Lack of Ghrelin Secretion in Response to Fasting in Cholecystokinin-A (-1), -B (-2) Receptor–Deficient Mice

Chihiro SAKURAI1,2, Minoru OHTA1, Setsuko KANAI1, Hiroshi UEMATSU2, Akihiro FUNAKOSHI3, and Kyoko MIYASAKA1

1Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, 173-0015 Japan; 2Department of Gerodontology, Division of Gerontology and Gerodontology, Graduate School, Tokyo Medical and Dental University, Tokyo, 113-8549 Japan; and 3Department of Gastroenterology, Kyushu Cancer Center, Fukuoka, 811-1395 Japan

Abstract: Cholecystokinin receptors (CCK-Rs) have been classified into two subtypes: CCK-AR (1R) and -BR (2R). We generated CCK-AR(-/-), CCK-BR(-/-), and CCK-AR(-/-)BR(-/-) mice and found that the gastric emptying of a liquid meal was increased in CCK-BR(-/-) and AR(-/-)BR(-/-) mice, compared with wild-type and CCK-AR(-/-) mice. Given that enhanced gastric emptying leads to eating, food intake after overnight fasting was examined, as was the effect of CCK-8S on food intake. Male mice 6–8 months of age were deprived of food for 16 h with free access to water, after which they were injected intraperitoneally (0.1 ml/mouse) with either vehicle or CCK-8 (0.3, 1.0, or 3.0 nmol/mouse), and their food intake was monitored for 4 h. CCK-8S inhibited food intake in wild-type and CCK-BR(-/-) mice, but not in CCK-AR(-/-) or AR(-/-)BR(-/-) mice. Unexpectedly, we observed a lower food intake in CCK-AR(-/-)BR(-/-) mice treated with vehicle than in mice of the other genotypes. To examine the mechanism of decrease in food intake in CCK-AR(-/-)BR(-/-) mice, the involvement of ghrelin was determined in wild-type and CCK-AR(-/-)BR(-/-) mice. Fasting plasma ghrelin levels were significantly lower in CCK-AR(-/-)BR(-/-) mice than in wild-type mice, and no increase in response to fasting was observed in CCK-AR(-/-)BR(-/-) mice. An administration of acyl-ghrelin produced a small increase in food intake in CCK-AR(-/-)BR(-/-) mice, but not to the levels of wild-type mice. In conclusion, CCK-AR(-/-)BR(-/-) mice showed lower food intake as well as lower response to exogenous ghrelin, and a lower plasma ghrelin level after fasting, though which receptor is more important is unknown.

Key words: CCK-A (1) receptor, CCK-B (2) receptor, ghrelin, food intake, knockout mice.

Multiple gastrointestinal (GI) hormones and neuropeptides have been implicated in feeding regulations. Cholecystokinin (CCK) is a common GI hormone and is also one of the most abundant neurotransmitter peptides in the brain [1, 2]. CCK was first reported as a neuropeptide mediating satiety signal [3]. In 1994, leptin was isolated from adipose tissue [4] and was found to inhibit feeding, increase thermogenesis, and decrease body weight. The first appetite-stimulating peptide, neuropeptide Y (NPY), was discovered in 1982 [5]. Orexin and ghrelin were discovered in 1998 and 1999, respectively [6,7]. All of these peptides and/or proteins are known to interact with one another. CCK and leptin interact synergistically to reduce food intake in mice [8], and an intracerebroventricular injection of leptin inhibits NPY mRNA levels in the rat hypothalamus [9]. Also, orexin-receptor and CCK-A receptor (R) are colocalized in the nodosal ganglia of rats and humans, and orexin inhibits the response to CCK [10]. Centrally administered ghrelin regulates feeding and energy homeostasis through the direct activation of the orexin and NPY pathways [11–13], and the peripheral administration of CCK prevents the appetite-stimulating effect of ghrelin [14]. In a recent study using CCK(-/-)gastrin (-/-) mice, Friis-Hansen et al. [15] found that a lack of gastrin plus CCK reduced ghrelin secretion in response to fasting, though a lack of gastrin alone did not. Two types of CCK receptors (CCK-AR and CCK-BR) have been cloned [1]. CCK-AR and CCK-BR were renamed as CCK-1R and CCK-2R following the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification [16]. However, in this study, the terms “CCK-AR and BR” are used as before because they are already familiar to most readers [17]. CCK and gastrin possess identical COOH-terminal pentapeptide sequences with differences in their sulfations at the sixth (gastrin) and seventh (CCK) tyrosyl residues. CCK-AR binds sulfated CCK with 500- to 1,000-fold higher affinity than gastrin and nonsulfated CCK,
although the CCK-BR interacts with gastrin and CCK with almost the same affinity. Thus the phenotype of the CCK(–/–)gastrin(–/–) mice reported by Friis-Hansen et al. [15] resembles that of the CCK-AR(–/–)BR(–/–) mice developed in our laboratory [18, 19].

The sulfated form of CCK-8 (CCK-8S) binds to CCK-AR and produces satiety in mammals, including humans [20–25]. Moreover, CCK-8S acts on CCK-sensitive afferent fibers distributed in the stomach and duodenum [26]. CCK released from the intestine after a meal activates CCK-AR on the vagal afferent nerve to transmit the sensation of fullness to the brain [3, 27]. The CCK-sensitive satiety signals reach the nucleus tractus solitarius (NTS) and project to the hypothalamus, which subsequently terminates feeding behaviors.

Although previous pharmacological studies have used CCK-R antagonists [23, 28], they have been unable to exclude cross-reactivity; that is, the case in which substances that should react to CCK-AR also react to CCK-BR and vice versa. Moreover, CCK-ARs are present in the brain only in certain regions, including the hippocampus, NTS, posterior nucleus accumbens, ventral tegmental area, substantia nigra, hypothalamus, and raphe nucleus, but CCK-BRs are distributed widely throughout the central nervous system [28–31]. Furthermore, it has been reported that satiety signals are mediated via CCK-BR in rats [32]. Therefore mice deficient in CCK-AR, BR, and ARBR [CCK-AR(–/–), BR(–/–) and AR(–/–)BR(–/–) mice] could be useful in determining more conclusively the physiological role of CCK-Rs.

We reported in a previous study [33] that the administration of CCK-8S inhibited the gastric emptying of a liquid meal in CCK-BR(–/–) and wild-type mice, but it failed to do so in CCK-AR(–/–) and CCK-AR(–/–)BR(–/–) mice. Thus CCK-AR is necessary to reveal the inhibitory action of CCK-8S on gastric emptying. Delayed gastric emptying prolongs satiety; however, when a vehicle instead of CCK-8S was administered, gastric emptying was enhanced significantly in mice without CCK-BR, that is, in CCK-BR(–/–) and CCK-AR(–/–)BR(–/–) mice, though the precise mechanism of the enhanced gastric emptying has not yet been clarified.

In the present study, we first compared food intake under the same conditions as those described previously [33] to examine whether enhanced gastric emptying correlated to an increase in food intake in CCK-AR(–/–), BR(–/–), AR(–/–)BR(–/–), and wild-type mice. Second, we examined the effect on the food intake of different doses of CCK-8S, including which doses could have bound to both CCK-AR and BR, comparing our results for the 4 genotypes to clarify which receptor was involved in satiety. Unexpectedly, we observed that food intake in CCK-AR(–/–)BR(–/–) mice was not higher, but instead was significantly lower than in wild-type and CCK-BR(–/–) mice. Therefore, based also on the data presented previously by Friis-Hansen et al. [15], we hypothesized that the regulation of ghrelin secretion in CCK-AR(–/–)BR(–/–) mice might differ from that in wild-type mice.

Ghrelin, a 28-amino acid peptide originally found in the stomach, was identified as the endogenous ligand for growth hormone secretagogue receptor (GHS-R) [7, 11]. Ghrelin stimulates appetite, food intake, and GH secretion [7, 11]. Because plasma ghrelin levels are elevated after fasting and reduced after feeding, blood ghrelin levels of CCK-AR(–/–)BR(–/–) mice were measured and compared with those of wild-type mice with or without fasting. A portion of ghrelin possesses a unique fatty acid modification, n-octanoylation, at Ser 3 [34–36]. We examined the plasma levels of acylated and desacyl ghrelin by enzyme-linked immunosorbent assay (ELISA). Finally, we examined whether an administration of ghrelin could increase food intake in CCK-AR(–/–)BR(–/–) mice.

MATERIALS AND METHODS

All animal procedures were conducted in accord with the Guiding Principles for the Care and Use of Animals approved by the Physiological Society of Japan. They were also approved by the Ethical Committee for Animal Experimentation of the Tokyo Metropolitan Institute of Gerontology. The animals were fed commercial chow (CRF-1, Charles River Japan, Inc., Atsugi, Japan) and water ad libitum. They were maintained in a controlled environment at 23 ± 1°C, under a 12-h light/12-h dark photocycle (on at 8:00, off at 20:00).

Animals and chemicals. The progenitor strain for CCK-AR(–/–) and BR(–/–) mice was C57BL/6J. Backcrossing was carried out for more than seven generations. Three male CCK-AR(–/–)BR(–/–) mice were bred with 12 female CCK-BR(–/–) mice to yield F1 progeny with the genotype CCK-AR(+/+)BR(+/+). Male and female F1 mice were then bred to yield progeny with nine genotypes: CCK-AR(+/+)BR(+/+), CCK-AR(+/-)BR(+/+), CCK-AR(+/+)BR(+/-), CCK-AR(+/-)BR(+/–), CCK-AR(+/-)BR(–/–), CCK-AR(–/–)BR(+/-), CCK-AR(–/–)BR(+/–), and CCK-AR(–/–)BR(–/–). Male CCK-AR(–/–)BR(–/–) mice were then bred with female CCK-AR(–/–)BR(–/–) mice to obtain double knockout mice. CCK-AR(–/–) and CCK-BR(–/–) mice were selected from the above lines, and wild-type mice [CCK-AR(+/+)BR(+/+)] were selected at random from both the CCK-AR and CCK-BR lines. Mice at 6–8 months of age were used for the present experiments. Each mouse was kept in a cage by itself for 1 week before the experiments.

Synthetic CCK-8S and acyl-ghrelin were purchased from the Peptide Institute (Osaka, Japan). Two major forms of ghrelin, acyl-ghrelin and desacyl-ghrelin, were measured using Active Ghrelin ELISA and Desacyl-Ghrelin ELISA Kits (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan).
Experimental protocols. Each experiment was conducted after a 16-h fast with free access to water. At 9:00–10:00, the body weight of the subjects and the weight of chow were measured. CCK was dissolved in 1% bovine serum albumin (BSA/saline). Different doses of CCK-8S (0.3, 1.0, or 3.0 nmol/mouse) were injected intraperitoneally (0.1 ml/mouse). Physiological saline was administered as a vehicle. The animals were then returned to their individual cages, which contained the preweighed food. Any remaining chow was weighed at 20 min, 1 h, 2 h, and 4 h after injection to estimate food intake at each of those time points.

Measurement of blood ghrelin concentrations in wild-type and CCK-AR(–/–)BR(–/–) mice. The animals were decapitated at 10:00–11:00, either without fasting or after a 16-h fast with free access to water. Blood samples were collected in chilled polypropylene tubes containing EDTA-2Na (1.25 mg/tube) and crushed protease inhibitor cocktail tablets (complete, Mini, EDTA Free; Roche Diagnostics Gmbh, Mannheim, Germany; 1 tablet/7 ml). The tubes were rocked immediately to centrifuge the blood sample (1500 × g, 15 min, 4°C). Separated plasma was immediately added to a 10% volume of 1 nmol/liter HCL, and the mixture was stored at –80°C for the subsequent assay. Acyl-ghrelin and desacyl-ghrelin were measured using an Active Ghrelin ELISA Kit and a Desacyl-Ghrelin ELISA Kit, respectively, following the manufacturer’s protocol.

Effect of administration of acyl-ghrelin on food intake in wild-type and CCK-AR(–/–)BR(–/–) mice. The experimental protocol was the same as described above. Acyl-ghrelin was dissolved in 1% bovine serum albumin (BSA/saline). Different doses of acyl-ghrelin (0.3, 1.0, or 3.0 nmol/mouse) were injected intraperitoneally (0.1 ml/mouse) in wild-type and CCK-AR(–/–)BR(–/–) mice. Physiological saline was administered as a vehicle. We observed that these doses could produce significant and dose-dependent increases in food intake in wild-type mice [37].

Statistical analysis. All results were expressed as mean ± SE. Results were analyzed by a one-way or two-way analysis of variance (ANOVA) or by a multiple analysis of variance (MANOVA) with repeated measures, followed by Fisher’s protected least significant difference test. An analysis of variance for linear regression was employed to determine the effect of acyl-ghrelin. P < 0.05 was considered to be statistically significant.

RESULTS

Body weights before experiments (after overnight fasting) were 28.63 ± 0.36 g, mean ± SE for wild-type mice, 27.17 ± 0.30 g for CCK-AR(–/–), 26.77 ± 0.38 g for BR(–/–), and 26.18 ± 0.34 g for AR(–/–)BR(–/–) mice. When analyzed by one-way ANOVA, body weights differed among the groups [F (3,147) = 9.57, p < 0.0001], and the body weights of CCK-BR(–/–) and AR(–/–) BR(–/–) mice were significantly lower than those of wild-type mice by the multiple comparison test.

Food intake in CCK-R(–/–) mice treated with vehicle

The changes in integrated food intake in mice treated with vehicle during the 4-h experimental period are shown in Fig. 1. Significant differences were found with respect to genotype [F (3,29) = 5.59, p < 0.004], to time [F (3,3) = 291.4, p < 0.0001], and to the interaction between genotype and time [F (3,9) = 3.94, p < 0.0004] when the data were analyzed by MANOVA with repeated measures. The values of CCK-AR(–/–)BR(–/–) mice were significantly lower than those of the other mice by the multiple comparison test.

All mice consumed the most chow during the first 20 min regardless of their genotype; the values of consumption during the first 20 min are shown in Fig. 1. When the values of vehicle-treated mice during the first 20 min of feeding were analyzed by one-way ANOVA with respect to genotype, significant differences were found [F (3,29) = 4.48, p = 0.0105], and the values of CCK-AR(–/–)BR(–/–) mice were significantly lower than those of CCK-BR(–/–) and wild-type mice by the multiple comparison test (Fig. 1).

Effects of CCK-8S on food intake

To determine the effects of CCK-8S on food intake among the 4 genotypes, our results were analyzed by two-way ANOVA with respect to genotype and CCK treatment, showing significant differences with respect to genotype [F (3,135) = 5.983, p < 0.0007], to CCK [F (3,135) = 14.769, p < 0.0001] and to the interaction between gen-
otype and CCK [F (9,135) = 3.294, p = 0.0012] (Fig. 2).

The effects of various doses of CCK-8S were analyzed by one-way ANOVA within each genotype. The administration of CCK-8S significantly decreased food intake in wild-type mice [F (3,38) = 14.661, p < 0.0001] and in CCK-BR(–/–) mice [F (3,33) = 9.386, p < 0.0001] (Fig. 2, A and C); however, no tested dose of CCK-8S affected food intake significantly in CCK-AR(–/–) [F (3,23) = 0.969, p = 0.425] and CCK-AR(–/–)BR(–/–) mice (Fig. 2, B and D).

To examine whether the inhibitory effects of CCK-8S differed between CCK-BR(–/–) and wild-type mice, the results were analyzed by two-way ANOVA with respect to genotype and CCK doses. No significant differences were found between CCK-BR(–/–) and wild-type mice with respect to genotype [F (1,71) = 3.63, p = 0.061] or to the interaction between genotype and CCK [F (3,71) = 0.67, p = 0.576], even though CCK significantly affected food intake in both genotypes [F (3,71) = 22.59, p < 0.0001].

Although CCK-8S did not affect food intake in either CCK-AR(–/–) or AR(–/–)BR(–/–) mice, there were significant differences with respect to genotype [CCK-AR(–/–) > AR(–/–)BR(–/–)] [F (1,64) = 15.17, p = 0.0002], but no differences with respect to either CCK [F (3,64) = 1.44, p = 0.24] or to the interaction between genotype and CCK [F (3,64) = 0.53, p = 0.67].

**Plasma ghrelin concentrations**

The plasma concentrations of acyl-ghrelin were low in all animals examined, compared with those of desacyl-ghrelin (Table 1). These concentrations were not affected by fasting and did not differ significantly between the two genotypes (p = 0.36–0.67).

On the other hand, the plasma concentrations of desacyl-ghrelin in response to fasting were found to differ significantly between wild-type and CCK-AR(–/–)BR(–/–) mice based on two-way ANOVA [F (1,33) = 9.36, p = 0.0044 for the interaction between genotype and fasting]. The plasma desacyl-ghrelin concentration was significantly increased by fasting in wild-type mice, but no increase was observed in CCK-AR(–/–)BR(–/–) mice.  

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**Fig. 2.** Food intake for the first 20 min. A: wild-type; B: CCK-AR(–/–); C: CCK-BR(–/–); D: CCK-AR(–/–)BR(–/–). The amounts of food intake of CCK-AR(–/–)BR(–/–) mice were significantly lower than those of CCK-BR(–/–) and wild-type mice. CCK inhibited the food intake of wild-type mice and CCK-BR(–/–) mice, but this peptide did not affect CCK-AR(–/–) or CCK-AR(–/–)BR(–/–) mice. *Significantly different from the corresponding values of mice treated with vehicle; **significantly different from the corresponding values of mice treated with 0.3 nmol CCK-8S; ***significantly different from the corresponding values of mice treated with 1.0 nmol CCK-8S; †significantly different from the corresponding values of wild-type and CCK-BR(–/–) mice; ††significantly different from the corresponding values of CCK-AR(–/–) mice. Food intake is shown as relative to body weight. Numbers in parentheses indicate the numbers of subject animals. All values are means ± SE.
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Changes in total ghrelin (sum of acyl-ghrelin and deacyl-ghrelin) were similar to those in desacyl-ghrelin. There were significant differences with respect to fasting \([F (1,33) = 9.38, \ p = 0.0043]\) and with respect to the interaction between genotype and fasting \([F (1,33) = 6.03, \ p = 0.0195]\); however, no significant differences were observed with respect to genotype \([F (1,33) = 0.09, \ p = 0.76]\).

**Effect of the administration of acyl-ghrelin on food intake in wild-type and CCK-AR(–/–)BR(–/–) mice**

The administration of acyl-ghrelin significantly increased food intake in both wild-type and CCK-AR(–/–)BR(–/–) mice \(F(1,33) = 9.38, \ p = 0.0043\) and with respect to ghrelin doses (Fig. 3). The responses were significantly different between wild-type and CCK-AR(–/–)BR(–/–) mice \(F(1,33) = 6.03, \ p = 0.0195\); however, no significant differences were observed with respect to genotype \(F(1,33) = 0.09, \ p = 0.76\).

![Fig. 3. Food intake for the first 20 min in wild-type and CCK-AR(–/–)BR(–/–) mice treated with graded doses of ghrelin. A: wild-type; B: CCK-AR(–/–)BR(–/–). Food intake is shown as relative to body weight. Numbers in parentheses indicate the numbers of subject animals. All values are means ± SE. The results of statistical analysis are shown in the text. *Significantly lower than the corresponding value of wild-type mice.](image)

Plasma concentrations of desacyl-ghrelin were significantly increased after overnight fasting in wild-type mice (*), but not in CCK-AR(–/–)BR(–/–) mice. The results of statistical analysis are given in the text.

| Table 1. Plasma ghrelin concentrations (pmol/liter) in CCK-AR(–/–)BR(–/–) and wild-type mice with or without overnight fasting. |
|---------------------------------|------------------|------------------|
|                                 | Without fasting  | Overnight fasting |
| Wild-type mice                  | (n = 10)         | (n = 10)         |
| acyl-ghrelin                    | 16.2 ± 4.1       | 18.1 ± 3.7       |
| desacyl-ghrelin                 | 317.5 ± 46.0     | 1,043.8 ± 161.0* |
| total ghrelin                   | 333.6 ± 45.9     | 1,061.9 ± 161.9  |
| CCK-AR(–/–)BR(–/–) mice         | (n = 8)          | (n = 9)          |
| acyl-ghrelin                    | 22.8 ± 4.1       | 17.8 ± 3.3       |
| desacyl-ghrelin                 | 674.9 ± 150.4    | 599.8 ± 139.7    |
| total ghrelin                   | 697.7 ± 150.2    | 617.4 ± 142.4    |

Changes in total ghrelin (sum of acyl-ghrelin and deacyl-ghrelin) were similar to those in desacyl-ghrelin. There were significant differences with respect to fasting \([F (1,33) = 9.38, \ p = 0.0043]\) and with respect to the interaction between genotype and fasting \([F (1,33) = 6.03, \ p = 0.0195]\); however, no significant differences were observed with respect to genotype \([F (1,33) = 0.09, \ p = 0.76]\).

**DISCUSSION**

We confirmed that CCK-8S inhibited food intake dose-dependently in mice having CCK-AR, i.e., in wild-type and CCK-BR(–/–) mice. Two different mechanisms have been reported for the inhibitory effect of CCK on food intake, a central action on brain feeding centers and a peripheral action mediated by abdominal vagal afferents. The higher dose may be required to produce a central action than to activate peripheral vagal afferent \([26, 38]\). The doses of CCK-8S used in the present study were considered to bind either peripheral or central CCK-ARs. Although the mean values of food intake after treatment with CCK-8S in CCK-BR(–/–) mice (Fig. 2C) tended to be lower than the corresponding values in wild-type mice, two-way ANOVA showed no significant difference between the two genotypes. Therefore the contribution of CCK-BR to the transmission of the satiety signal pro-
duced by CCK-8S could be negligible, even if it is substantial. CCK-8S is a full agonist for CCK-BR, and it also activates CCK-AR, which does not bind gastrin. We therefore examined the effect of CCK-8S but not of gastrin in the present study.

Unexpectedly, food intake during the first 20 min of feeding in CCK-AR(−/−)BR(−/−) mice treated with vehicle was significantly lower than that in the other genotypes, and the food intake of CCK-BR(−/−) mice was not significantly different from that of wild-type or CCK-AR(−/−) mice. In a previous study, we reported that enhanced gastric emptying could lead to eating, we had expected an increase in food intake after overnight fasting in both CCK-BR(−/−) and AR(−/−)BR(−/−) mice. However, no increase occurred.

CCK-BR(−/−) and CCK-AR(−/−)BR(−/−) mice both showed lower body weights before the experiment (after overnight fasting) than wild-type and CCK-AR(−/−) mice. In previous experiments, we usually observed a lower body weight in CCK-AR(−/−)BR(−/−) mice than in other genotypes [18, 19]. Rodents have nocturnal habits; thus they eat during nighttime and sleep during daylight. In a previous report [18], we examined food intake during the nighttime and found that CCK-BR(−/−) and CCK-AR(−/−)BR(−/−) mice showed higher food intake than wild-type or CCK-AR(−/−) mice did. Moreover, we observed higher energy consumption during a 24-h period in CCK-AR(−/−)BR(−/−) mice than in CCK-AR(−/−) or wild-type mice. That is, mice without CCK-BR [CCK-BR(−/−) and AR(−/−)BR(−/−) mice] showed enhanced energy turnover with higher food intake during the nighttime and higher energy consumption over a 24-h period resulting in constant body weight. Therefore it is possible that the absence of food intake during the nighttime together with the higher energy consumption of these mice may be responsible for the lower body weight of CCK-BR(−/−) and CCK-AR(−/−)BR(−/−) mice, although we determined only overall energy expenditure without distinguishing between daytime and nighttime expenditures [18].

However, the reason for the significant decrease in food intake in CCK-AR(−/−)BR(−/−) in spite of increased gastric emptying and energy consumption remains unknown. Based on a recent report [15] that a lack of both CCK and gastrin decreases ghrelin secretion, we hypothesized that ghrelin might be involved. Indeed, plasma levels of ghrelin after overnight fasting were found to be significantly lower in CCK-AR(−/−)BR(−/−) mice than in wild-type mice. Moreover, the induction of desacyl-ghrelin secretion by fasting was not observed in CCK-AR(−/−)BR(−/−) mice, though fasting significantly increased plasma desacyl-ghrelin levels in wild-type mice.

A portion of ghrelin possesses a unique fatty acid modification, n-octanoylation, at Ser 3 [34, 36]. This acylation is thought to be essential for ghrelin biological activity. In the present study, plasma levels of acyl-ghrelin were very low in both CCK-AR(−/−)BR(−/−) and wild-type mice; however, these values were compatible with those reported in a previous study [36]. The plasma levels of acyl-ghrelin did not differ between wild-type and CCK-AR(−/−)BR(−/−) mice. Although desacyl-ghrelin has been believed to be devoid of any endocrine activities [36], a recent study [39] reports that it increases feeding by the activation of orexin neurons in the lateral hypothalamic area. Therefore it is suggested that desacyl-ghrelin also has a physiological role for feeding.

The administration of acyl-ghrelin, which showed a substantial bioactivity in wild-type mice, revealed a weak stimulatory effect in CCK-AR(−/−)BR(−/−) mice. Therefore the mechanism of the lack in ghrelin secretion and of its function in CCK-AR(−/−)BR(−/−) mice is still unknown, though it is suggested that an impaired regulation of ghrelin function might play a role in hypophagia in CCK-AR(−/−)BR(−/−) mice after overnight fasting. The vagal afferent fibers are responsible for both CCK and ghrelin, and colocalizations of GHS-R and CCK-AR have been observed in rats [14]. Friis-Hansen et al. [15] were unable to examine CCK(−/−) mice because they were unavailable. Unfortunately, CCK-AR(−/−) and BR(−/−) mice were unavailable for the present experiment. A comparison of ghrelin concentrations in CCK-AR(−/−) and CCK-BR(−/−) mice remains to be performed to shed light on the regulatory mechanism of ghrelin secretion by CCK.

In conclusion, the CCK-AR, not BR, mediates satiety. CCK-AR(−/−)BR(−/−) mice showed lower food intake as well as lower response to exogenous ghrelin and lower plasma ghrelin level after fasting, though which receptor is more important is unknown.

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