Characterization of mice lacking the gene for cholecystokinin


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Lo CM, Samuelson LC, Chambers JB, King A, Heiman J, Jandacek RJ, Sakai RR, Benoit SC, Raybould HE, Woods SC, Tso P. Characterization of mice lacking the gene for cholecystokinin. Am J Physiol Regul Integr Comp Physiol 294: R803–R810, 2008. First published December 26, 2007; doi:10.1152/ajpregu.00682.2007.—CCK acts peripherally as a satiating peptide released during meals in response to lipid feeding and centrally functions in the modulation of feeding, exploratory, and memory activities. The present study determined metabolic parameters, food intake, anxiety-like behaviors, and cognitive function in mice lacking the CCK gene. We studied intestinal fat absorption, body composition, and food intake of CCK knockout (CCK-KO) mice by using the noninvasive measurement of intestinal fat absorption along with quantitative magnetic resonance (QMR) imaging and the DietMax system, respectively. Additionally, exploratory and memory capacities were assessed by monitoring running wheel activity and conducting elevated plus-maze and Morris water-maze tests with these mice. Compared with wild-type (WT) littermate controls, CCK-KO mice had normal food intake, fat absorption, body weight, and body mass. CCK-KO mice ate more food than control animals during the light period and less food during the dark period. Energy expenditure was unchanged between the genotypes; however, CCK-KO mice displayed greater fatty acid oxidation. CCK-KO mice spent more time in the closed arms of an elevated plus-maze, were as active as WT animals in the running wheel test. CCK-KO mice were as active as WT animals in the running wheel test. CCK-KO mice spent more time in the closed arms of an elevated plus-maze, indicative of increased anxiety. Additionally, CCK-KO mice exhibited attenuated performance in a passive avoidance task and impaired spatial memory in the Morris water maze test. We conclude that CCK is involved in metabolic rate and is important for memory and exploration. CCK is intimately involved in multiple processes related to cognitive function and food intake regulation.

cholecystokinin 1 receptor; cholecystokinin 2 receptor; cognitive behaviors

CCK occurs as a carboxyl terminally amidated peptide that exists in several possible lengths (43, 58, 66). In rats and mice, the predominant circulating forms include cholecystokinin octapeptide (CCK-8) and CCK-22, whereas larger molecular forms (CCK-33 and CCK-58) are also present in human and canine plasma (42, 61, 38, 63, 16). These molecular forms are processed from a 115-amino acid preprohormone product of the gene residing in chromosome 3 in rodents (13, 57). Intestinal endocrine cells secrete a mixture of medium-sized CCK forms, while central and peripheral neurons mainly release sulfated forms of CCK-8 (59, 62). In response to consumption of either lipid and protein, CCK is produced and secreted by intestinal I cells (41). The release of CCK from neurons is caused by potassium ion-induced depolarization (15). Peripheral CCK is involved in modulating intestinal motility, stimulating pancreatic enzyme secretion, enhancing gall-bladder contraction, and regulating meal size (11, 21, 24, 29, 48, 56, 64, 69).

CCK is also a neurotransmitter in many areas of the nervous system (6). Intraperitoneal or central administration of either purified CCK extracts or synthetic CCK-8 to fasted rats reduces food intake (22, 33, 37, 54), and this is manifested by a reduction of meal size as opposed to reduced frequency of meals (22, 37). Two types of CCK receptors (CCK1 and CCK2) have been cloned (5, 7). Pretreatment of animals with a CCK1 receptor antagonist increases meal size, whereas a CCK2 receptor antagonist has no effect (5, 7). In addition, administration of exogenous CCK-8 does not reduce food intake in CCK1 receptor-deficient mice and rats, suggesting that the satiation effect of CCK-8 is mediated via CCK1 receptors (36, 49). The satiating effect of systemic CCK is attenuated by subdiaphragmatic vagotomy, by selective vagal deafferentation, or by deactivation of vagal afferents with capsaicin (5, 44, 48). The generally accepted model is that exogenous CCK-8 elicits a short-term satiation effect by stimulating CCK1 receptors on vagal afferent nerves projecting to the brain (5, 7, 22, 33, 37, 44, 48).

Macronutrients in the diet, and especially fat and protein, stimulate a four- to seven-fold increase in CCK release to the circulation, and macronutrients also enhance memory (23, 34). Perhaps analogously, neuronal CCK, besides being involved in the control of food intake, also influences memory and exploratory behaviors (6). CCK receptors are distributed in many areas of the brain, including the frontal cortex, hippocampus, nucleus tractus solitarius (NTS), and the hypothalamus, areas associated with behaviors, including ingestion, memory, and anxiety (2, 6, 36, 52), and three forms of CCK (CCK-4, CCK-8, and CCK-33) interact with these brain CCK receptors (40, 45, 50, 65). Intraventricular or intravenous administration of CCK-8 modulates exploratory behavior and memory, and rats lacking functional CCK1 receptors have impaired learning and memory (8, 45). These observations collectively imply that endogenous CCK is a major controller of many behaviors and especially of cognitive function and food intake. In the present investigation, we examined metabolic parameters, food intake, and cognitive function in mice lacking the gene for CCK.
MATERIALS AND METHODS

Animals. The generation of the CCK-KO mouse has been described in detail by Lacourse et al. (39). Using a gene-targeting strategy, which replaced part of the mouse CCK gene with lacZ reporter gene, resulted in the complete removal of the NH2 terminus of CCK, including the signal sequence (39). The cholecystokinin-null (CCK-KO) mice have no functional CCK peptide fragments. CCK-KO mice had been back-crossed for more than 10 generations on the pure C57BL/6J genetic background. All of the mice used in these experiments were genotyped by PCR analysis of tail DNA to determine their genotype (39). Male CCK-KO mice and their wild-type (WT) littermates controls (C57BL/6J background) were generated from crosses between heterozygote mice (CCK+/-) in an American Association for the Accreditation of Laboratory Animal Care-accredited facility with corn cob bedding and under conditions of controlled illumination (12:12-h light-dark cycle, lights from 0600 to 1800). Mice had free access to standard rodent chow (5.6% fat) and water unless otherwise stated. All experiments used 5 to 11 male mice per genotype.

CCK-KO and WT mice were housed together in groups of four after weaning, and subsequently, they were placed into individual cages with corn cob bedding for monitoring 24-h food intake and body weight. CCK-KO and WT mice had free access to chow and water. Body weight was recorded daily, and water and food intakes were measured by weighing the food cups and water bottles at 1000 every morning (±0.01 g, Adventurer SL, Ohaus Corp, Pine Brook, NJ). All animal protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Materials. Olestra was a gift from Procter & Gamble. Sucrose was ordered from Sigma (St. Louis, MO), and nonfat dry milk was purchased from Kroger (Cincinnati, OH). Safflower oil was obtained from Holland (Boulder, CO). Boron trifluoride in methanol (BF3-methanol, 14% boron trifluoride and 86% methanol) was obtained from Pierce (Brockford, IL).

Noninvasive determination of fat absorption. Intestinal fat absorption was assessed by the noninvasive sucrose polybenehan method (30). Briefly, powdered diet was prepared with the following ingredients: 16% fat, 45% nonfat dry milk, and 39% sucrose. The fat was a mixture of 95% safflower oil and 5% Olestra (sucrose polybenehan, Procter & Gamble, Cincinnati, OH). Animals were transferred to individual cages with clean cob bedding for this assessment. Animals were fed the powdered diet for 3 days, and fecal pellets were collected for analysis after the 2nd and 3rd day of diet administration. The fecal pellets (≈15 mg) were extracted with methanolic sodium hydroxide, and fatty acids were methylated with boron trifluoride in methanol (46). Two milliliters of saturated saline and 10 ml of hexane were added, and the hexane fraction was collected after separation into two layers. The hexane fraction was injected into a gas chromatography system (Shimadzu GC 17-A) equipped with a DB-23 Column (J & W Scientific, Folsom, CA), an autosampler, and an autoinjector. Analysis of fatty acid methyl esters was calculated with Schimadzu Class VP 43 software. Percent fat absorption was determined from the ratios of total fatty acids to benenic acid in the diet and in the feces.

Fat mass/lean body mass. Fat mass and lean body mass of CCK-KO and WT animals were determined by the quantitative magnetic resonance imaging (EchoMRI) Quantitative magnetic resonance method (70). After system calibration, each mouse was weighed and then placed in the MRI restraint tube. Fat mass and lean body mass were determined using an EchoMRI whole body composition analyzer (Houston, TX).

Meat patterns. CCK-KO and WT mice (n = 8/group) were acclimatized to individual metabolic cages (Accuscan Instruments, Columbus, OH) for 3 days prior to the start of data collection. The mice had free access to powdered standard chow (Harlan Teklad, Madison, WI), and food intake was recorded at single-minute intervals for 3 days using the DietMax Food/Liquid consumption system (Accuscan Instruments).

Energy expenditure and respiratory quotient. A Physioscan open circuit calorimeter (Accuscan Instruments) was used to monitor oxygen (O2) and carbon dioxide (CO2) gas fractions at both the inlet and outlet ports to each of 8 acrylic test chambers, which were supplied with corn cob bedding. The airflow was 0.5 l/min, and air from each chamber was sampled and analyzed every 10 s. Experimental mice were either fasted or had free access to food, while in the individual test chambers for 23 h (from 1200 to 1100). Oxygen consumption was measured in mice either after 18-h fasting to determine basal metabolic rate (BMR) or after 24-h feeding to determine resting metabolic rate (RMR) (31). Gas flow measurements and gas fractions were used to compute respiratory quotient (RQ) and heat. RQ value was represented as the ratio of expired CO2 to inspired O2.

Running wheel activity. WT and CCK-KO mice were transferred to individual cages (9.3 × 13.9 × 7.7 cm) fitted with an anodized aluminum wheel (diameter 5 cm and 0.4 m/revolution), and a computerized monitor to measure activity (Lafayette Instrument, Lafayette, IN). Each mouse had free access to a food hopper and water bottle for the entire time in the running wheel cage. Total revolutions of the wheels were recorded at 10-min intervals for four continuous days, and food intake was assessed daily.

Elevated plus maze. The elevated plus maze was constructed of 1/8 polypropylene plastic. Each of the four arms (10 × 50 cm) was adjoined by a 10 × 10 cm intersection. The base of the maze was constructed such that the arms were elevated 50 cm above the floor. Five 60-W red lights were placed 1 m above the maze for lighting. Animals were placed in the center of the apparatus facing an open arm and allowed to freely explore the apparatus for 5 min. The total number of entries into the open and closed arms and the percentage of time spent in each arm were recorded.

Passive avoidance behavior. Passive avoidance behavior was assessed in a one-trial learning paradigm. The testing apparatus consisted of a 2-sided test chamber separated by a stainless-steel door, and both sides of the chamber had identical dimensions (Gemini avoidance system, San Diego Instruments, San Diego, CA). Animals were initially confined to one side of the chamber, which was kept completely dark. After 15 s, the chamber was brightly illuminated, and a door opened giving access to the other chamber, which remained unlit. The mouse was allowed to freely explore the entire apparatus, but the general tendency of mice was to escape the brightly lit side. Latency to enter the dark chamber was recorded. Upon full entrance to the dark side, the door was closed and a 0.4-mA shock (2-s duration) was administered via the metal floor grid. After this single learning trial, the rats were immediately removed from the apparatus. Retention was tested 24 h after the initial learning trial. The retention trial was conducted without electric shock, and latency to enter the unlit side of the chamber was recorded.

Morris water-maze test. The Morris water maze is a hippocampal-dependent memory task (9, 52, 53). Morris water-maze training was conducted in a circular, fiberglass pool (122 cm in diameter, 60 cm height; Rowland Fiberglass, Ingleside, TX) filled with cool tap water (17–19°C, 43 cm deep) in a clear glass platform (10.5 cm × 10.5 cm square) that was submerged 0.5 cm below the water surface. The pool was situated in a room that contained assorted extra-maze cues (42 cm square) was submerged 0.5 cm below the water surface. The pool was situated in a room that contained assorted extra-maze cues (42 cm square) and a door opened giving access to the other chamber, which remained unlit. The mouse was allowed to freely explore the entire apparatus, but the general tendency of mice was to escape the brightly lit side. Latency to enter the dark chamber was recorded. Upon full entrance to the dark side, the door was closed and a 0.4-mA shock (2-s duration) was administered via the metal floor grid. After this single learning trial, the rats were immediately removed from the apparatus. Retention was tested 24 h after the initial learning trial. The retention trial was conducted without electric shock, and latency to enter the unlit side of the chamber was recorded.

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individual mouse being placed into the water and allowed to swim freely for 90 s or until it found the platform and climbed onto it, at which time the trial was terminated, and the latency to find the platform was recorded. If the mouse failed to reach the platform within 90 s, the trial was terminated, and the mouse was guided onto the platform for 5 s. The mouse was then placed back into its home cage until all other mice received one trial, resulting in an intertrial interval of ~20 min. On the 5th day, each mouse received a final 90-s probe trial in which the platform was removed from the pool. The probe trial was recorded and analyzed by Cleversystem Topscan software (Reston, VA).

Statistical analysis. All values are expressed as means ± SE. Parametric statistical analyses and two-way repeated ANOVA were performed for comparison of all groups of animals subjected to the studies. The statistical analyses were performed using GraphPad Prism (ver. 3.0; San Diego, CA), and differences were considered significant if $P$ values were $<0.05$.

RESULTS

Body weight, fat absorption, and fat/lean body mass of CCK-KO mice. Male CCK-KO mice weighed 31.0 ± 0.7 g, and male WT mice weighed 31.9 ± 0.7 g (Fig. 1A; $P > 0.05$). There was also no statistical difference in percent lean or fat mass between the two genotypes ($P > 0.05$; Fig. 1B).

To examine intestinal fat absorption, a noninvasive method (30) was used. Fat absorption of CCK-KO mice (94.4 ± 1.13%) did not differ from that of WT mice (95.9 ± 0.63%) ($P > 0.05$). These findings suggest that CCK is not necessary for normal fat absorption, nor is it necessary to maintain the normal proportion of lean to fat mass in mice.

Food intake. When placed into individual cages, CCK-KO mice ate less chow (3.95 ± 0.23 g) than did WT animals (4.97 ± 0.12 g) on day 1 ($P < 0.026$; Fig. 2A). On day 2, food consumption did not differ between groups (data not shown). Mice were placed in the DietMax chambers and intake of powdered chow was precisely monitored for three days; after an initial 3-day acclimatization period, the CCK-KO mice consumed a comparable amount of food (4.22 ± 0.15 g) as the WT mice (4.47 ± 0.22 g) on the first day of assessment. Analysis of cumulative values on day 6 revealed that CCK-KO mice consumed significantly more food than WT mice during the light periods ($P < 0.038$) and less food during the dark periods, but the difference was not statistically significant ($P > 0.05$). Total food consumption did not differ (CCK-KO mice: 3.52 ± 0.27 g; WT mice: 3.52 ± 0.22 g (Fig. 2B). Hence, the meal pattern of CCK-deficient mice differed from that of WT mice in that they consumed more food during the light period and tended to compensate during the dark period by eating less.

Energy expenditure and RQ. During fasting, CCK-KO mice expended 17.38 ± 0.42 kcal/h, the same as WT mice (17.75 ± 0.41 kcal/h, $P > 0.05$) (Fig. 3A). CCK-KO mice also had comparable energy expenditure (18.25 ± 0.49 kcal/h) as WT mice (18.5 ± 0.32 kcal/h) during feeding (Fig. 3B). BMR and RMR for the two genotypes were also comparable over the 24-h cycle. No difference in RQ values was observed between the two genotypes during fasting, with mean values of ~0.7 (Fig. 3C). However, during ad libitum feeding, the RQ in CCK-KO mice was significantly lower than that in WT mice at some time points before and during the dark period ($P < 0.001$) (Fig. 3D). This result suggests that CCK-KO mice use a higher percentage of fat than WT mice at some time points before and during the dark period.

Running wheel activity. Figure 4 depicts running wheel activity for four continuous days. CCK-KO and WT mice had a comparable diurnal cycle of running wheel activity, with most running activity during the dark cycle. KO and WT mice also exhibited similar overall levels of running wheel activity.

Elevated plus-maze. CCK-KO mice spent a significantly higher percentage of time in the closed arms of the elevated plus maze (70.6 ± 3.9%) compared with WT mice (57.0 ± 4.2%) ($P < 0.036$) (Fig. 5). CCK-KO mice also had a nonsignificant tendency to spend less time in the open arm than WT mice (data not shown). These data are consistent with an interpretation of increased anxiety in KO mice relative to WT mice.

Passive avoidance task. During training trials of the passive avoidance task, CCK-KO mice entered the dark compartment with latencies comparable to WT mice (data not shown). These data imply either that CCK-KO mice have an impaired memory or that they are less sensitive to foot shock in general.

Morris water-maze test. CCK-KO mice had an attenuated ability to locate the escape platform, as demonstrated by an increased amount of total time spent swimming (64.2 ± 11.5 s vs. 26.0 ± 8.1 s for WT mice; $P < 0.04$; Fig. 6B). Additionally, CCK-KO mice spent less time (39.1 ± 4.6%) searching in

![Fig. 1. A: mean body weight of CCK-knockout (KO) and wild-type (WT) mice. Experimental mice (n = 8) and control animals (n = 8) were weighed at 6 mo of age. Data are expressed as means ± SE. The groups did not differ ($P > 0.05$). B: body composition of CCK-KO and WT mice. CCK-KO (n = 8) and WT (n = 8) mice were placed in the Echo MRI instrument. Percentages of fat and lean mass were calculated based on body weight. Data are expressed as means ± SE. The groups did not differ ($P > 0.05$).](https://www.ajpregu.org/content/294/3/R805/F1.large.jpg)
DISCUSSION

The present series of experiments indicates that the absence of CCK has little effect on body weight, body fat, or fat absorption, implying that CCK does not play a necessary role in the overall maintenance of energy balance in mice. These results are therefore consistent with earlier observations made by Samuelson and colleagues (39). The apparent lack of difference in food intake between the CCK-KO and WT animals is consistent with the conclusion derived from the CCK1 receptor knockout (CCK1 receptor KO). CCK1 receptor KO mice also have normal body weight when compared with WT controls (36). In contrast, CCK2 receptor KO mice increase daily food intake and develop obesity (4, 73). Gastrin has been reported by Clerc and associates (4) to inhibit food intake via CCK2 receptors in the brain. Consequently, the lack of CCK2 receptors for gastrin action results in increased food intake and body weight in the CCK2 receptor-deficient mice (4). Thus, how can we reconcile the apparent lack of effect of CCK2 receptor in our animals, since CCK-deficient mice have

the quadrant of the maze that previously contained the escape platform than the WT mice (69.4 ± 9.3% ; P < 0.025; Fig. 6C). These data suggest that mice lacking CCK are deficient in spatial memory function, relative to WT mice.
a comparable amount of gastrin in duodenum and stomach (39) We do not have an answer for this important question at the moment, but there could be interaction between CCK and gastrin with the CCK receptors, and how this interaction impacts food intake warrants further investigation.

In the present study, CCK-KO mice consumed less food than WT animals only on the first day that they were placed into individual cages. In contrast, food intake was comparable between groups in subsequent days. We speculate that increased anxiety of CCK-KO mice, perhaps because of placement in the new environment, may have contributed to this difference. The lack of a difference of total food intake on most days is consistent with a previous report (39). With regard to meal patterns, CCK-KO mice consumed significantly more food than WT mice during the light period and compensated during the dark such that total daily intakes were comparable. In contrast, mice lacking CCK1 receptors consumed significantly more food than the WT animals during the dark (3). These observations indicate that CCK may influence meal patterns via CCK2 receptors, although this remains to be assessed directly.

Using indirect calorimetry, we determined that CCK-KO mice had normal BMR and RMR. CCK-KO mice with ad libitum feeding did have a significantly lower RQ than WT mice at some time points before and during the dark period. These data indicate that CCK-KO mice use more energy from fat during these periods. One possible explanation might be that CCK-KO mice consume less food than WT mice during...
the dark, thus having to rely to a greater extent upon stored energy in the form of fat.

The running wheel activity of CCK-KO mice and WT mice did not differ on any parameter. Otsuka Long-Evans Tokushima Fatty (OLETF) rats, on the other hand, have considerably increased running wheel activity (2). Furthermore, OLETF rats are able to normalize their body weight and adjust their meal patterns when allowed to exercise in the running wheel activity and compared with either OLETF animals having no running wheel access or to control animals (2). Additional investigation is required to understand the mechanism that explains the differences between running activity of CCK-KO mice and OLETF rats. Nonetheless, our observations indicate that CCK-KO mice are equally as active as WT mice and that this may contribute to their normal body weight.

Exploratory activity in an elevated plus-maze is a traditional assessment of anxiety in laboratory animals (75). CCK-deficient mice spent significantly less time in the open arms than did WT mice in the present study, implying that they are more anxious. This observation is consistent with earlier reports that CCK receptor-deficient rodents exhibited increased anxiety behavior (47, 75). The data thus strongly imply that CCK receptors are normally involved in an overall anxiogenic effect. However, it is not clear from the present data which CCK receptors are involved in anxiety-related behaviors. Rats with reduced CCK2 receptor binding in the amygdala reportedly demonstrate more high-anxiety responses; hence, the CCK2 receptor in the amygdala may be involved in the anxiogenic effect (74). Furthermore, CCK2 receptor knockout (CCK2R KO) mice have increased anxiety in the elevated plus-maze test (47). In contrast, some conflicting reports indicate that CCK2R KO mice or rats administered a CCK2 receptor antagonist have reduced anxiety-related behaviors (71, 26). In addition, OLETF rats lacking the CCK1 receptor display an increase in anxiety behaviors (75). Therefore, CCK-KO mice in the present study have no CCK to bind to the CCK receptors, and this deficiency in CCK receptor stimulation may therefore explain the increase in anxiety-like responses of these animals.

CCK-KO mice had a significantly decreased latency to enter the dark chamber on the test day in the passive avoidance task compared with the control mice. Consistent with this, earlier pharmacological studies indicate that intraventricular or intraperitoneal administration of CCK-8 increases the latency on passive avoidance tasks (17, 18, 32). Furthermore, CCK antiserum or proglumide, a CCK1 receptor antagonist, individually prevents the acquisition and recovery of memory tasks, including passive avoidance tasks (18, 35). A previous study demonstrated that OLETF rats have longer latencies in hot-plate paw-lick task, indicating that CCK1R-deficient rats have an impaired response to painful stimuli (27). It is possible that the reduced latency exhibited by CCK-deficient mice can be explained by either impaired memory of the foot shock stimulus or reduced sensitivity to the foot shock. Another possibility is that the CCK-KO mice have normal pain sensitivity and normal memory but are less perturbed by the shock. However, the observation that they are more anxious argues against this possibility.

In the Morris water-maze task, CCK-KO mice required more time to locate the escape platform and spent less time in the area near the hidden platform than WT controls, suggesting that CCK-KO mice have impaired spatial memory. Spatial memory involves several areas of the brain, and especially the amygdala and hippocampus (1, 10, 12, 13, 50, 55, 60). Further, these brain areas express CCK1/CCK2 receptors (72, 74). Stimulation of the vagus nerve results in increased electrical activity in the central nucleus of the amygdala and in the hippocampus (12, 51). Previous studies indicate that systemically administered CCK facilitates memory performance via CCK1 receptors located on vagal afferent nerves (14, 19, 20, 60, 15). Vagal fibers enter the hindbrain at the level of NTS, and CCK1 receptors are abundant in the area postrema and NTS (25, 28, 67). Hence, CCK may act directly in the brain and/or peripherally via the vagus nerves to facilitate learning and memory. Earlier studies report that CCK1/CCK2 receptors are involved in the regulation of memory performance (45, 55, 68). OLETF rats and CCK2R KO mice exhibit impaired performance in various learning and memory tasks (45, 55, 68). In addition, a selective CCK2 receptor agonist when injected directly into the hippocampus improves memory performance (68). Therefore, CCK-KO mice may lack critical activity of CCK1/CCK2 receptors in these brain structures that is important in the formation of spatial memory.

Conclusion. Cholecystokinin is thought to be important in several peripheral metabolic actions related to the digestion and absorption of food, and there is solid evidence that it reduces meal size. In the brain, CCK additionally has widespread actions involving not only food intake but cognitive and emotional behaviors as well. In the present experiments, we assessed the phenotype of mice lacking the gene for CCK. CCK-KO mice ate the same amount of food and absorbed fat as well as WT mice, and they had normal body weight and body composition relative to the WT control mice. CCK-KO mice had slightly altered meal patterns over the day-night cycle, but the lack of CCK was not a factor in regulating total food intake. CCK-KO also had no effect on energy expenditure or running wheel activity in the present study. All of these results imply that the functions of CCK related to energy homeostasis are likely redundant with other signals such that CCK is not necessary for many of its actions. In contrast, CCK-deficient mice appeared more anxious and had impaired spatial memory and passive avoidance. While these observations will require further experimentation, we conclude that these central neurotransmitter actions of CCK are more critical than the systemic metabolic effects for normal functioning.

Perspectives and Significance

CCK is secreted from small intestine and brain. Exogenous CCK is involved in the secretion of pancreatic enzymes, satiety, anxiety-like behavior, and cognitive functions such as memory. The present study demonstrates that endogenous CCK is probably not required in lipid absorption in the lumen. Despite similar overall food intake, the CCK-KO mice had a different food intake pattern relative to the WT controls (more during the light period but less during the dark period). Metabolically, the CCK KO animals had greater fatty acid oxidation than the WT animals. Although the CCK-KO animals were as active as the WT animals, they have higher anxiety level and poorer memory than the WT animals. In future studies, we will study how the lack of CCK is compensated and whether this affects the expression of other peptides involved in food intake, energy homeostasis, and cognitive functions.
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