METABOLIC SIGNALS IN SLEEP REGULATION: THE ROLE OF CHOLECYSTOKININ

By

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A thesis for the degree of

DOCTOR OF PHYLOSOPHY

(Ph. D.)

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2010
Szeged

To the memory of Ferenc Obál Jr.

Peer-reviewed papers directly related to the thesis

1. **Kapás, L.**, F. Obál, Jr., P. Alföldi, G. Rubicsek, B. Penke, and F. Obál. Effects of nocturnal intraperitoneal administration of cholecystokinin in rats: simultaneous increase in sleep, increase in EEG slow-wave activity, reduction of motor activity, suppression of eating, and decrease in brain temperature. *Brain Res*. 438: 155-164, 1988.

2. **Kapás, L.**, F. Obál, Jr., I. Farkas, L. C. Payne, G. Sáry, G. Rubicsek, and J. M. Krueger. Cholecystokinin promotes sleep and reduces food intake in diabetic rats. *Physiol. Behav*. 50: 417-420, 1991.

3. **Kapás, L.**, F. Obál, Jr., M. R. Opp, L. Johannsen, and J. M. Krueger. Intraperitoneal injection of cholecystokinin elicits sleep in rabbits. *Physiol. Behav*. 50: 1241-1244, 1991.

4. Chang, H.-Y. and **L. Kapás**. The effects of CCK-4 and non-sulfated CCK-8 on sleep, EEG slow-wave activity and brain temperature in rats, *Physiol. Behav*., 62: 175-179, 1997.

5. Shemyakin, A. and **L. Kapás**. L-364,718, a cholecystokinin-A receptor antagonist, suppresses feeding-induced sleep in rats. *Am. J. Physiol*., 280: R1420-R1426, 2001.

Reviews and book chapters directly related to the thesis

1. **Kapás, L.**, F. Obál, Jr., and J. M. Krueger. Humoral regulation of sleep. Int. Rev. Neurobiol. 35: 131-160, 1993.

2. **Kapás, L.** and É. Szentirmai. Sleep regulatory factors. In: Monti, J., Sinton, C. and Pandi-Perumal, S. R. (Eds.), The Neurochemistry of Sleep and Wakefulness. Cambridge University Press, UK, 2008, pp. 315-336.

3. Szentirmai, É., **L. Kapás** and J. M. Krueger. Interactive regulation of sleep and feeding. In: Kryger, M. H. (Ed.), Atlas of Clinical Sleep Medicine, Philadelphia: Saunders Elsevier, 2010, pp. 53-56.

4. Szentirmai, É., **L. Kapás** and J. M. Krueger. Hormones and sleep. In: Breed, M. D. and Moore, J. (Eds.), Encyclopedia of Animal Behavior, Oxford: Academic Press, 2010, *in press*.

Abstracts directly related to the thesis

1. **Kapás, L.**, F. Obál, Jr., P. Alföldi, G. Rubicsek, B. Penke, and F. Obál. Sleep elicited by peripheral injection of cholecystokinin in rats. Neuroscience 22: p. S841, 1987.

2. **Kapás, L.**, M. R. Opp, L. Johannsen, and J. M. Krueger. Divergent effects of central and peripheral injections of cholecystokinin (CCK) on sleep-wake activity in rabbits. Sleep Res. 19: p. 18, 1990.

3. **Kapás, L.**, F. Obál, Jr., I. Farkas, L. C. Payne, G. Sáry, G. Rubicsek, and J. M. Krueger. Hypnogenic and anorectic effects of CCK persist in vagotomized and diabetic rats. Eur. Sleep Res. Soc. Abstracts. p. 93, 1990.

4. **Kapás, L.** LY-288,513, a cholecystokinin (CCK)-B receptor antagonist, inhibits CCK-induced sleep. Sleep Res. 25: p. 12, 1996.

5. **Kapás, L.** Cholecystokinin (CCK)-induced sleep is suppressed by LY-288,513, a CCK-B receptor antagonist. J. Sleep Res. 5 (suppl. 1): p. 103, 1996.

6. Chang, H.-Y. and **L. Kapás**. The effects of CCK-8NS and CCK-4 on sleep, slow wave activity of the EEG and brain temperature in rats. Soc. Neurosci. Abstr., Vol. 22., Part 1., p. 147, 1996.

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9. Shemyakin, A. and **L. Kapás**. Starvation suppresses NREMS and EEG slow-wave activity in rats. Sleep 22, Suppl. 1, S247, 1999.

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Summary

Acute metabolic changes in response to feeding or starvation as well as long-term metabolic shifts due to increased or decreased adiposity are influences that greatly affect the amount and the quality of sleep. We posit that hormones of the gastrointestinal (GI) system and adipose tissue play a key role in signaling for these adaptive sleep responses. Eating is followed by a characteristic postprandial behavioral sequence which ends with sleep. Several GI hormones which are released after eating, e.g., cholecystokinin and gastric leptin, are known to suppress food intake and bring about satiety. The specific hypothesis tested in the present work is that cholecystokinin is a sleep-inducing hormone which contributes to signaling for postprandial sleep.

We tested this hypothesis in six sets of experiments. We determined that systemic administration of cholecystokinin octapeptide sulfate ester (CCK) elicits dose-dependent and selective increases in non-rapid-eye-movement sleep (NREMS) in rats. The lowest effective dose is 10 μg/kg when administered intraperitoneally. The sleep responses are accompanied by a decrease in brain temperature and suppressed feeding. Similar sleep and thermoregulatory effects are observed in rabbits after systemic injection of 10 and 50 μg/kg CCK. Central administration of CCK does not affect sleep in rabbits. CCK2 receptor specific analogues, CCK tetrapeptide and nonsulfated CCK octapeptide, lack somnogenic and hypothermic activities when given systemically. Both the somnogenic and thermoregulatory effects of exogenously administered CCK are blocked by L-364,718, a selective CCK1 receptor antagonist. Lesion of pancreatic beta cells by streptozotocin does not prevent the somnogenic effects of CCK. Enhanced feeding results in increases in NREMS in control rats. The postprandial sleep responses are prevented by CCK1 receptor antagonist treatment.

We conclude that CCK has somnogenic activities in rats and rabbits. Selective activation of the CCK2 receptors is not sufficient for the effects whereas the activation of CCK1 receptors is required; these strongly suggest the involvement of CCK1 receptors both in the somnogenic and hypothermic actions of CCK. CCK strongly stimulates insulin secretion by the pancreas but pancreatic insulin is not a mediator of CCK-induced sleep. Endogenously released CCK after feeding is likely a key factor for signaling postprandial sleep responses.

Present results are consistent with the hypothesis that CCK is a component of a complex signaling mechanism which modulates sleep-wake activity according to the metabolic status of the body.

Abbreviations

BBB	Blood-brain barrier
CCK	Cholecystokinin; cholecystokinin octapeptide sulfate ester
CCK-4	CCK tetrapeptide
CCK-8-NS	Nonsulfated CCK octapeptide
CCK-8-SE	CCK octapeptide sulfate ester
CNS	Central nervous system
EEG	Electroencephalogram
EMG	Electromyography
GI	Gastrointestinal
icv	Intracerebroventricular
ip	Intraperitoneal
iv	Intravenous
LH	Lateral hypothalamus
NREMS	Non-rapid-eye-movement sleep
NTS	Nucleus tractus solitarius
PBN	Parabrachial nucleus
REMS	Rapid-eye-movement sleep
sc	Subcutaneous
SCN	Suprachiasmatic nucleus
SE	Sulfate ester; standard error
Tbr	Brain temperature
TNF	Tumor necrosis factor
VMH	Ventromedial hypothalamus

Introduction

1. Sleep Regulation.

Questions about the nature and function of sleep have interested a great number of scientists, philosophers and common people across cultures and millennia. Aristotle wrote the following about sleep 2,400 years ago:

> *"WITH regard to sleep and waking, we must consider what they are: whether they are peculiar to soul or to body, or common to both; and if common, to what part of soul or body they appertain: further, from what cause it arises that they are attributes of animals, and whether all animals share in them both, or some partake of the one only, others of the other only, or some partake of neither and some of both."* (Aristotle: "On Sleep and Sleeplessness", translated by J.I. Beare, Electronically Enhanced Text Copyright 1991, World Library, Inc.)

Replacing the word 'soul' with 'brain' leads to some of the most fundamental questions in sleep research that are still being debated in the 21st century. Is sleep for the brain or for the body? Which part(s) of the brain is sleep related to? What is that sleeps in the brain and what is that regulates sleep? Do all animals sleep?

All animals exhibit some form of rest-activity cycle. All mammals that have been studied so far exhibit sleep similar to humans' in that two basic forms of vigilance states alternate during the sleep period: non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS). The proportion of NREMS and REMS in total sleep time shows species differences; both in humans and rats about 80% of sleep is NREMS and the rest is REMS. There are no known differences in the fundamental nature of sleep among mammals, including humans. Sleep of humans and rats, the most widely studied species in sleep research, shows some difference only in its timing. Healthy young adults typically have a single, ~8 h sleep episode at night. In the laboratory, rats sleep both during the day and night but more sleep occurs during the light period, characteristic of nocturnal species. Rat sleep is polyphasic, i.e., multiple short sleep and wake episodes alternate during the nychthemeron, the length of single wake periods rarely exceeds one hour.

Most in the field of sleep research maintain that sleep is by the brain and for the brain. It is also evident that specific changes occur in the activities of most organs during sleep. Some

of the sleep-related physiological events are slight adjustments, such as the 5-10% decline in energy expenditure during NREMS, others are fundamental changes, such as the near complete loss of homeothermic thermoregulation during REMS. There are three aspects of vigilance-related alterations in physiological functions. One, most physiological adjustments are thought to be caused by sleep or the lack thereof. For example, the decline in energy expenditure and increases in growth hormone secretion are due to sleep itself. Two, some changes are not caused by sleep *per se* but are independent manifestations of the action of a common regulatory mechanism which they share with sleep/wakefulness. Increased feeding and wakefulness at the beginning of the behaviorally active phase are thought to be parallel outputs of such a shared hypothalamic circuit of sleep and feeding regulation. We posit that there is a third aspect of body-sleep interaction: physiological changes outside of the brain affect complex brain functions, including sleep. Part of these somatic changes is related to eating, adiposity or changes in metabolism. Our long-term goal is to understand how the metabolic status of the body affects brain in general and vigilance in particular. We aim to decipher the mechanisms involved in signaling to integrative sleep centers under various metabolic conditions. The present work focuses on one of the putative peripheral messengers involved in signaling between the body and sleep centers, the hormone cholecystokinin (CCK).

Sleep appears to be a robust and distributed function of the brain. Multiple brain structures are proposed to be involved in triggering and maintaining sleep and wakefulness. While lesions or stimulations of various structures often lead to transient changes in sleep, there is not a single structure the lesion of which would permanently eliminate sleep if the animal survives. Ascending arousal systems arising from the brain stem and basal forebrain as well as arousal mechanisms originating in the lateral hypothalamus and thalamus are implicated in the maintenance of wakefulness (Jones, 2003). Sleep-promoting regions reside within the hypothalamus, mainly the anterior and the ventro- and dorsomedial regions (McGinty and Szymusiak, 2003). Though much has been learned about these structures in regard to sleep regulation, the exact function of these regions and the interaction among them are still poorly understood and widely debated.

Our understanding of sleep regulation is more complete when we view it from a more theoretical perspective. The most widely accepted model of sleep regulation is the “two-process model” (Borbély, 1982). It describes the onset of sleep and waking and the intensity

of sleep as the function of two independent processes, the sleep pressure and sleep threshold. In short, sleep occurs when sleep pressure exceeds threshold.

Sleep threshold (Process C), is the circadian component of sleep regulation driven by the main biological clock, the suprachiasmatic nucleus (SCN). Sleep pressure (Process S) is independent of circadian influences, its intensity solely determined by and proportional to prior wakefulness. Changes in sleep threshold show a 24-h cycle. In essence, these changes increase the probability of wakefulness during those hours of the day when active engagement with the environment is likely to be the most advantageous for a given species. Sleep pressure is the homeostatic component of sleep regulation, a mechanism that aims to keep the amount of sleep optimal. After extended periods of wakefulness, increases in sleep pressure lead to more prolonged and deeper sleep. In humans, high sleep pressure and low sleep threshold normally coincide around the usual bedtime leading to sleepiness and sleep. During sleep, sleep pressure dissipates and sleep threshold increases. Low sleep pressure coinciding with increased sleep threshold at the habitual waking time leads to arousal.

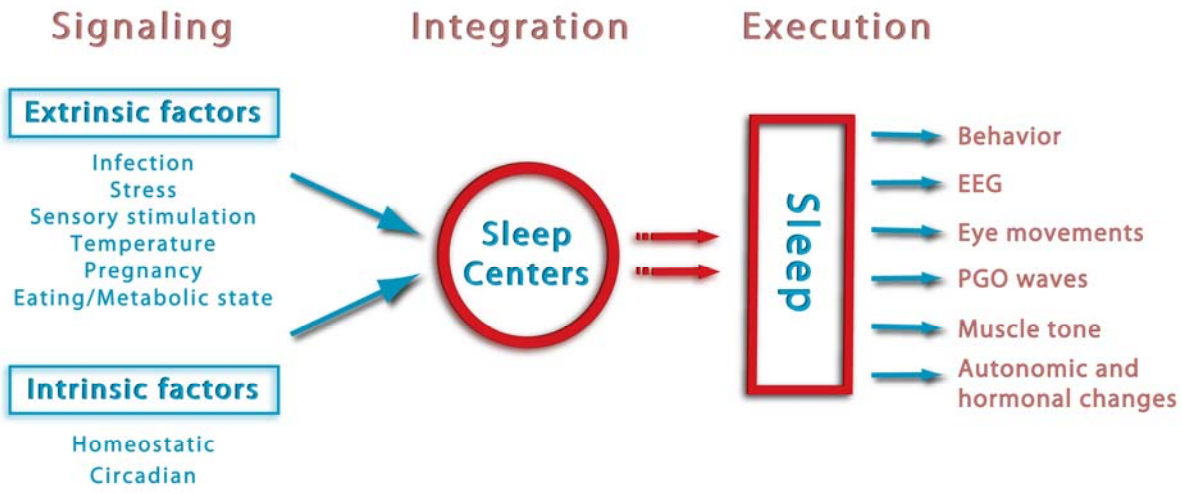

Fig. 1. Intrinsic and extrinsic factors in signaling sleep.

The two-process model gives a reliable statistical approximation of sleep timing in a large population of subjects under controlled, identical conditions but does not account for changes in vigilance driven by acute changes in the external of internal environment of the individual.

Both Process S and Process C are functions of the brain itself. Reflecting the fundamental nature of these processes that they arise from within the brain, we propose to consider them intrinsic factors in sleep regulation. The actual vigilance state of an individual subject, however, is also a function of influences that are not inherent to the brain but arise from outside of the central nervous system (CNS). These extrinsic factors include inputs from sensory organs, infections, stress and others (Fig. 1). Neural and hormonal signals in response to the external influences convey information to integrative sleep centers, which, in turn, bring about adaptive changes in sleep-wake activity. Acute metabolic changes in response to feeding or starvation as well as long-term metabolic shifts due to increased or decreased adiposity are extrinsic influences that greatly affect the amount and the quality of sleep. We posit that hormones of the gastrointestinal (GI) system and adipose tissue play a key role in signaling for these sleep changes.

2. Sleep, feeding and metabolism.

There is a strong bidirectional interaction between sleep/vigilance and metabolism/feeding. It has long been recognized that sleep is associated with characteristic changes in energy expenditure and metabolism (Garby et al., 1987). Cross-species correlational studies in mammals revealed a robust relationship between daily sleep amounts and resting metabolic rate (Zepelin and Rechtschaffen, 1974; Allison and Cicchetti, 1976). A growing body of evidence indicates that changes in metabolism and feeding lead to adaptive responses in sleep. Rats are nocturnal, about 80-90% of their daily feeding takes place at night when they are mostly awake and lipogenesis dominates their metabolic profile. In the light period, they sleep more, feeding is minimal and energy is mainly supplied by increased lipolysis. Reversing the lipolytic and lipogenic phases by sequential administration of lipolytic and lipogenic hormones (Danguir and Nicolaidis, 1980a) or by restricting feeding to the light period (Roky et al., 1999) leads to an almost complete reversal of the sleep-wake pattern of rats. The naturally nocturnal animals become diurnal, mostly awake during the day and sleep at night.

Acute, transient changes in the amount and/or content of food profoundly affect sleep-wake activity in several species, including humans. In general, starvation induces marked sleep loss (Borbély, 1977; Danguir and Nicolaidis, 1979; Szentirmai et al., 2010) while spontaneously or experimentally increased caloric intake leads to increased sleep. In 1964, Hockman reported that the electroencephalogram (EEG) of food-satiated animals shows a

marked increase in amount of high-voltage low-frequency activity, changes characteristic of sleep (Hockman, 1964). Introduction of milk into the duodenum leads to sedation in cats (Fara et al., 1969) and intragastric injection of eggnog results in postprandial EEG synchronization in rats (Bernstein, 1974). There is a positive correlation between meal size and the subsequent duration of sleep in normally feeding rats during the dark period (Danguir et al., 1979). Refeeding after food deprivation in adult (Jacobs and McGinthy, 1971; Borbely, 1977) or suckling rats (Lorenz, 1986), enhances sleep. The calorie-rich "cafeteria diet" induces hyperphagia and increases the amount of sleep in rats (Danguir, 1987; Hansen et al., 1998). Intravenous (iv) administration of highly nutritive composite solution greatly enhances both NREMS and REMS in rats (Danguir and Nicolaidis, 1980b).

In humans, enhanced postprandial sleepiness is not only our every day experience but it is also well-documented experimentally (Stahl et al., 1983; Smith et al., 1991; Zammit et al., 1992). Fat-rich meals have a more potent effect on subjective feelings of sleepiness than isocaloric meals in which fat is replaced by carbohydrate (Lloyd et al., 1994; Wells et al., 1995). In humans, both sleep and plasma CCK levels are enhanced after high-fat/low-carbohydrate diet as compared to low-fat/high-carbohydrate food (Wells et al., 1997). Nighttime protein- and fat-rich drinks prolong sleep (Southwell et al., 1972; Brezinova and Oswald, 1972). Intravenous infusion of amino acid mixture solutions promotes stage 3 and 4 NREMS (Lacey et al., 1978). Deep sleep profoundly increases during refeeding periods in anorexia nervosa patients when they are gaining weight but rapidly falls back to previous levels when normal weight is reached and stabilized (Lacey et al., 1975).

The regulation of sleep, feeding and metabolism overlaps on a structural level. Several hypothalamic areas, such as the SCN, lateral hypothalamus (LH) and ventromedial hypothalamic nucleus (VMH) are implicated in the regulation of both sleep and metabolism/food intake (Grill, 2006). We propose that there is also overlap on a second, signaling level as well; as certain signaling mechanisms, particularly GI hormones, may be involved both in sleep and feeding/metabolism regulation. **Our broad hypothesis is that feeding-related GI hormones play a key role as metabolic signals in aligning vigilance with the current metabolic state of the body.** Fasting is accompanied by marked increases in wakefulness and overall behavioral activity. There is strong evidence that ghrelin, a gastrointestinal peptide produced by the stomach during fasting, plays a role in fasting-induced arousal responses (Szentirmai et al., 2010). Eating is followed by a characteristic

postprandial behavioral sequence, called the satiety syndrome (Antin et al., 1975). Satiety syndrome entails the cessation of eating, transiently increased non-feeding activities such as grooming and exploration followed by reduced behavioral activity and social withdrawal ending with complete behavioral rest (Antin et al., 1975). Several GI hormones which are released after eating, e.g., CCK and gastric leptin, are known to suppress food intake and are thought to bring about satiety (Wren and Bloom, 2007). Blood transfusion experiments suggest that the increased postprandial EEG slow-wave activity (SWA), a characteristic sign of sleep, is due to the presence of a humoral factor in the plasma (Rosen et al., 1971). This supports the notion that increased sleep after eating is also signaled by humoral/hormonal factors. **The specific hypothesis tested in the present work is that CCK is a sleep-inducing hormone which contributes to signaling for postprandial sleep.**

3. Cholecystokinin.

The first, classic gastrointestinal effects of CCK were identified as the stimulatory effects of small intestinal extracts on gall bladder contraction (Ivy and Oldberg, 1928) and pancreatic exocrine secretion (Harper and Raper, 1943). Initially, the presence of two separate hormones was assumed, one named cholecystokinin and the other pancreozymin. The isolation and characterization of the active component of the intestinal extract led to the recognition that a single peptide is responsible for both effects (Jorpes et al., 1964). The name CCK prevailed and remained in general use.

There are two major, independent pools of CCK-producing cells, one in the gastrointestinal tract and the other in the nervous system (Crawley, 1985). Intestinal CCK serves as a GI hormone and paracrine agent while neuronal CCK is a neurotransmitter/neuromodulator (Crawley and Corwin, 1994). CCK is synthesized first as a 115-amino acid pre-prohormon which, in turn, is cleaved to various CCK forms of different sizes. Three of the four tyrosine residues of proCCK are sulfated in the trans-golgi network; sulfation of the CCK octapeptide is essential for its ability to bind to CCK1 receptors (Beinfeld, 2003). Posttranslational processing of pre-proCCK shows significant tissue- and species-specificity. In the brain of rats and mice, the predominant form is CCK octapeptide (Larsson and Rehfeld, 1979) while in the circulation longer forms, such as CCK-22 and CCK-33 also exist (Beinfeld, 2003). CCK octapeptide is the shortest form with full biological activity. Two G protein-coupled CCK receptor subtypes, CCK1 and CCK2 receptors (formerly known as CCK-A and CCK-B receptors, respectively), have been identified (Innis and Snyder, 1980; Jensen et al., 1980;

Saito et al., 1980). CCK1 receptors are mainly found in the GI tract but also present in select brain regions such as the nucleus tractus solitarius (NTS), area postrema, interpeduncular nucleus, posterior hypothalamic nuclei and posterior accumbens (Moran et al., 1986; Hill et al., 1987). CCK1 receptors are also expressed by peripheral and central axon terminals of vagal neurons (Lin and Miller, 1992; Corp et al., 1993) as well as by perikarya of nodose cells (Broberger et al., 2001). CCK2 receptors, which are identical to the gastrin receptor (Pisegna et al., 1992), are present in both the central (Innis and Snyder, 1980; Miceli and Steiner, 1989) and peripheral nervous system, e.g., the vagus nerve (Lin and Miller, 1992; Corp et al., 1993), as well as in various organs of the GI system.

The presence of a gastrin-like peptide in the brain was first reported in 1975 (Vanderhaeghen et al., 1975); subsequently it was determined that mainly sulfated CCK octapeptide accounts for the gastric-like activity (Dockray, 1976; Rehfeld, 1978). In the brain, especially high CCK peptide and mRNA (Cain et al., 2003) concentrations occur in the cortex, hippocampus, hypothalamic (Vanderhaeghen et al., 1980; Beinfeld and Palkovits, 1981) and thalamic (Beinfeld and Palkovits, 1981; Hunt et al., 1987; Bhatnagar et al., 2000) nuclei, striatum (Larsson and Rehfeld, 1979) and brain stem (Mantyh and Hunt, 1984); some of these areas are involved in sleep regulation. Well-defined ascending, descending and intranuclear CCK-ergic pathways have been described. Intrinsic CCK-ergic neurons are found in the hippocampus and the cortex (Handelmann et al., 1981). Ascending CCK-ergic projections originate from brain stem nuclei such as the parabrachial nucleus (PBN), dorsal raphe and periaqueductal gray matter and innervate various thalamic and hypothalamic nuclei (Bhatnagar et al., 2000). There is an extensive descending corticostriatal CCK-ergic pathway which is thought to interact with striatal dopaminergic terminals (Morino et al., 1992). CCK also co-localizes with classic neurotransmitters in various parts of the brain. Most notably, mesolimbic and mesostriatal dopaminergic neurons synthesize CCK (Hokfelt et al., 1980). CCK is also present in peripheral nerves, e.g., vagus afferents and primary spinal afferents (Dockray et al., 1981; Dalsgaard et al., 1982).

Intestinal CCK is secreted postprandially in response to dietary fat and protein by the “I” enteroendocrine cells of the small intestines (Liddle et al., 1985). CCK elicits a set of coordinated GI and behavioral responses characteristic of postprandial phase. CCK creates an alimentary environment favorable for fat and protein digestion by stimulating bile ejection and pancreatic enzyme secretion into the duodenum. CCK inhibits gastric emptying and

secretion, thereby delaying the delivery of undigested chyme into the small intestines. These autonomic actions in the GI system during the post-meal period are complemented by postprandial behavioral responses, also triggered by CCK.

The best characterized behavioral effect of CCK is its suppressive action on feeding. Administration of CCK decreases food intake in various species including rat, rabbit, mouse, sheep, and human (Crawley and Corwin, 1994). Administration of CCK antagonists stimulates eating (Lotti et al., 1987; Dourish et al., 1989; Weller et al., 1990). These basic observations led to postulate a role for CCK in the short-term regulation of feeding as a satiety hormone (Crawley and Corwin, 1994). Vagotomy prevents the food intake-suppressing effects of systemically administered CCK (Smith et al., 1981). According to the generally accepted view, CCK is released from the enteroendocrine cells after a meal and, by acting in a paracrine fashion, it binds to vagal CCK 1 receptors to stimulate vagus afferents. This leads to the activation of NTS – PBN – ventromedial hypothalamus (VMH) circuit resulting in the inhibition of feeding. CCK is present in NTS – PBN projection neurons as well as in the neurons from the PBN to the VMH suggesting that both peripheral, intestinal and central, neuronal CCK may contribute to signaling satiety. CCK is released in the hypothalamus after eating (McLaughlin et al., 1985; Schick et al., 1986). The role of central CCK in satiety is further supported by the notions that microinjections of CCK into the NTS and PBN and VMH suppress (Blevins et al., 2000) and centrally acting CCK receptor antagonists facilitate eating.

In addition to its effects on feeding, CCK has a wide variety of behavioral and autonomic actions. CCK suppresses exploratory behavior (Crawley et al., 1981b), modulates learning and memory (Flood et al., 1987; Gulpinar and Yegen, 2004), and elicits both hypothermia (Kapás et al., 1987; Kapás et al., 1989; Szelényi et al., 1994) and fever (Szelényi et al., 1994; Székely et al., 1994; Szelényi et al., 2004), has antiopioid activity (Faris et al., 1983; Kapás et al., 1989; Mollereau et al., 2005) and plays a role in opioid tolerance (Xie et al., 2005). CCK plays a key role in anxiety (Wang et al., 2005), dopamine-mediated reward (Rotzinger and Vaccarino, 2003) and psychostimulant sensitization (Rotzinger and Vaccarino, 2003).

4. Aims of the present studies.

At the outset of our studies, several lines of evidence suggested that CCK might signal for postprandial sleep increases. It was known that CCK administration to fasted rats not only

suppresses eating, but it leads to the complete sequence of behavioral events characteristic of rats after eating. This "satiety syndrome" terminates with resting. Since reduction of motor activity does not necessarily represent sleep, resting elicited by CCK might be a manifestation of behavioral sedation without sleep. Short episodes of sleep can often be observed after eating periods in rats. Supposing that postprandial sleep is a component of the behavioral manifestation of satiety, we postulated that resting observed after the injection of CCK may correspond to sleep. The few attempts to clarify the effects of CCK on sleep in rats produced controversial findings. Based on these observations, we set out to perform a series of experiments to determine the effects of CCK on sleep-wake activity and its role in postprandial sleep responses.

The following specific hypotheses were tested:

1. Systemic administration of CCK elicits sleep responses in rats.
2. Systemic but not central administration of CCK elicits sleep responses in rabbits.
3. The selective activation of CCK2 receptors by CCK tetrapeptide (CCK-4) or non-sulfated CCK octapeptide (CCK-8-NS) is not sufficient to induce sleep in rats.
4. The activation of CCK1 receptors is required for sleep responses in rats.
5. Sleep responses to systemically administered CCK are mediated by pancreatic insulin.
6. Intact CCK signaling on the CCK1 receptors is required for feeding-induced sleep responses.

Materials and Methods

1. General Methods

Animals. Forty-three Pasteurella-free New Zealand White rabbits, 60 CFY (Experiment 1), 54 Wistar (Experiment 5) and 99 Sprague-Dawley rats (Experiments 3,4 and 6) were used. All animals were male. Rabbits weighed 3-5 kg and the rats 260-420 g at the time of the experiments. Institutional guidelines for the care and use of research animals were followed and protocols were approved by the respective institutional committees when applicable.

Surgeries. The surgeries were performed using pentobarbital [50 mg/kg intraperitoneally (ip), Experiment 1] or ketamine-xylazine (rats: 87 and 13 mg/kg ip, respectively; rabbits: 35 and 5 mg/kg) anesthesia. For sleep recordings, animals were implanted with stainless steel screw EEG electrodes over the parietal and frontal cortices and above the cerebellum and electromyographic (EMG) electrodes in the nuchal muscle. With the exception of Experiment 5, a thermistor was also implanted over the dura above the parietal cortex to record brain temperature. A guide cannula for icv injections was also implanted into the left lateral ventricle for rabbits. Insulated leads from the EEG and EMG electrodes and the thermistor were routed to a plastic pedestal and cemented to the skull with dental adhesive.

Experimental conditions. After surgeries, the animals were placed into individual sleep-recording cages inside sound-attenuated and temperature-controlled environmental chambers for a minimum of a 1-week recovery followed by a 5-7-day habituation period. During the habituation period and the sleep recordings, the pedestal mounted on the animal's head was connected to a commutator through a flexible tether. The tether allowed the animals to move freely in their home cages. Cables from the commutator were connected to amplifiers (Grass 7D polygraphs or Coulbourn Instruments), the EEG signal was filtered below 0.5 and above 30 Hz. In Experiment 2, EEG and EMG signals were recorded on a polygraph, for the other experiments signals were digitized (100 or 128 Hz) and collected by a computer. In all experiments, a dark-light cycle of 12:12 h was maintained. Ambient temperature was set between 21 and 24°C and maintained within 1°C range for the entire duration of an experiment. Food and water were available *ad libitum*, unless noted otherwise.

Data analysis. Vigilance states were determined off-line by visually scoring the records in 10-30-s epochs or by an automatic analyzer (Experiment 1). Wakefulness, NREMS and REMS were distinguished. Wakefulness was defined as low-amplitude, high-frequency

irregular EEG and high EMG activity, NREMS as high-amplitude, low-frequency EEG waves with minimal EMG activity, and REMS as low-amplitude, relatively regular EEG waves with pronounced theta-wave activity and the complete lack of muscle tone. The amounts of NREMS, REMS and wakefulness were expressed as percent time spent in the given vigilance state over a 1-, 2-, 4- or 12-h period. In four experiments, spectral analysis of the EEG by fast-Fourier transformation (FFT) was also performed in 10-s intervals on 2-s segments of the EEG in the 0.5- to 4-Hz (delta) frequency range. For Experiments 3, 4 and 6, EEG power density values in the delta range were calculated separately for the three vigilance states. Delta-wave activity of the EEG during NREMS (also called slow-wave activity, SWA) is a measure of sleep intensity. For Experiment 1, all vigilance states were pooled for FFT analysis; separate analyses for NREMS, REMS and wakefulness were not performed.

Materials. Cholecystokinin octapeptide sulfate ester (synthesized by Botond Penke, University of Szeged for Experiment 1; purchased from Bachem Inc., Torrance, CA for Experiment 2 and Peninsula, Belmont, CA for Experiments 4 and 5), cholecystokinin tetrapeptide (Peninsula), nonsulfated cholecystokinin octapeptide (Peninsula), L-364,718 (Merck Research Laboratories, Rahway, NJ), streptozotocin (Sigma, St. Louis, MO), insulin radioimmunoassay kit (Incstar Corp., Stillwater, MN). L-364,718 was suspended in 4% methylcellulose and streptozotocin was dissolved in a mixture of citric acid and Na_2HPO_4 at pH 4; all other chemicals were dissolved in isotonic NaCl. Injection volumes were 2 ml/kg for systemic treatments, and 25µl for intracerebroventricular (icv) injections in rabbits.

2. Experimental Design

When feasible, repeated measures experimental design was used. On the baseline day(s) sleep-wake activity, temperature and motor activity were recorded; the same animals were subjected to the experimental challenge on the test day. For statistical analysis, ANOVA for repeated measures was applied; in most cases two factors were used (time effect and treatment effect, both repeated measures). Paired *t*-test was used *post hoc* when appropriate. In Experiment 5, a mixed repeated and independent measures design was used. Group effect (control vs. diabetic) was treated as independent factor, treatment effect (baseline vs. CCK) and time effect as repeated measures. Student's *t*-test was used *post hoc* for independent samples.

Experiment 1. *Effects of systemic injection of CCK in rats*.

a. Food intake measurements. Three groups of rats (n = 8, each) were injected with saline ip, 10 min before dark onset on the baseline day and 4, 10 or 50 μg/kg CCK on the test day. Pre-weighed food was placed in the cages at dark onset; after 1 h, spillage was recovered and reweighed.

b. Sleep, temperature and motor activity measurements. Three groups of rats (n = 12, each) were injected ip with saline on the baseline day and 4, 10 or 50 μg/kg CCK on the test day. The order of the saline and CCK treatments was balanced. The animals were fasted for 12 h before injections; treatments were done 5-10 min before dark onset. Recordings were obtained for 24 h after each injection.

Experiment 2. *Effects of ip and icv injection of CCK in rabbits*.

Six groups of rabbits were used. On the baseline day, isotonic NaCl was injected icv or ip. On the test day, 0.05, 0.5 or 2 μg CCK was injected icv (n = 7, 9 and 5, respectively) or 2.5, 10 or 40 μg/kg CCK was given ip (n = 11, 4 and 7, respectively). The order of saline and CCK treatments was balanced. Injections were performed 3 h after light onset. Sleep and brain temperature were recorded for 6 h.

Experiment 3. *Effects of CCK2 receptor agonists in rats*.

Six groups of rats were used. On the baseline day, the animals were injected with isotonic NaCl ip. On the test day, 3 groups of rats received 3 different doses of CCK-8-NS ip (10, 50 and 250 μg/kg, n = 7, 11 and 6, respectively) and the other 3 groups were injected with CCK-4 (10, 50 and 250 μg/kg, n = 7 for each). The order of the baseline and test days was balanced. Injections were done 5-10 min before dark onset, recordings continued for 12 h after lights-off.

Experiment 4. *Effects of CCK1 receptor antagonist on CCK-induced sleep in rats*.

Five groups of rats were used. On the baseline day, rats were injected with vehicle for L-364,718 and with isotonic NaCl 20 min later. Animals were also injected twice on the test day as follows. Group 1 received vehicle for L-364,718 followed by 10 μg/kg CCK (n = 10). Groups 2 and 3 received 100 μg/kg (n = 9) and 500 μg/kg (n = 6) L-364,718, respectively, followed by saline. Groups 4 and 5 received 100 μg/kg (n = 6) and 500 μg/kg (n = 7) L-364,718, respectively, followed by 10 μg/kg CCK. All injections were given ip during the

last 30 min of the light period. The order of the baseline and test days was balanced. Recordings started at dark onset and continued for 12 h.

Experiment 5. *Effects of CCK in diabetic rats*.

Diabetes was induced by iv injection of 65 mg/kg streptozotocin; control rats received vehicle. Sleep, food intake and serum insulin measurements were done on the second and third days after the streptozotocin treatment in three separate experiments in separate groups of animals.

a. Food intake measurements. Groups of diabetic (n = 4) and control (n = 6) rats were injected with saline on the baseline day and with 10 μg/kg CCK on the test day 5-10 min before dark onset. Pre-weighed food was placed in the cages at dark onset; after 1 h, spillage was recovered and reweighed.

b. Serum insulin measurements. Rats were implanted with chronic intra-atrial cannula through the jugular vein. Five days after the surgery, 5 rats were injected with streptozotocin and 7 with vehicle through the cannula. On days 2 and 3 after streptozotocin injections, baseline and test sampling was performed in a balanced order. Rats were injected with saline on the baseline and 50 μg/kg CCK on the test day 5 min before dark onset. Immediately before treatments (time 0), and 5, 15, 30 and 60 min after the injections blood samples (0.5 ml) were taken from the freely moving animals through the intraatrial cannula.

c. Sleep and temperature measurements. Two diabetic and two control groups of rats were used (n = 8 for each group). On the baseline day, all animals were injected with isotonic NaCl. On the test day, one control and one diabetic group received 10 μg/kg CCK, the other control and diabetic group was injected with 50 μg/kg CCK. All injections were given ip 5-10 min before dark onset. Recordings started at dark onset and continued for 12 h. At the end of the experiment, fasting plasma levels of glucose were determined.

Experiment 6. *Effects of a CCK1 receptor antagonist on feeding-induced sleep*.

The experiment consisted of 2 baseline days followed by 4 days of starvation and 2 days of refeeding. To induce starvation, food was removed at the end of the second baseline day (i.e., at dark onset of day 3); rat chow was returned to the animals 96 h later. The average weight loss during starvation was 13.2 ± 1.0% of the initial body weight. Two groups of rats were used (n = 8 for both). The control group received vehicle for L-364,718 on all 8 days.

The experimental group was injected with vehicle on the baseline and starvation days and with 500 µg/kg L-364,718 on both refeeding days. The injections were given ip 10-20 min before light onset. Sleep was recorded on the baseline and refeeding days; on these days, 12-h food intake was also measured separately for the dark and the light periods.

Results

Experiment 1. ***Effects of systemic injection of CCK in rats.***

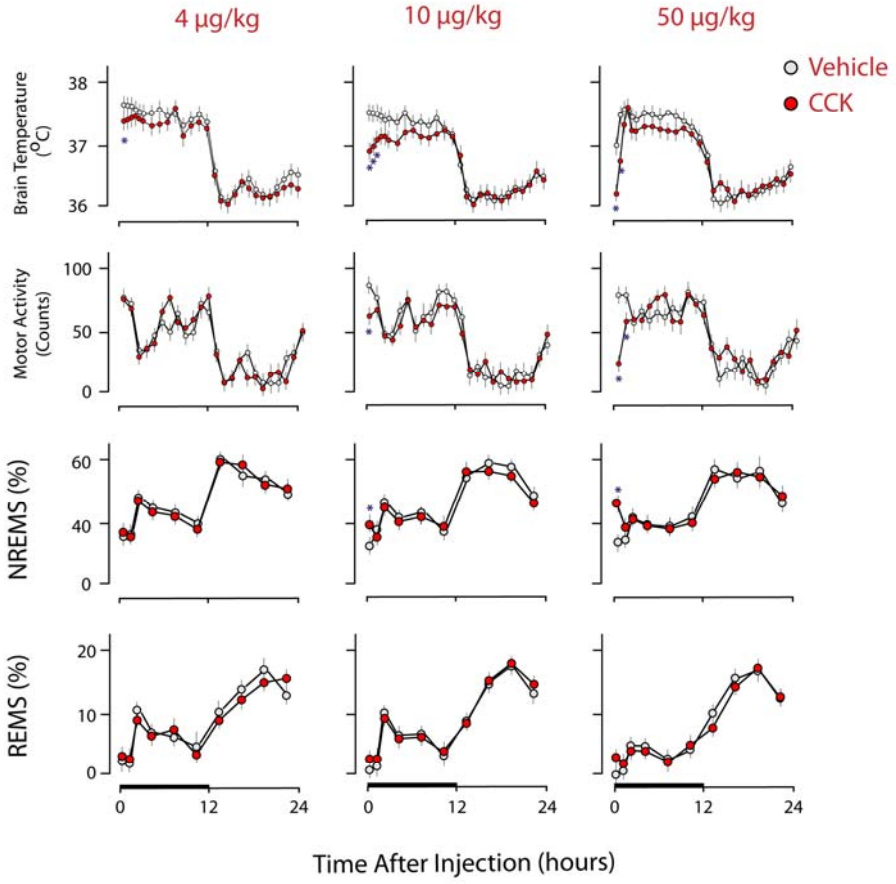

Fig. 2. The effects of the intraperitoneal (ip) injection of cholecystokinin octapeptide sulfate ester (CCK) on brain temperature (T_{br}), motor activity, non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in rats. Motor activity is shown in 1-h data blocks; T_{br} is shown in 30-min blocks for the first 2 h and in 1-h blocks for the rest of the recording. Sleep is expressed as percent of recording time in 1-h data blocks for the first three hours and in 3-h time blocks for the rest of the recording period. Horizontal dark bars: dark phase. Time 0: time of injections. Asterisks: significant difference between baseline and CCK treatment, $p < 0.05$, paired *t*-test. Error bars: SE. Modified from Kapás et al., 1988.

a. *Food intake*. Intraperitoneal injection of CCK suppressed eating dose-dependently [ANOVA treatment effect: $F(2,21) = 4.0$, $p < 0.05$; Attachment 1, Fig.1]. Ten and 50 µg/kg CCK reduced food intake by 45% and 63%, respectively ($p < 0.05$ for both, paired *t*-test); the lowest dose, 4 µg/kg, did not have significant effects.

b. *Sleep, brain temperature and motor activity*. Systemic injection of CCK elicited dose-dependent increases in NREMS, decreases in brain temperature (T_{br}) and suppressions in motor activity (Fig. 2). Four µg/kg CCK was a subthreshold dose for all measured parameters. After the middle dose, 10 µg/kg CCK, there were significant increases in NREMS and decreases in T_{br} in the first h after the injection. NREMS increased at the expense of wakefulness, the amount of REMS was not affected. Increased NREMS was accompanied by suppressed motor activity. The highest dose of CCK, 50 µg/kg, caused a more than 200% increase in NREMS in the first hour (baseline: 7.2 ± 0.2 vs. CCK: 22.1 ± 1.7 min, $p < 0.05$). Motor activity was suppressed by ~73% and T_{br} dropped by ~0.9°C during this period. Increases in the EEG power in the 0.4-6 Hz range accompanied the sleep enhancement after CCK injections (Attachment 1, Fig. 3).

Experiment 2. ***Effects of ip and icv injection of CCK in rabbits.***

The experiment was designed to test a) if the sleep-promoting effects of CCK are specific to rats or they are present in a second species and b) if central injection of CCK has also effects on sleep-wake activity.

a. *Intraperitoneal injection of CCK*. Similar to the effects seen in rats,

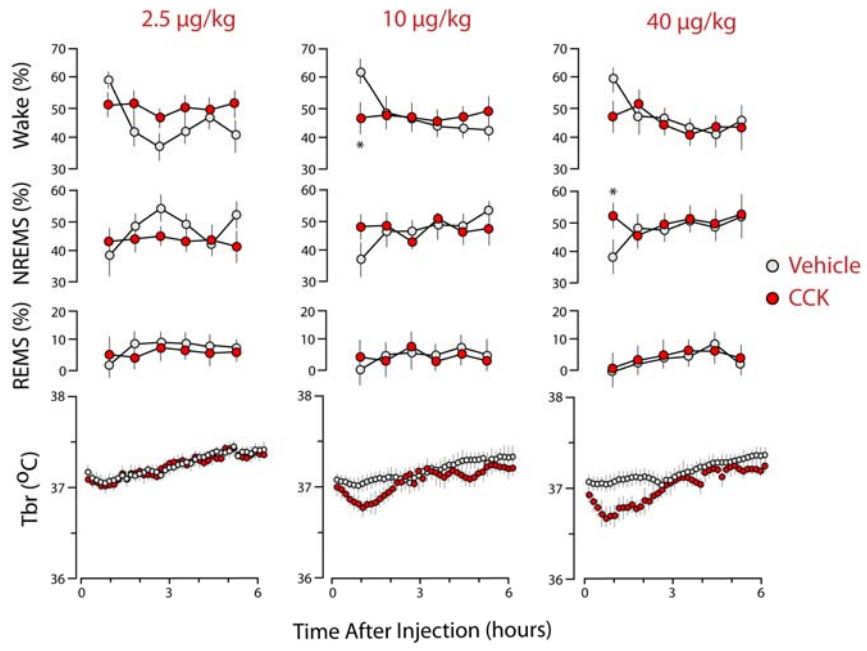

Fig. 3. The effects of ip injection of CCK on sleep and T_{br} in rabbits. Sleep is expressed as percent of recording time in 1-h data blocks, temperature is shown in 10-min intervals. Time 0: injection time. Asterisks: significant difference between baseline and CCK treatment, $p < 0.05$, paired *t*-test. Error bars: SE. Modified from Kapás et al., 1991.

ip injection of CCK caused dose-dependent increases in NREMS and decreases in T_{br} in rabbits (Fig. 3). Ten µg/kg CCK significantly decreased wakefulness and 40 µg/kg CCK significantly increased NREMS in the first h after injection. The lowest dose did not have significant effects on sleep or wakefulness. Maximal EEG delta-wave amplitudes during NREMS – a measure of NREMS intensity, analogous to SWA, see General Methods – was not affected by CCK treatment (Attachment 2, Table 1). The somnogenic effects of CCK were accompanied by dose-dependent decreases in T_{br}. While 2.5 µg/kg CCK did not affect T_{br}, 10 µg/kg slightly decreased T_{br} for about 2 h, and 40 µg/kg caused significant hypothermia lasting for about 3 h (Fig. 3).

b. *Intracerebroventricular injection of CCK*. In contrast to the effects of ip injections, icv administration of CCK did not cause any significant increase in NREMS in rabbits. Rather, 0.05 µg CCK reduced REMS across the 6-h recording period [ANOVA treatment effect: $F(1,6) = 4.2$, $p < 0.05$] and 0.5 µg CCK reduced NREMS in the first h after the injection (Fig. 4). There was a slight but significant decrease in T_{br} after the central injection of 0.05 and 2 µg CCK [ANOVA treatment effect for 0.05 µg: $F(1,18) = 7.1$, $p < 0.05$; for 2 µg: $F(1,18) = 4.7$, $p < 0.05$].

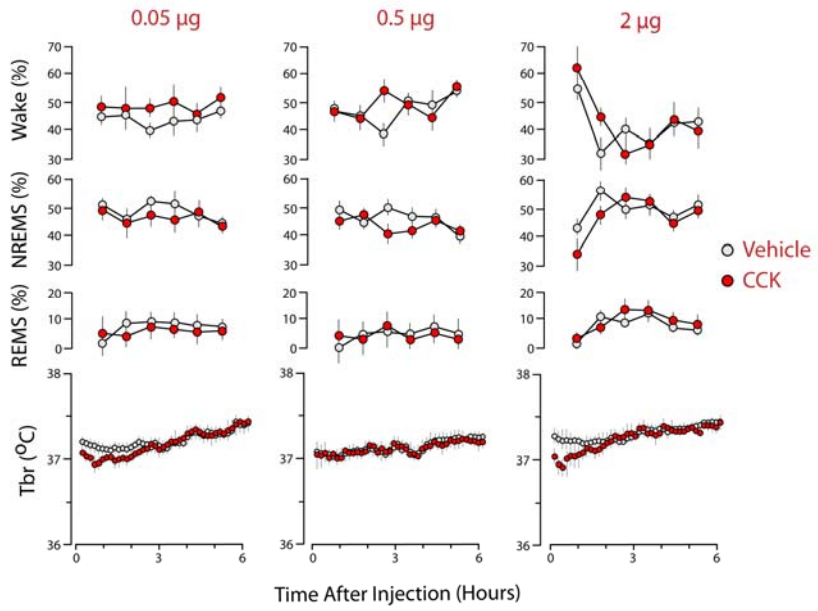

Fig. 4. The effects of intracerebroventricular injection of CCK on sleep and T_{br} in rabbits. See legend to Fig. 3 for details. Modified from Kapás et al., 1991.

Experiment 3. *Effects of CCK2 receptor agonists in rats.*

The experiments aimed to determine if selective activation of CCK2 receptors is sufficient to elicit sleep responses characteristic of CCK. CCK2 receptors are present both in the CNS and in peripheral tissues (Hokfelt et al., 1991). There are CCK2 receptor-selective CCK

analogues available, such as CCK-8-NS and CCK-4. The affinities of CCK-8-NS and CCK-4 to CCK2 receptors are about 500-1,000 fold higher than to the CCK1 receptor (Wank, 1998).

In rats, ip injection of neither CCK-4 (Fig. 5) nor CCK-8-NS (Attachment 3, Fig. 2) had significant effect on sleep or T_{br} in the first h; this is the time when the somnogenic and hypothermic effects of the sulfated CCK octapeptide are manifested. When the entire 12-h recording period is considered, the effects of 10 µg/kg CCK-4 on NREMS and T_{br} were significant [ANOVA for repeated measures, NREMS treatment effects: $F(1,6) = 12.0$, $p < 0.05$; T_{br} treatment effect: $F(1,5) = 9.4$, $p < 0.05$]. *Post hoc* paired *t*-test revealed significant decrease in NREMS in h 4; there were no significant changes in T_{br} at any time point by *post hoc* test (Fig. 5). The two higher doses of CCK-4 did not have any significant effect on sleep or T_{br}. CCK-8-NS did not have any significant effect on NREMS, SWA or T_{br} (Attachment 3, Fig. 2). There was a significant effect on REMS across the 12-h period after 10 µg/kg CCK-8-NS [ANOVA for repeated measures, treatment effect: $F(1,6) = 27.3$, $p < 0.05$]. REMS was elevated in h 3 (*post hoc* paired *t*-test, $p < 0.05$).

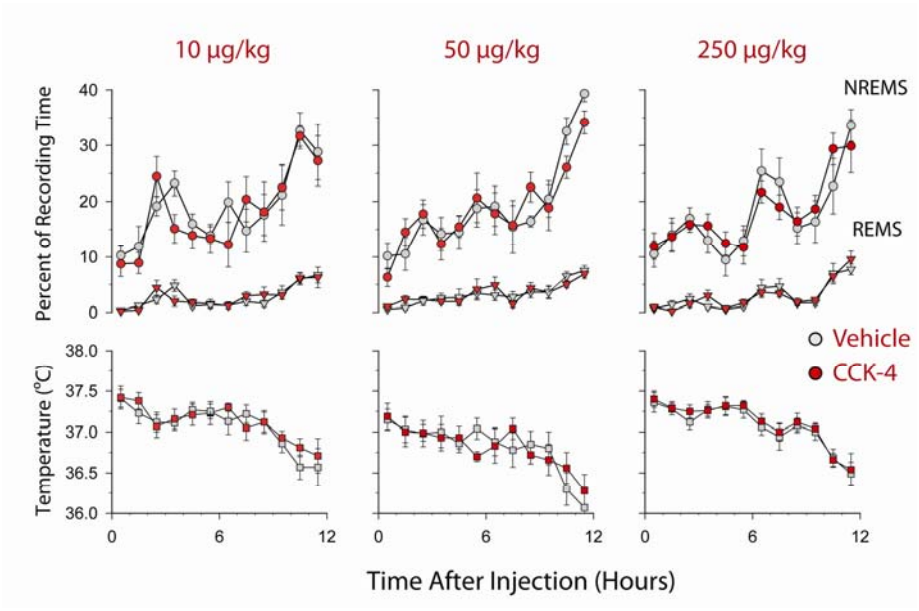

Fig. 5. The effects of ip injection of cholecystokinin tetrapeptide (CCK-4) on sleep and T_{br}. Sleep is expressed as percent of recording time in 1-h data blocks, temperature is shown as 1-h averages. Time 0: injection time. Error bars: SE. Modified from Chang and Kapás, 1997.

Experiment 4. ***Effects of CCK1 receptor antagonist on CCK-induced sleep in rats***.

The aim of the experiment was to determine if the activation of CCK1 receptors is necessary for the somnogenic effects of systemically administered CCK. CCK1 receptors are expressed in the brain, by neurons of the vagus nerve and by peripheral tissues (Hokfelt et al., 1991; Lin and Miller, 1992; Corp et al., 1993). The food intake-suppressing effects of CCK are mediated by the activation of CCK1 receptors on vagus nerve terminals (Dockray, 2009). L-364,718 is a widely-used and highly selective CCK1 receptor antagonist (Chang and Lotti, 1986; Lotti et al., 1987; Hewson et al., 1988; Soar et al., 1989).

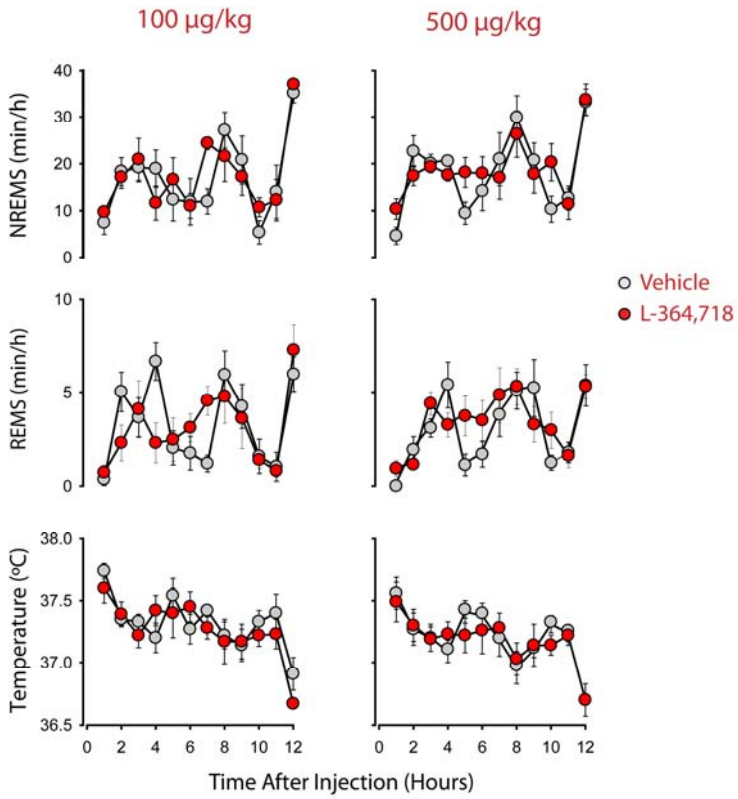

Fig. 6. The effects of ip injections of L-364,718, a CCK1 receptor antagonist on sleep and T_{br} in rats. See legend to Fig. 5 for details.

Intraperitoneal injection of L-364,718 alone did not have significant effects on spontaneous sleep, SWA and T_{br} (Fig. 6, Table 1). Ten µg/kg CCK, ip, elicited significant increases in NREMS and decreases in T_{br} in the first h after the injection (Fig. 7, Table 1). One hundred µg/kg L-364,718 attenuated but did not completely block CCK-induced sleep; NREMS was significantly increased across the 12-h and in the first 2-h time block as compared to baseline (Table 1). The same dose of L-364,718 completely blocked the hypothermic effects of CCK (Fig. 7). Five hundred µg/kg of L-364,718 completely abolished CCK-induced sleep and hypothermic responses (Fig. 7). L-364,718 pretreatment did not affect hourly SWA values. When, however, SWA values are averaged in 2-h time blocks, the combined treatment of CCK with either 100 or 500 µg/kg L-

364,718 caused significantly increased SWA in the first 2-h time block as compared to baseline (Fig. 7, Table 1). Neither dose of the antagonist, when given without CCK, caused significant changes in SWA in the first 2 h (data not shown, see Table 1 for statistical results).

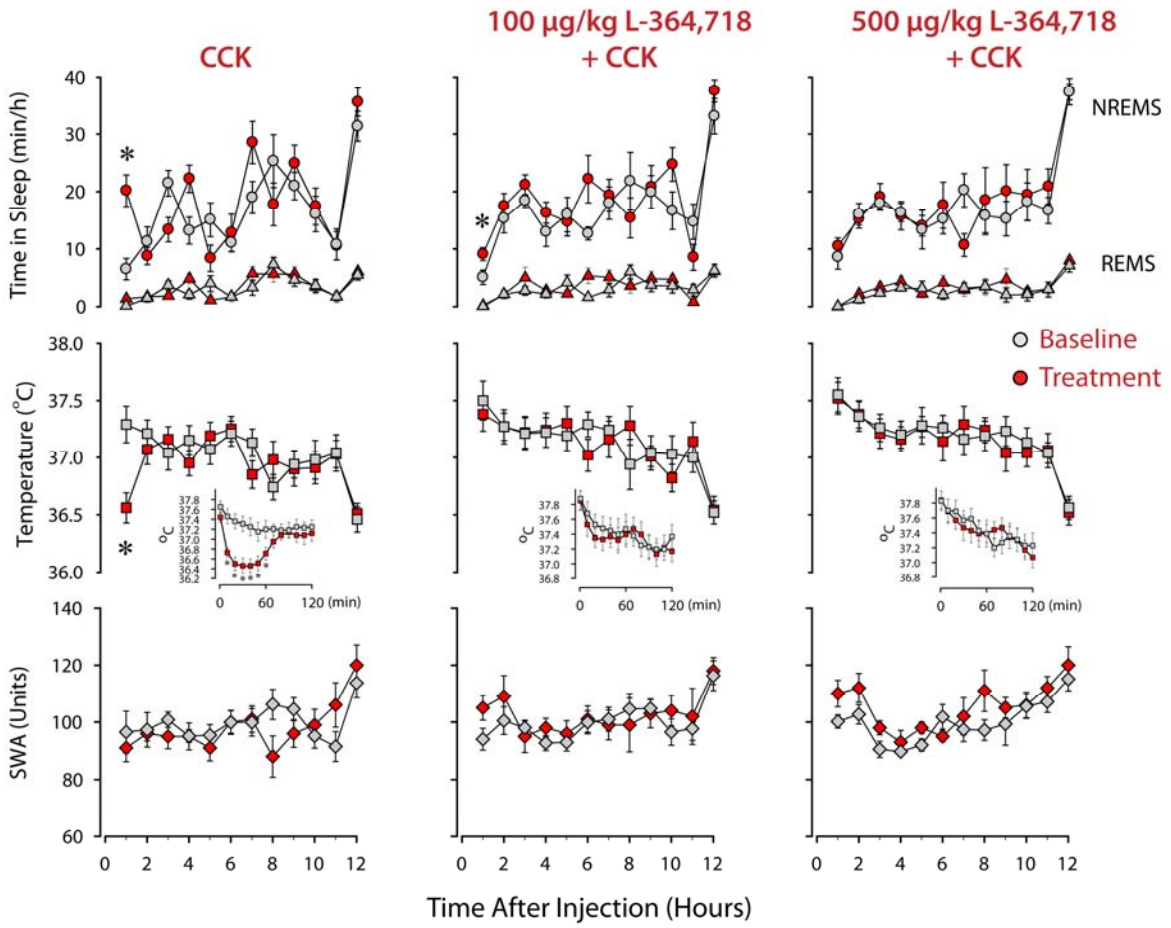

Fig. 7. The effects of L-364,718 pretreatment on CCK-induced sleep and hypothermic responses. Sleep and the slow-wave activity of the electroencephalogram during NREMS (SWA) are shown in 1-h data blocks. On the main T_{br} panel, average hourly temperatures are shown for 12 h. On the insets, T_{br} is plotted for the first 2 h after the injection in 10-min intervals. Baseline: ip vehicle for L-364,718 followed by ip saline; treatment: vehicle (left panels) or L-364,718 followed by CCK (middle and right panels). Asterisk: significant difference between baseline and treatment (paired *t*-test, $p < 0.05$). Error bars: SE.

Table 1.

The effects of cholecystokinin (CCK), L-364,718 (L) and combined administration of L-364,718 and CCK on sleep amounts, slow-wave activity of the electroencephalogram (SWA) during non-rapid-eye-movement sleep (NREMS) and brain temperature (T_{br}): Statistical results.

	Vehicle + CCK		**100 μg/kg L + CCK**		**500 μg/kg L + CCK**		**100 μg/kg L + Vehicle**		**500 μg/kg L + Vehicle**	
	h 1-12	h 1-2	h 1-12	h 1-2	h 1-12	h 1-2	h 1-12	h 1-2	h 1-12	h 1-2
NREMS	*F*(1,9) 3.14	*F*(1,9) **7.49***	*F*(1,8) **21.96***	*F*(1,8) **13.49***	*F*(1,5) 0.57	*F*(1,5) 0.26	*F*(1,5) 0.41	*F*(1,5) 0.15	*F*(1,6) 1.23	*F*(1,6) 0.02
REMS	*F*(1,9) 0.75	*F*(1,9) 1.91	*F*(1,8) 0.61	*F*(1,8) 0.05	*F*(1,5) **5.63***	*F*(1,5) 1.22	*F*(1,5) 0.09	*F*(1,5) 2.37	*F*(1,6) 1.29	*F*(1,6) 0.02
SWA	*F*(1,9) 1.96	*t* (7) 1.66	*F*(1,8) 0.41	*t* (8) **2.78***	*F*(1,5) 3.29	*t* (5) **3.46***	*F*(1,5) 0.81	*t* (5) 1.33	*F*(1,6) **5.36***	*t* (6) 0.92
T_{br}	*F*(1,7) 6.09	*F*(1,7) **13.67***	*F*(1,5) 0.09	*F*(1,5) 0.69	*F*(1,5) 3.23	*F*(1,5) 0.19	*F*(1,4) 0.45	*F*(1,4) 0.03	*F*(1,6) 0.09	*F*(1,6) 0.17

Two-way analysis of variance (ANOVA) for repeated measures was performed for NREMS, rapid-eye-movement sleep (REMS), SWA and T_{br}, between the treatment and corresponding baseline (vehicle + vehicle) days.

ANOVA across the specified hours was performed on 1-h time blocks for the amounts of NREMS and REMS and for T_{br}. For the statistical analysis of SWA, values were averaged in 2-h time blocks; paired *t*-test was performed between baseline and test days on the first 2-h time block and also ANOVA was performed across the 12-h recording period. For ANOVA, the degrees of freedom and *F*-values for the treatment effects are indicated; for paired *t*-tests, the degrees of freedom and *t* values are shown. Bold *F* and *t* values with asterisks indicate significance difference between control and test conditions.

Experiment 5. ***Effects of CCK in diabetic rats.***

CCK strongly stimulates pancreatic insulin secretion in rats (Szecowka et al., 1982) by acting on CCK1 receptors (Reagan et al., 1987). Insulin is known to enhance NREMS (Sangiah et al., 1982; Danguir and Nicolaidis, 1984). In this set of experiments, we tested if the sleep-promoting effects of CCK are mediated by pancreatic insulin. We tested the effects of systemic injection of CCK on sleep in streptozotocin-induced diabetic rats.

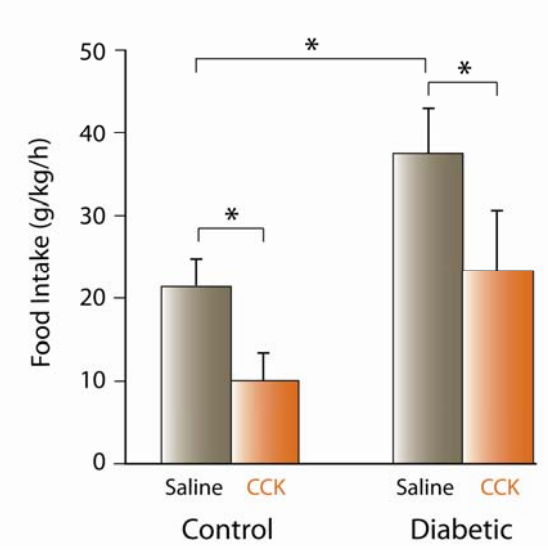

Fig. 8. The effects of ip injection of 10 µg/kg CCK on 1-h food intake in normal and streptozotocin-diabetic rats. Asterisks: $p < 0.05$, Student's *t*-test for between-group and paired *t*-test for within-group comparisons.

a. *Food intake*. Diabetic rats ate significantly more than controls on the baseline day. Intraperitoneal injection of 10 µg/kg CCK significantly suppressed feeding in both normal and diabetic rats by 53.6% and 37.5%, respectively (Fig. 8). Diabetic animals ate about the same amount after CCK treatment as normal rats after saline injection.

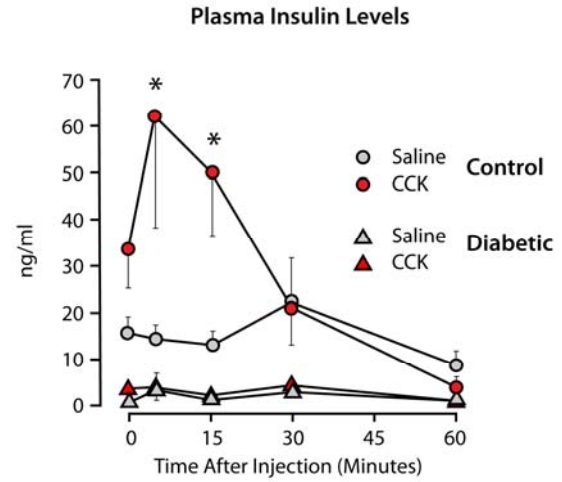

Fig. 9. The effects of ip injection of 10 µg/kg CCK on plasma insulin levels in normal and streptozotocin-diabetic rats. Error bars: SE. Asterisks: significant difference between saline and CCK treatments in the control group. Time 0: pre-injection baseline values. Modified from Kapás et al., 1991.

b. *Plasma insulin levels*. Baseline plasma insulin levels of diabetic rats were significantly lower than those of controls [Fig. 9; ANOVA across 60 min, streptozotocin effect: $F(1,40) = 19.21$, $p < 0.05$]. In the control group, insulin levels were slightly higher before CCK treatment compared to pre-saline levels; this difference was not

statistically significant. After saline treatment, there was no significant change in plasma insulin levels in either group of animals compared to pre-injection baseline. In control rats, CCK significantly increased plasma insulin levels 5 and 15 min after injection (paired *t*-test, $p < 0.05$). In diabetic rats, CCK did not have any significant effect on plasma insulin concentrations.

c. *Sleep, brain temperature and motor activity*. As expected, streptozotocin-induced diabetic rats had significantly higher plasma glucose levels compared to normal animals (17.6 ± 1.6 vs. 3.4 ± 0.2 mmol/l in diabetic and control animals, respectively). There were no significant differences in the baseline sleep-wake activity of control and diabetic rats. Neither time spent

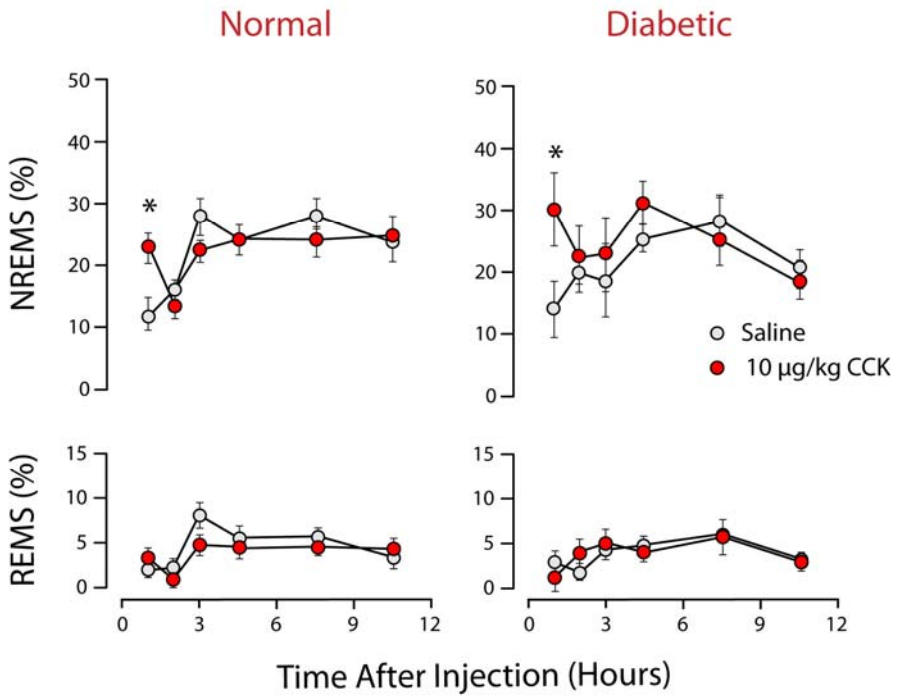

Fig. 10. The effects of ip injection of 10 µg/kg CCK on sleep in normal and streptozotocin-diabetic rats. Sleep is expressed as percent of recording time in 1-h blocks for the first three hours and in 3-h blocks from h 4 to 12. Asterisks: significant difference between saline and CCK treatment (paired *t*-test, $p < 0.05$). Error: SE. Modified from Kapás et al., 1991.

in NREMS during the 12-h recording period (diabetics: 25.6 ± 2.4%, controls: 25.7 ± 1.3%), nor REMS amounts differed between the two groups (diabetics: 3.7 ± 0.5%, controls: 3.5 ± 0.5%). Intraperitoneal injections of CCK induced selective increases in NREMS in both the control and the diabetic groups in the first h after the injection. Ten µg/kg CCK doubled the amount of NREMS in the first h in normal rats; similar increases were observed in

streptozotocin-pretreated animals (Fig. 10). Fifty µg/kg CCK had a slightly more pronounced NREMS-promoting activity both in the control and diabetic rats (Fig. 11).

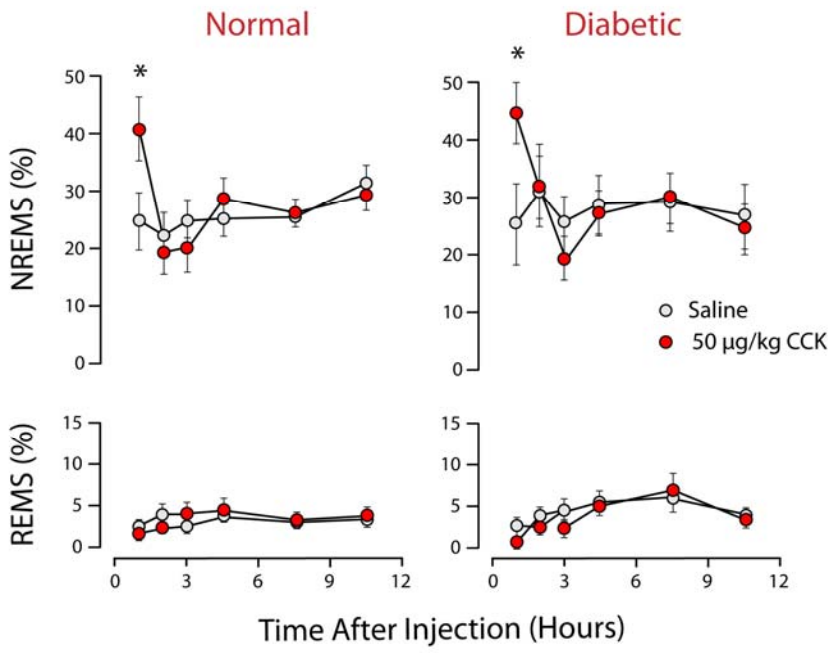

Fig. 11. The effects of ip injection of 50 µg/kg CCK in normal and streptozotocin-diabetic rats. See legends to Fig. 10 for details. Modified from Kapás et al., 1991.

<u>Experiment 6</u>. ***Effects of CCK1 receptor antagonist on feeding-induced sleep.***

Increased feeding stimulates both CCK secretion and NREMS in rats. We set out to test the hypothesis that feeding-induced sleep responses are mediated by endogenous CCK acting on CCK1 receptors. To induce increased feeding, a starvation-refeeding paradigm was used.

a. *<u>Body weight</u>*. Body weights did not differ significantly between control and L-364,718-treated groups [baseline: 421 ± 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 animals, respectively; two-way ANOVA treatment effect: $F(1,95) = 1.8$, not significant].

b. *<u>Food intake (Fig. 12)</u>*. There were no significant differences in food intake between control and L-364,718-treated rats throughout the experiment [three-way ANOVA, group

effect: $F(1,126) = 0.30$, not significant]. There was significant difference in feeding among baseline day, refeeding *day 1* and *2* [three-way ANOVA, day effect: $F(2,126) = 12.2$, $p < 0.05$]. Food intake significantly increased in the dark period of the first refeeding day in both treatment groups. In the following light phase, feeding in the control group decreased below baseline. Similar tendencies were present after the CCK antagonist treatment, but the changes were not significant. On the second refeeding day, both day- and night-time food intake returned to baseline levels in both groups.

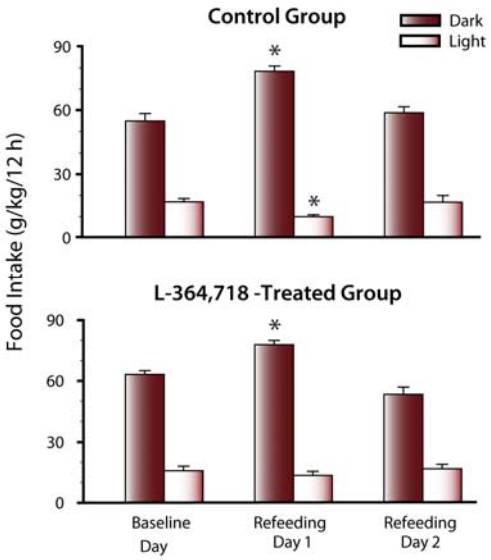

Fig. 12. Food intake of control and CCK1 antagonist (L-364,718)-treated rats under baseline conditions and on refeeding days 1 and 2 after food deprivation. Asterisk: significant difference from baseline (paired *t*-test, $p < 0.05$). Modified from Shemyakin and Kapás, 2001.

c. *The effects of refeeding on sleep and T_{br} in control, saline-treated rats*. Reintroducing food at the beginning of the dark period after 4 days of food deprivation led to delayed but long-lasting increases in NREMS indicative of postprandial sleep (Fig. 13). NREMS was significantly elevated during the second 12-h period (light phase) of the first refeeding day (baseline: 332 ± 23 min/12 h vs. refeeding *day 1*: 392 ± 7 min/12 h, $p < 0.05$). Strong tendencies toward increased NREMS continued throughout the next day, but the changes did not reach the level of significance. REMS was elevated during the light phase of the second refeeding day (baseline: 38 ± 5 min/12 h vs. refeeding *day 2*: 58 ± 7 min/12 h, $p < 0.05$). There were significant reductions in SWA during the second refeeding night; similar tendencies were present for the prior and subsequent 12-h periods. T_{br} was not affected by refeeding.

d. *The effects of refeeding on sleep and T_{br} in L-364,718-treated rats*. The NREMS-inducing effects of refeeding on the first and the REMS-promoting effects on the second refeeding day were completely abolished by the CCK1 receptor antagonist (Fig. 14). During the dark phase of the second refeeding day, increases in NREMS – that were present only as a tendency after saline injection – became significant; NREMS returned to baseline by the second part of refeeding *day 2*. L-364,718 completely abolished the SWA responses to refeeding.

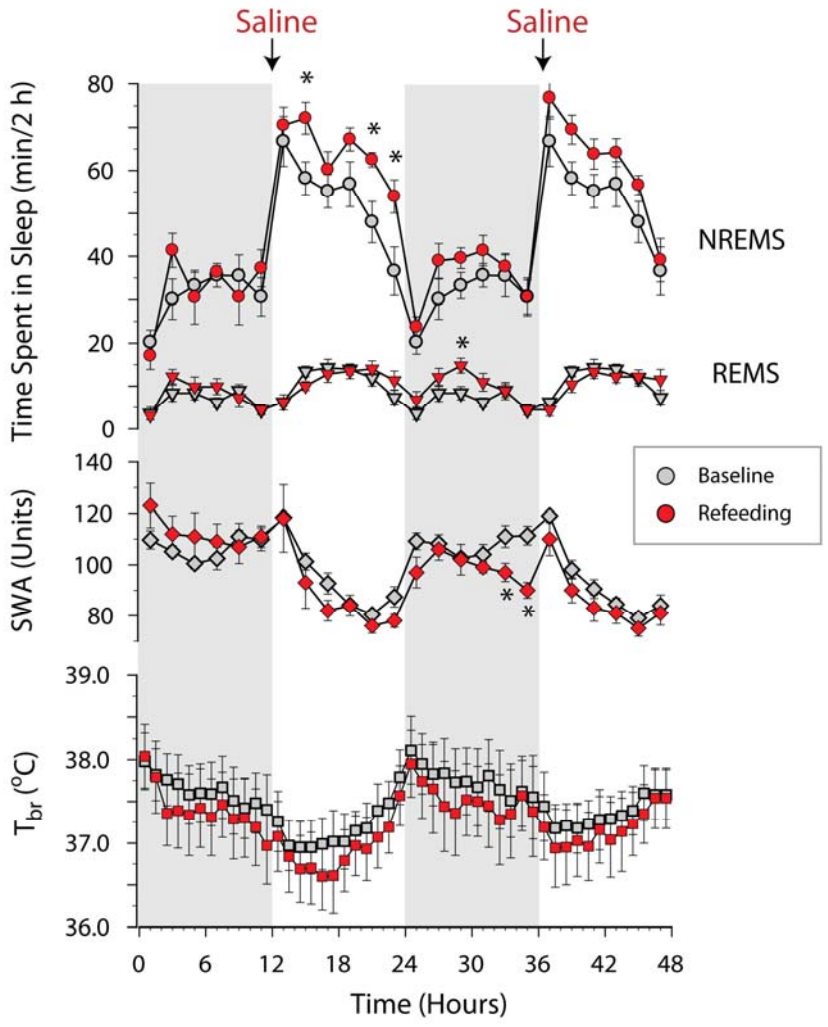

Fig. 13. The effects of refeeding on NREMS, REMS, SWA and T_{br} in control rats. Time spent in sleep is summed in 2-h blocks. SWA is averaged in 2-h and T_{br} in 1-h intervals. Error bar: SE. Gray shaded area: dark phase. Asterisks: significant difference from baseline ($p < 0.05$, paired *t*-test). Modified from Shemyakin and Kapás, 2001.

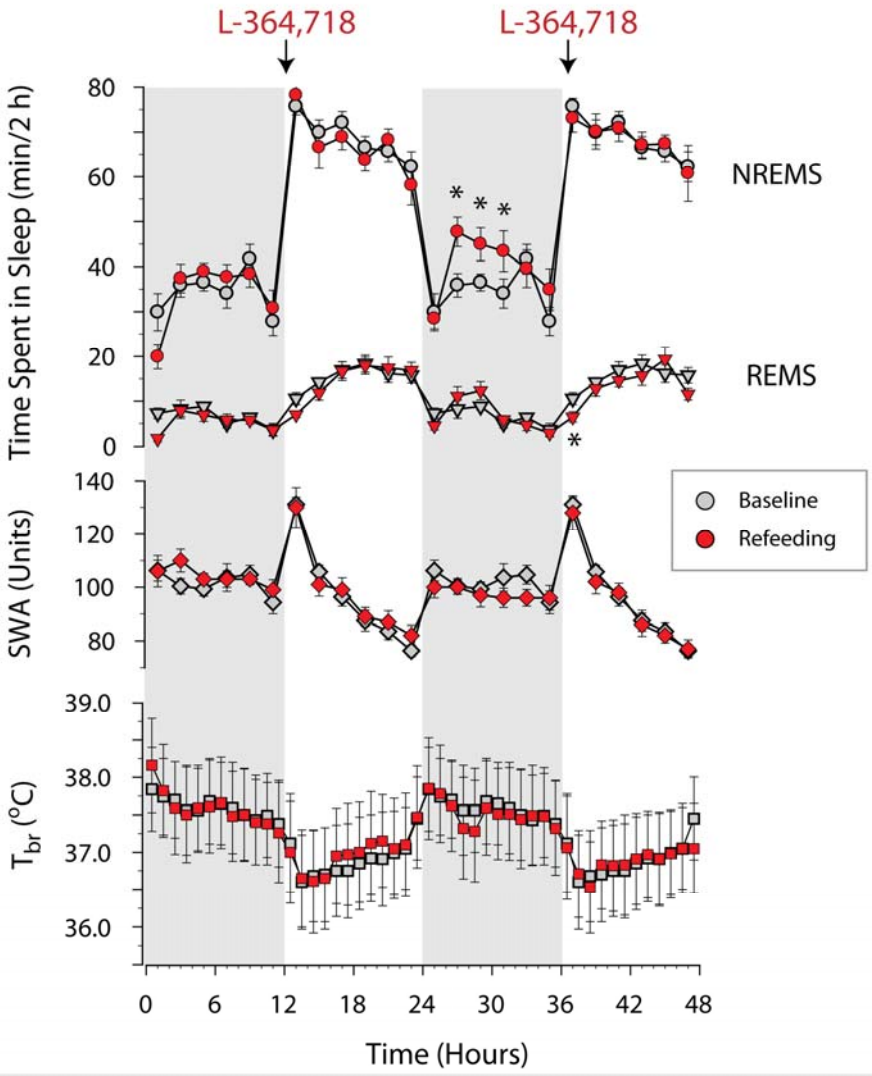

Fig. 14. The effects of refeeding on NREMS, REMS, SWA and T_{br} in L-364,718-treated rats. See legend to Fig. 13 for details. Modified from Shemyakin and Kapás, 2001.

Discussion

In the present experiments, we have shown that systemic injections of sulfated CCK octapeptide selectively and dose-dependently stimulate NREMS in rats and rabbits. Central injection of CCK in rabbits or systemic injection of CCK2 receptor agonists in rats did not have significant effects on sleep. The somnogenic effects of exogenously administered CCK as well as the sleep-inducing effects of refeeding after starvation were completely abolished by a selective CCK1 receptor antagonist. Systemic, but not central, administration of CCK elicited significant decreases in brain temperature, a response completely prevented by CCK1 receptor antagonist. The results are consistent with our hypothesis that CCK produced by the GI system in response to eating plays a key role in eliciting postprandial sleep and thus in aligning vigilance to the acute feeding/metabolic status of the body.

Effects of CCK on sleep

Prior to our studies, only sparse and mainly indirect data were available concerning the effects of CCK on sleep. The first experiments suggesting a possible somnogenic effect for CCK were performed in the late 1960s. In these studies, both intra-duodenal administration of fat and iv injection of CCK-rich duodenum extracts caused sedation in awake in cats (Fara et al., 1969). In 1982, Mansbach and Lorenz reported that CCK reduced sleep latency and 20 min after the ip injection of CCK, a significantly greater number of rats were asleep than after control treatments. These effects of CCK were indistinguishable from those induced by eating (Mansbach and Lorenz, 1983). There was one single report prior to our experiments where the effects of systemically administered CCK were quantitatively analyzed. A reduced latency to NREMS in rats after ip injection of 20 μg/kg CCK was reported without effects on the duration of sleep (Rojas-Ramirez et al., 1982). CCK injections and sleep recordings were performed during the first part of light phase in these experiments in *ad libitum* fed, therefore likely satiated, animals. The light period, however, is the rest phase in rats, physiological sleep-promoting mechanisms are fully engaged, sleep is already elevated; it is unlikely that a physiological sleep-promoting hormone in a presumably physiological dose range could further increase sleep amounts.

To avoid such a ceiling effect, we injected CCK immediately before dark onset in our experiments with rats. During the first part of the dark period, the spontaneous activity of sleep-promoting mechanisms, hence the amount of sleep, is minimal in rats. Also, in

Experiment 1, we fasted rats for 12 h before the CCK treatment to ensure that endogenous satiety mechanisms are not already activated. In our subsequent experiments it became apparent that such a prior fasting is not required for the manifestation of the sleep-promoting effects of ip administered CCK. As expected, the dose-dependent NREMS-promoting effects of CCK were mirrored by decreases in motor activity. In Experiment 1, sleep-increases were accompanied by increased SWA (i.e., delta wave activity) of the EEG. In this experiment, all artifact-free EEG segments were included in the SWA (FFT) analysis, including segments of NREMS, REMS and wakefulness. During NREMS, slow (delta) waves dominate the EEG; during REMS and wakefulness, slow waves are uncommon. Elevated SWA after CCK injections in Experiment 1 is simply the reflection of the increased amounts of NREMS; conclusions about the quality/intensity of NREMS cannot be drawn from the data. In our subsequent experiments, we restricted slow-wave analysis to the NREMS segments of the EEG. In this case, changes in SWA reflect qualitative changes in NREMS, mainly sleep intensity. We did not find any significant effect of CCK on SWA during NREMS suggesting that the intensity of NREMS is not affected by CCK.

The sleep-promoting effects of CCK in rats were confirmed by independent laboratories after our initial publication (de Saint Hilaire-Kafi et al., 1989; Posadas-Andrews et al., 1989). We also described the somnogenic actions of CCK in rabbits (Experiment 2) and mice (Szentirmai et al., 2007b) thereby demonstrating that its sleep-promoting effects are not species specific. In all three species, the lowest somnogenic ip dose was 10 μg/kg. Although we did not observe any appreciable effects on duration of rapid-eye-movement sleep (REMS) in rats, rabbits or mice (Szentirmai et al., 2007b), there are reports that in parachlorophenylalanine-induced insomniac cats CCK restores REMS (Prospero-Garcia et al., 1987), and in normal rats CCK increases REMS frequency (DeMesquita and Haney, 1986), and decreases REMS latency (Mansbach and Lorenz, 1983).

Effects of CCK on brain temperature

We previously found that systemic injections of CCK elicit dose-dependent hypothermia in rats (Kapás et al., 1987; Kapás et al., 1989). These results were confirmed by the present experiments and subsequently replicated by independent laboratories (South, 1992; Szelényi et al., 1994; Rezayat et al., 1999). We extended these finding by showing that ip injection of CCK also produces dose-dependent hypothermic responses in rabbits (Experiment 2). In rats, the dose-response relationships for the somnogenic, hypothermic and food intake-suppressing

effects of CCK were similar. The effects of ip CCK on thermoregulation and sleep were also in the same dose range in rabbits. Our data indicate that the hypothermic response to CCK is mediated by the CCK1 receptor subtype. First, CCK2 receptor-selective CCK analogues, CCK-4 and CCK-8-NS, did not have hypothermic activities in our present and previous (Kapás et al., 1987) experiments in rats. Consistent with this observation, sc injection of the same analogues did not affect body temperature in mice (Rezayat et al., 1999). Second, the hypothermic effects of CCK were completely abolished by pretreatment with L-364,718, a selective CCK1 receptor antagonist. Similarly, Szelényi and coworkers reported that the hypothermic effects of sc injected CCK were attenuated by a CCK1, but not a CCK2, receptor antagonist in rats (Szelényi et al., 1994).

While there is a broad consensus about the hypothermic effects of systemically injected CCK, there are conflicting data about the central effects of CCK on thermoregulation. Initially, it was found that icv (Morley et al., 1981; Katsuura et al., 1981) or intra-preoptic (Liu and Lin, 1985) injection of CCK reduces body temperature in rats. Subsequently, these effects were not confirmed, rather, a hyperthermic response was reported in the same species (Shido et al., 1989; Szelényi et al., 1994; Székely et al., 1994; Ghosh et al., 1997; Ghosh et al., 1998; Sugimoto et al., 1999). In rats, hyperthermia is elicited in the 0.02-10 μg/rat dose range. In our experiment, we did not find hyperthermic response to CCK in rabbits in the dose range of 0.05-2 μg/animal, rather, a modest, but statistically significant drop in brain temperature was evident. This may reflect true species-specific difference in the central effects of CCK or it may be due to differences in experimental conditions. In rabbits, we measured brain temperature in freely moving animals, while colonic temperature was recorded in restrained rats (Shido et al., 1989; Szelényi et al., 1994; Székely et al., 1994) or CCK was given in the form of chronic icv infusion with telemetric recording of the abdominal temperature (Szelényi et al., 2004). The hyperthermic response to centrally administered CCK is attenuated by a CCK2 receptor antagonist (Szelényi et al., 1994) suggesting the involvement of brain CCK2 receptors in CCK-induced fever. It is unlikely that the activation of peripheral CCK2 receptors also leads to hyperthermic responses since systemic injections of CCK2 receptor-selective analogues (CCK-4 and CCK-8-NS) did not cause fever in our previous (Kapás et al., 1987) or present (Experiment 3) studies in rats or in mice (Rezayat et al., 1999).

The mechanism of CCK-induced sleep

The two main questions regarding the mechanism of CCK-induced sleep are related to the involvement of CCK1 vs. CCK2 receptor subtypes and the anatomical location of the target. Our results with CCK2 agonists and CCK1 receptor antagonist indicate that CCK2 receptor activation is not sufficient but CCK1 receptor activation is necessary for the somnogenic effects of CCK.

CCK-8-SE binds to both CCK receptor subtypes with equal affinity. CCK2 receptors have similar high affinity for both sulfated CCK and nonsulfated analogues such as CCK-8-NS and CCK-4 (Wank, 1998). The affinities of CCK-8-NS and CCK-4 to CCK1 receptors are about 500-1,000 fold less than that of sulfated CCK octapeptide (Wank, 1998). If the somnogenic effects of CCK are due to the activation of CCK2 receptors then it is expected that equimolar amounts of sulfated CCK octapeptide, CCK-8-NS and CCK-4 would lead to similar sleep responses. This was not the case. The lowest effective somnogenic dose of systemically injected CCK-8-SE is 8.7 nmol/kg (10 μg/kg) in rats. In Experiment 3, the amount of NREMS did not increase in response to ip injection of 16.8-419.3 nmol/kg CCK-4 or 9.4-235.3 nmol/kg CCK-8-NS. These clearly show that the selective activation of CCK2 receptors is not sufficient to elicit somnogenic responses characteristic of CCK-8-SE. After the injection of 10 μg/kg (16.8 nmol/kg) CCK-4, NREMS decreased in h 3. The biological significance of such a delayed and slight effect is not clear, nevertheless, it is consistent with prior findings that BC-264, another CCK2 receptor agonist, slightly enhances wakefulness (de Saint Hilaire et al., 1991) and CCK-4 induces behavioral activation in open-field tests (Hsiao et al., 1984).

L-364,718 is a selective antagonist of the CCK1 receptor. It is void of CCK-like agonistic activities. CCK antagonism by L-364,718 lasts for at least 2-5 h (Lotti et al., 1987). In rats, systemic injection of 100 μg/kg L-364,718 prevents the effects of exogenous CCK on food intake (Hewson et al., 1988), locomotor activity (Soar et al., 1989) and gall bladder contraction (Chang and Lotti, 1986). We found that 100 μg/kg L-364,718 nearly completely while 500 μg/kg completely abolished the sleep-inducing effects of CCK. This indicates that the activation of CCK1 receptors is necessary for the manifestation of sleep-inducing effects of ip administered CCK. The CCK1 antagonist did not affect spontaneous sleep in normally fed animals when given at dark onset suggesting that tonic activation of CCK1 receptors by endogenous CCK plays minimal role in maintaining spontaneous sleep at the beginning of

the activity phase in rats. To make more definitive conclusions about the role of endogenous CCK in maintaining normal amounts of sleep in the dark and light periods, additional studies are needed by testing the effects of a wider dose range of both CCK1 and CCK2 receptor antagonists by various routes of administration at different times of the diurnal cycle. Regardless, we hypothesize that increased CCK secretion is likely a physiological signal for increased sleep under certain conditions (discussed below).

Our findings that CCK2 receptor activation is not sufficient but CCK1 receptor activation is necessary for CCK-induced sleep responses do not rule out the possibility that the activation of CCK2 receptors also contributes to the sleep effects. The co-activation or sequential activation of CCK1 and CCK2 receptors may be necessary for the manifestation of the somnogenic effects of CCK. There are known effects of CCK that require the activation of both receptor subtypes, e.g., suppression of acetylcholine release from cerebral cortex (Kimura et al., 1995) or the potentiation of the anticonvulsive actions of morphine (Legido et al., 1995).

The site of the somnogenic action of CCK.

The present experiments with L-364,718 do not address the question of the site of the somnogenic effects of CCK. CCK1 receptors are present both in the brain and in the periphery. L-364,718 crosses the BBB after systemic injection (Pullen and Hodgson, 1987) and binds to both central as well as peripheral CCK1 receptors. Systemically injected CCK does not cross the BBB (Passaro E Jr et al., 1982; Zhu et al., 1986) and likely acts on peripheral targets or brain structures that lack the BBB.

Regarding peripheral targets, we considered the possibility that the sleep effects of CCK are mediated through the release of another peripheral hormone stimulated by CCK. We considered insulin as a potential mediator of CCK's somnogenic action since CCK is a potent stimulator of insulin secretion (Unger et al., 1967; Szecowka et al., 1982) and the effects of insulin on sleep and feeding are similar to those of CCK. Exogenous administration of insulin stimulates NREMS (Sangiah et al., 1982; Danguir and Nicolaidis, 1984), suppresses feeding (Woods and Porte, Jr., 1983) while diabetic rats show diminished sleep (Danguir, 1984; Kapás et al., 1991) and increased feeding (Kumaresan and Turner, 1965; Booth, 1972). To test the role of pancreatic insulin in the sleep-promoting action of CCK, we studied the effects of CCK in streptozotocin-diabetic rats. Our findings of virtually undetectable plasma

insulin levels, elevated plasma glucose concentrations and increased feeding under baseline conditions confirmed the lack of pancreatic insulin in streptozotocin-treated animals. Confirming our prior results, spontaneous sleep in diabetic rats was unaltered during the dark phase (Kapás et al., 1991). The known sleep deficiency in diabetic animals (Danguir, 1984) is confined to the light period of the day (Kapás et al., 1991). In line with the known stimulatory effects of CCK on insulin secretion, ip injection of CCK caused increases in plasma insulin levels in control rats but not in diabetics. In spite of the lack of insulin response, diabetic rats mounted similar sleep responses to CCK injection as normal animals indicating that insulin is not involved in the sleep actions of CCK. As in normal rats, 10 μg/kg CCK suppressed 1-h food intake in diabetic animals. This confirms prior studies suggesting that CCK-induced satiety is independent of pancreatic insulin (Vanderweele, 1982).

The role of CCK in postprandial sleep

Increased feeding or postingestive satiety elicits postprandial sleep (Mansbach and Lorenz, 1983). Delivery of nutrients into the stomach or duodenum elicits EEG synchronization in rats (Bernstein, 1974) and cats (Fara et al., 1969). Increased eating induced by palatable, high-energy diet (Danguir, 1987; Hansen et al., 1998) or by the lesion of the VMH (Danguir and Nicolaidis, 1978) leads to increases of daily NREMS and REMS amounts. Genetically hyperphagic and obese Zucker rats have large amounts of NREMS (Danguir, 1989). There is a positive correlation between meal size and the length of the subsequent sleep period in rats (Danguir et al., 1979). Excess eating induced by prior food deprivation for 80 or 96 h results in sleep increases (Borbély, 1977; Danguir and Nicolaidis, 1979).

In Experiment 6, we induced excess eating by reintroducing food after 96 h of food deprivation. In control animals, refeeding elicited significant increases in NREMS and REMS, predominantly during the light phase of the refeeding days. Similarly, NREMS is elevated only during the light period in cafeteria diet-fed rats (Hansen et al., 1998). Eating and sleep cannot take place simultaneously, therefore an increase in feeding behavior may 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 food-induced increases in sleep pressure; as a result, sleep amounts did not change. In the following light period eating was below baseline which allowed the somnogenic effect of previously consumed food to be manifested. In the

dark of the second refeeding day, feeding behavior was less robust than the first night. This decline in feeding activity allowed for sleep increases in the dark, i.e., increases in REMS and a strong tendency toward increased NREMS. Also, since less food was consumed, sleep enhancement in the following light period were less pronounced, in fact, increases in NREMS were not statistically significant in the light phase of refeeding *day 2*.

SWA was reduced during the dark period of the second refeeding day in control rats. This is likely due to the fact that re-fed 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 be the consequence of this reduced pressure. Previously, we described similar decreases in SWA activity that accompanied feeding-induced sleep in the cafeteria diet model (Hansen et al., 1998).

The CCK1 receptor antagonist, L-364,718, was administered at light onset on both refeeding days, i.e., the first injection was done 12 h after reintroducing the food. We did not inject the antagonist immediately after the end of the starvation period for two reasons. 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 (Reidelberger and O'Rourke, 1989); postponing treatments allowed the animals to eat according to their natural needs during the first dark period after starvation. L-364,718 completely abolished the NREMS increases during the light periods both on refeeding *day 1* and 2. This, together with the known increase of plasma CCK in response to feeding and our observation that L-364,718 abolishes exogenous CCK-induced sleep, strongly indicate a role of endogenously produced CCK in feeding-induced sleep responses.

<u>A model of feeding and metabolism and sleep - perspectives</u>

The present work represents a segment of an ongoing broad project to test our model on the integration of metabolism and sleep (Fig. 15). We hypothesize that in addition to the well-established wake-dependent homeostatic and SCN-driven circadian factors, metabolic signals also play a fundamental role in determining sleep-wake activity. We posit that CCK is such a metabolic signal. These signals may modulate the activity of arousal mechanisms or may modulate circadian influences by acting through the food-entrainable oscillator, an endogenous clock independent of SCN (Antle and Silver, 2009). Some signals trigger acute changes in sleep in response to short-term negative energy balance such as during starvation

(ghrelin) or positive energy balance such as postprandial states (CCK, gastric leptin). Different signaling mechanisms set sleep amounts in response to long-term changes in adiposity (adipocyte-secreted leptin and TNF) or food availability (FEO). We hypothesize that both short- and long-term signals converge on a common integrative center in the hypothalamus. A ghrelin-NPY-orexin circuit is thought to be a key component of this integrative center (Szentirmai et al., 2007a; Szentirmai et al., 2009; Szentirmai et al., 2010).

Circulating CCK may modulate the activity of this hypothalamic circuit by acting through a peripheral or central target or both. In Experiment 5, we ruled out pancreatic insulin as a possible peripheral mediator of CCK-induced sleep. Another potential peripheral target for CCK to induce sleep is the vagus nerve. Peripheral sensory nerve endings in the vagus abundantly express CCK1 and CCK2 receptors (Dufresne et al., 2006). Numerous effects of systemic CCK on brain functions are, indeed, mediated by vagal afferents. Surgical vagotomy, or selective chemical destruction of vagal afferents by capsaicin prevents the effects of CCK on food intake (Smith et al., 1981), memory (Flood et al., 1987), exploratory behavior (Crawley et al., 1981a), and oxytocin secretion (Verbalis et al., 1986). Further, sensory inputs from vagal afferents modulate sleep, the activation of vagus - NTS complex results in generation of NREMS (Puizillout and Foutz, 1977). In our prior studies, however, surgical vagotomy did not prevent the somnogenic effects of ip injected CCK (Kapás, 1997). This finding makes it unlikely that peripheral vagal CCK receptors are the target for the somnogenic action of CCK although the role of vagus in CCK-induced sleep cannot be ruled it out completely for two main reasons. One, the fact CCK is capable of inducing sleep in the absence of functional vagus does not necessarily mean that intact vagus does not contribute to the effects of CCK in normal animals. Two, nodose cells express CCK1 receptors and, in general, respond to CCK (Widdop et al., 1993). They also survive subdiaphragmal vagotomy (Lieberman, 1971), although with a markedly reduced CCK1 receptor expression (Broberger et al., 2001). Surviving nodose cells are not sufficient to mediate food intake-suppressing effects of CCK as those effects are completely prevented by subdiaphragmatic vagotomy; nevertheless, it cannot be precluded that CCK acts on surviving cell populations to elicit sleep. While its role in signaling CCK-induced sleep is questionable, the vagus is likely to play a role in signaling increased adiposity-induced sleep. We have shown that cafeteria-diet induced adiposity is accompanied by increased sleep, a response that is prevented in vagotomized animals (Hansen et al., 1998). TNF is a major adipokine, most circulating TNF is of adipose origin in normal healthy rats (Kershaw and Flier, 2004). TNF also has potent

somnogenic actions (Kapás et al., 1992; Kapás and Krueger, 1992) that are abolished in vagotomized rats (Kubota et al., 2001).

The findings that lateral ventricular injection of CCK does not induce sleep in rabbits (Experiment 2) or rats (Gilligan et al., 1998) seemingly contradict a central target for CCK. The possibility, however, remains that there is a central target site which can be reached by circulating CCK easily but less accessible for CCK injected into the lateral ventricle. When administered into the lateral ventricle, CCK can potentially reach all neurons in the brain without encountering barriers such as the BBB. This, however, does not imply that CCK indeed reaches all neurons in a physiologically meaningful concentration since, clearly, there is a gradient for injected molecules in the cerebrospinal fluid from the site of the injection to distant neuron populations such as in the brain stem. Those brain stem sites that are supplied by fenestrated capillaries can be reached by circulating CCK unabatedly.

We hypothesize that circulating CCK acts on CCK receptors within NTS to elicit sleep. NTS is the primary projection target for vagal visceral afferents arising from the GI tract thus a major relay sites for vagus-mediated CCK effects. We posit that circulating CCK reaches the NTS directly and may act on the NTS directly by bypassing peripheral vagal sensory nerves. CCK1 receptors are abundantly expressed in the NTS, mainly restricted to its medial subnucleus (Hill et al., 1987; Corp et al., 1993; Qian et al., 1997). The medial subnucleus is also rich in highly fenestrated capillaries similar to those in the area postrema (Gross et al., 1990). The lack of BBB makes neurons and CCK receptors in the NTS accessible to circulating CCK and other large blood-borne molecules. CCK by acting on NTS CCK1 receptors has known physiological actions such as eliciting satiety (Blevins et al., 2000) and modulating glutamate release from vagus afferents (Appleyard et al., 2005). The NTS may serve as a key interface between metabolic signals, both blood-born and vagus mediated, and sleep regulatory centers in the brain. NTS has extensive projections to the PBN (Loewy and Burton, 1978; Saper and Loewy, 1980). PBN itself is implicated in sleep regulation, most of its neurons show sleep-dependent activity pattern (Saito et al., 1977; Gilbert and Lydic, 1994). From the PBN, dense projections arise to the VMH, DMH, posterior LH and preoptic hypothalamus (Bester et al., 1997), areas thought to play key role in the regulation of sleep and wakefulness.

In summary, we have shown that systemic injection of CCK elicits dose-dependent somnogenic and hypothermic responses in rats and rabbits. The sleep effects are

accompanied by suppressed feeding and motor activity. Selective activation of CCK2 receptors is not sufficient to elicit the responses while the activation of CCK1 receptors is required suggesting CCK1 receptors as a primary target. Pancreatic insulin does not play a role in CCK-induced sleep and thermoregulatory responses. Eating-induced sleep is prevented by CCK1 receptor antagonist treatment indicating a role for CCK in the postprandial modulation of vigilance. Present results are consistent with the hypothesis that CCK is a component of a complex signaling mechanism which modulates sleep-wake activity according to the metabolic status of the body.

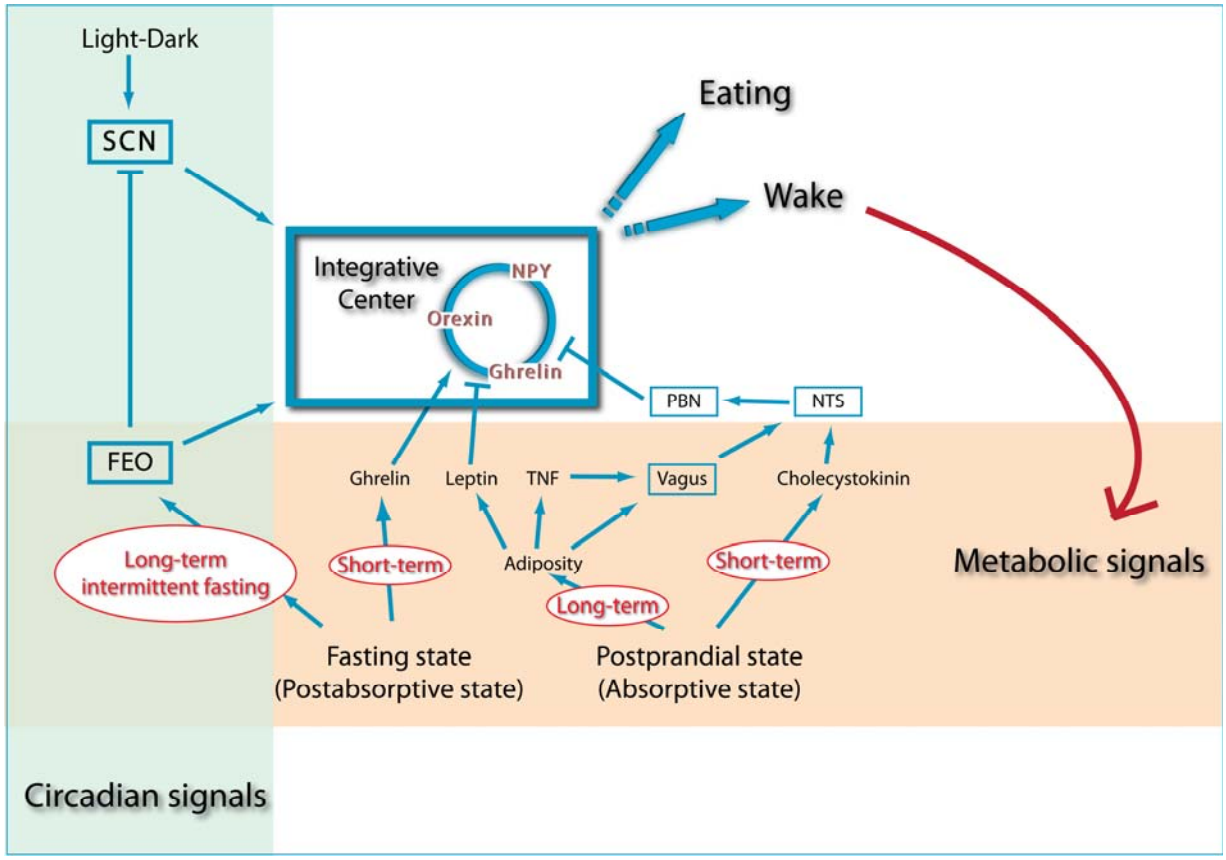

Fig. 15. Integration of metabolic and circadian signals in the regulation of sleep, wakefulness and feeding.

Acknowledgements

I thank the Chairs of the Department of Physiology in Szeged for their unwavering support over the years. Professor Ferenc Obál recruited me to work in the Department as a student and offered my first post-doctoral position. Professor György Benedek gave me the opportunity to work in his laboratory during my student years; he has been encouraging my scientific endeavors and sharing his knowledge and wisdom generously ever since. Professor Gábor Jancsó gave invaluable support by retaining me in the PhD program, without his help this thesis could not have been completed.

Much of the present work was aided by my mentors, Drs. Ferenc Obál, Jr. and James M. Krueger. Their contribution to shaping my understanding of science reaches far beyond this thesis. Their role in my life as a mentors and friends can never be overstated.

I would like to acknowledge my coauthors' contribution to the studies. It would not have been possible to carry out the studies without their generous help. I thank Drs. Péter Alföldi, Hee-Yoon Chang, Ibolya Farkas, Lars Johannsen, Mark R. Opp, Linda C. Payne, Botond Penke, György Rubicsek, Gyula Sáry and Alexei Shemyakin.

I thank the skilled technical assistance by Klára Ormos, Ilona Ponicsán, László Kiss, Sandy Johnson, Donna Maxwell and Gail Richmond. Special thanks to Péter Liszli for his help with computerized data collection in Szeged and Dr. Zoltán Lelkes for his continuing help and advice in the last thirty years.

Extended thanks to Dr. Éva Szentirmai for the inspiring discussions that helped shape the interpretation of past and the conception of present and future studies. She gave invaluable feedback on early versions of this thesis.

I am indebted to my parents for their selfless support, understanding and endless patience.

This work was supported by grants from the Hungarian Academy of Sciences, Hungarian Ministry of Health, National Institutes of Health (USA), Office of Naval Research (USA), US Army and Medical Research and Development Command and Fordham University, New York.

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Attachment 1.

Brain Research, 438 (1988) 155–164
Elsevier

BRE 13183

Effects of nocturnal intraperitoneal administration of cholecystokinin in rats: simultaneous increase in sleep, increase in EEG slow-wave activity, reduction of motor activity, suppression of eating, and decrease in brain temperature

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(Accepted 23 June 1987)

Key words: Cholecystokinin; Sleep; Brain temperature; Feeding

Rats received an i.p. injection of cholecystokinin-octapeptide sulfate ester (CCK; 4, 10 or 50 μg/kg) or physiological saline at dark onset, and the 24-h sleep–wake cycle (12-h-dark and 12-h-light phases), spontaneous motor activity and brain temperature (T_{br}) were recorded. EEG activity was studied through spectral analysis for 2.5 h, and food intake was measured at the end of postinjection hour 1. In response to CCK, non-REM sleep increased at the expense of wakefulness, and the sleep-promoting effect was substantiated by an increase in EEG slow-wave activity. Motor activity, T_{br} and food intake decreased. The effects vanished in postinjection hour 2; the diurnal rhythms were not modified. The changes varied as a function of the dose: the effects were significant following 10 μg/kg, and even higher in response to 50 μg/kg CCK. The results indicate that i.p. CCK definitely promotes non-REM sleep. This effect may belong to the behavioral sequence elicited by the peptide, which is often attributed to satiety. As evidenced by the reduction of T_{br}, CCK also exerts strong autonomic actions, which might interfere with the behavioral responses.

INTRODUCTION

Besides the well-known effects of cholecystokinin (cholecystokinin-octapeptide sulfate ester, CCK) as a gut hormone, exogenously administered CCK elicits a great number of behavioral responses. The reduction of food intake[17] is possibly the best documented effect; it is generally attributed to satiety induced by the peptide[38]. It is assumed that endogenous CCK released after the ingestion of food promotes satiety too, and thereby contributes to the cessation of feeding (see refs. 3 and 24 for reviews). Satiety is therefore regarded as a physiological action of CCK, and the peptide has been implicated in the regulation of food intake. Although this concept has been supported by several lines of evidence, it should be noted that the role of endogenous CCK awaits further experimental support; and some findings have suggested explanations different from satiety for the mechanism of inhibition of eating after CCK (see ref. 3 for a review).

CCK administration to fasted rats is followed by a sequence of behavioral events characteristic of rats after eating[2]. This 'syndrome of satiety' terminates with resting. Since the pharmacological reduction of motor activity does not necessarily lead to sleep, resting elicited by CCK might represent behavioral sedation without sleep[8]. Short episodes of sleep can normally often be observed after eating periods in rats. Supposing that postprandial sleep is a component of the behavioral manifestation of satiety, the resting observed after the injection of CCK may correspond

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to sleep. The few attempts to clarify the effects of CCK on sleep in rats produced controversial findings. While i.c.v. injection of CCK did not promote sleep in two previous experiments[13,35] no increases in sleep (but a shortened sleep latency)[36] and an increased number of rats displaying sleep[23] have been reported following systemic administration of the peptide.

In the present experiments, the effects of intraperitoneal (i.p.) injection of 3 doses of CCK (4, 10 or 50 μg/kg) were studied on sleep, EEG slow-wave activity, spontaneous motor activity, brain temperature, and food intake in rats. An experimental schedule was used that has also been applied to test presumed sleep peptides[31,32]. This included efforts to habituate the animals to the experimental conditions and handling, and timing of the i.p. injection of CCK to the beginning of the dark cycle, the physiologically active cycle for the rat. Each rat was tested both with i.p. saline and with one of the CCK doses; the effects were evaluated with respect to the baseline records obtained after saline. Sleep–wake activity was recorded for 24 h (12-h-dark and subsequent 12-h-light cycles) following the injection of saline or CCK. EEG slow-wave activity has been proposed as a useful marker for the activity of sleep mechanisms[6,7]. The EEG was therefore also studied by means of spectral analysis for 2.5 h after the injections. A great number of experiments demonstrated that CCK reduced exploration and elicited long pauses of behavioral inactivity in rats exposed to a new environment (see ref. 8 for a review). In order to study the effects of CCK on the increased nocturnal motor activity, spontaneous motor activity was recorded throughout the 12-h-dark and 12-h-light cycle. CCK has been shown to reduce body temperature[18,19,26,43]. Nothing is known, however, about the relation of this effect to the behavioral actions of the peptide. Brain temperature (T_{br}) was therefore continuously recorded, together with the sleep–wake activity and motor activity. Previous experiments had revealed that the changes in T_{br} measured over the parietal cortex were a reliable indicator of the changes in body temperature recorded by means of an i.p. transducer[30]. Finally, the effects of the 3 doses of CCK used in these experiments were also studied in the classical test by measuring the food intake in the first hour of the dark period.

MATERIALS AND METHODS

Male CFY rats were used. The weights of the animals were between 300 and 350 g at the time of the CCK injections.

Under pentobarbital anesthesia (50 mg/kg), golden jewelery screws were implanted over the frontal and parietal cortices and the cerebellum for EEG recording. Diodes cemented over the parietal cortex served for T_{br} measurements as reported earlier[30].

The animals were adapted to the experimental conditions for 7–10 days after the surgery. They lived connected to the flexible recording cable in individual Plexiglass cages in the sound-attenuated recording chamber. The ambient temperature was regulated at 21 °C, and low-level continuous noise was provided by means of loudspeakers. The animals were raised on a light–dark cycle of 12 h each, with the light on from 08.30 to 20.30 h. The same light–dark cycle was maintained in the experimental chambers. Food was available only for the 12-h-dark phases.

CCK was synthetized by one of us (B.P.) as described earlier[33]. The peptide was dissolved in physiological saline and injected i.p. in a volume of 2.0 ml/kg. The effects of the 3 doses (4, 10 or 50 μg/kg) were tested on 3 different groups of animals, each including 12 rats.

The rats received i.p. saline injection for 5 days before the recording. Both during the habituation period and in the actual experiments, the injections were timed to 5–10 min before dark onset. Half of the animals in each group were injected with saline on day 6, and the sleep–wake activity, motor activity and T_{br} were recorded for 24 h. The next evening CCK was administered, and the recording was continued for another 24 h. The sequence of the injection of saline and CCK was reversed for the other animals in the groups, i.e., the CCK day preceded the baseline day. The records obtained following the injection of saline were regarded as baseline.

Through the EEG and motor activity (potentials generated in electromagnetic transducers activated by the movements of the recording cable), an automatic analyzer, using a modified program developed by Neuhaus and Brobély[27], scored the arousal states as wakefulness (W: relatively low EEG amplitudes, high level of θ- (6–10 Hz) activity, motor activity), non-REM sleep (NREMS: high EEG amplitudes,

low level of θ-activity, lack of motor activity), or REM sleep (REMS: relatively low EEG amplitudes, θ-activity exceeding that in W, lack of motor activity or occasional twitches) for each 10-s period. Motor activity counts were integrated for 10-s intervals (sampling rate: 100 Hz). This method of recording motor activity is as sensitive as the traditional methods in evaluating drug effects on gross motor activity in rats[41]. A value of T_{br} was also taken for each 10-s interval. The scores of the sleep–wake states, and the values of the integrated motor activity and T_{br} were calculated for consecutive 1-h and 3-h periods. All the baseline records and the records obtained after CCK were pooled for each group. The differences in the motor activity counts, in T_{br}, and in the percentages of the vigilance states were calculated between the CCK day and the baseline day. Analysis of variance followed by the paired t-test (two-sided) was used for statistical evaluation of the results.

For spectral analysis, the EEG signals (filtering: below 0.53 Hz and above 30 Hz at 6 dB/octave) in the first 2.5 h following the administration of saline or CCK were subjected to analog–digital conversion (sampling rate: 100 Hz), and fed into a computer to be processed through fast Fourier transformation. Power density values (μV^2/0.4 Hz) were computed for 2.5-s periods. The power density spectra were then averaged for 1-min periods, and the values were integrated for 2-Hz frequency ranges. Since power densities below 6 Hz have been reported to characterize slow-wave activity in the rat[7], the effects of CCK were studied on the power densities in the frequency range from 0.4 to 6.0 Hz (0.4–2 Hz, 2.4–4.0 Hz, and 4.4–6.0 Hz). The mean power densities were calculated for the consecutive 30-min periods. The effects of CCK were evaluated as the differences between the power densities on the CCK day and on the baseline day, expressed as percentages of the baseline power densities.

The changes in food intake in response to CCK were studied in a separate experiment. The three groups of animals, each including 8 rats, were habituated to the same experimental conditions as in the sleep studies. The rats received CCK on one evening, and saline on the other evening; the sequence of the injections was reversed for half of each of the groups. Food was available only for the dark periods. Rat food pellets (LATI, Hungary) were weighed and placed on the floor of the cage. After 1 h spillage was recovered and the food was reweighed.

RESULTS

Food intake

I.p. injection of CCK inhibited eating. The amount of food consumed in the first hour of the dark cycle was a function of the dose of CCK (analysis of variance: $F_{2,21} = 4.03$, $P < 0.05$). With respect to the food consumed following the administration of saline, the reduction in food intake was 27% after 4 μg/kg, 45% following 10 μg/kg, and 63% in response to 50 μg/kg CCK. The effects of 10 and of 50 μg/kg CCK were significant (Fig. 1).

Brain temperature

The course of T_{br} after the administration of saline showed the diurnal variations characteristic of rats, with high values at night and low values during the day. Injection of CCK resulted in a decrease of T_{br}. The effect was a function of the dose (analysis of variance for the first 3 h of the dark cycle: the time effect: $F_{2,99} = 5.649$, $P < 0.05$; dose effect: $F_{2,99} = 5.483$, $P < 0.05$; interaction: $F_{4,99} = 2.173$). The mean T_{br} calculated for 1-h periods did not differ from the baseline values after 4 μg/kg, while T_{br} was significantly reduced for 1 and 2 h after 10 and 50 μg/kg CCK, respectively (Table I, Fig. 2). Significant changes in T_{br} were not observed for the remainder of the dark cycle and in the light cycle. It might be worth noting, however, that T_{br} tended to be slightly lower than the

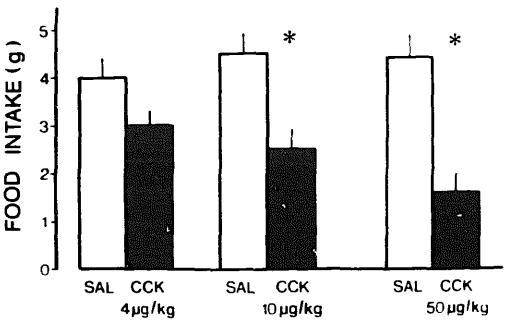


Fig. 1. Effects of i.p. injection of physiological saline (SAL) and CCK (4, 10 or 50 μg/kg) on food intake ($n = 8$ for each group) in postinjection hour 1, i.e., in the first hour of the dark period. Asterisks indicate significant differences between the food intakes measured after saline and CCK (paired t-test, two-sided, $P < 0.05$).

TABLE I

Percentages of the vigilance states (W, NREMS, REMS), and the brain temperature and motor activity in the first hour of the dark period

The values were computed in the first hour following the i.p. injection of physiological saline or CCK (4, 10 or 50 μg/kg). $n = 12$ for each group. Asterisks denote significant differences between the values (mean ± S.E.M.) obtained after the injection of saline and CCK (paired t-test, two-sided).

Group	*CCK 4 μg/kg*		*CCK 10 μg/kg*		*CCK 50 μg/kg*	
	Saline	*CCK*	*Saline*	*CCK*	*Saline*	*CCK*
T_{br} (°C)	37.7 ± 0.1	37.4 ± 0.1	37.5 ± 0.1	36.9 ± 0.2*	37.1 ± 0.1*	36.2 ± 0.1*
Motor activity (counts)	1480 ± 173	1413 ± 170	1654 ± 211	1094 ± 151*	1490 ± 256	396 ± 202*
W (%)	87.2 ± 3.2	84.9 ± 4.3	90.7 ± 2.8	76.3 ± 5.5*	86.6 ± 3.1	58.8 ± 4.7*
NREMS (%)	10.7 ± 2.7	13.1 ± 3.7	8.2 ± 2.6	20.9 ± 5.0*	12.0 ± 2.8	36.8 ± 2.8*
REMS (%)	2.1 ± 0.7	2.0 ± 0.9	1.1 ± 0.4	2.8 ± 0.8	1.4 ± 0.5	4.4 ± 1.4

*$P < 0.05$.

control values throughout the night following each dose of CCK.

Motor activity

As expected for the rat, a nocturnal animal, spontaneous motor activity was much higher during the dark cycle than during the light cycle on the day of control recording. Administration of CCK affected spontaneous motor activity in a dose-dependent manner (analysis of variance of the differences with respect to the baseline day for the first 3 h of the dark phase: time effect $F_{2,99} = 4.720$, $P < 0.05$; dose effect: $F_{2,99} = 3.984$, $P < 0.05$; and interaction: $F_{4,99} = 2.301$). While the mean motor activity level did not change significantly after 4 μg/kg, 10 and 50 μg/kg CCK caused increasing suppression in the first postinjection hour (Table I, Fig. 2). The baseline motor activity was recovered in postinjection hour 2, and from this time on, the motor activity level followed the normal diurnal course.

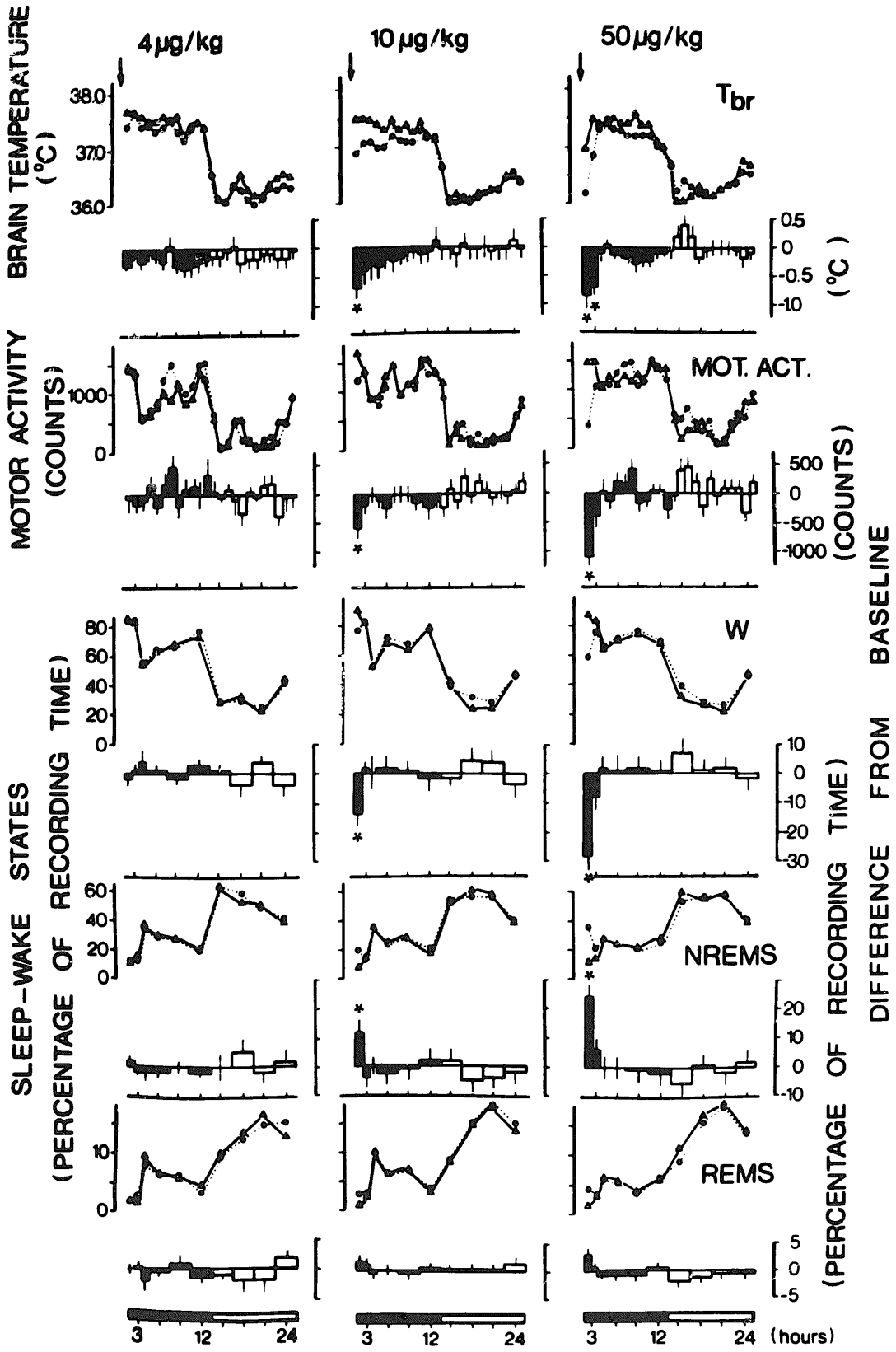
Sleep–wake activity

The diurnal variations in sleep–wake activity were characterized by a high percentage of wake periods and little sleep at night, and less W and more NREMS and REMS during the day. Depending on the dose, injection of CCK at dark onset promoted NREMS at the expense of W (analysis of variance for the first 3 h of the dark period for NREMS: time effect: $F_{2,99} = 6.413$, $P < 0.05$; dose effect: $F_{2,99} = 3.547$, $P < 0.05$; interaction: $F_{4,99} = 1.276$; and for W: time effect: $F_{2,99} = 6.261$, $P < 0.05$; dose effect: $F_{2,99} = 3.184$, $P < 0.05$; interaction: $F_{4,99} = 0.994$). The low dose of CCK failed to affect the sleep–wake activity, while 10 and 50 μg/kg CCK produced an increasing suppression of W and a comparable increase in NREMS in postinjection hour 1 (Table I, Fig. 2). Thereafter, the percentages of the vigilance states returned to the baseline levels and did not reveal significant changes for the remainder of the dark cycle and for the subsequent light cycle.

The percentage of REMS was not significantly affected by the administration of CCK (analysis of variance for the first 3 h: time effect: $F_{2,99} = 2.448$; dose effect: $F_{2,99} = 0.700$; interaction: $F_{4,9} = 0.381$), though a tendency to an increased REMS could be noted particularly following the 50 μg/kg dose (Table I, Fig. 2).

→

Fig. 2. Effects of i.p. injection of 4 μg/kg (left), 10 μg/kg (middle) and 50 μg/kg (right) CCK on brain temperature (T_{br}), motor activity, and sleep–wake activity. For each group $n = 12$. The curves show the diurnal rhythms in terms of mean values calculated for consecutive 1-h periods after the injection of physiological saline (triangles, continuous lines) or CCK (dots, dotted lines). For the vigilance states, 1-h values are presented for the first 3 h, and the means for 3-h periods are shown for the remainder of the 24-h records. Saline or CCK was administered at dark onset (arrows). Histograms show the mean differences (± S.E.M.) between the values after saline and after CCK (1-h periods for T_{br}, for motor activity, and for the first 3 h for the vigilance states; and 3-h periods for the remainder of the 24-h records for the vigilance states). Black columns: dark period; open columns: light period. Asterisks indicate significant differences between the values after saline and those after CCK (paired t-test, two-sided, $P < 0.05$).


4µg/kg
10µg/kg
50µg/kg
BRAIN TEMPERATURE (°C)
38.0
37.0
36.0
Tbr
0.5
0
-0.5
-1.0
(°C)
MOTOR ACTIVITY (COUNTS)
1000
0
MOT. ACT.
500
0
-500
-1000
(COUNTS)
SLEEP-WAKE STATES (PERCENTAGE OF RECORDING TIME)
80
60
40
20
0
W
10
0
-10
-20
-30
60
40
20
0
NREMS
20
10
0
-10
10
0
REMS
5
0
-5
DIFFERENCE FROM BASELINE (PERCENTAGE OF RECORDING TIME)
3
12
24
(hours)

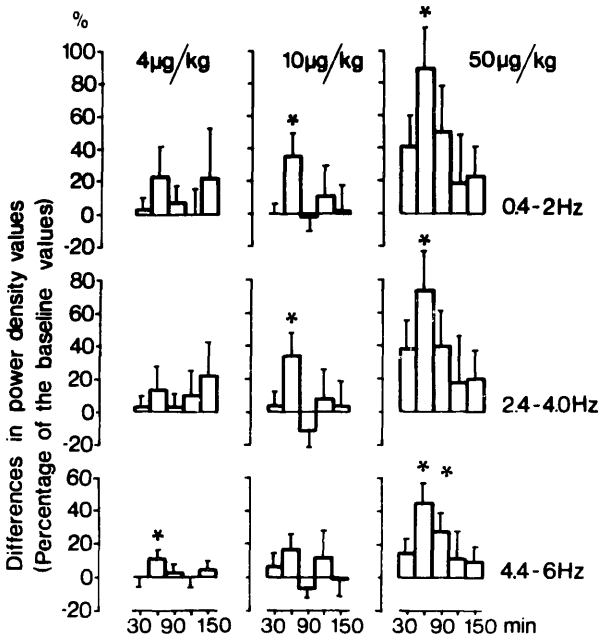


Fig. 3. Changes in EEG slow-wave activity as characterized by the differences in the power density values calculated from the EEG after the injection of saline and CCK (4 μg/kg: left; 10 μg/kg: middle; 50 μg/kg: right; $n = 12$ for each dose). Columns show the mean differences (± S.E.M.) in the power density values calculated for consecutive 30-min periods for 150 min after the injections. Saline or CCK was injected at zero time, at dark onset. Each row of columns refers to the canges in power density in the frequency range indicated on the right. Asterisks indicate significant changes elicited by CCK with respect to the baseline values obtained after the injection of physiological saline (paired t-test, two-sided, $P < 0.05$).

EEG slow wave activity

Spectral analysis of the EEG indicat d an increase in EEG slow-wave activity in response to CCK injection. The effect was a function of the dose (analysis of variance for the 0.4 to 2-Hz range: time effect: $F_{2,165} = 1.981$; dose effect: $F_{2,165} = 5.387$, $P < 0.05$; interaction: $F_{8,165} = 0.528$; for the 2.4 to 4-Hz range: time effect: $F_{4,165} = 1.685$; dose effect: $F_{4,165} = 5.154$, $P < 0.05$; interaction: $F_{8,165} = 0.698$; for the 4.4 to 6-Hz range: time effect: $F_{4,165} = 2.142$; dose effect: $F_{4,165} = 5.889$, $P < 0.05$; interaction: $F_{8,165} = 0.798$). The increase in slow wave activity was significant 30–60 min after the injection, and it was particularly pronounced following the administration of 50 μg/kg CCK (Fig. 3).

DISCUSSION

In agreement with previous reports, i.p. injection of CCK decreased food intake in rats. CCK also reduced the increased spontaneous motor activity characteristic of rats at night and promoted NREMS at the expense of W. Spectral analysis of the EEG substantiated the increase in NREMS by demonstrating an increase of slow-wave activity. Finally, CCK injection was followed by a decrease of T_{br}. All these effects were elicited at the beginning of the dark period, when both spontaneous activity and T_{br} show diurnal rises, the rats are awake for most of the time, and a large proportion of the daily food intake is consumed. As has been reported for other CCK actions (e.g. ref. 3), the effects declined rapidly, and vanished by postinjection hour 2. All functions were in the normal range during the remainder of the dark cycle. Sleep–wake activity, motor activity and T_{br} were not affected in the subsequent light cycle. Thus, the circadian rhythm of the various functions were not modified.

It seems that the dose–effect relationships were similar for all the behavioral and autonomic functions studied in the present experiments. Four μg/kg CCK was ineffective, though it cannot be excluded that analysis of time periods shorter than 1 h may have revealed some responses, as shown in previous studies on food intake[4]. Significant changes were obtained in all the recorded parameters after the administration of 10 μg/kg CCK, and the effects increased when 50 μg/kg CCK was injected. Although the reported threshold doses of CCK for reducing food intake are highly variable in rats, doses between 2 and 6 μg/kg have often been found to be effective when CCK was injected in the light phase (see ref. 3 for a review). The higher dose required in our experiments might be explained by the finding that CCK is less potent in inhibiting feeding when injected at night than during the day[20].

Models of sleep regulation predict a minimum activity for the endogenous sleep mechanisms and a high threshold for falling asleep at the beginning of the circadian active cycle[6]. Consequently, this period is not favorable for sleep. The reason why this period is nevertheless often chosen to test the effects of presumed sleep substances is that the interference between the effects of the exogenously applied substances and the activity of the endogenous sleep mechanisms can be avoided by timing of the injection of the substances to the diurnal active cycle. With re-

gard to the high capacity of CCK to overcome the physiological pressure for W and thereby to increase sleep at night, it is reasonable to suppose that the peptide might also be effective when injected during the light cycle. Mansbach and Lorenz[23], in fact, found an increased number of rats displaying sleep and an increased sleep duration in response to i.p. CCK in rats during the day. Rojas-Ramirez et al.[36], however, observed a reduction in sleep latency without any increases in sleep following i.p. CCK during the light cycle, though the baseline percentages of the vigilance states could easily allow increases in NREMS. The results were interpreted by suggesting that CCK induced an unusual state of behavioral sedation without concomitant EEG sleep[8].

The evident increase of sleep in our experiments suggests that other explanations might also be considered. Two points are worth mentioning. (1) Since the process of physiological sleep can easily be disturbed by any unusual stimulus, efforts were made to habituate the rats to the experimental conditions as far as possible both in the study by Mansbach and Lorenz[23] and in our own experiments. A habituation procedure was not mentioned in the report by Rojas-Ramirez et al.[36]. In non-habituated rats, drowsiness may decrease sleep latency, but an apparent increase of sleep may be absent because of frequent arousals. This explanation implies that CCK promotes physiological NREMS. In agreement with this suggestion, human subjects injected with CCK reported drowsiness, but irresistible sleepiness was not experienced[40]. (2) In the study by Mansbach and Lorenz[23], the rats received food first, and were injected with CCK after a period of eating. Food was also available in our experiments, and eating was not totally inhibited even after the largest dose of CCK. In contrast, Rojas-Ramirez et al.[36] used fasted rats without food. It cannot be excluded therefore, that the increase of sleep results from the combined effect of CCK and some other factor(s) closely related to food ingestion. An increased availability of nutrients in the blood together with insulin release, has also been shown to promote sleep[12], and may be an additional factor in the mechanism of postprandial sleep.

Various lines of evidence indicate that the suppression of eating and motor activity elicited by systemic administration of CCK are mediated through vagal afferents[9,11,22,25,39] though a direct effect of CCK on the area postrema, a region with a deficient blood–brain barrier, might also be involved[42]. Since i.c.v. administration of CCK failed to increase sleep[13,35] there is no reason to assume that the sleep effects of i.p. CCK injection were produced by a direct action of the peptide on the central nervous system, and possible peripheral sites of action should be considered. Introduction of fat into the duodenum induced sedation in the cat, and the effect could be mimicked by i.v. administration of CCK[15]. Electrical stimulation of the intestines has been shown to promote sleep, and the effect seemed to be mediated, at least in part, via vagal afferents[21]. Vagal afferents are acknowledged as an important input for sleep regulation (see ref. 34 for a review). The increase of sleep might therefore be mediated through the vagus nerve, like the other components of the behavioral sequence elicited by CCK. It has been suggested for both the reduction of exploratory activity and the suppression of food intake that the vagal information about the peripherally administered CCK is carried to the basal forebrain through the ascending projection of the solitary tract nuclei[10]. At the level of the basal forebrain, however, the mediation of the behavioral effects of CCK seems to be separated: the paraventricular and dorsomedial nuclei of the hypothalamus are involved in the inhibition of eating, while a different, albeit non-specified area may be responsible for the reduction of exploratory activity[8]. At least in the paraventricular nucleus, CCK appears to be the neurotransmitter delivering the signal for reducing food intake[24]. Another area in the basal forebrain, the olfactory tubercle, is known as one of the regions with the highest densities of CCK receptors[16]. Unit activity in the olfactory tubercle has been reported to be responsive to hypnogenic vagal stimulations[14]. Experiments with stimulations[5] and lesions[29] indicated that the olfactory tubercle might be involved in sleep regulation. It is possible, therefore, that the basal forebrain provides mechanisms for the mediation of all the behavioral events of the syndrome of satiety elicited by peripheral injection of CCK, including sleep. While feeding is inhibited in a few minutes following the administration of CCK, sleep-like resting behavior has been reported to terminate the behavioral sequence elicited by the peptide[2]. The changes in EEG slow-wave activity indicated that NREMS increased significantly only

30–60 min after CCK injection. It seems therefore that the effect of CCK on sleep has a much longer latency than on food intake which is in agreement with the findings reported by Rojaz-Ramirez et al.[36]. The possible temporal separation of these effects may support the notion that they are elicited by different central mechanisms.

While the sleep-promoting activity of CCK can be regarded as an integral part of the satiety syndrome attributed to the peptide, the mechanism of the decrease of T_{br} and the importance of this effect in the behavioral actions are not clear. Motor inactivity and sleep have been shown to reduce T_{br}; these changes, however, were much slighter than the relatively large fall found in response to CCK[30]. In fact, both intracerebral[19,26] and systemic[18,43] administrations of CCK have been reported to elicit hypothermia; the reduction of body temperature seemed to result from a decreased heat production[37] and an increased heat loss[18,37]. It has also been speculated that the hypothermic effect of CCK might be closely related to the mechanism of the suppression of food intake, either as a causal factor or as a component of the satiety syndrome[26,43]. These ideas, however, were not supported by the few experiments where the effects of systemic administration of CCK on body temperature were studied, because the threshold dose of CCK required for the thermoregulatory effects was several times higher than the dose of CCK effective on food intake[18,43]. The present results were therefore unexpected. The difference in the effects of CCK on T_{br} and rectal temperature may be due to differences in the recording techniques; either T_{br} is a more sensitive indicator of the effects of CCK than the rectal temperature, or the stress caused by the repeated insertion of the rectal probes interfered with the hypothermic action of low doses of CCK, and this could be avoided by means of the chronic recording technique.

CCK has been shown to elicit a reduction in body temperature by acting on the thermosensitive units in the preoptic region[37]. After systemic injection, however, CCK penetrates poorly through the blood–brain barrier, and a direct central action seems to be unlikely[44]. In fact, some differences in the hypothermia elicited by central and peripheral injection of CCK indicated that the structures involved were not the same[18]. Although heat-sensitive afferent fibers have been identified in the vagus nerve[1], there is no evidence suggesting that the thermosensitive vagal afferents are involved in the effects of CCK. Thus the mechanism of the decrease of body temperature in response to systemic administration of CCK remains to be clarified.

To summarize, in addition to reducing the high spontaneous motor activity at night and decreasing food intake, CCK promoted NREMS and increased EEG slow-wave activity. These effects may belong to the behavioral sequence of satiety, which has been proposed as the major behavioral action of the peptide. The effects of CCK on NREMS were similar to, or even more marked than those obtained in response to i.c.v. injection of presumed sleep peptides (δ-sleep-inducing peptide (DSIP)[31], DSIP analogs[28], vasoactive intestinal polypeptide[32], growth hormone-releasing factor[28]) in the same experimental schedule. Unlike these peptides, however, CCK also reduced T_{br}. The pronounced effect on T_{br} indicates that the autonomic actions of the peptide should be considered seriously in further studies on the behavioral responses to peripheral administration of CCK.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. A.A. Borbély for his continuous support during the experiments, including the provision of computer programs for the analysis of sleep–wake activity. The technical assistance by Mrs. K. Ormos, Mrs. I. Ponicsán, Mr. L. Kiss and Mr. P. Liszli is gratefully acknowledged. This work was supported by the Hungarian Academy of Sciences (OTKA 1104, OKKFT177/86) and the Ministry of Health (ETT 528).

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Attachment 2.

Physiology & Behavior, Vol. 50, pp. 1241–1244. © Pergamon Press plc, 1991. Printed in the U.S.A. 0031-9384/91 $3.00 + .00

Intraperitoneal Injection of Cholecystokinin Elicits Sleep in Rabbits

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Received 25 March 1991

KAPÁS, L., F. OBÁL, JR., M. R. OPP, L. JOHANNSEN AND J. M. KRUEGER. *Intraperitoneal injection of cholecystokinin elicits sleep in rabbits.* PHYSIOL BEHAV **50**(6) 1241–1244, 1991.—Cholecystokinin (CCK) reduces food intake and promotes non-rapid-eye-movement sleep (NREMS) in rats. The purpose of present experiments was to determine if CCK is somnogenic in rabbits; another species in which CCK suppresses feeding. White New Zealand rabbits were treated intracerebroventricularly (ICV; 0.05, 0.5 and 2 μg) or intraperitoneally (IP; 2.5, 10 and 40 μg/kg) with CCK or saline, and sleep-wake activity and brain temperature (T_{br}) were recorded for 6 h. Injections of 10 and 40 μg/kg CCK IP elicited a decrease in wakefulness and an increase in NREMS during the first hour postinjection. The hypnogenic effects were accompanied by a decrease in T_{br}. After the IP injection of a lower dose (2.5 μg/kg) a slight, nonsignificant increase in NREMS during the first hour postinjection was followed by a decrease in NREMS. ICV injections of CCK had relatively small inhibitory effects on sleep. We conclude that circulating, hormone CCK might be a hypnogenic signal with a peripheral site of action.

Sleep Cholecystokinin Rabbit Brain temperature

CHOLECYSTOKININ (CCK) is a gastrointestinal hormone/neuropeptide which has several autonomic and behavioral effects including suppression of food intake (10,11) and elicitation of the behavioral sequence of satiety (1). It is hypothesized that CCK contributes to the short-term regulation of feeding and it is often regarded as a satiety hormone [reviewed in (2)]. Another facet of the behavioral effects of CCK is its effects on sleep-wake activity. After systemic injection of CCK an increased number of rats were reported to display sleep which was similar to postprandial sleep (19). Intraperitoneal (IP) injections of CCK dose-dependently and selectively promote non-rapid-eye-movement sleep (NREMS) at dark onset in normal rats (15), and restore rapid-eye-movement sleep (REMS) in parachlorophenylalanine-pretreated cats (25). Much indirect evidence also indicates that the hypnogenic and satiety effects of CCK are closely associated. For example, after IP injections, the threshold doses to induce sleep and to suppress food intake are indistinguishable in rats (15), furthermore, neither feeding (5,12) nor sleep (7,27) is affected after intracerebroventricular (ICV) injection of CCK in rats.

Although the behavioral effects of CCK are widely investigated in rats, there are only a few reports on the effects of CCK in rabbits. Houpt et al. reported decreased food intake in rabbits after systemic injection of CCK (13), but there are no data concerning the effects of CCK on sleep-wake activity of rabbits. If the somnogenic and satiety effects of CCK are closely related then one could expect that sleep effects also occur in the same species in which CCK suppresses food intake. The sleep effects of CCK, however, have been systematically documented only in rats thus far. These considerations led us to determine the effects of CCK on sleep-wake activity of rabbits. We report herein, that IP, but not ICV, injections of CCK selectively promote NREMS in this species.

METHOD

Male New Zealand White Pasteurella-free rabbits (3–5 kg) were used. Stainless steel screw EEG electrodes were implanted over the frontal and parietal cortices upon the dura mater, a guide cannula for ICV injections was placed in the left lateral cerebral ventricle. A thermistor implanted over the parietal cortex measured brain temperature (T_{br}). Combined ketamine/xylazine (35 and 5 mg/kg, respectively, IM) anesthesia was used for the surgeries. A minimum of two weeks was allowed for recovery before the animals were habituated to the recording chambers.

The rabbits were housed in an animal facility on a 12:12-h light-dark cycle with a light onset at 0600 h. The ambient temperature was 21 ± 1°C, food and water were provided ad lib. Before experimentation, the animals were habituated to the recording chambers for at least two 24-h sessions. When sleep recordings were scheduled, the rabbits were placed in individual sleep recording chambers the preceding evening. In these sound-attenuated recording chambers the light-dark cycle and ambient temperature were identical to that in the animal facility. A flexi-

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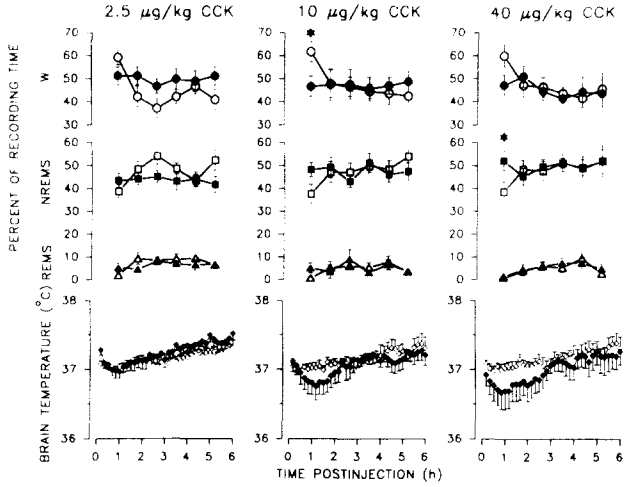

FIG. 1. The effects of IP injection of various doses of cholecystokinin (CCK, filled symbols) and saline (open symbols) on sleep-wake activity and brain temperature. The injections were done at time "0." Error bars indicate SE, asterisks indicate significant differences between the effects of CCK and saline (paired Student's *t*-test, $p<0.05$). The highest dose of CCK induced an enhancement of NREMS for about 1 h.

ble tether connected the Amphenol connector mounted on the rabbit's head to an electronic swivel allowing the rabbits to move freely. An accelerometer attached to the tether served for movement detection. The cables from the swivels and the movement detector were connected to Grass 7D polygraphs. The EEG was band-pass filtered with the 0.5–3.5 Hz (delta), 4.0–7.5 Hz (theta) frequency bands rectified by a Buxco model 24/32 data logger. EEG amplitudes in the delta frequency band during 1-min epoches of NREMS for the first postinjection hour were also determined. The 5 samples of greatest magnitude were averaged to obtain one value for each rabbit; such values were then used to calculate the means presented. The polygraphs recorded EEG, T_{br}, body movement and the ratio of theta to delta activity of EEG. The vigilance states were determined by visually scoring the polygraph records in 12-s epoches. Wakefulness (W), REMS and NREMS were distinguished according to the criteria reported earlier (17). T_{br}s were also recorded using data logger with values for each rabbit sampled at 10-min intervals.

On the experimental days, the rabbits were injected IP or ICV with saline (control) or cholecystokinin octapeptide sulphate ester (Bachem Inc., Torrance, CA) dissolved in isotonic saline. The injections were done between 0845 and 0915 h. The volume of the IP injection was 2 ml/kg. Each ICV injection (25 μl/rabbit) lasted for 1 min and an additional min was allowed before removing the injection cannula. After the treatments the sleep-wake activity and T_{br} were recorded for 6 h. Three doses were tested ICV: 0.05 μg/rabbit, 0.5 μg/rabbit, 2 μg/rabbit, three different doses were injected IP: 2.5 μg/kg, 10 μg/kg, 40 μg/kg. The experimental protocol followed a self-control design, i.e., each rabbit received saline treatment (control day) and 1–3 treatments of the various CCK doses. The differences between the effects of CCK and the saline injection were statistically evaluated. The experiments were separated by at least 2 days.

The average percent (±SE) of time spent in W, NREM, and REMS was calculated in each hour. These hourly values after CCK and saline injections were compared across 6 h by two-way ANOVA followed by an hour-by-hour comparison by paired Student's *t*-test. The maximal delta amplitudes during NREMS were compared after CCK and saline treatment in the first postinjection h by paired Student's *t*-test. The temperature data were averaged in 10-min intervals (±SE). Comparisons were made between the effects of saline and CCK injections for the first 3 postinjection h by two-way ANOVA. A p value <0.05 was taken to indicate significant difference.

TABLE 1

THE EFFECTS OF SALINE AND CCK INJECTIONS ON THE MAXIMAL EEG DELTA WAVE AMPLITUDES DURING NREMS IN THE FIRST POSTINJECTION HOUR

Dose	Route	n	Saline*	CCK
0.05 μg	ICV	7	306.9 ± 22.2	303.6 ± 27.2
0.5 μg	ICV	9	306.3 ± 27.3	232.7 ± 18.3
2 μg	ICV	5	259.5 ± 26.0	284.8 ± 33.1
2.5 μg/kg	IP	11	300.1 ± 13.3	287.2 ± 17.5
10 μg/kg	IP	4	316.1 ± 5.5	280.8 ± 18.6
40 μg/kg	IP	7	266.2 ± 22.4	259.9 ± 19.4

*The values represent average maximal delta wave amplitudes in μV ± SE.

RESULTS

Effects of IP Injections of CCK

Ten μg/kg CCK IP significantly decreased W and 40 μg/kg CCK significantly increased NREMS in the first postinjection h (Fig. 1), the time period within which CCK affects rat sleep (15). The sleep-wake activity returned to the baseline level by the second postinjection h, and there were not significant changes found in the sleep-wake activity across the 6-h period (ANOVA, $p>0.05$). After the injection of 2.5 μg/kg CCK there was a nonsignificant increase in sleep in the first postinjection h. Thereafter, NREMS was suppressed in the remainder of the experimental period (Fig. 1) [2.5 μg/kg CCK, IP: ANOVA for NREMS: drug effect: $F(1,6)=4.2$, $p<0.05$, time effect: $F(5,6)=1.5$, interaction: $F(5,6)=1.5$; ANOVA for REMS: drug effect: $F(1,6)=0.3$, time effect: $F(5,6)=0.1$, interaction, $F(5,6)=0.3$; ANOVA for W: drug effect: $F(1,6)=4.6$, $p<0.05$, time effect: $F(5,6)=2.2$, interaction: $F(5,6)=1.5$]. None of the tested doses of CCK affected the maximal EEG delta wave amplitudes during NREMS in the first postinjection h (Table 1).

The somnogenic effects of CCK were accompanied by dose-dependent decreases in T_{br}. While 2.5 μg/kg CCK did not affect T_{br}, 10 μg/kg CCK slightly decreased T_{br} for about two h, and 40 μg/kg CCK elicited a significant hypothermia for about three h (Fig. 1) [ANOVA for 40 μg/kg CCK: drug effect: $F(1,18)=15.8$ $p<0.05$, time effect: $F(17,18)=0.2$, interaction: $F(17,18)=0.2$].

Effects of ICV Injections of CCK

In contrast to the effects of IP injections, ICV injections of CCK did not cause any significant increase in NREMS. Rather, 0.05 μg CCK reduced REMS across 6 h [ANOVA, drug effect: $F(1,6)=4.2$ $p<0.05$, time effect: $F(5,6)=1.6$, interaction: $F(5,6)=0.2$] and 0.5 μg CCK slightly, but statistically significantly decreased NREMS during the first postinjection h (Fig. 2). The highest dose of CCK (2 μg ICV) did not cause significant changes in any of the measured sleep parameters. The

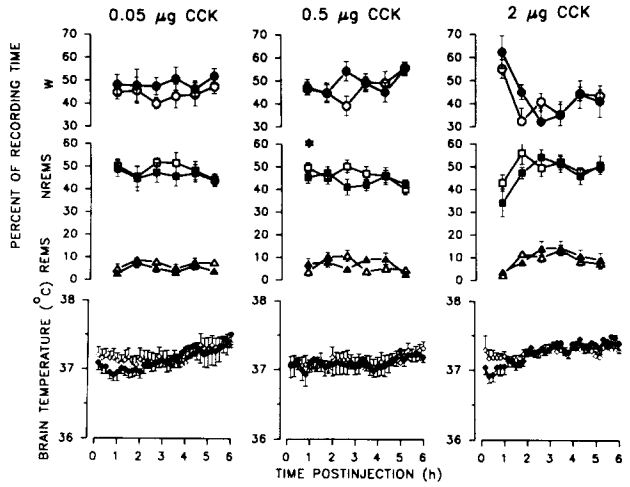

FIG. 2. The effects of ICV injection of various doses of CCK and saline on sleep-wake activity and T_{br}. See legend to Fig. 1 for details. Unlike high doses of CCK injected IP, ICV CCK failed to enhance sleep.

maximal EEG delta wave amplitudes were not affected by any doses of CCK (Table 1).

Injections of 0.05 μg and 2 μg, but not 0.5 μg CCK, elicited statistically significant decreases in T_{br} for about one h, although the amplitudes of these changes were minor (Fig. 2) [ANOVA for 0.05 μg CCK: drug effect: $F(1,18)=7.1$ $p<0.05$, time effect: $F(17,18)=0.2$, interaction: $F(17,18)=0.2$; and for 2 μg CCK: drug effect: $F(1,18)=4.7$ $p<0.05$, time effect: $F(17,18)=1.6$, interaction: $F(17,18)=0.7$].

DISCUSSION

The effects of CCK on sleep-wake activity of rats are well documented (6, 15, 19, 24). Although preliminary attempts to demonstrate somnogenic activity of CCK have been done in cats (8), our present work represents the first systematic description of the effects of exogenously administered CCK on normal sleep in rabbits.

We report here that 10 μg/kg CCK decreases W, and 40 μg/kg CCK selectively increases NREMS and elicits hypothermia in rabbits. In contrast, ICV injections of CCK or IP injection of 2.5 μg/kg CCK did not induce NREMS in rabbits, in fact, after 0.05 μg ICV and 2.5 μg/kg IP CCK NREMS was decreased and after 0.05 μg CCK REMS was reduced, although these effects were very small. The results are similar to those obtained in rats where increases in NREMS (15) and decreases in temperature (14,15) were also found after IP administration of CCK, furthermore, ICV bolus injection (27) or chronic ICV infusion of CCK (7) failed to promote sleep. The dose-range for the hypnogenic effects of CCK is similar in both species. A threshold dose of 10 μg/kg CCK in rats was reported (15), others also observed sleep increases after 10 μg/kg CCK (24), and in a third study 16, but not 8, μg/kg CCK promoted NREMS (6). IP injection of 4 μg/kg (15) or 2 μg/kg (27) CCK were ineffective on sleep of rats.

The enhanced sleep after CCK treatment was not accompanied with changes in EEG delta wave amplitudes. In contrast, recovery sleep following sleep deprivation (21), the sleep that accompanies acute infections (30,31) and the sleep elicited by several sleep-promoting substances such as IL-1 (17), muramyl dipeptide (16) or growth hormone-releasing hormone (20) are characterized by increased EEG delta wave amplitudes during NREMS. This suggests that the underlying mechanisms of CCK-induced sleep and sleep induced by the above mentioned interventions may be partially independent.

The dose-dependent hypothermic effects of systemically injected CCK are similar to those reported in rats (14,15) and mice (34). These thermoregulatory effects parallel the hypnogenic effects of CCK. ICV administration of 0.05 and 2 μg CCK also elicited a statistically significant decline in T_{br}. The magnitude of these changes, however, were so minor that the physiological significance of these responses seems questionable. Similarly, Lipton and Glyn reported that ICV injection of 5 μg CCK does not alter the body temperature in rabbits (18).

The mechanisms mediating the effects of exogenous CCK on sleep-wake activity are not known. Since the peripheral-circulating and the central-neuronal CCK pools are separated by the blood-brain barrier, which is practically impermeable to CCK (22), we assume that systemic injection of CCK mainly mimics the effects of the endogenous, peripherally circulating hormone, whereas ICV administration of CCK mimics the effects of the neurotransmitter/neuromodulator CCK. Since no somnogenic effects were observed after central injection of CCK it seems that the site of hypnogenic action of CCK is peripheral. It is known that sensory inputs conveyed by the n. vagus contribute to the regulation of sleep-wake activity (26). It has also been reported that several effects of CCK are mediated by the n. vagus (3, 9, 29, 33). It is possible, therefore, that the somnogenic effects of CCK are also mediated by n. vagus. Another possibility is that CCK causes the release of other sleep-promoting substance(s) from the periphery. The role of insulin seemed likely, since insulin elicits sleep in rats (4,28), and CCK is a potent stimulator of insulin release [e.g., (23,32)]. Experiments in rats indicate, however, that insulin plays little if any role in the hypnogenic effects of CCK (Kapás et al., submitted).

It is possible that the somnogenic doses of CCK are not physiological, although it is difficult to know since the concentration of CCK at the hypnogenic site, when its secretion is physiologically stimulated (e.g., after eating), is unknown. It is likely that when CCK is released within the small intestine it reaches high concentrations in circumscribed areas of the GI tract. To achieve such high concentrations experimentally, systemic injections of relatively large amounts of the peptide are needed. Regardless of the specific mechanism by which CCK affects sleep and thermoregulation, current results clearly show that the hypnogenic effects of CCK are not species specific. Furthermore, CCK enhances sleep in a species, in which it also suppresses food intake. It supports the idea that the sleep-promoting and food intake reducing effects are closely associated, presumably different manifestations of satiety. Finally, the experience of postprandial sleepiness, when the secretion of CCK is physiologically stimulated, further suggests that one of the primary actions of peripherally released CCK may be sleep promotion.

ACKNOWLEDGEMENTS

The authors thank Dr. Mayumi Kimura-Takeuchi for reviewing the manuscript. The technical assistance of Sandy Johnson, Donna Maxwell and Gail Richmond and the secretarial assistance of Collean Payne is gratefully acknowledged. This work was supported by the Office of Naval Research (N00014-90-J-1069), the U.S. Army Medical Research and Development Command (DAMD-17-86-C-6194) and the National Institutes of Health (NS-25378 and NS-27250).

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Attachment 3.

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Physiology & Behavior, Vol. 62, No. 1, pp. 175–179, 1997
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0031-9384/97 $17.00 + .00

PII S0031-9384(97)00034-6

Selective Activation of CCK-B Receptors Does Not Induce Sleep and Does Not Affect EEG Slow-Wave Activity and Brain Temperature in Rats[1]

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Received 3 December 1996; Accepted 14 February 1997

CHANG, H.-Y. AND L. KAPÁS. *Selective activation of CCK-B receptors does not induce sleep and does not affect EEG slow-wave activity and brain temperature in rats.* PHYSIOL BEHAV **62**(1) 175–179, 1997.—Systemic injections of cholecystokinin octapeptide sulfate ester (CCK-8-SE) elicit various behavioral and autonomic responses, such as increases in nonrapid-eye-movement sleep (NREMS) and hypothermia. There are two CCK receptors; both CCK-A and CCK-B receptors are stimulated by CCK-8-SE. The relative importance of the CCK-A and CCK-B receptors in the somnogenic and hypothermic effects of CCK-8-SE is not well understood. In the present experiments, we studied the effects of the selective activation of CCK-B receptors by CCK tetrapeptide (CCK-4) or nonsulfated CCK-8 (CCK-8-NS) on sleep and brain temperature (T_{br}). Rats were injected intraperitoneally with saline on the control day and with CCK-8-NS (10, 50, or 250 μg/kg) or CCK-4 (10, 50, or 250 μg/kg) on the test day 5–10 min before dark onset. Electroencephalogram, electromyogram, and T_{br} were recorded for 12 h. None of the treatments affected sleep or T_{br} significantly, with the exception of 10 μg/kg CCK-4, which transiently decreased the amount of NREMS, and 10 μg/kg CCK-8-NS, which slightly increased REMS. These results suggest that the activation of CCK-B receptors by systemic injection of CCK-4 or CCK-8-NS is not sufficient to elicit increased NREMS and hypothermia in rats. © 1997 Elsevier Science Inc.

Sleep Nonrapid-eye-movement sleep Rapid-eye-movement sleep Brain temperature EEG slow-wave activity CCK-4 CCK-8-NS CCK-B receptor Rat

CHOLECYSTOKININ (CCK) is a gastrointestinal hormone and a neurotransmitter/neuromodulator in the nervous system. CCK has a wide variety of autonomic and behavioral effects; it induces anxiety and hypolocomotion, inhibits food intake, and affects sexual behavior, memory, and thermoregulation [reviewed in (3)]. Exogenous administration of CCK octapeptide sulfate ester (CCK-8-SE) promotes physiological sleep resembling that which normally follows satiation (14); systemic injections of 10–50 μg/kg CCK-8-SE dose-dependently increase sleep in rats (9) and rabbits (10).

There are two CCK receptor subtypes. CCK-B receptors are abundant in various brain regions, such as the cerebral cortex, olfactory bulbs, hippocampus, amygdala, nucleus accumbens, and nucleus tractus solitarius [reviewed in (19)], but CCK-B receptors are also found in the periphery (e.g., on the vagus nerve) (13). CCK-A receptors are located mainly in the gastrointestinal tract; in the brain, CCK-A receptors are restricted only to a few regions such as area postrema, nucleus tractus solitarius, interpenduncular nucleus, and the posterior hypothalamus (15).

Both CCK-A and CCK-B receptors are involved in mediating the effects of CCK on the nervous system. For example, the anxiogenic (19) and febrile (18) actions of CCK are mediated by CCK-B receptors, whereas the suppressive effects of CCK on feeding are mediated by CCK-A receptors (7). The involvement of CCK-A and the CCK-B receptors in the somnogenic effects of CCK, however, remains unclear. The aim of the present study was to test the effects of CCK-B receptor activation by CCK tetrapeptide (CCK-4) and nonsulfated CCK-8 (CCK-8-NS) on spontaneous sleep in rats. CCK-8-NS and CCK-4 have similar affinities to the CCK-B receptor as CCK-8-SE, but their affinities to CCK-A receptors are 500–10,000-fold weaker than that of CCK-8-SE (19), thereby providing a useful tool for activating CCK-B receptors selectively. Our results suggest that the activation of CCK-B receptors is not sufficient to elicit sleep or

[1] Part of the present study results have been reported in abstract form (Soc. Neurosci. Abstr. 22, Part 1:147, 1996.)

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hypothermic responses. A part of the present results has been reported in abstract form (2).

MATERIALS AND METHODS

Materials

CCK-8-NS and CCK-4 (Peninsula Lab., Inc., Belmont, CA) were dissolved in isotonic NaCl solution immediately before the injections.

Animals

Male Sprague–Dawley rats (320–400 g) were implanted with chronic electrodes for cortical electroencephalogram (EEG) and nuchal electromyogram (EMG), and a thermistor for cortical brain temperature (T_{br}) recordings using combined ketamine (85 mg/kg) and xylazine (15 mg/kg) anesthesia. The EEG electrodes were placed over the frontal and parietal cortices; the thermistor was placed upon the dura over the parietal cortex. After an 8–10-day recovery period, the animals were placed into sound-attenuated individual experimental cages for adaptation to the experimental conditions. During this 6–8-day habituation period, the rats were connected to recording cables and injected with saline daily at dark onset. The animals were kept on a light-dark cycle of 12-12 h (dark onset at 2000 h) and at an ambient temperature of 26 ± 0.5°C for at least 2 weeks before surgeries, during the recovery, habituation, and the experimental periods. Water and food were available ad libitum throughout the experiment.

Recordings

EEG, EMG, and T_{br} were recorded by computer. The EMG recordings served as an aid for determining the vigilance states and were not further quantified. EEG was filtered below 0.1 and above 40 Hz, EMG was filtered below 250 and above 1000 Hz. The amplified signals were digitized at the frequency of 128 Hz for EEG, and at 2 Hz for EMG and T_{br}. Single T_{br} samples were saved on the hard disc in 10-s intervals. Online fast Fourier analysis of the EEG was also performed in 10-s intervals on 2-s segments of the EEG in 0.5 Hz bands of the 0.5–30 Hz frequency range. The vigilance states were determined offline in 10-s epochs. EEG, EMG, and T_{br} were displayed on the computer monitor in 10-s epochs, and also simultaneously in a more condensed form, in 12-min epochs. Wakefulness, nonrapid-eye-movement sleep (NREMS), and rapid-eye-movement sleep (REMS) were distinguished as described before in detail (12). Briefly, NREMS was characterized by high amplitude slow-wave EEG activity and low EMG activity; REMS was characterized by low-amplitude, fast-wave EEG activity with a regular, visible theta rhythmicity and a lack of muscle tone occasionally interrupted by muscle twitches; wakefulness was characterized by irregular, low-amplitude, fast-wave EEG activity and irregular, high EMG activity. The EEG power density values were measured in the delta (0.5–4 Hz) band for each 10-s epoch of NREMS and then averaged in 1-h time blocks. EEG delta activity during NREMS, also called slow-wave activity (SWA) is often regarded as an indicator of NREMS intensity (12).

Experimental Protocol

Six groups of rats were used. On the control day, all the animals were injected with vehicle intraperitoneally (IP). On the test day, 3 groups of rats received 3 different doses of CCK-8-NS [10, 50, and 250 μg/kg (9.4, 47.1, and 235.3 nmol/kg), $n = 7$, $n = 11$, and $n = 6$, respectively] and the other 3 groups were treated with CCK-4 [10, 50, or 250 μg/kg (16.8, 83.9, and 419.3 nmol/kg), $n = 7$, $n = 7$, and $n = 7$, respectively] IP. The order of the control and test days was counterbalanced. Saline, CCK-4, and the two lower doses of CCK-8-NS were injected in a volume of 2 ml/kg; 250 μg/kg CCK-8-NS and its control, vehicle treatment, were injected in a volume of 10 ml/kg. All the injections were done 5–10 min before dark onset. EEG, motor activity, and T_{br} recordings started at dark onset and continued for 12 h. Due to the malfunction of several thermistors, the animal numbers for T_{br} are lower (50 μg/kg CCK-8-NS, $n = 8$; 250 μg/kg CCK-8-NS, $n = 5$; 10, 50, and 250 μg/kg CCK-4, $n = 6$, $n = 4$, and $n = 6$, respectively). The recordings from the rats that had artifact contamination in their EEGs were excluded from the fast Fourier analysis. The sample size, therefore, for SWA analysis is lower than for sleep recordings (10 and 50 μg/kg CCK-8-NS, $n = 5$, $n = 8$, respectively; 10 and 50 μg/kg CCK-4, $n = 6$, $n = 5$, respectively).

Statistical Analysis

Comparisons were made between the control and experimental days by using two-way ANOVA for repeated measures and paired t-test a posteriori. Time spent in different vigilance states and the SWA were calculated in 1-h time blocks; ANOVA was performed on the 1-h time blocks across 12 h. For T_{br}, ANOVA was performed across 12 h on values averaged in 1-h intervals.

RESULTS

The lowest dose, 10 μg/kg CCK-4 had a significant effect on NREMS and T_{br} across the 12-h recording period, as indicated by ANOVA (Fig. 1; ANOVA for repeated measures, NREMS treatment effect: $F(1,6) = 11.99$, $p < 0.05$; T_{br} treatment effect: $F(1,5) = 9.42$, $p < 0.05$). Post hoc paired t-test showed a significant decrease in NREMS in Hour 4; for T_{br}, the post hoc analysis did not indicate any significant effect in any h. REMS and SWA were not affected by 10 μg/kg CCK-4. The other 2 doses, 50 and 250 μg/kg of CCK-4, did not affect NREMS, REMS, SWA, or T_{br}. CCK-8-NS did not have any significant effect on NREMS, SWA, or T_{br} at any of the tested doses (Fig. 2). Ten μg/kg CCK-8-NS, however, significantly affected REMS across the 12-h recording period [ANOVA for repeated measures, treatment effect: $F(1,6) = 27.32$, $p < 0.05$]. REMS was significantly increased in Hour 3 (paired t-test, $p < 0.05$).

DISCUSSION

Intraperitoneal injection of 10–50 μg/kg CCK-8-SE (8.7–43.7 nmol/kg) causes increased sleep during the first 1–2 h after treatment in rats (9) and rabbits (10). CCK-8-SE binds to both CCK-A and CCK-B receptors with similar affinities (20). The aim of the present experiments was to study if selective activation of CCK-B receptors is sufficient to elicit increases in sleep as observed after CCK-8-SE treatment. The affinities of CCK-4 and CCK-8-NS to the CCK-B receptors are ~10 times weaker than that of CCK-8-SE (20), but their affinities to CCK-A receptors are 500–10,000-fold less than that of CCK-8-SE (19). If the somnogenic effects of CCK-8-SE are due to the selective activation of CCK-B receptors, then 90 nmol/kg CCK-4 or CCK-8-NS would be expected to elicit similar somnogenic actions as 9 nmol/kg CCK-8-SE. In our experiments, the amount of NREMS and NREMS intensity (as indicated by SWA) were not increased after the injection of 16.8–419.3 nmol/kg CCK-4 or 9.4–235.3 nmol/kg CCK-8-NS; rather, NREMS was slightly decreased after the injection of 10 μg/kg (16.8 nmol/kg) CCK-4. Similarly, 8 μg/kg BC-264, another selective agonist of the CCK-B receptors, slightly increases wakefulness in

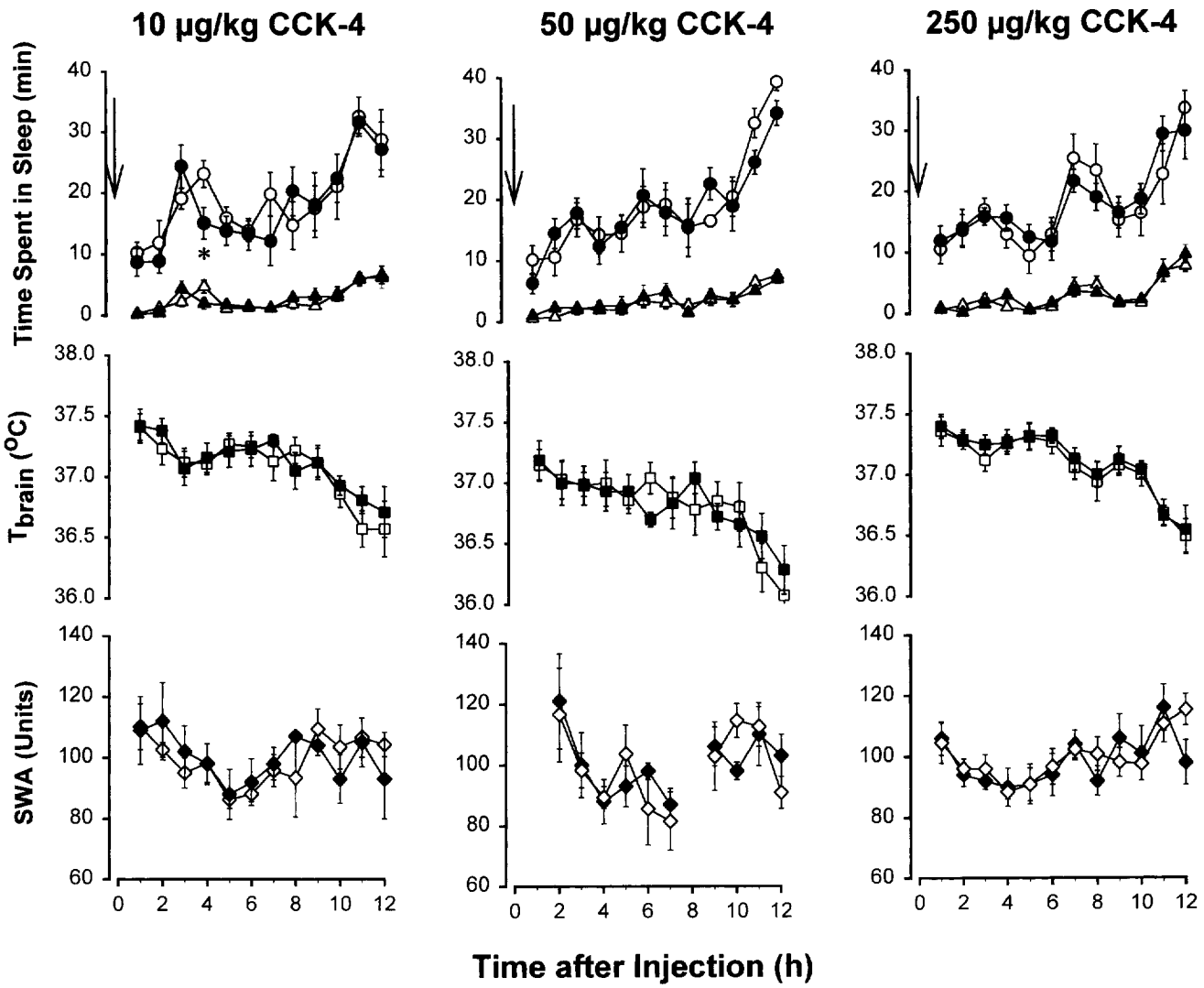

FIG. 1. The effects of cholecystokinin tetrapeptide (CCK-4) on nonrapid-eye-movement sleep (NREMS, ●; compared to vehicle ○), rapid-eye-movement sleep (REMS, ▲; compared to vehicle △), brain temperature (T_{br}, ■; compared to vehicle □), and slow-wave activity of the electroencephalogram during NREMS (SWA, ◆; compared to vehicle ◇). The data points represent means ± standard error; arrows, time of the injection; missing data points for SWA: 3 or less animals exhibited NREMS in a certain h and SWA was not calculated for that h because of the low sample size. CCK-4 did not affect any of the measured parameters with the exception of the 10 µg/kg dose, which slightly suppressed NREMS in Hour 4 [ANOVA for repeated measures, NREMS treatment effect: $F(1,6) = 11.99$, $p < 0.05$; * post hoc paired t-test, $p < 0.05$ in Hour 4; ANOVA for repeated measures, T_{br}: $F(1,5) = 9.42$, $p < 0.05$]. Sample size for 10 µg/kg dose: $n = 7$, 6, and 6 for sleep, T_{br}, and SWA, respectively; 50 µg/kg: $n = 7$, 4, and 5; 250 µg/kg: $n = 7$, 6, and 7.

rats (6) and CCK-4 induces behavioral activation in the open-field test (8). The lowest dose of CCK-8-NS induced a statistically significant increase in REMS. The biological significance of this effect is, however, unclear. The magnitude of the increase was rather small (1.4 min in 1 h) and, unlike the somnogenic effects of CCK-8-SE that occur immediately in the first h (9,10,14), it appeared in the third h after the injection. Taken together, our results indicate that the selective activation of CCK-B receptors is not sufficient to elicit somnogenic responses characteristic of CCK-8-SE. In accordance with this, preliminary data suggest that the somnogenic effects of CCK-8-SE are completely blocked by a CCK-A receptor antagonist (H.-Y. Chang and L. Kapás, unpublished observations).

In our experiments, neither CCK-8-NS nor the two higher doses of CCK-4 altered T_{br} significantly. Ten µg/kg CCK-4 had a significant effect on T_{br} as indicated by ANOVA, but the biological relevance of this effect is questionable because post hoc statistical analysis did not reveal any time point at which T_{br} significantly differed from that in the controls. These findings are consistent with previous reports that systemic injections of 100 or 200 µg/kg CCK-4 or 200 µg/kg CCK-8-NS (11) or intracerebroventricular (ICV) injection of CCK-4 and CCK-8-NS do not affect body temperature (17). In a recent study, ICV injection of CCK-8-SE induced fever-like thermoregulatory responses in female rats; these responses were inhibited by the CCK-B receptor blocker L-365,260 suggesting the involvement of CCK-B receptors in fever genesis (18). Our data suggest, however, that the stimulation of CCK-B receptors by systemic injections of CCK-B receptor agonists is not sufficient to induce fever in male rats. It is possible that, when injected systemically, CCK-4 and CCK-8-NS do not reach the brain regions that mediate the febrile ef-

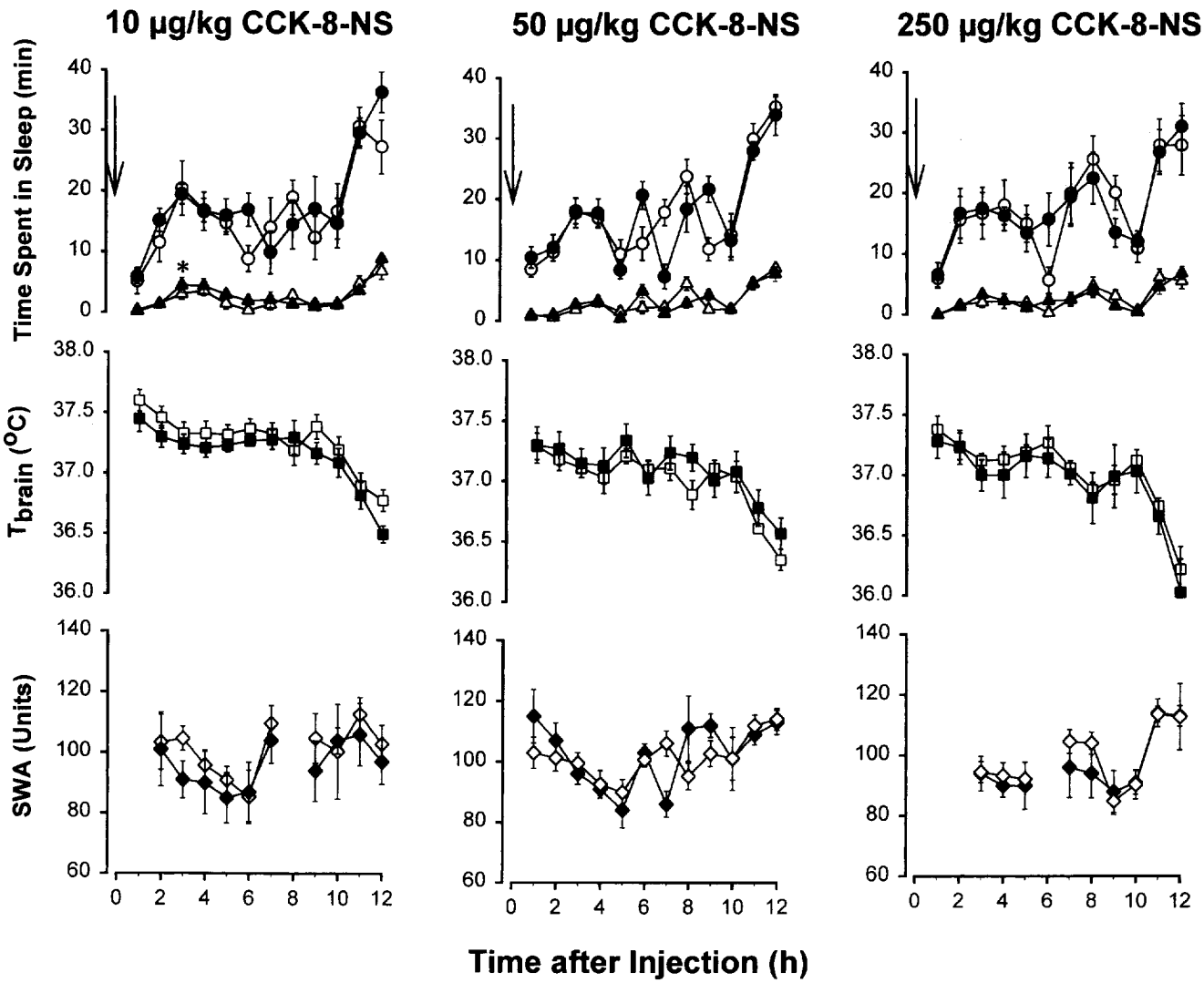

FIG. 2. The effects of nonsulfated CCK octapeptide (CCK-8-NS) on NREMS, REMS, T_{br}, and SWA. See legend to Fig. 1 for details. CCK-8-NS did not affect any of the measured parameters, with the exception of the 10 μg/kg dose that slightly increased REMS in Hour 3 [ANOVA for repeated measures, REMS treatment effect: $F(1,6) = 27.32$, $p < 0.05$; * post hoc paired t-test, $p < 0.05$ in Hour 3]. Sample size for 10 μg/kg dose: $n = 7$, 7, and 5 for sleep, T_{br}, and SWA, respectively; 50 μg/kg: $n = 11$, 8, and 8; 250 μg/kg: $n = 6$, 5, and 6.

fects of ICV-administered CCK-8-SE in sufficient amounts to initiate CCK-B receptor-mediated febrile responses.

Systemic injection of CCK-8-SE elicits the complete sequence of "satiety syndrome," including the cessation of eating, reduced exploration, social withdrawal, and sleep (1). Similar to the lack of sleep-promoting effects of CCK-B receptor agonists, the other components of the satiety syndrome cannot be elicited by the selective activation of CCK-B receptors. For example, systemic injection of CCK-4 (5) or CCK-8-NS (4) does not affect food intake and exploratory behavior. Furthermore, central injection of physiological doses of CCK-8-SE does not induce sleep (10,16). These results indirectly suggest that CCK may act on the periphery primarily on CCK-A receptors or simultaneously on both CCK-A and CCK-B receptors to elicit the complete sequence of the satiety syndrome, including sleep.

ACKNOWLEDGEMENTS

This study was supported, in part, by grants from the National Institute of Health (NS-30514) and Fordham University (12-07-1257).

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Attachment 4.

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Cholecystokinin Promotes Sleep and Reduces Food Intake in Diabetic Rats

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Received 18 January 1991

KAPÁS, L., F. OBÁL, JR., I. FARKAS, L. C. PAYNE, G. SÁRY, G. RUBICSEK AND J. M. KRUEGER. *Cholecystokinin promotes sleep and reduces food intake in diabetic rats.* PHYSIOL BEHAV **50**(2) 417–420, 1991.—It has been reported that systemic injections of cholecystokinin (CCK) elicit the behavioral characteristics of satiety, including sleep, in rats. CCK is a potent stimulator of insulin secretion, and insulin is hypothesized to be involved in sleep and feeding regulation. The purpose of the current experiments was to study the possible role of endogenous insulin in the food-intake-reducing and hypnogenic effects of intraperitoneally (IP) administered CCK. Normal and streptozotocin (STR)-diabetic rats were injected with isotonic saline or CCK (10 and 50 μg/kg) at dark onset, and sleep-wake activity was determined for the next 12 h. There were no significant differences between the baseline sleep-wake activity of normal and diabetic rats. IP injection of CCK elicited a selective increase in nonrapid-eye-movement sleep in both groups during the first postinjection hour. In a separate experiment, the effects of CCK (10 μg/kg) on food intake were determined in control and diabetic rats; CCK suppressed the 1-h food intake in both groups. In a third experiment, the effects of CCK treatment (50 μg/kg) on plasma insulin levels were determined. In normal rats, CCK elicited a two-fold increase in plasma insulin concentration, whereas diabetic rats had a significantly lower basal insulin level which was not affected by CCK treatment. We conclude that hypnogenic and food-intake-reducing effects of exogenously administered CCK are closely associated; however, pancreatic insulin does not play a significant role in either of these effects.

Cholecystokinin Sleep Food intake Insulin Streptozotocin Rat

IT is hypothesized that humoral factors are involved in the regulation of sleep-wake activity and feeding behavior. A variety of endogenous substances that promote sleep [sleep factors, reviewed in (5)] and/or reduce food intake [satiety peptides, reviewed in (28)] have been described. Among these substances, cholecystokinin (CCK) and insulin are of special interest, since both are implicated as sleep factors and satiety hormones. A single peripheral injection of CCK suppresses food intake (10,11), elicits the whole behavioral sequence of satiety (2), and promotes nonrapid-eye-movement sleep (NREMS) (13,18) resembling that observed after eating (18). Similarly, exogenous administration of insulin selectively increases NREMS (8,23) and decreases food intake (27), whereas diabetic rats exhibit disturbed sleep (6) and increased feeding (4,16). Since CCK is a major hormonal stimulatory factor of insulin secretion (1, 22, 24, 25, 29), it seemed possible that the effects of exogenously administered CCK on sleep and feeding are, in fact, mediated by endogenously released insulin. The aim of the current experiments was to study the possible role of pancreatic insulin in the food-intake-suppressive and sleep-promoting effects of exogenously administered CCK using a streptozotocin (STR)-induced diabetes model in rats. We report here that systemic injection of CCK promotes NREMS and reduces food intake, but it is unlikely that these effects are mediated by pancreatic insulin.

METHOD

Sleep Experiments

Effects of 50 μg/kg CCK on sleep-wake activity. Male rats of the Wistar strain were used weighing 250–350 g at the time of the CCK injections. Gold jewelry screws were implanted over the frontal and parietal cortices, and the cerebellum for electroencephalogram (EEG) recordings. The surgeries were performed under ketamine anesthesia (95 mg/kg). After the implantation, the animals were placed in the sleep-recording chamber and allowed to adapt to the experimental conditions for 7–10 days. They were connected to a flexible recording cable in individual cages in a sound-attenuated environment. A 12:12-h light:dark schedule was maintained with a dark onset at 2030 h during the adaptation and sleep-recording periods. The ambient temperature was 21 ± 0.5°C. Water was provided ad lib, whereas food was available only during the dark period. The habituation also included the gentle handling of the animals and daily IP injections of isotonic saline 5–10 min before dark onset.

To produce diabetic animals, rats provided with EEG electrodes were treated with STR (65 mg/kg) through the tail vein under light ether anesthesia. Control rats using vehicle were also prepared. The sleep recordings were done on the second and

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third days after the STR treatment. One day was used as a baseline day, and the animals received an IP injection of saline. On the other day, they received an IP injection of CCK. The order of the CCK and the saline treatment was such that in half of the animals of each group, the CCK day preceded the baseline day. The injections were given 5–10 min before dark onset. EEG and motor activity (potentials generated in electromagnetic transducers activated by the movements of the recording cable) were recorded on a polygraph for 12 h. The arousal states were determined by visually scoring the polygraph records in 40-s epochs according to standard criteria (13) as wakefulness (W), NREMS, or rapid-eye-movement sleep (REMS). The percent of time in the vigilance states was computed for consecutive 1-h and 3-h periods, and for the entire 12-h recording period. The differences between the CCK days and baseline days within the same groups of rats, differences of the baseline sleep-wake activities, and the responsiveness to CCK between normal and diabetic rats were calculated. After the sleep recordings, fasting plasma glucose levels were measured by the o-toluidin method using 20 μl blood obtained from the tail vein. STR-pretreated rats showed significantly higher plasma glucose concentrations than normal rats (17.6 ± 1.63 nmol/l vs. 3.4 ± 0.20 nmol/l, $p<0.001$).

Effects of 10 μg/kg CCK on sleep-wake activity. The experiments with the low dose of CCK were done basically under identical circumstances as listed above with the following exceptions. The surgery was performed under ketamine-xylazine anesthesia (87 and 13 mg/kg), and it included the implantation of a thermistor upon the dura mater above the parietal cortex for measuring brain temperature (T_{br}). The dark onset during the habituation and the experiments was at 2000 h. The polygraphs (Grass 7D) recorded EEG, T_{br}, body movement (detected by ultrasonic motion detector), and the ratio of theta-to-delta activity calculated from the band-pass filtered EEG (0.5–3.5 Hz for delta, and 4.0–7.5 Hz for theta frequency rectified by a Buxco 24/32 data logger). The polygraph records were visually scored in 10-s epochs for the duration of the states of vigilance according to the criteria reported earlier (20).

Food Intake Measurement

The effects of CCK on the food intake of diabetic ($n=4$) and control ($n=6$) animals were studied in separate experiments on rats previously tested for the sleep effects of 50 μg/kg CCK. After the sleep recordings, experimental conditions were maintained for an additional week and then, on two consecutive days, food intake measurements were performed. The rats were IP injected with saline on one day, and with CCK (10 μg/kg) on the other day 5–10 min before dark onset. Rat food pellets (LATI, Hungary) were weighed and placed into the cage at the start of the dark period. After 1 h, spillage was recovered and food was reweighed.

Serum Insulin Measurement

A separate set of rats was implanted with chronic intra-atrial cannula through the right jugular vein. After implantation, the animals were kept under the same conditions as in the sleep experiments. The patency of the cannula was maintained by daily flushing with heparinized saline. Five days after the surgery, five rats were treated with STR (65 mg/kg) and seven with vehicle through the cannula. Two days later, three of the STR-treated and three of the control rats were injected with CCK (50 μg/kg IP) or with saline at the dark onset. The next evening, the treatment was reversed. Immediately before treatment (time 0), and 5, 15, 30 and 60 min after the injections, blood samples (0.5 ml) were taken from the freely moving rats. The plasma was separated and was frozen at −20°C, and the blood cells were reinjected after the next blood-taking. Plasma insulin was measured by radioimmunoassay specific to rat insulin (INCSTAR Corp., Stillwater, MN).

Statistics

The hourly percents of vigilance states throughout 12 h were compared between the diabetic and normal groups after the identical treatments using ANOVA followed by unpaired Student's *t*-test. The percent of vigilance states within the same experimental group after saline and CCK treatment was compared by ANOVA followed by paired Student's *t*-test. The effects of saline and CCK on plasma insulin levels within the same groups were compared by ANOVA followed by paired Student's *t*-test, and the differences between the baseline insulin levels of normal and diabetic rats were analyzed by ANOVA followed by unpaired Student's *t*-test. The differences between the food intake of diabetic and control rats were compared by unpaired Student's *t*-test; the differences between the effects of saline and CCK were compared by paired Student's *t*-test.

Materials

Cholecystokinin octapeptide sulfate ester (Peninsula Laboratories, Inc., Belmont, CA) was dissolved in isotonic NaCl solution. Streptozotocin (Sigma, St. Louis, MO) was dissolved in a mixture of citric acid and Na_2HPO_4 at pH 4. Both of these substances were dissolved immediately before the experiments and were injected in a volume of 2 ml/kg.

RESULTS

Effects on Sleep-Wake Activity

There were no significant differences between the baseline sleep-wake activity of the diabetic and the normal rats. Neither the time spent in NREMS during the total 12-h recording period ($25.6 \pm 2.37\%$ and $25.7 \pm 1.32\%$, pooled data from the two diabetic groups and pooled data from two control groups, respectively), nor the time spent in REMS ($3.7 \pm 0.54\%$ and $3.5 \pm 0.51\%$) differed significantly.

IP injection of CCK selectively promoted NREMS in all of the experimental groups, and this effect was confined to the first postinjection hour in all cases. The lower dose of CCK (10 μg/kg) elicited a significant increase in NREMS of about 11% in normal rats, although the duration of REMS was not affected. This dose of CCK had the same effects in diabetic rats; the amount of NREMS was significantly elevated in the first hour, whereas the time spent in REMS was not changed (Fig. 1). Administration of 50 μg/kg CCK caused more pronounced changes in NREMS than the lower dose. In the first hour, the NREMS was elevated significantly in both normal and diabetic rats by 16 and 19%, respectively. The percent of REMS was not affected in either group (Fig. 2).

Effects on Food Intake

Diabetic rats ate significantly more in the baseline day than normal rats (37.3 ± 5.88 vs. 21.1 ± 3.57 g/kg/h, unpaired Student's *t*-test, $p<0.05$). In response to the IP injection of 10 μg/kg CCK, the food intake of the diabetic rats decreased significantly; this value (23.3 ± 7.32 g/kg/h, paired Student's *t*-test, $p<0.05$) approached the level observed in normal rats after saline treatment. CCK also suppressed the food intake in normal

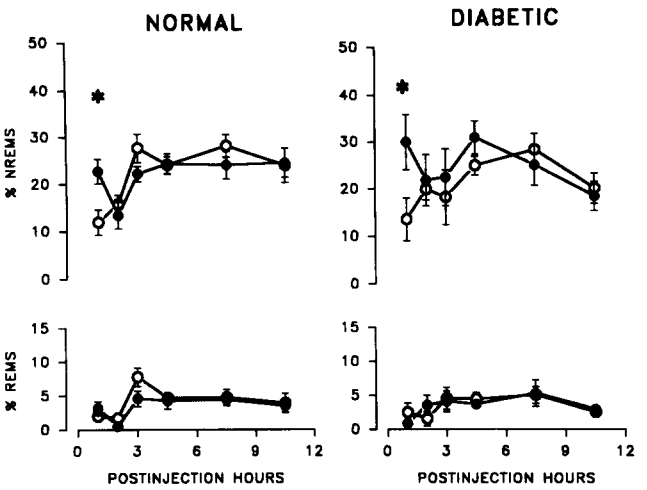

FIG. 1. The effects of IP injection of 10 μg/kg CCK (filled circles) and saline (open circles) on the percent of time spent in NREMS and REMS in normal and diabetic rats. The 1-h averages are shown for the first 3-h postinjection, and means for 3-h periods are shown for the remainder of 12-h records. Error bars represent the SE. Asterisks indicate significant differences between the effects of saline and CCK (paired Student's t-test, $p<0.05$).

rats by about 50% to a value of 9.8 ± 3.46 g/kg/h (paired Student's t-test, $p<0.05$).

Effects on Plasma Insulin Concentration

The baseline plasma insulin levels of the STR-pretreated rats were significantly lower than those of controls [Fig. 3; ANOVA across 60 min: drug effect: $F(1,40)=19.21$, $p<0.001$; time effect: $F(4,40)=1.01$; interaction: $F(4,40)=0.36$]. In the control group, the preinjection levels of plasma insulin were slightly but not significantly higher preceding the CCK treatment than prior to saline injection. While saline treatment did not cause any significant changes in plasma insulin level, IP injection of 50 μg/kg

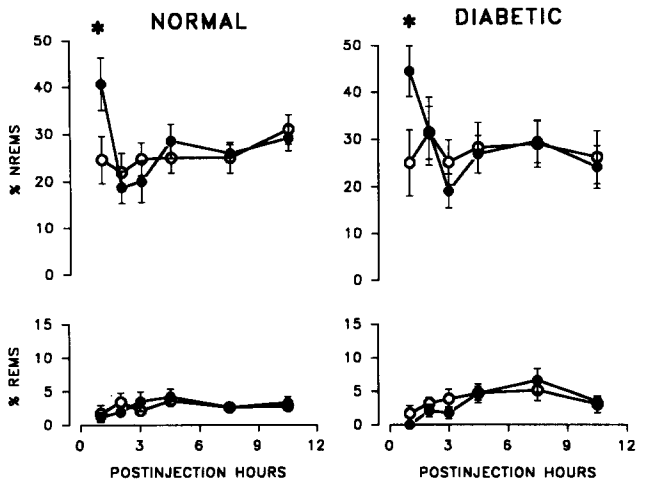

FIG. 2. The effects of IP injection of 50 μg/kg CCK and saline on NREMS and REMS in normal and diabetic rats. See legend to Fig. 1 for details.

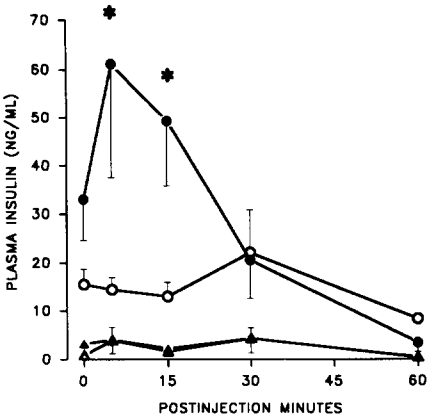

FIG. 3. The effect of IP injection of 50 μg/kg CCK and saline on plasma insulin levels of diabetic and normal rats. Filled circles: CCK treatment in normal rats; open circles: saline treatment in normal rats; filled triangles: CCK treatment in diabetics; open triangles: saline treatment in diabetics. The zero time is the time of injections, and the values at zero time correspond to the preinjection insulin levels. Error bars indicate SE. Asterisks designate significant difference within the group between CCK and saline treatment (paired Student's t-test, $p<0.05$).

CCK induced a significant increase in plasma insulin concentration in normal rats at 5 and 15 min postinjection (paired Student's t-test, $p<0.05$). In diabetic rats, there was no significant difference between the effects of saline and CCK treatment.

DISCUSSION

Present results confirm previous reports describing selective increases in NREMS after systemic administration of CCK in rat. Mansbach and Lorenz found an increased number of rats displaying sleep and increased sleep duration in response to CCK (18). In our previous experiments, 10 and 50 μg/kg, but not 4 μg/kg, intraperitoneal CCK promoted sleep during the night (13). De Saint Hilaire-Kafi et al. observed a selective enhancement of NREMS after 16 μg/kg CCK, whereas 8 μg/kg induced an unusual EEG in rats with their eyes open (9). Previous results indicated that, during the dark period, the threshold dose for the food-intake-suppressive and hypnogenic effects of CCK was 10 μg/kg in rats (13). We used this dose in the present experiments studying the effects of CCK on food intake, and the results confirm the previous observations that 10 μg/kg CCK significantly reduces food intake in normal rats after 12-h fasting (13). CCK is known to stimulate insulin secretion (1, 22, 24, 25, 29). In accordance with these observations in our experiments, IP injection of CCK elicited a short-latency increase in plasma insulin level in freely moving normal rats.

STR treatment significantly reduced the plasma insulin level and brought about other well-known consequences of diabetes mellitus such as hyperphagia (4,16) and increased blood glucose level (12), indicating that the rats were, in fact, diabetic. The baseline sleep-wake activity of diabetic rats at night did not differ from those of normals. Danguir reported a decreased NREMS in diabetic rats during a total 24-h period (6). Our previous observations indicated, however, that this sleep deficit is confined to the second half of the light phase; the sleep during the dark period is not affected by STR treatment (14). It indicates that endogenous insulin does not play a crucial role in the regulation of sleep-wake activity of rats during the behaviorally active phase of the circadian rhythm.

In previous reports in which CCK seemed to be particularly effective in promoting sleep, the rats were also provided food (13,18), and though food intake decreased after CCK treatment, it was not totally inhibited. It was speculated, therefore, that additional factors closely linked to eating might be involved in the sleep-promoting actions of CCK. Since food intake is followed by insulin release, a hormone that promotes sleep (7, 8, 23), and CCK stimulates insulin secretion, the involvement of insulin in the mediation of sleep-promoting action of CCK was postulated (19). According to the present results, however, this possibility can be ruled out, since, as in normal rats, CCK increased NREMS in diabetic rats. There was no effect on REMS. Although an increased β-cell responsiveness was reported to a CCK analogue, caerulein, in perifused pancreas of diabetic rats in vitro (21), we did not find any significant insulin response in vivo in diabetic animals even after a relatively high dose (50 μg/kg) of CCK. As in normal rats, 10 μg/kg CCK significantly suppressed the 1-h food intake in diabetic animals. This finding is in accordance with that of Vanderweele, who also reported food-intake-suppressive effects of CCK in STR-diabetic rats (26). The findings that CCK increases NREMS and decreases food intake in diabetic rats without stimulating pancreatic insulin release indicate that plasma insulin does not mediate the hypnogenic and food-intake-suppressive effects of CCK.

A similar conclusion was drawn recently concerning the hypnogenic effects of another sleep factor, interleukin 1 (IL-1). Among its biological activities, IL-1 suppresses feeding (17), enhances NREMS (15) and alters insulin release (3). Originally, we hypothesized that insulin might contribute to the hypnogenic effects of IL-1. STR pretreatment of rats, however, failed to alter the hypnogenic activity of IL-1, indicating that its hypnogenic effect is independent from pancreatic insulin (14).

ACKNOWLEDGEMENTS

The authors would like to thank Dr. M. Opp for reviewing the manuscript and Dr. L. Johannsen for providing his expertise in RIA. The technical assistance of Ms. Klára Ormos, Ms. Ilona Ponicsán, Ms. Gail Richmond, Ms. Donna Maxwell and Mr. P. Liszli is gratefully acknowledged. This work was supported by the Hungarian Academy of Sciences (OTKA 1104, OKKFT 177/86), Hungarian Ministry of Health (ETT 528) and the National Institutes of Health (USA) NS-27250.

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Attachment 5.

Am J Physiol Regulatory Integrative Comp Physiol
280: R1420–R1426, 2001.

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

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Received 14 January 2000; accepted in final form 25 September 2000

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 refeeding 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 refeeding 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 refeeding days). In the control group, refeeding 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 refeeding; 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 (16) and mammalian (29) 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 (T_{br}). Insulated leads from the EEG screw electrodes, EMG electrodes, and thermistor were routed to a plastic pedestal and ce-

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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 maintenance was conducted. On the baseline and refeeding days, preweighed rat chow (LM-485 Mouse/Rat Diet; Harlan Teklad, Madison, WI) was given to the animals twice a day, at light and dark onsets. 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 T_{br} were recorded on a computer. EMG 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 T_{br}. Single T_{br} values were saved on hard disk in 10-s intervals. T_{br} 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 each 10-s epoch of NREMS, and average activities were calculated in 2-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 T_{br} were displayed 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 T_{br} on entry; REMS: low-amplitude EEG and regular theta activity in the EEG, general lack of body movements with occasional twitches, and a rapid rise in T_{br} at onset; wakefulness: low-amplitude, fast EEG activity, lack of visible regular theta rhythm, high EMG activity, and a gradual increase in T_{br} 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 T_{br} 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 T_{br}, 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 T_{br} 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. T_{br} 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 T_{br} 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 T_{br} 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 T_{br} throughout the day.

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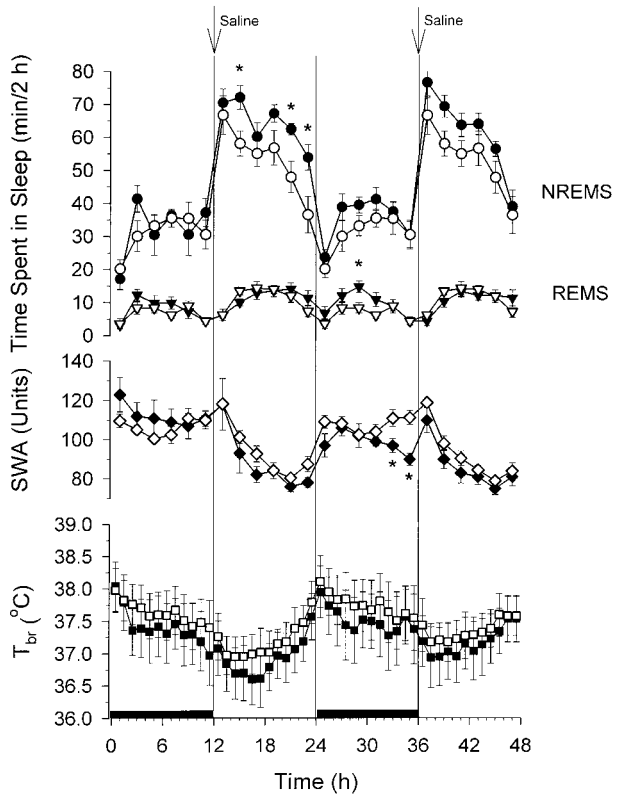

Fig. 1. The effects of refeeding on non-rapid eye movement sleep (NREMS, circles), rapid eye movement sleep (REMS, triangles), slow-wave activity (SWA) of the electroencephalogram (diamonds), and brain temperature (T_{br}, squares) in control rats. Time spent in sleep is summed in 2-h blocks. SWA values are averaged in 2-h, T_{br} values in 1-h intervals. Baseline day: open symbols, refeeding day: solid symbols. Horizontal solid bars: dark period of the day. Error bars: SE. Arrows indicate the time of injections. *Significant differences between baseline and refeeding conditions ($P < 0.05$, paired *t*-test). *Time 0*: the time of returning the food on the refeeding day. Refeeding after 4 days of starvation caused increases in NREMS and REMS and decreases in SWA.

Effects of Refeeding on Sleep, SWA, and T_{br} 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 postingestive 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-

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

	1–12 h	13–23 h	25–36 h	37–47 h
Control				
NREMS	$F(1,7) = 0.075$ NS	$F(1,7) = 10.161$ $P < 0.05$	$F(1,8) = 4.723$ NS	$F(1,8) = 3.574$ NS
REMS	$F(1,7) = 0.424$ NS	$F(1,7) = 0.017$ NS	$F(1,8) = 10.296$ $P < 0.05$	$F(1,8) = 0.222$ NS
SWA	$F(1,3) = 1.169$ NS	$F(1,5) = 1.487$ NS	$F(1,6) = 15.525$ $P < 0.05$	$F(1,7) = 1.477$ NS
T_{br}	$F(1,4) = 1.383$ NS	$F(1,4) = 1.573$ NS	$F(1,3) = 6.282$ NS	$F(1,3) = 1.953$ NS
L-364,718				
NREMS	$F(1,7) = 0.089$ NS	$F(1,7) = 0.928$ NS	$F(1,7) = 23.534$ $P < 0.05$	$F(1,7) = 0.116$ NS
REMS	$F(1,7) = 4.949$ NS	$F(1,7) = 0.657$ NS	$F(1,7) = 0.159$ NS	$F(1,7) = 8.008$ $P < 0.05$
SWA	$F(1,7) = 0.340$ NS	$F(1,7) = 0.134$ NS	$F(1,7) = 0.886$ NS	$F(1,7) = 0.294$ NS
T_{br}	$F(1,6) = 0.028$ NS	$F(1,6) = 0.678$ NS	$F(1,6) = 0.693$ NS	$F(1,6) = 0.051$ NS

Two-way ANOVA for repeated measures was performed for non-rapid eye movement sleep (NREMS), rapid eye movement sleep (REMS), and brain temperature (T_{br}), between the baseline and refeeding conditions. ANOVA across the specified hours was performed on 1-h time blocks for T_{br} and on 2-h time blocks for sleep and slow-wave activity (SWA). The F 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.

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 refed 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).

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, T_{br} was at baseline levels. For the remainder of the refeeding period, however, T_{br} was slightly but consistently below baseline. This slight decrease in T_{br} was absent in the CCK antagonist-treated group. It is possible that CCK, released during refeeding, contributed to the slight decrease in T_{br}, 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

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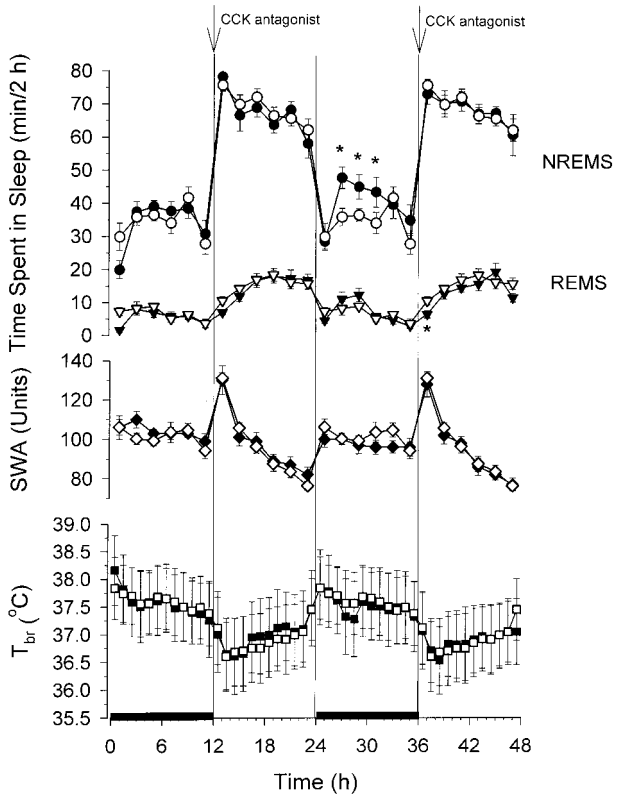

Fig. 2. The effects of refeeding on NREMS, REMS, SWA, and T_{br} in L-364,718-treated rats. Symbols same as in Fig. 1. L-364,718 completely abolished the REMS and SWA responses to refeeding and the NREMS-promoting effects of refeeding during the light periods of the refeeding days. *Significant differences between baseline and refeeding conditions ($P < 0.05$, paired t-test).

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

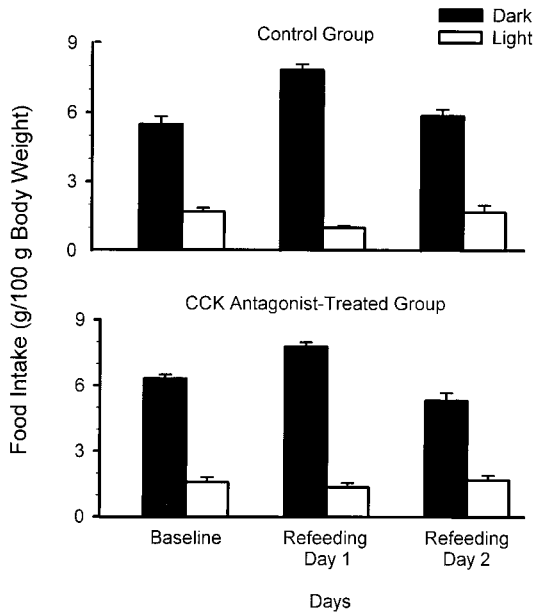

Fig. 3. Food intake under baseline conditions and on refeeding *days 1* and *2* after food deprivation in control and L-364,718-treated rats. Solid bars: food intake during the dark period; open bars: food intake during the light period. *Top*: food intake in control rats. *Bottom*: food intake in L-364,718-treated rats. Food intake is expressed as the amount of food consumed per 100 g body wt. Error bars: SE. Three-way ANOVA indicated that there is no significant difference between the food intake of the control and L-364,718-treated rats. The circadian period (light-dark effect) and the refeeding (day effect) have significant effects on food intake. See RESULTS for details.

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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 afferents to the effects of CCK (5); the effects of IL-1 on the vagus (27) and on food intake (17) are suppressed by CCK-A receptor antagonists; and CCK stimulates the production of IL-1, tumor necrosis factor-α, and granulocyte/monocyte-colony-stimulating factor from monocytes (11). Increased feeding stimulates the secretion of CCK as well as the expression of IL-1β mRNA in the liver and the brain. It is possible that CCK and cytokines form an intertwined humoral network to induce sleep in response to eating and infections or under other conditions.

L-364,718 was a generous gift by Dr. William Henckler (Merck Sharp and Dohme).

This work was supported by National Institutes of Health Grant NS-30514.

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