ghrelin secretion is as strongly coupled to feeding as the diurnal rhythm of leptin. Finally, glucocorticoids have been proposed as a stimulator of leptin secretion (8, 17, 37). The diurnal rhythm of corticosterone in rats is such that the peak occurs around dark onset, and then corticosterone concentration declines and reaches a trough after light onset followed by gradual increases in the light period (5). Food restriction to 2 (29, 30) or 4 h (21, 61) of the light period shifts the corticosterone peak to the onset of the eating period. A corticosterone peak is, however, not detected when food availability is extended to 6 h or more in this condition; the rats are able to consume the amount of food normally ingested in 24 h (21). In agreement with this, corticosterone was unaltered in our RF studies. Dissociation between the diurnal rhythms of cortisol and leptin was also observed in human subjects when the leptin rhythm was altered by meal timing (44). That corticosterone might not be fundamental in modulating the diurnal rhythm of leptin is shown by the finding that adrenalectomy and corticosterone replacement do not alter the leptin rhythm (25).

Entrainment of the diurnal rhythms of ghrelin and leptin by RF is in sharp contrast with the resistance of the diurnal rhythm of NREMS to the same condition. That RF has little impact on NREMS in rats was previously reported (43). The RF condition is also a model of the subtle sleep alterations experiencing by Muslims during the diurnal fasting in the lunar month of Ramadan (41, 42). The homeostatic and circadian aspects of NREMS regulation are described by the two-process model (7). The homeostatic process determines sleep propensity as the function of previous wakefulness or neuronal use. The activity of homeostatic process is characterized by the intensity of NREMS described by EEG delta power during NREMS (1). This function was practically unaltered by RF. The circadian control of NREMS was also markedly resistant to RF. NREMS time decreased only when the rats were occupied with eating in the light phase, and this modest loss was compensated with more sleep at night.

Unlike NREMS, REMS was fundamentally altered in RF condition. The responsiveness of REMS to RF was also previously reported (34, 43). The mechanism through which RF modulates REMS is not clear, but it has been suggested that changes in REMS might be related to changes in T_{crt} or body temperature, another parameter markedly influenced by RF. Alterations in T_{crt} can be linked to metabolic activity determined by feeding: higher metabolism results in higher T_{crt} during the light period in rats on RF, whereas T_{crt} drops at night when the rats have to fast. REMS duration is inversely proportional to body temperature in humans (15), and lowering body temperature promotes REMS in pontine cats (23). Diurnal leptin secretion may also contribute to REMS suppression in RF conditions because leptin is posited to decrease REMS

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

(49). Theoretically, hypersecretion of glucocorticoids may also be implicated in the RF-induced sleep alterations (42), but this is unlikely considering the lack of corticosterone response to RF in our experiments.

The dissociation of the NREMS response and the plasma hormonal response to RF suggests that the links between sleep regulation and leptin are relatively weak. Results from the SD experiments provide serious challenge to such putative links: thus, if there are links between sleep and leptin, then one might anticipate alterations in leptin secretion during SD, during recovery sleep, or both. SD failed to alter plasma leptin concentrations in our experiments. This finding is in agreement with reports in human subjects (44, 48). Another paper reported a reduced amplitude of plasma leptin during SD in humans (35). However, direct comparisons between humans and rats are difficult because in the rat, the rest phase is associated with the diurnal trough of leptin, and thus suppressions of leptin levels would be hard to detect. SD stimulated corticosterone secretion, which apparently did not influence leptin secretion. In contrast to leptin, plasma ghrelin responded to SD with an increase in *hour 1*. This response is the opposite of that observed in human subjects where the nocturnal rise in plasma ghrelin was blunted during SD (16). The increase in ghrelin secretion in *hour 1* of SD may signal hunger and eating in the rat, and in fact, our rats did consume food during SD.

Interestingly, hypothalamic ghrelin, exhibiting biphasic changes during and after SD, was much more responsive to SD than plasma ghrelin. Various functions display biphasic changes in association with SD; the list includes the amounts of NREMS and REMS, which decrease during SD and increase during recovery, and T_{crt} and feeding activity, which increase during SD and decrease during recovery. The problem with linking alterations in hypothalamic ghrelin to feeding activity is that rapid rises in peptide contents generally indicate accumulation, i.e., inhibition of ghrelin release, which is not consistent with increased feeding during SD, and decreases in peptide contents suggest enhanced release, which is not anticipated during recovery sleep when feeding decreases. GH secretion via stimulating GHRH neurons in the arcuate nucleus is another well-documented action of GHSs, including ghrelin. GH secretion correlates with the observed changes in intrahypothalamic ghrelin because GH secretion is inhibited during SD and tends to enhance during recovery in the rat (26). GHRH neurons also promote NREMS. Therefore, SD-induced changes in hypothalamic ghrelin may reflect the first sign of sleep-related alterations in ghrelin in our experiments.

Previous findings suggesting that leptin and ghrelin secretions have sleep-associated components were predominantly obtained in human subjects. A comparison of the observations between humans and rats, however, shows significant differences in the relationship between sleep and these hormones. Both humans (17, 35, 44, 48) and rats exhibit a nocturnal peak in leptin, but this rise is opposite in terms of the rest phase of the day in the two species. In addition to the diurnal rhythm, a sleep-associated increase in leptin secretion is also suggested in humans (48) that is obviously missing in rodents. A negative correlation was reported between plasma leptin rhythm and rectal temperature in humans (48) that does not occur in rats. The diurnal rhythm of ghrelin is in phase with that of leptin in humans (11), i.e., both leptin and ghrelin rise at night, whereas the rhythms of ghrelin and leptin are shifted by 180° in rats. Thus, at least ghrelin peaks in the rest phase in both humans and rats. However, plasma ghrelin concentration increases in sleeping subjects during the early part of the night, and there is a positive correlation between plasma ghrelin and the peak of GH secretion during the first hours of sleep, suggesting that sleep-associated processes modulate nocturnal ghrelin secretion (16). In contrast, the peak of the ghrelin rhythm is in the middle of the rest phase in the rat, and by this time the NREMS-promoting GHRH activity is over (19), and correlation is not observed between ghrelin pulses in the plasma and GH secretion (54). Although the differences in the relationship between sleep and plasma leptin and ghrelin rhythms do not support a universal role of these hormones in sleep regulation, sleep responses can be elicited by exogenous administration of leptin and ghrelin (38, 49, 58). These responses might represent pharmacological actions, but it is also possible that endogenous leptin and ghrelin contribute to sleep promotion in special conditions. For example, ghrelin released before feeding may stimulate postprandial sleep. Further experiments may determine the significance of intrahypothalamic ghrelin in sleep regulation.

ACKNOWLEDGMENTS

We thank S. Toth for technical assistance.

GRANTS

This work was supported, in part, by National Institutes of Health Grants NS-25378 and NS-27250 and grants from the Hungarian National Foundation (OTKA-T-043156) and Ministry of Health (ETT 103 04/2003).

REFERENCES

1. **Achermann P and Borbély AA.** Mathematical models of sleep regulation. *Front Biosci* 8: S683–S693, 2003.
2. **Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, and Flier JS.** Role of leptin in the neuroendocrine response to fasting. *Nature* 382: 250–252, 1996.
3. **Ahrén B.** Diurnal variation in circulating leptin is dependent on gender, food intake and circulating insulin in mice. *Acta Physiol Scand* 169: 325–331, 2000.
4. **American Physiological Society.** Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol* 283: R281–R283, 2002.
5. **Atkinson HC and Waddell BJ.** Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. *Endocrinology* 138: 3842–3848, 1997.
6. **Bagnasco M, Kalra PS, and Kalra SP.** Ghrelin and leptin pulse discharge in fed and fasted rats. *Endocrinology* 143: 726–729, 2002.
7. **Borbély AA.** A two process model of sleep regulation. *Hum Neurobiol* 1: 195–204, 1982.
8. **Bradley RL and Cheatham B.** Regulation of *ob* gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. *Diabetes* 48: 272–278, 1999.
9. **Cocchi D, De Gennaro Colonna V, Bagnasco M, Bonacci D, and Müller EE.** Leptin regulates GH secretion in the rat by acting on GHRH and somatostatinergic functions. *J Endocrinol* 162: 95–99, 1999.
10. **Copinschi G, Leproult R, Van Onderbergen A, Caufriez A, Cole KY, Schilling LM, Mendel CM, De Lepeleire I, Bolognese JA, and Van Cauter E.** Prolonged oral treatment with MK-677, a novel growth hormone secretagogue, improves sleep quality in man. *Neuroendocrinology* 66: 278–286, 1997.
11. **Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, and Weigle DS.** A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50: 1714–1719, 2001.
12. **Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, and Nakazato M.** Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 141: 4255–4261, 2000.

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

13. **Date Y, Murakami N, Kojima M, Kuroiwa T, Matsukura S, Kangawa K, and Nakazato M.** Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. *Biochem Biophys Res Commun* 275: 477–480, 2000.
14. **Dickson SL and Luckman SM.** Induction of *c-fos* messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. *Endocrinology* 138: 771–777, 1997.
15. **Dijk DJ and Czeisler CA.** Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle. *J Neurosci* 15: 3526–3538, 1995.
16. **Dzaja A, Dalal MA, Himmerich H, Uhr M, Pollmächer T, and Schuld A.** Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *Am J Physiol Endocrinol Metab* 286: E963–E967, 2004.
17. **Elimam A and Marcus C.** Meal timing, fasting and glucocorticoids interplay in serum leptin concentrations and diurnal profile. *Eur J Endocrinol* 147: 181–188, 2002.
18. **Frieboes RM, Murck H, Maier P, Schier T, Holsboer F, and Steiger A.** Growth hormone-releasing peptide-6 stimulates sleep, growth hormone, ACTH and cortisol release in normal man. *Neuroendocrinology* 61: 584–589, 1995.
19. **Gardi J, Obal F Jr, Fang J, Zhang J, and Krueger JM.** Diurnal variations and sleep deprivation-induced changes in rat hypothalamic GHRH and somatostatin contents. *Am J Physiol Regul Integr Comp Physiol* 277: R1339–R1344, 1999.
20. **Havel PJ.** Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* 13: 51–59, 2002.
21. **Honma KJ, Honma S, and Hiroshige T.** Critical role of food amount for prefeeding corticosterone peak in rats. *Am J Physiol Regul Integr Comp Physiol* 245: R339–R344, 1983.
22. **Hwa JJ, Ghibaudi L, Compton D, Fawzi AB, and Strader CD.** Inracerebroventricular injection of leptin increases thermogenesis and mobilizes fat metabolism in *ob/ob* mice. *Horm Metab Res* 28: 659–663, 1996.
23. **Jouvet M, Buda C, Debilly G, Dittmar A, and Sastre JP.** Central temperature is the principal factor of regulation of paradoxical sleep in pontine cats. *C R Acad Sci (Paris)* 306: 69–73, 1988.
24. **Kalra SP, Bagnasco M, Otukonyong EE, Dube MG, and Kalra PS.** Rhythmic, reciprocal ghrelin and leptin signaling: new insight in the development of obesity. *Regul Pept* 111: 1–11, 2003.
25. **Kalsbeek A, Fliers E, Romjin JA, La Fleur SE, Wortel J, Bakker O, Endert E, and Buis RM.** The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685, 2001.
26. **Kimura F and Tsai CW.** Ultradian rhythm of growth hormone secretion and sleep in the adult male rat. *J Physiol* 353: 305–315, 1984.
27. **Kohno D, Gao HZ, Muroya S, Kikuyama S, and Yada T.** Ghrelin directly interacts with neuropeptide-Y-containing neurons in rat arcuate nucleus. *Diabetes* 52: 948–956, 2003.
28. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, and Kangawa K.** Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660, 1999.
29. **Krieger DT.** Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* 95: 1195–1201, 1974.
30. **Leal AMO and Moreira AC.** Feeding and the diurnal variation of the hypothalamic-pituitary-adrenal axis and its responses to CRH and ACTH in rats. *Neuroendocrinology* 64: 14–19, 1996.
31. **Lu S, Guan JL, Wang QP, Uehara K, Yamada S, Goto N, Date Y, Nakazato M, Kojima M, Kangawa K, and Shioda S.** Immunocytochemical observations of ghrelin-containing neurons in the rat arcuate nucleus. *Neurosci Lett* 321: 157–160, 2002.
32. **Maheshwari HG, Rahim A, Shalet SM, and Baumann G.** Selective lack of growth hormone (GH) response to the GH-releasing peptide hexarelin in patients with GH-releasing hormone receptor deficiency. *J Clin Endocrinol Metab* 84: 956–959, 1999.
33. **Moreno-Reyes R, Kerkhofs M, L'Hermite-Balériaux M, Thorner MO, Van Cauter E, and Copinschi G.** Evidence against a role for the growth hormone-releasing peptide axis in human slow-wave sleep regulation. *Am J Physiol Endocrinol Metab* 274: E779–E784, 1998.
34. **Mouret JR and Bobillier P.** Diurnal rhythm of sleep in the rat: augmentation of paradoxical sleep following alterations in the feeding schedule. *Int J Neurosci* 2: 265–270, 1971.
35. **Mullington JM, Chan JL, Van Dongen HPA, Szuba MP, Samaras J, Price NJ, Meier-Ewert HK, Dinges DF, and Mantzoros CS.** Sleep loss reduces diurnal rhythm amplitude of leptin in healthy men. *J Neuroendocrinol* 15: 851–854, 2003.
36. **Murakami N, Hayashida T, Kuroiwa T, Nakahara K, Ida T, Mondal MS, Nakazato M, Kojima M, and Kangawa K.** Role for central ghrelin in food intake and secretion profile of stomach ghrelin in rats. *J Endocrinol* 174: 283–288, 2002.
37. **Newcomer JW, Selke G, Melson AK, and Newcomer JW.** Dose-dependent cortisol-induced increases in plasma leptin concentration in healthy humans. *Arch Gen Psychiatry* 55: 995–1000, 1998.
38. **Obal F Jr, Alt J, Taishi P, Gardi J, and Krueger JM.** Sleep in mice with nonfunctional growth hormone-releasing hormone receptors. *Am J Physiol Regul Integr Comp Physiol* 284: R131–R139, 2003.
39. **Obal F Jr. and Krueger JM.** Biochemical regulation of non-rapid-eye-movement sleep. *Front Biosci* 8: D520–D550, 2003.
40. **Popovic V, Damjanovic S, Micic D, Djurovic M, Dieguez C, and Casanueva FF.** Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: evidence that GHRP-6 main action is exerted at the hypothalamic level. *J Clin Endocrinol Metab* 80: 942–947, 1995.
41. **Roky R, Chapotot F, Benchekroun MT, Benaji B, Hakkou F, Elkhalifi H, and Buguet A.** Daytime sleepiness during Ramadan intermittent fasting: polysomnographic and quantitative waking EEG study. *J Sleep Res* 12: 95–101, 2003.
42. **Roky R, Chapotot F, Hakkou F, Benchekroun MT, and Buguet A.** Sleep during Ramadan intermittent fasting. *J Sleep Res* 10: 319–327, 2001.
43. **Roky R, Kapás L, Taishi P, Fang J, and Krueger JM.** Food restriction alters the diurnal distribution of sleep in rats. *Physiol Behav* 67: 697–703, 1999.
44. **Schoeller DA, Cella LK, Sinha MK, and Caro JF.** Entrainment of the diurnal rhythm of plasma leptin to meal timing. *J Clin Invest* 100: 1882–1887, 1997.
45. **Seoane LM, Lopez M, Tovar S, Casanueva FF, Senaris R, and Dieguez C.** Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* 144: 544–551, 2003.
46. **Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF, and Dieguez C.** Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *Eur J Endocrinol* 143: R7–R9, 2000.
47. **Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, and Nakao K.** Ghrelin, and endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 50: 227–232, 2001.
48. **Simon C, Gronfier C, Schlienger JL, and Brandenberger G.** Circadian and ultradian variations of leptin in normal man under continuous enteral nutrition: relationship to sleep and body temperature. *J Clin Endocrinol Metab* 83: 1893–1899, 1998.
49. **Sinton CM, Fitch TE, and Gershenfeld HK.** The effects of leptin on REM sleep and slow wave delta in rats are reversed by food deprivation. *J Sleep Res* 8: 197–203, 1999.
50. **Strubbe JH and Van Dijk G.** The temporal organization of ingestive behaviour and its interaction with regulation of energy balance. *Neurosci Biobehav Rev* 26: 485–498, 2002.
51. **Sugino T, Hasegawa Y, Kikkawa Y, Yamaura J, Yamagishi M, Kurose Y, Kojima M, Kangawa K, and Terashima Y.** A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. *Biochem Biophys Res Commun* 295: 255–260, 2002.
52. **Tannenbaum GS, Epelbaum J, and Bowers CY.** Interrelationship between the novel peptide ghrelin and somatostatin/growth hormone-releasing hormone in regulation of pulsatile growth hormone secretion. *Endocrinology* 144: 967–974, 2003.
53. **Tannenbaum GS, Gurd W, and Lapointe M.** Leptin is a potent stimulator of spontaneous pulsatile growth hormone (GH) secretion and the GH response to GH-releasing hormone. *Endocrinology* 139: 3871–3875, 1998.
54. **Tolle V, Bassant MH, Zizzari P, Poindessous-Jazat F, Tomasetto C, Epelbaum J, and Bluet-Pajot M.-T.** Ultradian rhythmicity of ghrelin secretion in relation with GH, feeding behavior, and sleep-wake patterns in rats. *Endocrinology* 143: 1353–1361, 2002.

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017

55. **Tschöp M, Smiley DL, and Heiman ML.** Ghrelin induces adiposity in rodents. *Nature* 407: 908–913, 2000.
56. **Vuagnat BAM, Pierroz DD, Lalaoui M, Englaro P, Pralong FP, Blum WF, and Aubert ML.** Evidence for a leptin-neuropeptide Y axis for the regulation of growth hormone secretion in the rat. *Neuroendocrinology* 67: 291–300, 1998.
57. **Watanobe H and Habu S.** Leptin regulates growth hormone-releasing factor, somatostatin, and α-melanocyte-stimulating hormone but not neuropeptide Y release in rat hypothalamus in vivo: relation with growth hormone secretion. *J Neurosci* 22: 6265–6271, 2002.
58. **Weikel JC, Wichniak A, Ising M, Brunner H, Friess E, Held K, Mathias S, Schmid DA, Uhr M, and Steiger A.** Ghrelin promotes slow-wave sleep in humans. *Am J Physiol Endocrinol Metab* 284: E407–E415, 2003.
59. **Wren AM, Small CJ, Fribbens CV, Neary NM, Ward HL, Seal LJ, Ghatei MA, and Bloom SR.** The hypothalamic mechanisms of the hypophysiotropic action of ghrelin. *Neuroendocrinology* 76: 316–324, 2002.
60. **Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DGA, Ghatei MA, and Bloom SR.** The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 141: 4325–4328, 2000.
61. **Xu B, Kalra PS, Farmerie WG, and Kalra SP.** Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. *Endocrinology* 140: 2868–2875, 1999.

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 18, 2017