METABOLIC SIGNALS IN SLEEP REGULATION: THE ROLE OF CHOLECYSTOKININ

By

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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.

7. Chang, H.-Y. and **L. Kapás**. L-364,718, a cholecystokinin (CCK)-A receptor antagonist, inhibits the sleep-inducing effects of CCK. Sleep Res. 26:138, 1997.

8. **Kapás, L.** Subdiaphragmatic vagotomy does not prevent the somnogenic and hypothermic effects of cholecystokinin (CCK). Sleep Res. 26:76, 1997

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

10. Shemyakin, A. and **L. Kapás**. A CCK-A receptor antagonist inhibits sleep responses to feeding in rats. Sleep 23, Suppl. 2, A121, 2000.

11. Shemyakin, A. and **L. Kapás**. L-364,718, a CCK-A receptor antagonist, suppresses sleep responses to feeding in rats. J. Sleep Res. 9, Suppl. 1, P175, 2000.

12. Shemyakin, A. and **L. Kapás**. L-364,718, a CCK-A receptor antagonist does not prevent the somnogenic effects of interleukin in rats. Sleep 24, A145, 2001.

Table of Content

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.

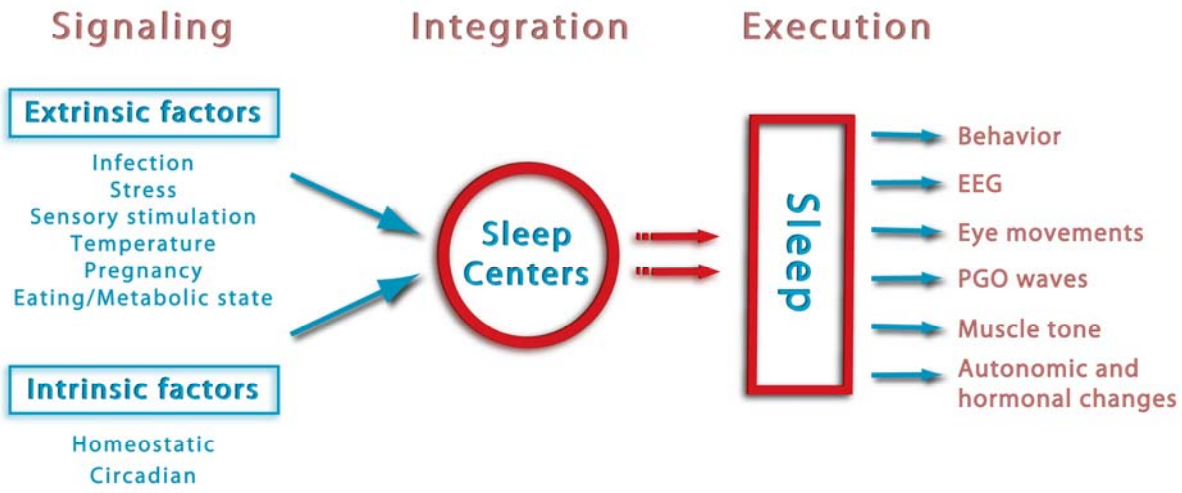

Fig. 1. Intrinsic and extrinsic factors in signaling sleep.

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.***

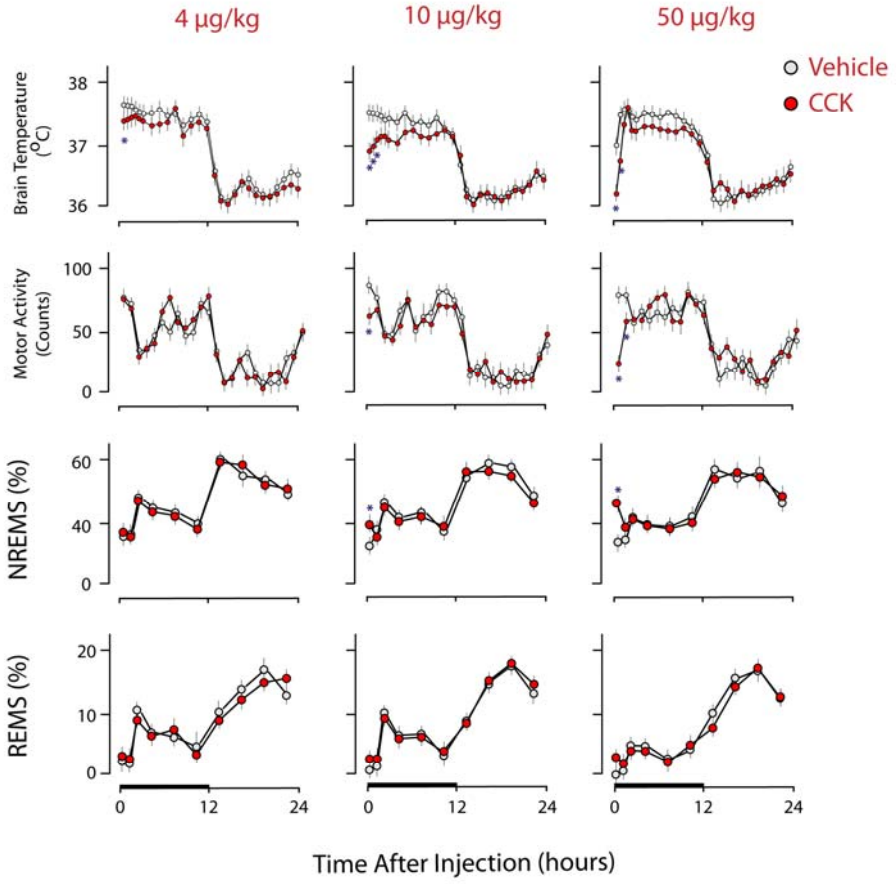

Fig. 2. The effects of the intraperitoneal (ip) injection of cholecystokinin octapeptide sulfate ester (CCK) on brain temperature (T_{br}), motor activity, non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in rats. Motor activity is shown in 1-h data blocks; T_{br} is shown in 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,

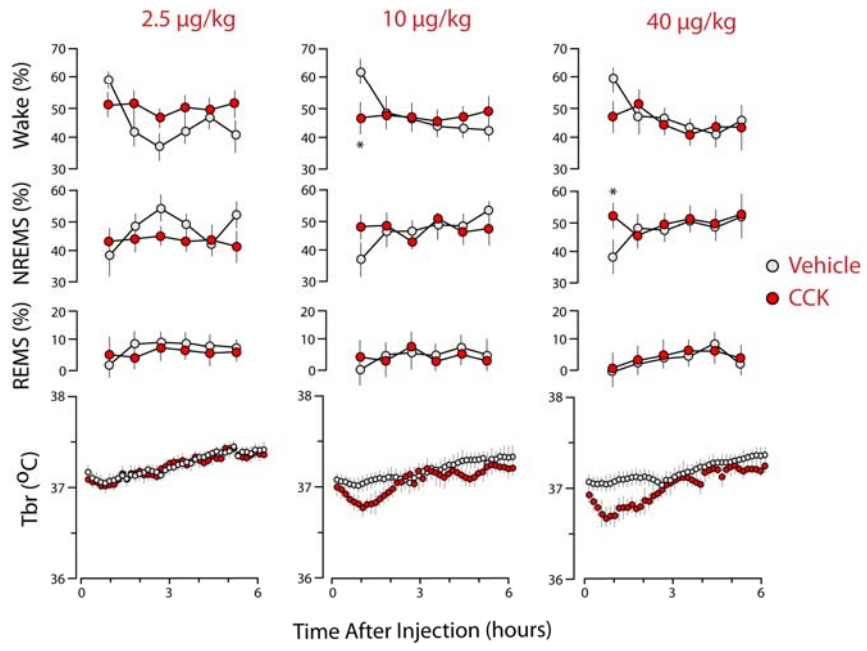

Fig. 3. The effects of ip injection of CCK on sleep and T_{br} in rabbits. Sleep is expressed as percent of recording time in 1-h data blocks, temperature is shown in 10-min intervals. Time 0: injection time. Asterisks: significant difference between baseline and CCK treatment, $p < 0.05$, paired *t*-test. 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: $F(1,18) = 4.7$, $p < 0.05$].

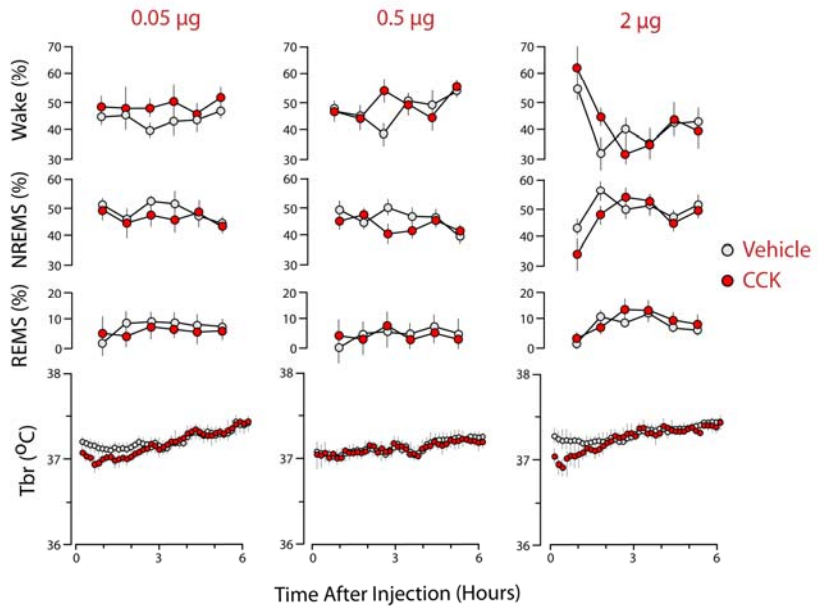

Fig. 4. The effects of intracerebroventricular injection of CCK on sleep and T_{br} in rabbits. See legend to Fig. 3 for details. Modified from Kapás et al., 1991.

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, $p < 0.05$).

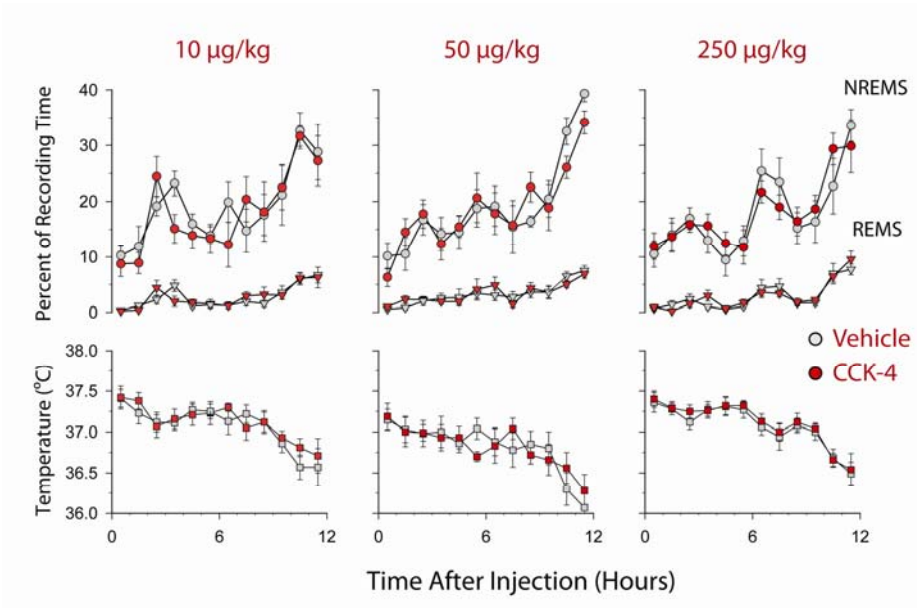

Fig. 5. The effects of ip injection of cholecystokinin tetrapeptide (CCK-4) on sleep and T_{br}. Sleep is expressed as percent of recording time in 1-h data blocks, temperature is shown as 1-h averages. Time 0: injection time. Error bars: SE. Modified from Chang and Kapás, 1997.

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).

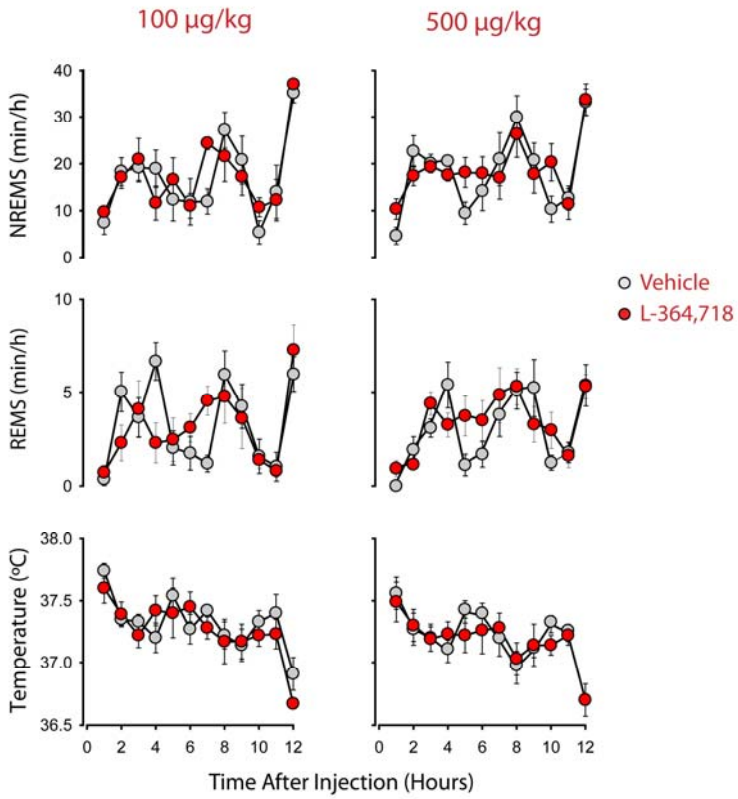

Fig. 6. The effects of ip injections of L-364,718, a CCK1 receptor antagonist on sleep and T_{br} in rats. See legend to Fig. 5 for details.

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).

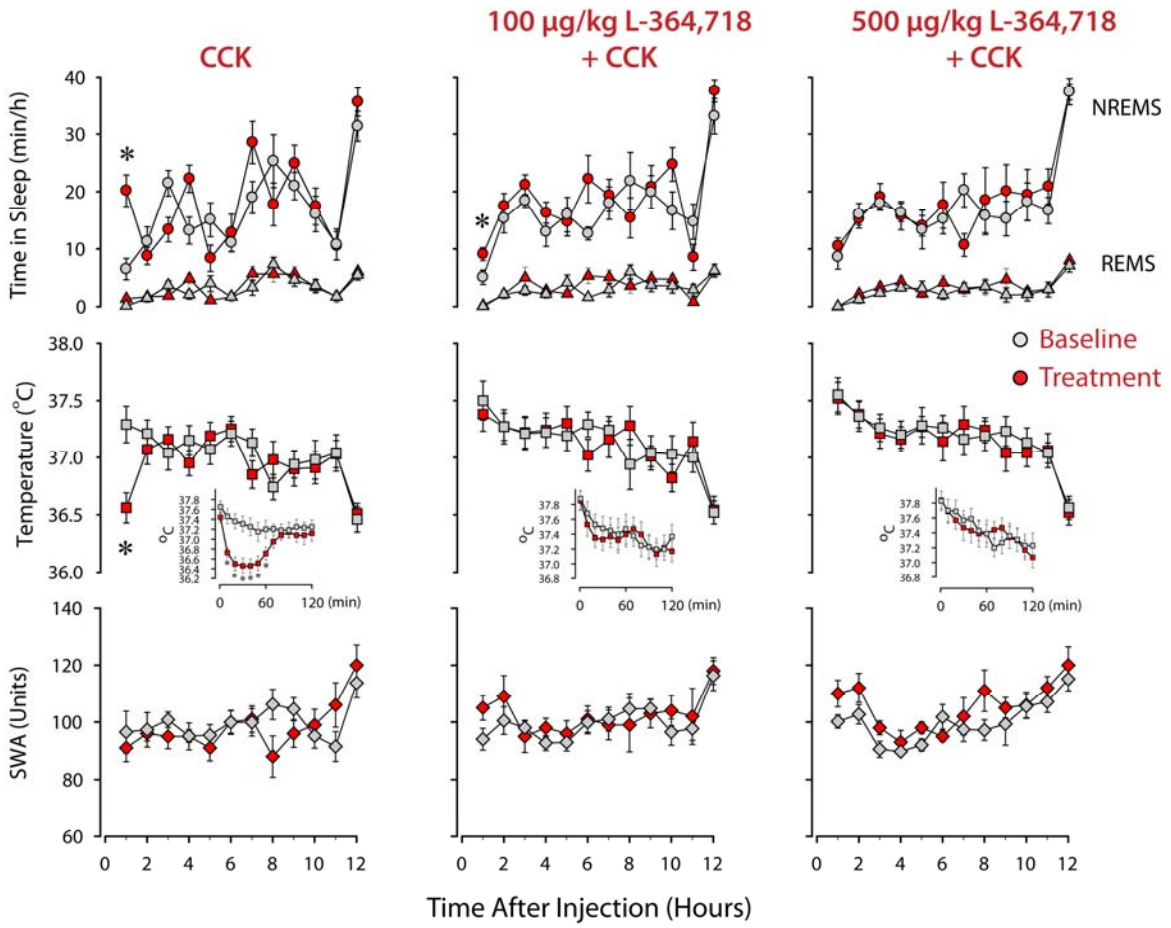

Fig. 7. The effects of L-364,718 pretreatment on CCK-induced sleep and hypothermic responses. Sleep and the slow-wave activity of the electroencephalogram during NREMS (SWA) are shown in 1-h data blocks. On the main T_{br} panel, average hourly temperatures are shown for 12 h. On the insets, T_{br} is 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.

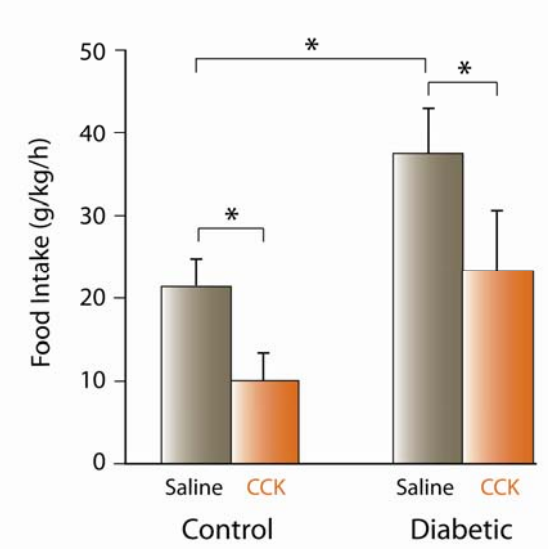

Fig. 8. The effects of ip injection of 10 µg/kg CCK on 1-h food intake in normal and streptozotocin-diabetic rats. Asterisks: $p < 0.05$, Student's *t*-test for between-group and paired *t*-test for within-group comparisons.

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.

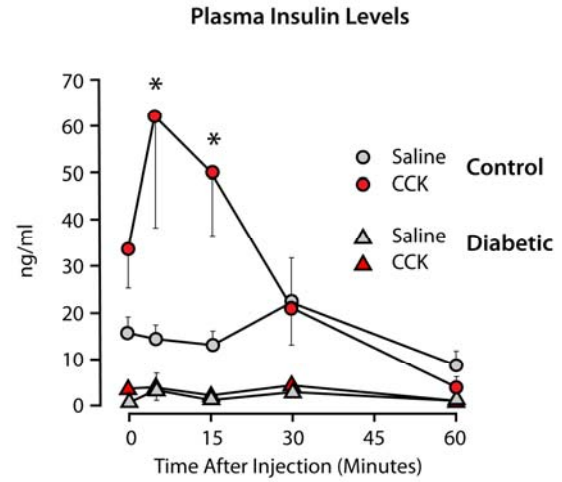

Fig. 9. The effects of ip injection of 10 µg/kg CCK on plasma insulin levels in normal and streptozotocin-diabetic rats. Error bars: SE. Asterisks: significant difference between saline and CCK treatments in the control group. Time 0: pre-injection baseline values. Modified from Kapás et al., 1991.

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

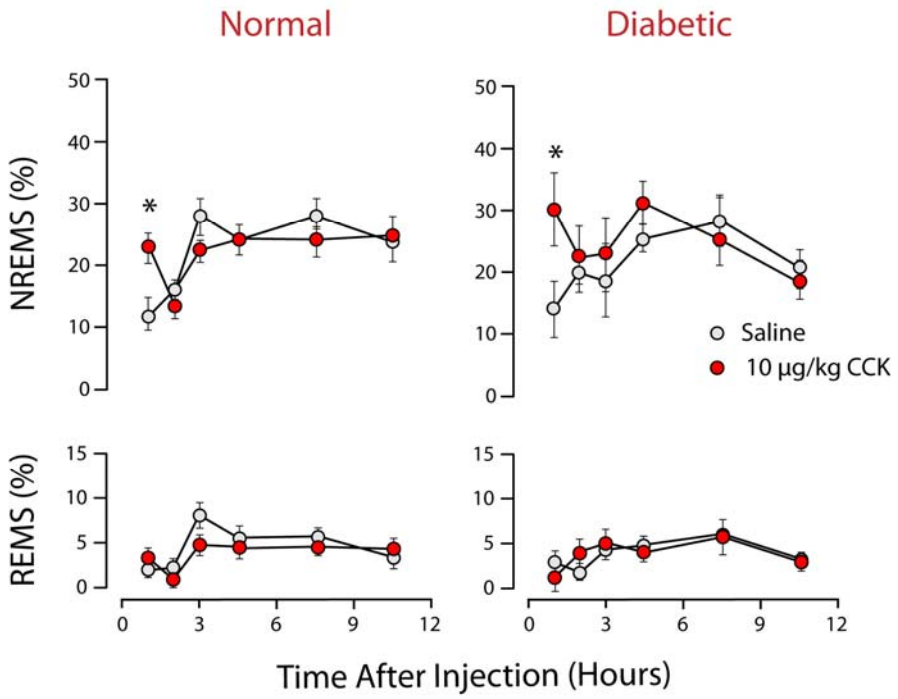

Fig. 10. The effects of ip injection of 10 µg/kg CCK on sleep in normal and streptozotocin-diabetic rats. Sleep is expressed as percent of recording time in 1-h blocks for the first three hours and in 3-h blocks from h 4 to 12. Asterisks: significant difference between saline and CCK treatment (paired *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).

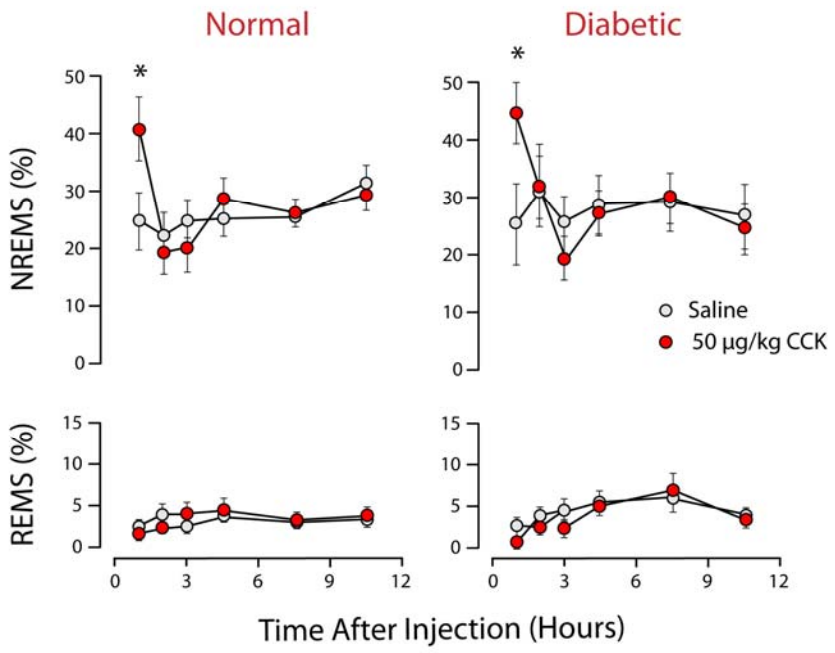

Fig. 11. The effects of ip injection of 50 µg/kg CCK in normal and streptozotocin-diabetic rats. See legends to Fig. 10 for details. Modified from Kapás et al., 1991.

<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.

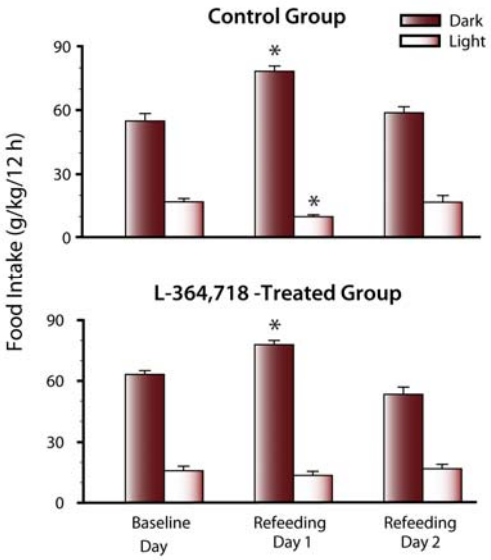

Fig. 12. Food intake of control and CCK1 antagonist (L-364,718)-treated rats under baseline conditions and on refeeding days 1 and 2 after food deprivation. Asterisk: significant difference from baseline (paired *t*-test, $p < 0.05$). Modified from Shemyakin and Kapás, 2001.

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.

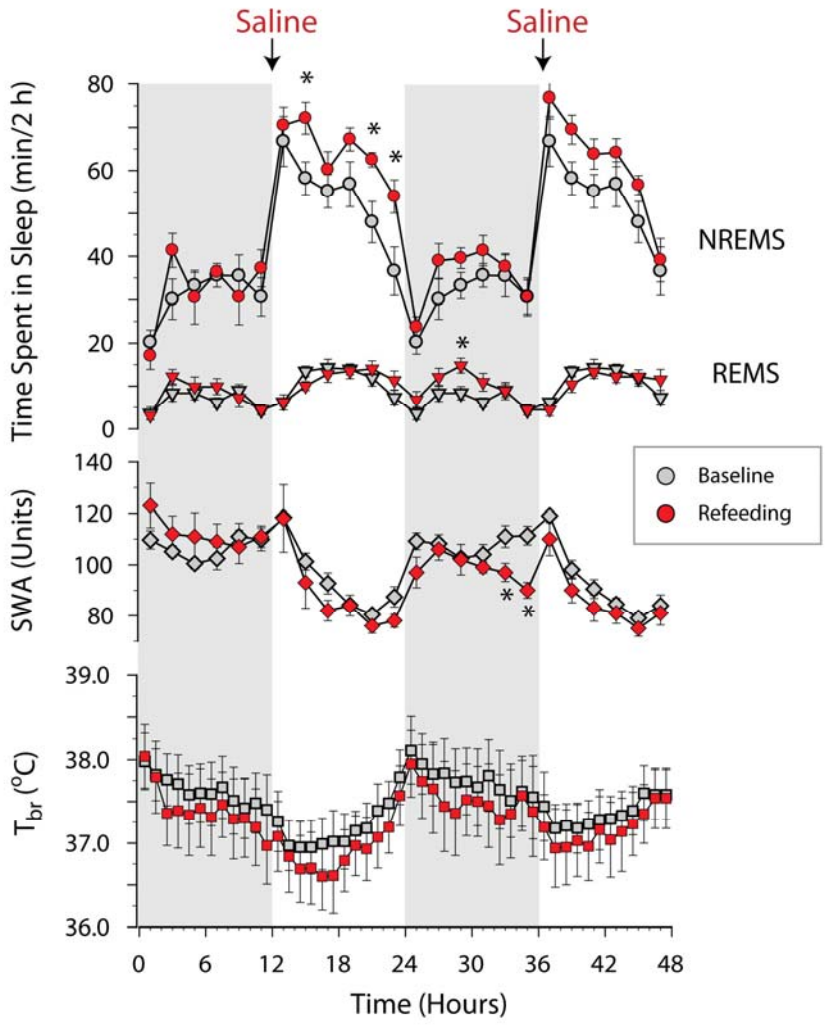

Fig. 13. The effects of refeeding on NREMS, REMS, SWA and T_{br} in control rats. Time spent in sleep is summed in 2-h blocks. SWA is averaged in 2-h and T_{br} in 1-h intervals. Error bar: SE. Gray shaded area: dark phase. Asterisks: significant difference from baseline ($p < 0.05$, paired *t*-test).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

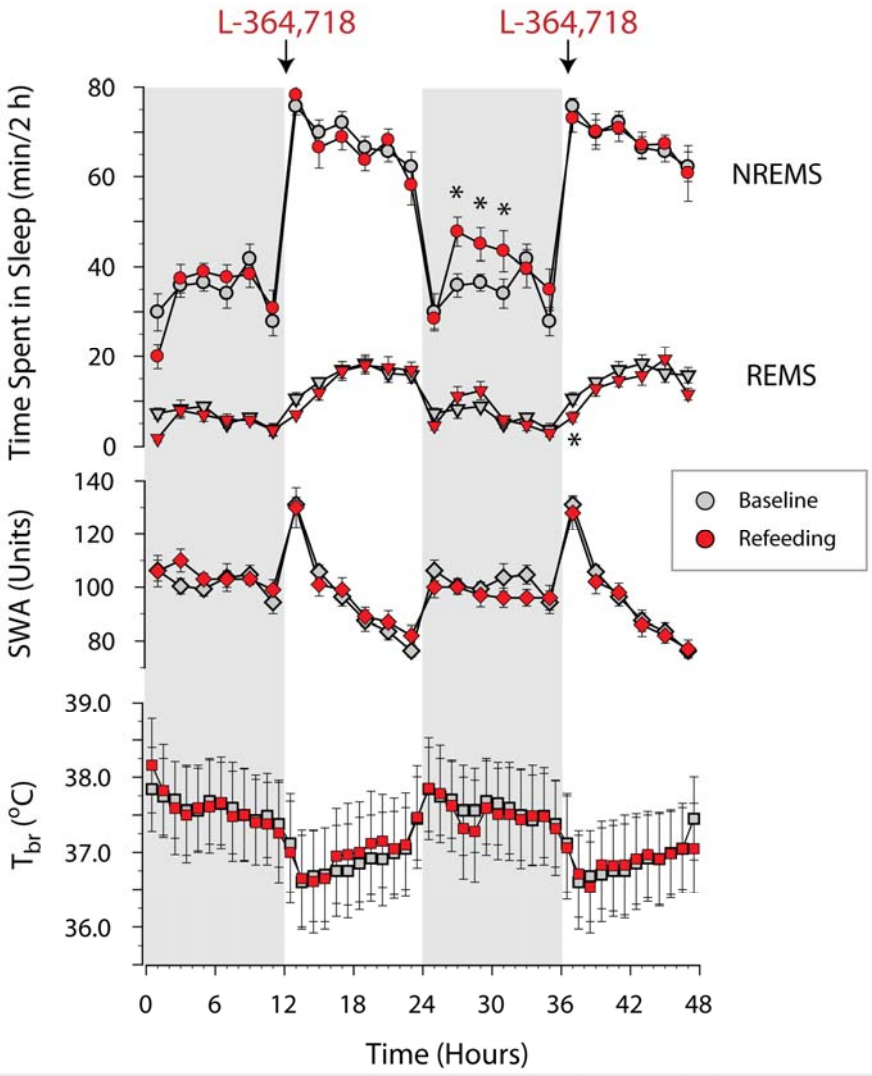

Fig. 14. The effects of refeeding on NREMS, REMS, SWA and T_{br} in L-364,718-treated rats. See legend to Fig. 13 for details. Modified from Shemyakin and Kapás, 2001.

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.

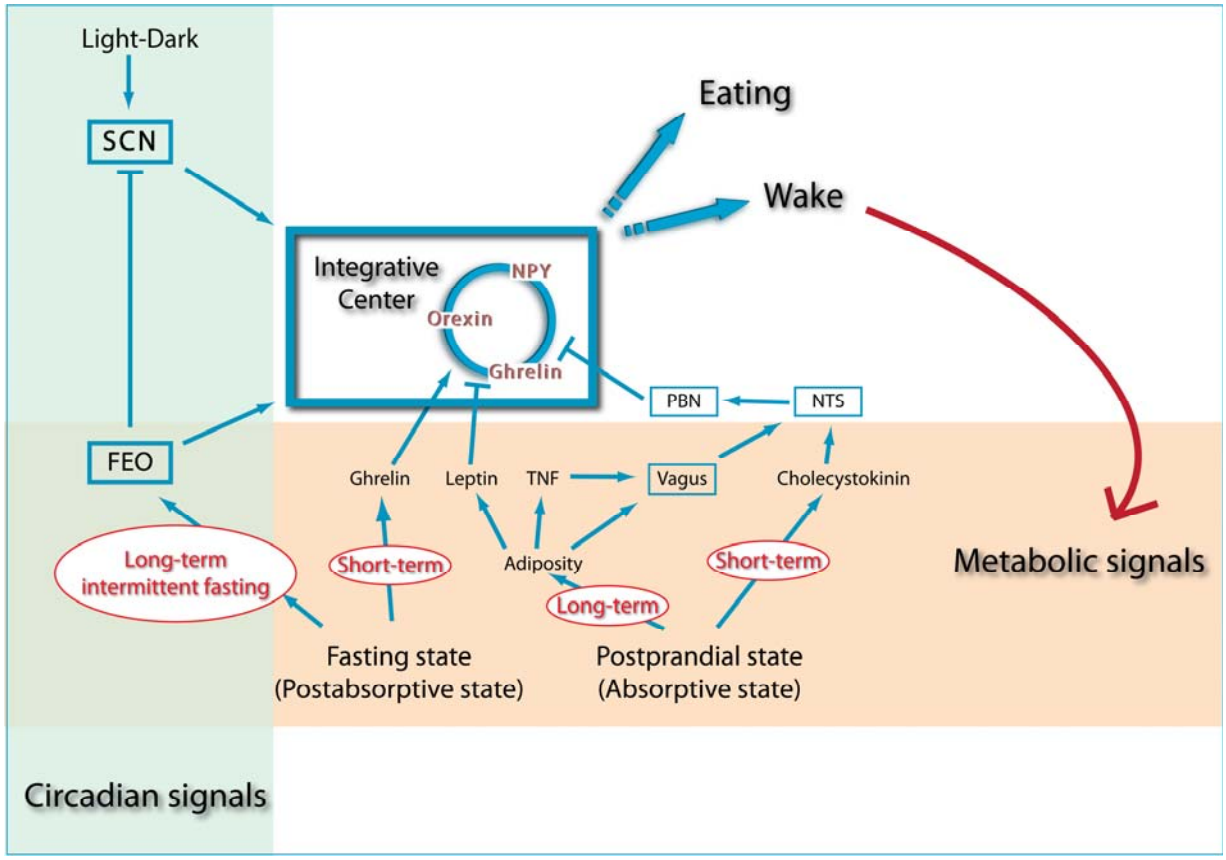

Fig. 15. Integration of metabolic and circadian signals in the regulation of sleep, wakefulness and feeding.

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References

Allison, T., Cicchetti, D.V., 1976. Sleep in mammals: ecological and constitutional correlates. Science 194, 732-734.

Antin, J., Gibbs, J., Holt, J., Young, R.C., Smith, G.P., 1975. Cholecystokinin elicits the complete behavioral sequence of satiety in rats. J Comp Physiol Psychol 89, 784-790.

Antle, M.C., Silver, R., 2009. Neural basis of timing and anticipatory behaviors. Eur J Neurosci 30, 1643-1649.

Appleyard, S.M., Bailey, T.W., Doyle, M.W., Jin, Y.H., Smart, J.L., Low, M.J., Andresen, M.C., 2005. Proopiomelanocortin neurons in nucleus tractus solitarius are activated by visceral afferents: regulation by cholecystokinin and opioids. J Neurosci 25, 3578-3585.

Beinfeld, M.C., 2003. Biosynthesis and processing of pro CCK: recent progress and future challenges. Life Sci 72, 747-757.

Beinfeld, M.C., Palkovits, M., 1981. Distribution of cholecystokinin (CCK) in the hypothalamus and limbic system of the rat. Neuropeptides 2, 123-129.

Bernstein, I.L., 1974. Post-prandial EEG synchronization in normal and hypothalamically lesioned rats. Physiol Behav 12, 535-545.

Bester, H., Besson, J.M., Bernard, J.F., 1997. Organization of efferent projections from the parabrachial area to the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. J Comp Neurol 383, 245-281.

Bhatnagar, S., Viau, V., Chu, A., Soriano, L., Meijer, O.C., Dallman, M.F., 2000. A cholecystokinin-mediated pathway to the paraventricular thalamus is recruited in chronically stressed rats and regulates hypothalamic-pituitary-adrenal function. J Neurosci 20, 5564-5573.

Blevins, J.E., Stanley, B.G., Reidelberger, R.D., 2000. Brain regions where cholecystokinin suppresses feeding in rats. Brain Res 860, 1-10.

Booth, D.A., 1972. Some characteristics of feeding during streptoxotocin-induced diabetes in the rat. J Comp Physiol Psychol 80, 238-249.

Borbély, A.A., 1977. Sleep in the rat during food deprivation and subsequent restitution of food. Brain Res124, 457-471.

Borbély, A.A., 1982. A two process model of sleep regulation. Hum Neurobiol 1, 195-204.

Brezinova, V., Oswald, I., 1972. Sleep after a bedtime beverage. Br Med J 2, 431-433.

Broberger, C., Holmberg, K., Shi, T.J., Dockray, G., Hokfelt, T., 2001. Expression and regulation of cholecystokinin and cholecystokinin receptors in rat nodose and dorsal root ganglia. Brain Res 903, 128-140.

Cain, B.M., Connolly, K., Blum, A., Vishnuvardhan, D., Marchand, J.E., Beinfeld, M.C., 2003. Distribution and colocalization of cholecystokinin with the prohormone convertase enzymes PC1, PC2, and PC5 in rat brain. J Comp Neurol 467, 307-325.

Chang, R.S., Lotti, V.J., 1986. Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc Natl Acad Sci USA 83, 4923-4926.

Corp, E.S., McQuade, J., Moran, T.H., Smith, G.P., 1993. Characterization of type A and type B CCK receptor binding sites in rat vagus nerve. Brain Res 623, 161-166.

Crawley, J.N., 1985. Clarification of the behavioral functions of peripheral and central cholecystokinin: two separate peptide pools. Peptides 6 Suppl 2, 129-136.

Crawley, J.N., Corwin, R.L., 1994. Biological actions of cholecystokinin. Peptides15, 731-755.

Crawley, J.N., Hays, S.E., Paul, S.M., 1981a. Vagotomy abolishes the inhibitory effects of cholecystokinin on rat exploratory behaviors. Eur J Pharmacol 73, 379-380.

Crawley, J.N., Hays, S.E., Paul, S.M., Goodwin, F.K., 1981b. Cholecystokinin reduces exploratory behavior in mice. Physiol Behav 27, 407-411.

Dalsgaard, C.J., Vincent, S.R., Hokfelt, T., Lundberg, J.M., Dahlstrom, A., Schultzberg, M., Dockray, G.J., Cuello, A.C., 1982. Coexistence of cholecystokinin- and substance P-like peptides in neurons of the dorsal root ganglia of the rat. Neurosci Lett 33, 159-163.

Danguir, J., 1984. Sleep deficits in diabetic rats: restoration following chronic intravenous or intracerebroventricular infusions of insulin. Brain Res Bull 12, 641-645.

Danguir, J., 1987. Cafeteria diet promotes sleep in rats. Appetite 8, 49-53.

Danguir, J., 1989. Sleep patterns in the genetically obese Zucker rat: effect of acarbose treatment. Am J Physiol 256, R281-R283.

Danguir, J., Nicolaidis, S., 1978. Sleep and feeding patterns in the ventromedial hypothalamic lesioned rat. Physiol Behav 21, 769-777.

Danguir, J., Nicolaidis, S., 1979. Dependence of sleep on nutrients' availability. Physiol Behav 22, 735-740.

Danguir, J., Nicolaidis, S., 1980a. Circadian sleep and feeding patterns in the rat: possible dependence on lipogenesis and lipolysis. Am J Physiol 238, E223-E230.

Danguir, J., Nicolaidis, S., 1980b. Intravenous infusions of nutrients and sleep in the rat: an ischymetric sleep regulation hypothesis. Am J Physiol 238, E307-E312.

Danguir, J., Nicolaidis, S., 1984. Chronic intracerebroventricular infusion of insulin causes selective increase of slow wave sleep in rats. Brain Res 306, 97-103.

Danguir, J., Nicolaidis, S., Gerard, H., 1979. Relations between feeding and sleep patterns in the rat. J Comp Physiol Psychol 93, 820-830.

de Saint Hilaire, Z., Roques, B.P., Nicolaidis, S., 1991. Effect of a highly selective central CCK-B receptor agonist: BC-264 on rat sleep. Pharmacol Biochem Behav 38, 545-548.

de Saint Hilaire-Kafi, Z., Depoortere, H., Nicolaidis, S., 1989. Does cholecystokinin induce physiological satiety and sleep? Brain Res 488, 304-310.

DeMesquita, S., Haney, W.H., 1986. Effect of chronic intracerebroventricular infusion of cholecystokinin on respiration and sleep. Brain Res 378, 127-132.

Dockray, G.J., 1976. Immunochemical evidence of cholecystokinin-like peptides in brain. Nature 264, 568-570.

Dockray, G.J., 2009. Cholecystokinin and gut-brain signalling. Regul Pept 155, 6-10.

Dockray, G.J., Gregory, R.A., Tracy, H.J., Zhu, W.Y., 1981. Transport of cholecystokinin-octapeptide-like immunoreactivity toward the gut in afferent vagal fibres in cat and dog. J Physiol 314, 501-511.

Dourish, C.T., Rycroft, W., Iversen, S.D., 1989. Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors. Science 245, 1509-1511.

Dufresne, M., Seva, C., Fourmy, D., 2006. Cholecystokinin and gastrin receptors. Physiol Rev 86, 805-847.

Fara, J.W., Rubinstein, E.H., Sonnenschein, R.R., 1969. Visceral and behavioral responses to intraduodenal fat. Science 166, 110-111.

Faris, P.L., Komisaruk, B.R., Watkins, L.R., Mayer, D.J., 1983. Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. Science 219, 310-312.

Flood, J.F., Smith, G.E., Morley, J.E., 1987. Modulation of memory processing by cholecystokinin: dependence on the vagus nerve. Science 236, 832-834.

Garby, L., Kurzer, M.S., Lammert, O., Nielsen, E., 1987. Energy expenditure during sleep in men and women: evaporative and sensible heat losses. Hum Nutr Clin Nutr 41, 225-233.

Ghosh, S., Geller, E.B., Adler, M.W., 1997. Interaction of cholecystokinin and somatostatin with a selective mu-opioid agonist and mu- and kappa-antagonists in thermoregulation. Brain Res 745, 152-157.

Ghosh, S., Handler, C.M., Geller, E.B., Adler, M.W., 1998. Effect of a mu-selective opioid antagonist on CCK-8-induced changes in thermoregulation in the rat. Pharmacol Biochem Behav 59, 261-264.

Gilbert, K.A., Lydic, R., 1994. Pontine cholinergic reticular mechanisms cause state-dependent changes in the discharge of parabrachial neurons. Am J Physiol 266, R136-R150.

Gilligan, J.G., Milanes, L., Chang, H.Y., Ribeiro, A.C., Kapás, L., 1998. Intracerebroventricular injections of cholecystokinin in rats does not elicit changes in sleep or brain temperature. Sleep7 (Suppl. 2), 30.

Grill, H.J., 2006. Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. Obesity 14 Suppl 5, 216S-221S.

Gross, P.M., Wall, K.M., Pang, J.J., Shaver, S.W., Wainman, D.S., 1990. Microvascular specializations promoting rapid interstitial solute dispersion in nucleus tractus solitarius. Am J Physiol 259, R1131-R1138.

Gulpinar, M.A., Yegen, B.C., 2004. The physiology of learning and memory: role of peptides and stress. Curr Protein Pept Sci 5, 457-473.

Handelmann, G., Meyer, D.K., Beinfeld, M.C., Oertel, W.H., 1981. CCK-containing terminals in the hippocampus are derived from intrinsic neurons: an immunohistochemical and radioimmunological study. Brain Res 224, 180-184.

Hansen, M.K., Kapás, L., Fang, J., Krueger, J.M., 1998. Cafeteria diet-induced sleep is blocked by subdiaphragmatic vagotomy in rats. Am J Physiol 274, R168-R174.

Harper, A.A., Raper, H.S., 1943. Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J Physiol 102, 115-125.

Hewson, G., Leighton, G.E., Hill, R.G., Hughes, J., 1988. The cholecystokinin receptor antagonist L364,718 increases food intake in the rat by attenuation of the action of endogenous cholecystokinin. Br J Pharmacol 93, 79-84.

Hill, D.R., Campbell, N.J., Shaw, T.M., Woodruff, G.N., 1987. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J Neurosci 7, 2967-2976.

Hockman, C.H., 1964. EEG and behavioral effects of food deprivation in the albino rat. Electroencephalogr Clin Neurophysiol 17, 420-427.

Hokfelt, T., Cortes, R., Schalling, M., Ceccatelli, S., Pelto-Huikko, M., Persson, H., Villar, M.J., 1991. Distribution patterns of CCK and CCK mRNA in some neuronal and non-neuronal tissues. Neuropeptides19 Suppl, 31-43.

Hokfelt, T., Rehfeld, J.F., Skirboll, L., Ivemark, B., Goldstein, M., Markey, K., 1980. Evidence for coexistence of dopamine and CCK in meso-limbic neurones. Nature 285, 476-478.

Hsiao, S., Katsuura, G., Itoh, S., 1984. Cholecystokinin tetrapeptide, proglumide and open-field behavior in rats. Life Sci 34, 2165-2168.

Hunt, C.A., Seroogy, K.B., Gall, C.M., Jones, E.G., 1987. Cholecystokinin innervation of rat thalamus, including fibers to ventroposterolateral nucleus from dorsal column nuclei. Brain Res 426, 257-269.

Innis, R.B., Snyder, S.H., 1980. Distinct cholecystokinin receptors in brain and pancreas. Proc Natl Acad Sci U S A 77, 6917-6921.

Ivy, A.C., Oldberg, E., 1928. A hormone mechanism for gall-bladder contraction and evacuation. Am J Physiol 86, 599-613.

Jacobs, B.L., McGinthy, D.J., 1971. Effects of food deprivation on sleep and wakefulness in the rat. Exp Neurol 30, 212-222.

Jensen, R.T., Lemp, G.F., Gardner, J.D., 1980. Interaction of cholecystokinin with specific membrane receptors on pancreatic acinar cells. Proc Natl Acad Sci U S A 77, 2079-2083.

Jones, B.E., 2003. Arousal systems. Front Biosci 8, s438-s451.

Jorpes, E., Mutt, V., Toczko, K., 1964. Further purification of cholecystokinin and pancreozymin. Acta Chem Scand 18, 2408-2410.

Kapás, L., 1997. Subdiaphragmatic vagotomy does not prevent the somnogenic and hypothermic effects of cholecystokinin (CCK). Sleep Res 26, 76.

Kapás, L., Benedek, G., Penke, B., 1989. Cholecystokinin interferes with the thermoregulatory effect of exogenous and endogenous opioids. Neuropeptides14, 85-92.

Kapás, L., Hong, L., Cady, A.B., Opp, M.R., Postlethwaite, A.E., Seyer, J.M., Krueger, J.M., 1992. Somnogenic, pyrogenic, and anorectic activities of tumor necrosis factor-alpha and TNF-alpha fragments. Am J Physiol 263, R708-R715.

Kapás, L., Krueger, J.M., 1992. Tumor necrosis factor-beta induces sleep, fever, and anorexia. Am J Physiol 263, R703-R707.

Kapás, L., Obál, F., Jr., Penke, B., Obál, F., 1987. Cholecystokinin-octapeptide-induced hypothermia in rats: dose-effect and structure-effect relationships, effect of ambient temperature, pharmacological interactions and tolerance. Neuropharmacology 26, 131-137.

Kapás, L., Payne, L., Obál, F., Jr., Opp, M., Johannsen, L., Krueger, J.M., 1991. Sleep in diabetic rats: effects of interleukin 1. Am J Physiol 260, R995-R999.

Katsuura, G., Hirota, R., Itoh, S., 1981. Cholecystokinin-induced hypothermia in the rat. Experientia 37, 60.

Kershaw, E.E., Flier, J.S., 2004. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89, 2548-2556.

Kimura, I., Wakasono, S., Kimura, M., 1995. Loxiglumide, L-364,718 and L-365,260 prevent the inhibition of spontaneous acetylcholine release from the frontal cerebral cortex of freely moving rat peripherally administered with cholecystokinin-8S. Jpn J Pharmacol 68, 129-132.

Kubota, T., Fang, J., Guan, Z., Brown, R.A., Krueger, J.M., 2001. Vagotomy attenuates tumor necrosis factor-alpha-induced sleep and EEG delta-activity in rats. Am J Physiol 280, R1213-R1220.

Kumaresan, P., Turner, C.W., 1965. Effect of alloxan on feed consumption in rats. Proc Soc Exp Biol Med 119, 400-402.

Lacey, J.H., Crisp, A.H., Kalucy, R.S., Hartmann, M.K., Chien, C.N., 1975. Weight gain and the sleeping electroencephalogram: study of 10 patients with anorexia nervosa. Br Med J 4, 556-558.

Lacey, J.H., Stanley, P., Hartmann, M., Koval, J., Crisp, A.H., 1978. The immediate effects of intravenous specific nutrients on EEG sleep. Electroencephalogr Clin Neurophysiol 44, 275-280.

Larsson, L.I., Rehfeld, J.F., 1979. Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Res 165, 201-218.

Legido, A., Adler, M.W., Karkanias, C., Geller, E.B., Bradley, E., Greenstein, J.I., Grover, W.D., 1995. Cholecystokinin potentiates morphine anticonvulsant action through both CCK-A and CCK-B receptors. Neuropeptides 28, 107-113.

Liddle, R.A., Goldfine, I.D., Rosen, M.S., Taplitz, R.A., Williams, J.A., 1985. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. J Clin Invest 75, 1144-1152.

Lieberman, A.R., 1971. The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol 14, 49-124.

Lin, C.W., Miller, T.R., 1992. Both CCK-A and CCK-B/gastrin receptors are present on rabbit vagus nerve. Am J Physiol 263, R591-R595.

Liu, H.J., Lin, M.T., 1985. Cholecystokinin-induced hypothermia: possible involvement of serotoninergic mechanisms in the rat hypothalamus. Pharmacology 31, 108-114.

Lloyd, H.M., Green, M.W., Rogers, P.J., 1994. Mood and cognitive performance effects of isocaloric lunches differing in fat and carbohydrate content. Physiol Behav 56, 51-57.

Loewy, A.D., Burton, H., 1978. Nuclei of the solitary tract: efferent projections to the lower brain stem and spinal cord of the cat. J Comp Neurol 181, 421-449.

Lorenz, D.N., 1986. Alimentary sleep satiety in suckling rats. Physiol Behav 38, 557-562.

Lotti, V.J., Pendleton, R.G., Gould, R.J., Hanson, H.M., Chang, R.S., Clineschmidt, B.V., 1987. In vivo pharmacology of L-364,718, a new potent nonpeptide peripheral cholecystokinin antagonist. J Pharmacol ExpTher 241, 103-109.

Mansbach, R.S., Lorenz, D.N., 1983. Cholecystokinin (CCK-8) elicits prandial sleep in rats. Physiol Behav 30, 179-183.

Mantyh, P.W., Hunt, S.P., 1984. Neuropeptides are present in projection neurones at all levels in visceral and taste pathways: from periphery to sensory cortex. Brain Res 299, 297-312.

McGinty, D., Szymusiak, R., 2003. Hypothalamic regulation of sleep and arousal. Front Biosci 8, s1074-s1083.

McLaughlin, C.L., Baile, C.A., Della-Fera, M.A., Kasser, T.G., 1985. Meal-stimulated increased concentrations of CCK in the hypothalamus of Zucker obese and lean rats. Physiol Behav 35, 215-220.

Miceli, M.O., Steiner, M., 1989. Novel localizations of central- and peripheral-type cholecystokinin binding sites in Syrian hamster brain as determined by autoradiography. Eur J Pharmacol 169, 215-224.

Mollereau, C., Roumy, M., Zajac, J.M., 2005. Opioid-modulating peptides: mechanisms of action. Curr Top Med Chem 5, 341-355.

Moran, T.H., Robinson, P.H., Goldrich, M.S., McHugh, P.R., 1986. Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res 362, 175-179.

Morino, P., Herrera-Marschitz, M., Meana, J.J., Ungerstedt, U., Hokfelt, T., 1992. Immunohistochemical evidence for a crossed cholecystokinin corticostriatal pathway in the rat. Neurosci Lett 148, 133-136.

Morley, J.E., Levine, A.S., Lindblad, S., 1981. Intraventricular cholecystokinin-octapeptide produces hypothermia in rats. Eur J Pharmacol.74, 249-251.

Passaro E Jr, Debas, H., Oldendorf, W., Yamada, T., 1982. Rapid appearance of intraventricularly administered neuropeptides in the peripheral circulation. Brain Res 241, 335-340.

Pisegna, J.R., de, W.A., Huppi, K., Wank, S.A., 1992. Molecular cloning of the human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization. Biochem Biophys Res Commun 189, 296-303.

Posadas-Andrews, A., Prospero-Garcia, O., Rojas-Ramirez, J.A., 1989. CCK-8 effects on sleep and feeding. Soc Neurosci Abst15, part 2, 1068.

Prospero-Garcia, O., Ott, T., Drucker-Colin, R., 1987. Cerebroventricular infusion of cholecystokinin (CCK-8) restores REM sleep in parachlorophenylalanine (PCPA)-pretreated cats. Neurosci Lett 78, 205-210.

Puizillout, J.J., Foutz, A.S., 1977. Characteristics of the experimental reflex sleep induced by vago-aortic nerve stimulation. Electroencephalogr Clin Neurophysiol 42, 552-563.

Pullen, R.G., Hodgson, O.J., 1987. Penetration of diazepam and the non-peptide CCK antagonist, L-364,718, into rat brain. J Pharm Pharmacol 39, 863-864.

Qian, M., Johnson, A.E., Kallstrom, L., Carrer, H., Sodersten, P., 1997. Cholecystokinin, dopamine D2 and N-methyl-D-aspartate binding sites in the nucleus of the solitary tract of the rat: possible relationship to ingestive behavior. Neuroscience 77, 1077-1089.

Reagan, J.E., Robinson, J.L., Lotti, V.J., Goldman, M.E., 1987. Fasting and L-364,718 prevent cholecystokinin-induced elevations of plasma insulin levels. Eur J Pharmacol 144, 241-243.

Rehfeld, J.F., 1978. Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J Biol Chem 253, 4022-4030.

Reidelberger, R.D., O'Rourke, M.F., 1989. Potent cholecystokinin antagonist L 364718 stimulates food intake in rats. Am J Physiol 257, R1512-R1518.

Rezayat, M., Ravandeh, N., Zarrindast, M.R., 1999. Cholecystokinin and morphine-induced hypothermia. Eur Neuropsychopharmacol 9, 219-225.

Rojas-Ramirez, J.A., Crawley, J.N., Mendelson, W.B., 1982. Electroencephalographic analysis of the sleep-inducing actions of cholecystokinin. Neuropeptides 3, 129-138.

Roky, R., Kapás, L., Taishi, T.P., Fang, J., Krueger, J.M., 1999. Food restriction alters the diurnal distribution of sleep in rats. Physiol Behav 67, 697-703.

Rosen, A.J., Davis, J.D., ladove, R.F., 1971. Electrocortical activity: modification by food ingestion and a humoral satiety factor. Comm Behav Biol 6, 323-327.

Rotzinger, S., Vaccarino, F.J., 2003. Cholecystokinin receptor subtypes: role in the modulation of anxiety-related and reward-related behaviours in animal models. J Psychiatry Neurosci 28, 171-181.

Saito, A., Sankaran, H., Goldfine, I.D., Williams, J.A., 1980. Cholecystokinin receptors in the brain: characterization and distribution. Science 208, 1155-1156.

Saito, H., Sakai, K., Jouvet, M., 1977. Discharge patterns of the nucleus parabrachialis lateralis neurons of the cat during sleep and waking. Brain Res 134, 59-72.

Sangiah, S., Caldwell, D.F., Villeneuve, M.J., Clancy, J.J., 1982. Sleep: sequential reduction of paradoxical (REM) and elevation of slow-wave (NREM) sleep by a non-convulsive dose of insulin in rats. Life Sci 31, 763-769.

Saper, C.B., Loewy, A.D., 1980. Efferent connections of the parabrachial nucleus in the rat. Brain Res 197, 291-317.

Schick, R.R., Yaksh, T.L., Go, V.L., 1986. An intragastric meal releases the putative satiety factor cholecystokinin from hypothalamic neurons in cats. Brain Res 370, 349-353.

Shido, O., Yoneda, Y., Nagasaka, T., 1989. Changes in brown adipose tissue metabolism following intraventricular vasoactive intestinal peptide and other gastrointestinal peptides in rats. Jpn J Physiol 39, 359-369.

Smith, A., Ralph, A., McNeill, G., 1991. Influences of meal size on post-lunch changes in performance efficiency, mood, and cardiovascular function. Appetite 16, 85-91.

Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R., Simansky, K.J., 1981. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science213, 1036-1037.

Soar, J., Hewson, G., Leighton, G.E., Hill, R.G., Hughes, J., 1989. L364,718 antagonizes the cholecystokinin-induced suppression of locomotor activity. Pharmacol Biochem Behav 33, 637-640.

South, E.H., 1992. Cholecystokinin reduces body temperature in vehicle- but not capsaicin-pretreated rats. Am J Physiol 263, R1215-R1221.

Southwell, P.R., Evans, C.R., Hunt, J.N., 1972. Effect of a hot milk drink on movements during sleep. Br Med J 2, 429-431.

Stahl, M.L., Orr, W.C., Bollinger, C., 1983. Postprandial sleepiness: objective documentation via polysomnography. Sleep 6, 29-35.

Sugimoto, N., Simons, C.T., Romanovsky, A.A., 1999. Vagotomy does not affect thermal responsiveness to intrabrain prostaglandin E2 and cholecystokinin octapeptide. Brain Res 844, 157-163.

Szecowka, J., Lins, P.E., Efendic, S., 1982. Effects of cholecystokinin, gastric inhibitory polypeptide, and secretin on insulin and glucagon secretion in rats. Endocrinology110, 1268-1272.

Székely, M., Szelényi, Z., Balasko, M., 1994. Cholecystokinin participates in the mediation of fever. Pflugers Arch 428, 671-673.

Szelényi, Z., Barthó, L., Székely, M., Romanovsky, A.A., 1994. Cholecystokinin octapeptide (CCK-8) injected into a cerebral ventricle induces a fever-like thermoregulatory response mediated by type B CCK-receptors in the rat. Brain Res 638, 69-77.

Szelényi, Z., Hummel, Z., Székely, M., Petervari, E., 2004. CCK-8 and PGE1: central effects on circadian body temperature and activity rhythms in rats. Physiol Behav 81, 615-621.

Szentirmai, É., Kapás, L., Krueger, J.M., 2007a. Ghrelin microinjection into forebrain sites induces wakefulness and feeding in rats. Am J Physiol 292, R575-R585.

Szentirmai, E., Kapás, L., Sun, Y., Smith, R.G., Krueger, J.M., 2009. The preproghrelin gene is required for the normal integration of thermoregulation and sleep in mice. Proc Natl Acad Sci U S A 106, 14069-14074.

Szentirmai, E., Kapás, L., Sun, Y., Smith, R.G., Krueger, J.M., 2010. Restricted feeding-induced sleep, activity, and body temperature changes in normal and preproghrelin-deficient mice. Am J Physiol 298, R467-R477.

Szentirmai, É., Kapás, L., Sun, Y., Smith, R.G., Krueger, J.M., 2007b. Sleep response to ghrelin, leptin and cholecystokinin in ghrelin knockout mice. Sleep 30, A18.

Unger, R.H., Ketterer, H., Dupre, J., Eisentraut, A.M., 1967. The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. J Clin Invest 46, 630-645.

Vanderhaeghen, J.J., Lotstra, F., De, M.J., Gilles, C., 1980. Immunohistochemical localization of cholecystokinin- and gastrin-like peptides in the brain and hypophysis of the rat. Proc Natl Acad Sci U S A 77, 1190-1194.

Vanderhaeghen, J.J., Signeau, J.C., Gepts, W., 1975. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 257, 604-605.

Vanderweele, D.A., 1982. CCK, endogenous insulin condition and satiety in free-fed rats. Physiol Behav 29, 961-964.

Verbalis, J.G., McCann, M.J., McHale, C.M., Stricker, E.M., 1986. Oxytocin secretion in response to cholecystokinin and food: differentiation of nausea from satiety. Science 232, 1417-1419.

Wang, H., Wong, P.T., Spiess, J., Zhu, Y.Z., 2005. Cholecystokinin-2 (CCK2) receptor-mediated anxiety-like behaviors in rats. Neurosci Biobehav Rev 29, 1361-1373.

Wank, S.A., 1998. G protein-coupled receptors in gastrointestinal physiology. I. CCK receptors: an exemplary family. Am J Physiol 274, G607-G613.

Weller, A., Smith, G.P., Gibbs, J., 1990. Endogenous cholecystokinin reduces feeding in young rats. Science 247, 1589-1591.

Wells, A.S., Read, N.W., Craig, A., 1995. Influences of dietary and intraduodenal lipid on alertness, mood, and sustained concentration. Br J Nutr 74, 115-123.

Wells, A.S., Read, N.W., Uvnas-Moberg, K., Alster, P., 1997. Influences of fat and carbohydrate on postprandial sleepiness, mood, and hormones. Physiol Behav 61, 679-686.

Widdop, R.E., Krstew, E., Mercer, L.D., Carlberg, M., Beart, P.M., Jarrott, B., 1993. Electrophysiological and autoradiographical evidence for cholecystokinin A receptors on rat isolated nodose ganglia. J Auton Nerv Syst 46, 65-73.

Woods, S.C., Porte, D., Jr., 1983. The role of insulin as a satiety factor in the central nervous system. Adv Metab Disord 10, 457-468.

Wren, A.M., Bloom, S.R., 2007. Gut hormones and appetite control. Gastroenterology132, 2116-2130.

Xie, J.Y., Herman, D.S., Stiller, C.O., Gardell, L.R., Ossipov, M.H., Lai, J., Porreca, F., Vanderah, T.W., 2005. Cholecystokinin in the rostral ventromedial medulla mediates opioid-induced hyperalgesia and antinociceptive tolerance. J Neurosci 25, 409-416.

Zammit, G.K., Ackerman, S.H., Shindledecker, R., Fauci, M., Smith, G.P., 1992. Postprandial sleep and thermogenesis in normal men. Physiol Behav 52, 251-259.

Zepelin, H., Rechtschaffen, A., 1974. Mammalian sleep, longevity, and energy metabolism. Brain Behav Evol 10, 425-470.

Zhu, X.G., Greeley, G.H., Jr., Lewis, B.G., Lilja, P., Thompson, J.C., 1986. Blood-CSF barrier to CCK and effect of centrally administered bombesin on release of brain CCK. J Neurosci Res 15, 393-403.