L-364,718, a cholecystokinin-A receptor antagonist, suppresses feeding-induced sleep in rats

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Shemyakin, Alexei, and Levente Kapás. L-364,718, a cholecystokinin-A receptor antagonist, suppresses feeding-induced sleep in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1420–R1426, 2001.—Feeding induces increased sleep in several species, including rats. The aim of the study was to determine if CCK plays a role in sleep responses to feeding. We induced excess eating in rats by 4 days of starvation and studied the sleep responses to refueling in control and CCK-A receptor antagonist-treated animals. Sleep was recorded on 2 baseline days when food was provided ad libitum. After the starvation period, sleep was recorded on 2 refueling days when the control rats (n = 8) were injected with vehicle and the experimental animals (n = 8) received intraperitoneal injections of L-364,718 (500 μg/kg, on both refueling days). In the control group, refueling caused increases in rapid eye movement sleep (REMS) and non-REMS (NREMS) and decreases in NREMS intensity as indicated by the slow-wave activity (SWA) of the electroencephalogram. CCK-A receptor antagonist treatment completely prevented the SWA responses and delayed the NREMS responses to refueling; REMS responses were not simply abolished, but the amount of REMS was below baseline after the antagonist treatment. These results suggest that endogenous CCK, acting on CCK-A receptors, may play a key role in eliciting postprandial sleep.

Electroencephalogram slow-wave activity; food intake; brain temperature; food deprivation; non-rapid eye movement sleep

There is a well-documented relationship between feeding, satiety, and sleep. Postprandial sleep has been described in several avian species and mammalian species, including humans (35). Excessive eating induced by cafeteria diet (12,21) or refeeding after food deprivation (14) induces sleep in rats. After eating, a complex behavioral syndrome, the so-called satiety syndrome, develops. Decreased motor activity, social withdrawal, and increased sleep are components of the satiety syndrome (1). The mechanisms of feeding-induced sleep are not well understood. Somnogenic signals from the gastrointestinal tract can be carried to the brain by sensory nerves (21) or by humoral factors. Gastrointestinal hormones provide a humoral communication link between the intestines and the central nervous system.

One of the best-characterized gastrointestinal hormones is CCK. CCK has two basic functions in mammals. It is a neurotransmitter/neuromodulator in the brain and a classic hormone released from the upper small intestines in response to fat- and protein-rich meals (reviewed in Ref. 10). There are two CCK receptor subtypes. CCK-A receptors are found in the gastrointestinal tract and select brain areas, such as area postrema and the nucleus of the solitary tract (reviewed in Ref. 10). CCK-B receptors are widely distributed in the central nervous system, and they are also present on the vagus nerve (9,28). Exogenously administered CCK elicits the complete behavioral syndrome of satiety, including sleep (1). The food intake-suppressive effects of CCK are mediated by peripheral CCK-A receptors (18,32). Intraperitoneal injection of CCK stimulates non-rapid eye movement sleep (NREMS) in rats (22,29), rabbits (23), and cats (19). The somnogenic effects of CCK are likely to be mediated by CCK-A receptors, because a CCK-A receptor antagonist abolishes the effect of CCK (6) and CCK-B receptor agonists do not have somnogenic actions (7).

We hypothesized that CCK, acting on CCK-A receptors, may play a central role in sleep responses to feeding. To test this hypothesis, we studied the effects of L-364,718, a selective and potent CCK-A receptor antagonist (8), on sleep responses to excess feeding. Excess feeding was induced by a starvation-refeeding paradigm. Our results show that the CCK-A receptor antagonist inhibits sleep responses to feeding, suggesting a key role for endogenous CCK in signaling postprandial sleep.

METHODS

Adult male Sprague-Dawley rats weighing 350–450 g were anesthetized using ketamine-xylazine (87 and 13 mg/kg, respectively) and implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes and a brain thermistor. Stainless steel screws for EEG recordings were implanted into the skull over the frontal and parietal cortices. EMG electrodes were implanted in the dorsal neck muscles. A thermistor was placed on the dura over the parietal cortex and used to measure brain temperature (Tbr). Insulated leads from the EEG screw electrodes, EMG electrodes, and thermistor were routed to a plastic pedestal and connected to a data acquisition system.
mented to the skull with dental adhesive. The animals were placed into individual sleep-recording cages inside a sound-attenuated chamber for adaptation to the experimental conditions for a 1-wk recovery period followed by 5- to 7-day habituation period, during which the animals were connected to recording cables. The animals were kept on a 12:12-h light-dark cycle (lights on at 0500) and at 24 ± 1°C ambient temperature for at least 1 wk before surgeries, during the recovery, habituation, and the recordings.

The experimental protocol included 2 baseline days followed by 4 starvation days and 2 days of refeeding. A control group of animals (n = 8) received vehicle on the baseline, starvation, and refeeding days. The experimental group (n = 8) was injected with vehicle during baseline and starvation days, and with the CCK-A receptor antagonist (L-364,718, Merck Research Laboratories, Rahway, NJ, 500 μg/kg suspended in 4% methylcellulose) on the refeeding days. The injections were given intraperitoneally 10–20 min before light onset in a volume of 2 ml/kg.

Sleep was recorded on the baseline and refeeding days. All recording sessions started at dark onset and lasted for 23 h. During the last hour of the light periods, body weights were measured and maintained during light and dark periods. The leftover was collected and weighed 12 h later, and food intake for the light and dark periods was calculated. To induce starvation, food was removed at the end of the second baseline day (i.e., at dark onset of day 3). After 4 days of starvation, food was returned at the beginning of refeeding day 1 (i.e., dark onset of refeeding day 1). A 4-day starvation protocol was chosen because, based on pilot experiments, it proved to be optimal to cause significant increases in eating and sleep on refeeding. Water was available ad libitum throughout the starvation period. The rats were observed every 12 h, i.e., during the daily maintenance and body weight measurements (1700) and at the time of injections (0500).

The loss of 20% of body weight or apparent signs of sickness (e.g., decreased locomotion, increased irritability, reduced grooming, sanguinopurulent exudate from nares) were the criteria for stopping the starvation. However, such weight losses or signs of sickness were never observed. The average weight loss during starvation was 13.2 ± 1.0%.

EEG, EMG, and Tbr were recorded on a computer. EEG activity served as an aid in determining the vigilance states and was not further quantified. EEG was filtered <0.1 and >40 Hz. The amplified signals were digitized at a frequency of 128 Hz for EEG and EMG and 2 Hz for Tbr. Single Tbr values were saved on hard disk in 10-s intervals. Tbr values were averaged in 1-h blocks. Online fast Fourier analysis of the EEG was performed in 10-s intervals on 2-s segments of the EEG in 0.5- to 4.0-Hz frequency range. The EEG power density values in the delta frequency range were summed for the EEG in 0.5- to 4.0-Hz frequency range. The EEG power density values were averaged in 1-h blocks. The delta activity during NREMS also called slow-wave activity (SWA) is often regarded as a measure of NREMS intensity. The vigilance states were determined offline in 10-s epochs. EEG, EMG, and Tbr were recorded on the computer monitor in 10-s epochs and also simultaneously in a more condensed form in 12-min epochs. Wakefulness, NREMS, and rapid eye movement sleep (REMS) were distinguished. Briefly, the criteria for vigilance states are as follows. NREMS: high-amplitude EEG slow waves, low level EMG activity, and declining Tbr on entry; REMS: low-amplitude EEG activity and regular theta activity in the EEG, general lack of body movements with occasional twitches, and a rapid rise in Tbr at onset; wakefulness: low-amplitude, fast EEG activity, lack of visible regular theta rhythm, high EMG activity, and a gradual increase in Tbr after arousal. Time spent in each vigilance state was calculated in 2-h time blocks.

Statistical Analysis

For SWA and sleep amounts, the values were compared between the average of 2 baseline days and each of refeeding days by using ANOVA for repeated measures on 2-h time blocks across the 12-h dark and 11-h light periods. We compared Tbr between the average of 2 baseline days and each of refeeding days by using ANOVA for repeated measures on temperature values averaged in 1-h time blocks across the 12-h dark and 11-h light periods. For the analysis of food intake, three-way ANOVA was performed on the 12-h food intake values (g/100 body wt) during the light and dark periods of the baseline day (average of 2 baseline days), refeeding day 1, and day 2 [factor A: group effect (control vs. CCK-antagonist-treated group), factor B: day effect, factor C: light-dark effect (the effect of the circadian phase of the day)]. Tukey test was performed post hoc for food intake, and paired t-test for Tbr, SWA, and sleep measures when ANOVA revealed significant effects. In all tests, an α-level of P < 0.05 was taken as an indication of statistical significance.

RESULTS

In both control and CCK antagonist-treated rats, the distribution of NREMS and REMS showed normal diurnal patterns, with high percentages of sleep during the light and less sleep during the dark period. SWA and Tbr also showed a circadian pattern. SWA had middle range values throughout the dark period followed by an increase in the first hour of the light period with a subsequent gradual decrease. Tbr was the highest at the beginning of the dark period, slightly decreased thereafter reaching a minimum at light onset, and increased throughout the rest of a day.

Effects of Refeeding on Sleep, SWA, and Tbr in Control, Saline-Treated Rats

Baseline versus refeeding day 1. Reintroducing food after food deprivation caused statistically significant increases in the amount of NREMS during light period compared with baseline levels (ANOVA, P < 0.05; Fig. 1, Table 1). On the baseline day, rats spent 332 ± 23 min in NREMS during light period compared with 392 ± 7 min on the first refeeding day. There were no significant differences in REMS amount or SWA (Fig. 1, Table 1). There was a tendency toward lower Tbr on refeeding day 1.

Baseline versus refeeding day 2. During the second refeeding day, an increase in time spent in REMS (ANOVA, P < 0.05) and decrease in SWA (ANOVA, P < 0.05) during dark period were observed (Fig. 1, Table 1). On the baseline day, rats spent 38 ± 5 min in REMS during dark period compared with 58 ± 7 min on the second refeeding day. There were strong tendencies toward increased NREMS and decreased Tbr throughout the day.
Effects of Refeeding on Sleep, SWA, and Tbr in CCK Antagonist-Treated Rats

Baseline versus refeeding day 1. The effects of refeeding on NREMS were completely abolished by L-364,718 treatment (Fig. 2, Table 1).

Baseline versus refeeding day 2. There were significant increases in the amount of time spent in NREMS during dark (ANOVA, P < 0.05) and decreases in REMS during light period (ANOVA, P < 0.05; Fig. 2, Table 1). On the baseline day, rats spent 206 ± 11 min in NREMS during dark period and 92 ± 7 min in REMS during light period compared with 239 ± 13 min and 81 ± 8 min, respectively, on the second refeeding day. The significant reduction in SWA that was observed in the control animals was completely prevented by the antagonist treatment.

Effects of Refeeding on Body Weight and Food Intake

Body weights did not differ significantly between control and CCK antagonist-treated rats throughout experiment (baseline: 421.7 ± 19.2 and 435.9 ± 12.0 g; refeeding day 1: 363.3 ± 18.6 and 374.0 ± 10.0 g; refeeding day 2: 393.2 ± 19.9 and 414.4 ± 10.8 g, in control and L-364,718-treated rats, respectively; 2-way ANOVA, group effect: F(1,95) = 1.792, not significant (NS]).

There were no significant differences in food intake between control and CCK antagonist-treated rats throughout the experiment [3-way ANOVA, group effect: F(1,126) = 0.298, NS; Fig. 3]. There was a significant difference in consumed food between days [3-way ANOVA, day effect: F(2,126) = 12.221, P < 0.05] and between light and dark periods [3-way ANOVA, light-dark effect: F(1,126) = 1064.353, P < 0.05]. Food intake was increased on the first refeeding day (8.99 ± 0.26 g, data pooled from the 2 groups) compared with baseline levels (7.54 ± 0.34 g, data pooled from the 2 groups). On the second refeeding day, food intake returned to a level similar to baseline (7.29 ± 0.36 g, data pooled from the 2 groups). There was a significant interaction between days and dark-light periods throughout the experiment [3-way ANOVA: F(2,126) = 31.792, P < 0.05]: food intake during dark and light periods of all 3 days significantly differed from each other (Tukey test, P < 0.05). Food intake was increased during the dark period and decreased during the light period of refeeding day 1 compared with baseline levels. On refeeding day 2, food intake returned to levels close to baseline during both dark and light periods.

DISCUSSION

Our results are consistent with the well-documented findings that 4 days of food deprivation cause 10–15% decrease in body weight (e.g., Ref. 30) and a rebound increase in food intake (e.g., Ref. 14). On the first and second refeeding day, NREMS and REMS, respectively, were increased above baseline. This is in line with previous findings that increased feeding or postgestive satiety elicits postprandial sleep (29). For example, intragastric or intraduodenal administration of nutrients elicits postprandial EEG synchronization in rats (2) and cats (19). Increased eating induced by palatable, high-energy diet (cafeteria diet) results in increases of daily NREMS and REMS amounts (12). There is a positive correlation between meal size and the length of the following sleep period in rats (15). Hyperphagia, induced by ventromedial hypothalamic lesion, is accompanied by increases in both NREMS and REMS (13). In the present study, we induced excess eating by reintroducing food after a 96-h food deprivation. Refeeding elicited significant increases in NREMS and REMS. In previous studies, food restitution after a 96-h food deprivation also caused rebound increases in both NREMS and REMS (14), and refeeding after an 80-h food deprivation caused a strong tendency to increased duration of NREMS and total sleep episodes in rats, although the changes were not statistically significant (3).

NREMS increased predominantly during the light phase of the refeeding days in control rats. Similarly, NREMS was elevated only during the light period in cafeteria diet-fed rats (21). The reason why sleep in-
creases were restricted to the light period is likely due to the circadian rhythm of feeding behavior in rats. Under normal conditions as well as after food deprivation, 80–85% of daily food is consumed during the dark. Because eating and sleep cannot take place simultaneously, an increase in feeding behavior per se will interfere with the possible somnogenic effects of the ingested food. In the dark phase of the first refeeding day, food intake was increased by 43% above baseline. It is likely that the increased behavioral activity interfered with an increased food-induced pressure for sleep, and, as a result of the two opposing forces, sleep amounts did not change. During the following light period, food intake was below baseline. This decrease in feeding activity allowed the somnogenic effect of food consumed during the previous dark phase to prevail. In the dark period of the second refeeding day, feeding behavior was less robust than in the first dark period; food intake was increased only by 7.3% above baseline. This decline in feeding had two consequences. First, it allowed sleep increases in the dark, i.e., the development of a strong tendency toward increased NREMS and a significantly increased REMS. Second, because less food was consumed, sleep responses in the following light period were less pronounced, in fact, NREMS was not significantly increased in the light phase of refeeding day 2. Timing of the refeeding is an important factor that may determine whether sleep responses occur during the light or dark period. In a previous study, sleep rebound after 4 days of starvation occurred predominantly during the dark phase (14). In that study, however, food was reintroduced in the light period and rebound eating occurred during the light. In the following dark period, feeding activity returned to baseline levels, therefore allowing sleep increases to take place.

The two-process model of sleep regulation describes SWA as an indicator of homeostatic pressure for NREMS and a measure of NREMS intensity (4). For example, homeostatic increases in NREMS and SWA occur after sleep loss and, vice versa, increased amounts of NREMS cause sleep pressure to dissipate and SWA to decline. In fact, increased SWA is a more sensitive indicator of the homeostatic changes in sleep pressure than the duration of NREMS itself (reviewed in Ref. 4). In our experiments, SWA was significantly reduced during the dark period of the second refeeding day in control rats. This is likely due to the fact that refeed rats spent extra time in NREMS during the first light and second dark periods. The excess sleep likely reduced the homeostatic pressure for subsequent NREMS, and the decreased SWA may indicate this reduced pressure. Previously, similar decreases in SWA activity that accompanied feeding-induced sleep were described, e.g., cafeteria diet induces increases in time spent in NREMS but suppresses SWA (21).

Table 1. Effects of refeeding on the amounts of NREMS and REMS, SWA of the electroencephalogram during NREMS, and Tbr in control and L-364,718-treated rats

<table>
<thead>
<tr>
<th></th>
<th>1–12 h</th>
<th>13–23 h</th>
<th>25–36 h</th>
<th>37–47 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NREMS</td>
<td>F(1,7) = 0.075</td>
<td></td>
<td>F(1,8) = 4.723</td>
<td>F(1,8) = 3.574</td>
</tr>
<tr>
<td>REMS</td>
<td>F(1,7) = 0.424</td>
<td></td>
<td>F(1,8) = 10.296</td>
<td>F(1,8) = 0.222</td>
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<tr>
<td>SWA</td>
<td>F(1,3) = 1.169</td>
<td></td>
<td>F(1,6) = 15.525</td>
<td>F(1,7) = 1.477</td>
</tr>
<tr>
<td>Tbr</td>
<td>F(1,4) = 1.383</td>
<td></td>
<td>F(1,3) = 6.282</td>
<td>F(1,3) = 1.953</td>
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<tr>
<td><strong>L-364,718</strong></td>
<td></td>
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<tr>
<td>NREMS</td>
<td>F(1,7) = 0.089</td>
<td></td>
<td>F(1,7) = 23.534</td>
<td>F(1,7) = 0.116</td>
</tr>
<tr>
<td>REMS</td>
<td>F(1,7) = 4.949</td>
<td></td>
<td>F(1,7) = 0.159</td>
<td>F(1,7) = 8.008</td>
</tr>
<tr>
<td>SWA</td>
<td>F(1,7) = 0.340</td>
<td></td>
<td>F(1,7) = 0.886</td>
<td>F(1,7) = 0.294</td>
</tr>
<tr>
<td>Tbr</td>
<td>F(1,6) = 0.028</td>
<td></td>
<td>F(1,6) = 0.693</td>
<td>F(1,6) = 0.051</td>
</tr>
</tbody>
</table>

Two-way ANOVA for repeated measures was performed for non-rapid eye movement sleep (NREMS), rapid eye movement sleep (REMS), and brain temperature (Tbr), between the baseline and refeeding conditions. ANOVA across the specified hours was performed on 1-h time blocks for Tbr and on 2-h time blocks for sleep and slow-wave activity (SWA). The P values for the treatment effects are indicated. P < 0.05: significant difference between the baseline and refeeding conditions. NS, nonsignificant difference between the baseline and refeeding conditions.

Starvation results in reduced basal metabolic rate (30) and, depending on the duration of the food restriction and the ambient temperature, no change (30) or a slight decrease in body temperature (34). We did not measure body temperature during the starvation period. During the first 2 h of the first refeeding day, Tbr was at baseline levels. For the remainder of the refeeding period, however, Tbr was slightly but consistently below baseline. This slight decrease in Tbr was absent in the CCK antagonist-treated group. It is possible that CCK, released during refeeding, contributed to the slight decrease in Tbr, because systemic administration of CCK causes hypothermia in rats (24), which is completely abolished by L-364,718 treatment (6).

There are several possible mechanisms that may contribute to the somnogenic effects of feeding. For
example, the activation of gastrointestinal sensory nerves causes increases in NREMS (26). In fact, subdiaphragmal dissection of the vagus nerve prevents the somnogenic effects of cafeteria diet (21). Another possibility is that increased sleep is a consequence of metabolic changes after eating (31). A third possibility is that feeding-induced sleep is due to the release of somnogenic hormones from the gastrointestinal tract. One of the most studied somnogenic gastrointestinal hormones is CCK. Systemic injection of CCK causes the complete behavioral sequence of satiety (1), including sleep (19, 22, 23, 29). We hypothesized that CCK, acting on CCK-A receptors, plays a key role in eliciting sleep responses to feeding.

L-364,718 was administered at light onset on both refeeding days. That is, the first injection of L-364,718 was done 12 h after reintroducing the food. There are two reasons why we did not inject the antagonist immediately after the end of the starvation period. First, sleep responses to refeeding started only after a latency of 12 h in control rats. Second, L-364,718 itself stimulates feeding in rats (32); delaying the injection allowed the animals to eat according to their natural needs during the first dark period after starvation. Our major finding is that L-364,718 completely abolished the NREMS increases on refeeding day 1. During the dark period of the following day, however, NREMS was significantly increased in CCK antagonist-treated rats, a response that was present only as a tendency in the control group. It is not likely that the observed effects in the dark period are directly related to the action of the antagonist at that time, because the duration of the action is shorter than 12 h. There are two explanations for this increase in NREMS. First, during the light period of refeeding day 1, antagonist-injected animals ate ~50% more than animals in the control group. Increased feeding may have contributed to NREMS increases in the subsequent dark period. Second, the NREMS increase seen in control rats during the light period on refeeding day 1 was absent in CCK antagonist-injected rats. It is possible that the antagonist-treated animals accumulated undischarged sleep pressure that was carried over to the dark period of refeeding day 2. Furthermore, in contrast to decreased SWA in control rats, SWA in the antagonist-treated animals was not significantly suppressed in the dark period on refeeding day 2 likely because the antagonist-treated rats did not accumulate excess sleep during refeeding day 1.

In summary, we found that the CCK-A receptor antagonist, L-364,718, suppresses NREMS and REMS responses to feeding. This is consistent with the hypothesis that endogenous CCK plays a role in eliciting postprandial sleep. L-364,718 acts on both central and peripheral CCK-A receptors. It is likely that the role of peripherally located CCK-A receptors is more important for the somnogenic effects of feeding, because...
intracerebroventricular injection of CCK does not elicit changes in sleep (20).

Perspectives

Although sleep is generated by the brain, there are several factors arising from outside the brain that contribute to the regulation of sleep. For example, somatosensory stimuli, ambient temperature, systemic infections, and feeding all affect sleep. There are signal mechanisms from the periphery that stimulate or inhibit somnogenic brain structures according to somatic needs. Hormones and various neurotransmitters are part of these signaling mechanisms. For example, cytokines have a key role in triggering sleep responses during systemic infections (reviewed in Ref. 25). Nitric oxide may have a role in signaling homeostatic needs for sleep (33). Cytokines and CCK have overlapping biological activities and have mutual stimulatory effects on each other’s secretion or function. For example, administration of interleukin (IL)-1 increases CCK plasma levels (17, 27), IL-1 sensitizes peripheral vagus nerves (18), and IL-1 decreases the activity of the gastric vagal afferents to cholecystokinin in rat (19). Intraperitoneal injection of CCK does not elicit changes in sleep or brain temperature (Abstract). Sleep 21, Suppl 3: 30, 1998.

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