Effect of peripheral administration of cholecystokinin on food intake in apolipoprotein AIV knockout mice

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Apo AIV and CCK-8 significantly reduces food intake relative to apo AIV or CCK-8 alone. In this study, we determined whether CCK-8 (1, 3, or 5 μg/kg ip) reduces food intake in fasted apo AIV knockout (KO) mice as effectively as in fasted wild-type (WT) mice. Food intake was monitored by the DietMax food system. Apo AIV KO mice had significantly reduced 30-min food intake following all doses of CCK-8, whereas WT mice had reduced food intake only at doses of 3 μg/kg and above. Post hoc analysis revealed that the reduction of 10-min and 30-min food intake elicited by each dose of CCK-8 was significantly larger in the apo AIV KO mice than in the WT mice. Peripheral CCK 1 receptor (CCK1R) gene expression (mRNA) in the duodenum and gallbladder of the fasted apo AIV KO mice was comparable to that in WT mice. In contrast, CCK1R mRNA in nodose ganglia of the apo AIV KO mice was upregulated relative to WT animals. Similarly, upregulated CCK1R gene expression was found in the brain stem of apo AIV KO mice by in situ hybridization. Although it is possible that the increased satiating potency of CCK in apo AIV KO mice is mediated by upregulation of CCK 1R in the nodose ganglia and nucleus tractus solitarius, additional experiments are required to confirm such a mechanism.

MATERIALS AND METHODS

Adult male wild-type (WT) and apo AIV knockout (KO) mice (20–24 wk of age, C57Bl/6j background) were used. These WT and apo AIV KO (backcrossed at least 12 generations to the C57Bl/6j background) mice were bred in house in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care and were housed with corn cob bedding under conditions of controlled illumination (12:12-h light-dark cycle, lights from 0600 to 1800). All mice had free access to standard rodent chow (5% fat, Harlan Laboratories, Indianapolis, IN) and water unless otherwise stated. All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Food intake study. Apo AIV KO and WT mice (n = 7–8/group) were individually acclimatized in metabolic cages (Accuscan Instruments, Columbus, OH) for 3 days prior to the start of data collection. Animals were randomly assigned into four groups. For the CCK test, mice were fasted for 18 h (from 1500 to 0900) with
continuous access to water; 5 min prior to refeeding, experimental mice received 1, 3, or 5 μg/kg sulfated CCK-8 dissolved in 0.9% saline intraperitoneally, while control animals received saline alone (200 μl) (26). Food intake was recorded at single-minute intervals for 24 h by use of the DietMax Food/Liquid consumption system (Accuscan Instruments).

**Real-time-PCR for relative mRNA measurement.** Apo AIV KO and WT mice \( (n = 6/\text{group}) \) were fasted for 18 h (from 1500 to 0900) with water access. The duodenum and gallbladder of fasted mice were collected on dry ice next morning. Total RNA was isolated with RNeasy Mini kit (Qiagen, Germantown, MD), and the concentration was determined spectrophotometrically at 260 nm. In the nodose ganglia study, apo AIV KO and WT mice \( (n = 4/\text{per group}) \) were fasted for 18 h (from 1500 to 0900) with water access. The right and left nodose ganglia were collected on dry ice and total RNA was isolated with RNAqueous Micro kit (Ambion, Grand Island, NY) according to the manufacturer’s protocol. First-strand cDNA was synthesized from 1 μg total RNA with Scripts cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative PCR was performed in a 25-μl final reaction volume with an iCycler iQ Detection System by use of iQ SYBR Green Supermix (Bio-Rad Laboratories). Real-time PCR conditions were conducted as follows: 95°C for 3 min

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>Cyclophilin</td>
<td>5'-ATTCAATTTGCAAGGGTGTTGACT-3'</td>
<td>5'-TCAGCTCTTGGCAATGCAATGAGAT-3'</td>
</tr>
<tr>
<td>mCCK1R</td>
<td>5'-AAGAGATGAGGACGTGCA-3'</td>
<td>5'-CAGACCGGAGATTGAGG-3'</td>
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Fig. 1. Feeding behavior of apolipoprotein AIV (apo AIV) knockout (KO) and wild-type (WT) mice over a 3-day period (WT: \( n = 6 \), apo AIV KO: \( n = 8 \)). A: histograms of the daily feeding patterns of apo AIV KO and WT mice with food consumption averaged per 60 min for each mouse. B: food intake of free-feeding mice over 24 h. C: light-phase food intake of ad libitum fed mice. D: dark-phase food intake of ad libitum fed mice. Data are expressed as means ± SE.
Table 2. Body weight in WT and apo AIV KO mice following fasting and re-feeding

<table>
<thead>
<tr>
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<th>Before Fast, g</th>
<th>18-h Fasted, g</th>
<th>24-h Refed, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT mice</td>
<td>27.8 ± 0.49</td>
<td>25.1 ± 0.48</td>
<td>27.0 ± 0.55</td>
</tr>
<tr>
<td>Apo AIV KO mice</td>
<td>27.9 ± 0.34</td>
<td>25.2 ± 0.36</td>
<td>27.3 ± 0.39</td>
</tr>
</tbody>
</table>

Values represent means ± SE of body weight. WT, wild type; Apo AIV KO, apolipoprotein AIV knockout.

Effect of fasting and refeeding on body weight change. Table 2 presents the body weight for apo AIV KO and WT animals at three time points during the experiment: 1) before fasting; 2) 18-h fasted; and 3) 24 h after refeeding. Apo AIV KO mice (27.9 ± 0.34 g) had comparable body weight to WT mice (27.9 ± 0.49 g) before fasting. Both the WT and apo AIV KO mice lost 10% of their initial body weight after 18-h fasting and regained comparable amounts of weight after refeeding (Table 2). Therefore, there was no significant difference in body weight between WT and apo AIV KO mice following either fasting or refeeding.

Effect of fasting and refeeding on body weight change.

**RESULTS**

**Daily food consumption and feeding patterns.** Figure 1A depicts a histogram of 60-min feeding over a 3-day period; there were no significant differences in the 24-h feeding patterns of WT vs. apo AIV KO mice. These findings are consistent with the lack of a significant difference in the 24-h cumulative food intake levels of apo AIV KO and WT animals (Fig. 1B). The average daily food intake of apo AIV KO mice was 3.79 ± 0.09 g, compared with 3.82 ± 0.06 g for WT mice (P > 0.05; Fig. 1B). Apo AIV KO mice consumed slightly less food than WT mice during the dark period, but the difference was not significant (dark phase: WT mice, 2.41 ± 0.04 g; apo AIV KO mice, 2.29 ± 0.06 g, P > 0.05; light phase: WT mice, 1.41 ± 0.07 g; apo AIV KO mice, 1.50 ± 0.08 g, P > 0.05; Fig. 1, C and D).

**Effect of fasting and refeeding on body weight change.** Table 2 presents the body weight for apo AIV KO and WT animals at three time points during the experiment: 1) before fasting; 2) 18-h fasted; and 3) 24 h after refeeding. Apo AIV KO mice (27.9 ± 0.34 g) had comparable body weight to WT mice (27.9 ± 0.49 g) before fasting. Both the WT and apo AIV KO mice lost 10% of their initial body weight after 18-h fasting and regained comparable amounts of weight after refeeding (Table 2). Therefore, there was no significant difference in body weight between WT and apo AIV KO mice following either fasting or refeeding.

**Effect of CCK on food intake.** Following an overnight fast, noninjected apo AIV KO mice consumed significantly more food (0.78 ± 0.03 g) than WT mice (0.69 ± 0.03 g) over 30 min (two-way ANOVA, P < 0.0001, Fig. 2A). Apo AIV KO mice had significantly reduced 30-min food intake following each dose of CCK-8 (1, 3, and 5 μg/kg) (Fig. 2C) whereas WT mice had significantly reduced food intake in response to doses of 3 μg/kg CCK-8 and higher (P < 0.05, Fig. 2B). Cumulative
food intake over 24 h did not differ between the two genotypes prior to or following the CCK administration at each CCK dose (1, 3, and 5 μg/kg, respectively) (Fig. 3, A–C).

To determine whether CCK-8 is more efficacious at reducing acute food intake in apo AIV KO mice than in WT controls, we calculated the percent decrease from saline for each mouse following each dose of CCK-8 at two time points (10 and 30 min). The 10-min point was analyzed since the cumulative ingestion curves (Fig. 2C) suggest that the greatest reduction caused by CCK-8 occurs early and that the curves appear parallel after that until 30 min when the test was ended. A 2 × 4-factor ANOVA followed by the post hoc partial least significant difference test on the 10-min data revealed that, relative to intake following saline, the suppression of food intake in the KO mice was greater than that in the WT mice at each dose of CCK-8, attaining significance after 3 (P < 0.01) and 5 μg/kg (P < 0.01) and nearing significance after 1 μg/kg.

After 30 min, the differences were significant following 1 (P < 0.01) and 5 μg/kg (P < 0.01) CCK-8 and approached significance after 3 μg/kg (P = 0.061). When analyzed as absolute intakes, the outcomes were the same at both time points. Thus CCK-8 was more efficacious at reducing food intake in apo AIV KO than in WT mice.

CCK1R gene expression in duodenum, gallbladder, and nodose ganglia. Following an overnight fast, CCK1R mRNA in the duodenum and gallbladder as measured by real-time PCR in apo AIV KO mice were comparable to that in WT animals (Fig. 4A). In contrast, fasted apo AIV KO mice had a fourfold increase of CCK1R mRNA in the nodose ganglia relative to their fasted WT control group (Fig. 4B). These findings suggest that the upregulation of CCK1R mRNA in the nodose ganglia of the apo AIV KO animals relative to the WT animals was specific since it was not observed in the duodenum or gallbladder.

In situ hybridization. As depicted in Fig. 5, A–E, the intensity of labeled CCK1R mRNA in the nucleus tractus solitarius (NTS) of apo AIV KO mice after an overnight fast was significantly higher than the level in WT mice (P < 0.05). In contrast, CCK1R mRNA in the apo AIV KO animals following refeeding was decreased, such that they had a comparable level as WT animals (Fig. 5E). As depicted in Fig. 6, A–D, the intensity of CCK1R in the PVN in both the WT and apo AIV KO animals was comparable following either feeding or fasting (Fig. 6E). These findings suggest that increased CCK1R mRNA increased in the NTS, but not in the PVN, in fasted apo AIV KO mice. Apo AIV KO mice had increased satiation potency in response to CCK, and this may be mediated via an increased CCK1R in the NTS.

DISCUSSION

Apo AIV is an anorectic protein synthesized in the intestine and hypothalamus that reduces food intake (10, 22, 25). Mice with apo AIV deficiency (apo AIV KO mice) exhibit normal food intake and normal body weight during ad libitum feeding.
Exogenous administration of apo AIV dose dependently inhibits food intake in fasted rats (10). Using rats, we found that coadministration of CCK and apo AIV at individual subthreshold doses causes a significant reduction of food intake via a peripheral CCK1R pathway (25). We have also found that combinations of CCK and apo AIV reduce food intake in mice (24). The present study asked whether CCK would suppress food intake in the absence of apo AIV. In fact, CCK-8 inhibited food intake more potently in apo AIV KO than in WT animals over a range of doses, including a low dose that is ineffective in WT mice (1 μg/kg body wt), indicating that the ability of CCK to suppress acute food intake is increased in apo AIV KO animals. Although at first glance these observations are hard to reconcile with the additive nature of CCK and apo AIV in normal animals, it is possible that the greater sensitivity may be a compensation for the deficiency of normal apo AIV signaling. However, the present study cannot distinguish whether a lack of intestinal apo AIV, hypothalamic apo AIV, or both is responsible for the increased potency of CCK on food intake. Future experiments with organ-specific knockout will be needed to evaluate these possibilities.

CCK is a peptide released by endocrine cells from the duodenal mucosa and is widely distributed in nerves, including in both myenteric and mucosal neurons (38). Peripheral CCK1R are present in the enteric neurons of duodenal mucosal (predominantly involved in secretory and mucosal functions), myenteric plexus (predominantly involved in the control of motor activity), and nodose ganglia (2, 29, 33, 40). CCK directly binds with CCK1R on vagal afferent neurons in the intestinal enteric plexuses whose cell bodies are in the nodose ganglia, and the signals are relayed to vagal nerve endings in the NTS (2, 27, 29, 39). We hypothesized that the increased sensitivity to CCK-8 in apo AIV KO mice relative to that in WT animals might be mediated by an increase in vagal afferent and/or possibly NTS CCK1R. This possibility was supported partly by an upregulation of CCK1R mRNA in the nodose ganglia of KO vs. WT animals. This upregulation is certainly specific since we failed to detect a similar upregulation in the duodenum or gallbladder where peripheral CCK1R are abundantly present (6). These observations suggest that the increased sensitivity to CCK-induced satiation in KO animals could be partly mediated by increased CCK1R on vagal terminals in the duodenum as reflected by the increased expression of CCK1R in the nodose ganglia. How apo AIV (or its absence) regulates the gene expression of CCK-1R in nodose ganglia is unknown.
CCK1R receptors are also distributed in the hypothalamus, including the PVN, arcuate nucleus, and DMH, as well as in the hindbrain and the area postrema (14, 15, 29). Neuropeptide Y (NPY) gene expression in DMH in Otsuka Long-Evans Tokushima Fatty (OLETF) rats with deficient CCK1R is different from their controls, Long-Evans Tokushima Otsuka (LETO) rats (1), implying a role for forebrain CCK activity in metabolism. Because mice do not express NPY in the DMH, we assessed CCK-1R mRNA in PVN and NTS in the present study. After an overnight fast, CCK1R gene expression was upregulated in the NTS of the apo AIV KO mice compared with that in WT animals. In contrast, there was no difference in CCK1R mRNA in the PVN between the two genotypes following an overnight fast. These findings imply that upregulation of CCK1R mRNA in the PVN between the two genotypes following an overnight fast. These findings imply that upregulation of CCK1R in the NTS, but not in the PVN, may be important in compensating for the apo AIV deficiency. We propose that since both apo AIV and CCK are secreted by the small intestine in response to fatty meals, they provide an integrated signal to regulate food intake via peripheral and central CCK1R pathways. In the event that apo AIV is absent, as occurs in apo AIV KO animals, CCK1R expression is upregulated in the neural circuit leading from the duodenum to the brain that influences satiation. Consistent with this mechanism, CCK1R mRNA was elevated in the nodose ganglia as well as in NTS of apo AIV KO mice, and they are more sensitive to the action of exogenous CCK to reduce food intake.

In conclusion, Apo AIV KO mice have a comparable 24-h food intake and body weight during ad libitum feeding, although they consume more food than controls when food is first made available following an overnight fast. In addition, exogenous CCK-8 is more effective at suppressing food intake in apo AIV KO mice than in WT animals; this might be mediated via increased CCK1R gene expression in nodose ganglia and the NTS, but additional studies will be needed to support this mechanism.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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**Fig. 6.** ISH for CCK-1R mRNA in the paraventricular nucleus (PVN) of fed (A) and fasted (B) WT mice and fed (C) and fasted (D) apo AIV KO mice. E: mean CCK1R mRNA in the PVN of apo AIV KO and WT mice following either feeding or 18-h fast. Data are expressed as means ± SE.
REFERENCES


