Anagliptin suppresses diet-induced obesity through enhancing leptin sensitivity and ameliorating hyperphagia in high-fat high-sucrose diet fed mice

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Abstract. Obesity is a major risk factor for type 2 diabetes, and weight loss is beneficial to diabetic patients who are obese or overweight. Dipeptidyl peptidase-4 (DPP-4) inhibitors are anti-diabetic drugs. Although it has been known that the effect of most of the DPP-4 inhibitors on body weight is neutral, several studies suggested that some DPP-4 inhibitors suppressed body weight. Nonetheless, the mechanisms underlying DPP-4 inhibitor-induced weight loss are not fully understood. In this study, the mice fed high-fat high sucrose diet (HFHSD) containing a DPP4 inhibitor, anagliptin, showed reduced food intake and body weight compared to the mice fed non-treated HFHSD, but oxygen consumption and respiratory exchange ratio (RER) were not altered. Sequential administration of leptin suppressed food intake and body weight more apparently in anagliptin treated HFHSD fed mice than non-treated HFHSD fed mice. Oxygen consumption and RER were comparable between anagliptin treated and non-treated mice after leptin administration. The number of phospho STAT3 expressed cells in the arcuate nucleus after leptin administration was increased in anagliptin treated mice compared to non-treated mice. These data suggested that anagliptin ameliorated leptin resistance induced by HFHSD and thereby decreased food intake and body weight. These effects of anagliptin could be beneficial to the treatment of obese diabetic patients.

Key words: DPP-4 inhibitor, Anagliptin, Obesity, Leptin, Food intake

Material and Methods

Animals and treatment

All animal experiments were approved by Institutional Animal Care and Use Committee of Gunma University. C57BL/6J mice at 6 weeks old were purchased from Charles River Laboratories Japan (Yokohama, Japan). Mice were kept at room temperature (22–24°C) with 12-h light/dark cycle. Food and water were provided ad libitum. Mice were fed standard chow diet (CE-2, CLEA Japan, Tokyo Japan) until 8 weeks old, and either the high-fat high-sucrose (HFHS) diet (30% fat and 20% sucrose (wt/wt), F2HFHSD; Oriental Yeast, Suita, Japan) or HFHS diet containing 0.3% (wt/wt) anagliptin (HFHS-A) were fed from 8 to 14 weeks old.
**Body weight, food intake, oxygen consumption, and locomotor activity**

Body weight was measured weekly from 8 to 14 weeks old. Food intake, oxygen consumption, respiratory exchange ratio (RER) and locomotor activity were measured when mice were 14 weeks old. Oxygen consumption and locomotor activity were measured simultaneously after 3 days of acclimation period. Oxygen consumption and RER were measured every 18 min using oxymax (Columbus Instruments, Columbus, OH). Locomotor activity was measured by ACTIMO-100 monitoring system (Shinfactory, Fukuoka, Japan).

**Leptin sensitivity test**

Leptin sensitivity test was performed as reported previously with a little modifications [8]. Leptin (2 μg per body weight (g)) was administered intraperitoneally (IP) to 14 weeks old mice at zeitgeber time (ZT) 01:30 and ZT 11:30. Body weight, food intake, and oxygen consumption were measured for 3 days before and during leptin administration.

**Hormone levels**

Blood was collected to a tube treated with aprotinin (018-18111, FUJIFILM Wako Pure Chemical, Osaka, Japan) and DPP4 inhibitor (DPP4-010, Merck, Kenilworth, NJ) using heparin treated syringe from heart after IP administration of 10% pentobarbital (7 μL/g body weight) at ZT 8–10. GLP-1 and leptin levels in plasma were measured using a LBIS GLP-1 (Active) ELISA Kit (AKMGP-011, FUJIFILM Wako Shibayagi, Gunma, Japan) and a mouse leptin ELISA kit (AKRLP-011, FUJIFILM Wako Shibayagi), respectively, according to the instructions of the manufacturer.

**Immunohistochemistry**

Leptin (2 μg per body weight (g)) was administered intraperitoneally to 24 hour fasted mice at ZT9. One hour after leptin administration, mice were anesthetized with 10% pentobarbital and then perfused transcardially with saline and 10% formalin (062-01661, FUJIFILM Wako Pure Chemical). Brains were post-fixed overnight at 4°C. Then, they were transferred to phosphate-buffered saline (PBS, pH 7.4) containing 20% sucrose. The brains were frozen and kept at −80°C until sectioning. Coronal sections were cut at 25 μm using a cryostat (1:5 series). Sections were collected and transferred to a cryoprotectant solution, and stored at −30°C. Sections were rinsed with PBS, and then treated with 0.3% H₂O₂ diluted in PBS for 30 min, 0.3% glycine in PBS for 25 min, and then 0.03% SDS in PBS for 25 min. Sections were then blocked with 1% NGS and 1% BSA diluted in PBS containing 0.1% Triton X-100 for 30 min. Then, sections were incubated in rabbit anti-phosphorylated STAT3 (pSTAT3) (Tyr705) antibody (1:300, #9131, Cell Signaling Technology, Beverly, MA) diluted in blocking solution overnight. After rinsing with PBS, sections were incubated with biotinylated goat anti-rabbit IgG (1:400, Vector Laboratories, VA-1000) for 40 min and incubated with ABC reagent (Vector Laboratories) for 40 min. Sections were rinsed with PBS and sodium acetate buffer (pH 5.6), and color was developed with a nickel-diaminobenzidine solution (10 g/liter nickel ammonium sulfate, 0.2 g/liter DAB, and 0.006% H₂O₂ in sodium acetate buffer). The number of pSTAT3 immunopositive cells was counted from one side of ARC at 1.58 mm posterior to the bregma.

**Quantitative PCR analysis**

Total RNA was extracted from dissected ARC nuclei with QIAzol lysis reagent (QIAGEN, Hilden, Germany) and chloroform. RNA samples were treated with DNsase I and then reverse transcribed using ReverTra Ace qPCR RT Master Mix with gDNA Remover (FSQ-301, TOYOBO, Osaka, Japan). TaqMan assays were performed using primers and probes for Npy (Mm03048253_m1), Agp (Mm00475829_g1), Pomc (Mm00435874_m1), and 18S (Hs99999901_s1) from Thermo Fisher Scientific (Waltham, MA). The ddCT method was used to express mRNA levels in arbitrary units.

**Statistics**

Data were presented as mean ± SEM. Statistical analyses were performed using IBM SPSS Statistics 23 (IBM) software. After confirming normal distribution of data, comparisons between two groups were made by the unpaired Student’s t test. p < 0.05 was considered significant.

**Results**

**Anagliptin suppressed HFHSD-induced body weight gain due to decreased food intake but not due to increased energy expenditure**

The mice were fed regular diet until 8 weeks old, and then, food was switched from regular diet to either HFHSD with (HFHS-A) or without (HFHS) 0.3% anagliptin (Fig. 1A). This concentration of anagliptin was selected based on the information by which high-fat diet induced-body weight gain was suppressed [7]. Administration of anagliptin significantly increased the plasma GLP-1 levels (HFHS: 23.47 ± 8.68 pg/mL, n = 7 versus HFHF-A: 54.53 ± 8.71 pg/mL, n = 8, p < 0.05). The body weight of the mice fed HFHS-A diet was significantly lower than those of mice fed HFH at 13 and 14 weeks old (Fig. 1B). The daily food intake of the mice
fed HFHS-A was also significantly lower than that of mice fed HFHS (Fig. 1C). Oxygen consumption and RER were unaltered in the mice fed HFHS-A compared to the mice fed HFHS (Fig. 1D and 1E). While locomotor activity of the mice fed HFHS-A tended to be decreased, there was no significant difference between the mice fed HFHS and HFHS-A (Fig. 1F). These data indicated that oral administration of anagliptin partly prevented HFHS diet-induced obesity by decreasing food intake.

Plasma level of leptin, a major hormone that reduces food intake and body weight, was not altered in HFHS-A fed mice (HFHS: 795.15 ± 176.38 pg/mL, n = 7 versus HFHS-A: 1,040.13 ± 322.29 pg/mL, n = 8). RNA expression levels of neuropeptides controlling feeding, Npy, Agrp, and Pomc, in the arcuate nucleus (ARC) of the hypothalamus were not significantly altered in the mice fed HFHS-A compared to the mice fed HFHS (Fig. 1G).

**Anagliptin ameliorated leptin resistance and thereby enhanced leptin’s effects on food intake and body weight in HFHS fed mice**

We next examined the leptin sensitivity in the mice administered anagliptin. Leptin was administered twice a day, and alterations of body weight, food intake and oxygen consumption were examined. The body weight was much more significantly reduced by leptin administration in HFHS-A fed mice (around 5% reduction) than that in HFHS fed mice (Fig. 2A). At day 3 of leptin administration, the food intake in HFHS fed mice was reduced up to 20% from the initial levels, by contrast, the food intake in HFHS-A fed mice was reduced by 50% (Fig. 2B). On the other hand, oxygen consumptions and RERs were comparable between HFHS and HFHS-A fed mice (Fig. 2C and 2D). Because ARC is a center of leptin’s anorexigenic action [9], we examined the leptin signaling in ARC by counting pSTAT3 immunopositive cells. The number of pSTAT3 expressing cells after leptin administration was significantly increased in the ARC of HFHS-A fed mice (Fig. 2E and 2F). These data indicated that anagliptin administration enhanced leptin sensitivity and thereby ameliorated HFHS diet-induced hyperphagia.

**Discussion**

In this study, we found that chronic oral administration of anagliptin suppressed HFHSD induced body weight gain by decreasing food intake. We also found that leptin resistance induced by HFHSD was ameliorated by anagliptin. These findings indicate that anagliptin ameliorates diet-induced obesity (DIO) through the improvement of leptin-sensitivity.

Because some other DPP-4 inhibitors including teneligliptin and KR-66195 also decrease body weight of DIO mice [5, 6], pathways mediated by DPP-4 inhibition could be the mechanisms underlying anagliptin induced reduction of food intake and body weight. A major DPP-4 substrate, GLP-1, is produced both in periphery and in the brain and reduces food intake and body weight [10-12]. In this study, GLP-1 levels at least in the plasma were increased after administration of anagliptin. In addition, it has been reported that the concentration of anagliptin in the brain is less than one tenth of that in blood after an oral administration of anagliptin [13]. Therefore, the increase of GLP-1 level in periphery may primarily mediate the reduction of food intake and body weight. Further studies will be required to address the effect of anagliptin in the brain.

Central nervous system including ARC and vagus nerve mediates food intake reductions induced by a peripheral administration of a GLP-1 receptor agonist, liraglutide, or native GLP-1 [14-17], suggesting that central nervous system is the key place for controlling food intake at the downstream of GLP-1 signaling. Npy, Agrp, and Pomc mRNA expression levels in the ARC were not altered in anagliptin treated mice (Fig. 1G). This is similar to obscure alteration of Npy, Agrp, and Pomc mRNA expression levels after administration of liraglutide [15]. Unaltered expression of these neuropeptides is considered to be effects of GLP-1 that are not directly related to NPY/AgRP and POMC neurons [18].

Leptin-induced anorectic effect and STAT3 activation in ARC were improved in anagliptin treated mice. There are some potential mechanisms underlying the improvement of leptin sensitivity. DPP-4 is also involved in immune system through the activation of T cells [19], and DPP4 inhibitors including anagliptin suppress vascular inflammation [20, 21]. Suppression of inflammatory signaling could also underlie amelioration of leptin-resistance by anagliptin. Another possibility is the secondary effect of body weight reduction, which is caused by the increase of GLP-1 level, because leptin resistance in ARC is known to be reversible depending on body weight [22].

Improvement of leptin sensitivity could also enhance GLP-1’s anorexic effect. In leptin receptor deletion rats, GLP-1 action to reduce food intake is abolished [23], suggesting that leptin supports GLP-1’s anorexic effect. The synergistic interaction between GLP-1 and leptin may contribute to the reduction of food intake and body weight.

In this study, plasma leptin level was not changed after 7 weeks administration of anagliptin. Plasma concentrations of leptin correlates with body fatness and nutritional status [24]. Anagliptin administration concomitantly with
Fig. 1  Body weight, food intake, oxygen consumption, respiratory exchange ratio and locomotor activity of mice fed HFHS diet containing 0.3% anagliptin

A. Mice were fed regular diet until 8 weeks. At 8 weeks old, food was switched either to high-fat high-sucrose (HFHS) diet or to HFHS diet containing 0.3% anagliptin (HFHS-A). Food intake, energy expenditure, locomotor activity, and leptin sensitivity were measured at 14 weeks old. B. Weekly body weights of mice fed HFHS (n = 25) or HFHS-A (n = 26). C. Daily food intake of 14-week-old mice fed HFHS (black, n = 7) or HFHS-A (red, n = 8). D. Oxygen consumption of mice fed HFHS (n = 19) or HFHS-A (n = 20) (left) and the average oxygen consumption during light and dark cycle (right). E. Respiratory exchange ratio (RER) of mice fed HFHS (n = 15) or HFHS-A (n = 16) (left), and the average RER during light or dark cycle (right). F. Locomotor activity of mice fed HFHS (n = 19) or HFHS-A (n = 20) (left), and the average locomotor activity during light or dark cycle (right). G. Relative mRNA expression of Npy, Agrp, and Pomc in the arcuate nucleus (ARC). * p < 0.05, *** p < 0.001
Fig. 2  Body weight, food intake, oxygen consumption, and RER after repeated administration of leptin
Alteration of body weight (n = 5) (A) and daily food intake (n = 5) (B) of mice fed HFHS or HFHS-A after administration of leptin. C. Oxygen consumption before and after administration of leptin (left), and average oxygen consumption during every light (L) and dark (D) phase (right) (n = 8). D. RER before and after administration of leptin (left), and average RER during every light and dark phase (right) (n = 8). Leptin was administered at points indicated by arrowheads. E. Immunohistochemistry for phospho STAT3 (pSTAT3) in the ARC of the mice fed HFHS or HFHS-A after administration of leptin. Scale bar: 30 μm. 3V: third ventricle. F. The number of pSTAT3 expressing cells in the ARC (n = 4). * p < 0.05, **** p < 0.001
metformin and miglitol to type 2 diabetes patients decreased plasma leptin levels without altering body weight after 24–52 weeks of treatment [25]. Similarly, diabetic model, high-fat diet fed glucokinese haploefficient mice, also showed decreased plasma leptin levels after anagliptin administration [7]. The difference of adiposity between anagliptin treated group and control group and the diet condition used in this study, which contained higher amount of fat and sucrose, may have caused the obscured alteration in plasma leptin level.

Oxygen consumption and RER were not altered by anagliptin treatment with or without leptin injection when comparing the HFHSD fed mice with HFHSD-A fed mice. However, both groups showed increased RER when leptin was injected, suggesting that leptin sensitivity was still maintained even after 7 weeks of HFHSD feeding. Therefore, relatively mild leptin resistance in regard to energy expenditure could account for unaltered energy expenditure by anagliptin. In addition, the dose of anagliptin could also be related, because another DPP-4 inhibitor, teneligliptin, increased oxygen consumption only when high dose of teneligliptin was administered [6].

In this study, we found that a DPP-4 inhibitor, anagliptin, ameliorates leptin resistance and attenuates food intake and body weight in DIO mice. These effects of anagliptin could be beneficial to the treatment of obese diabetic patients.

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Disclosure

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