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Olanzapine-activated AMPK signaling in the dorsal vagal complex is attenuated by histamine H1 receptor agonist in female rats

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Abstract
Weight gain and its related metabolic disorders are major side-effects associated with second generation antipsychotic drug (SGA) treatment. The dorsal vagal complex (DVC) and AMP-activated protein kinase (AMPK) are implicated in the regulation of food intake and body weight. Blocking the histamine H1 receptor (H1R) contributes to antipsychotic-induced weight gain. The present study investigated the time-dependent effect of olanzapine treatment (8, 16 and 36 days) on DVC AMPK signaling in olanzapine-induced weight gain, and whether these changes are associated with olanzapine-induced H1 receptor antagonism. During the 8-day olanzapine treatment the rats were hyperphagic and rapidly gained weight. The phosphorylation of AMPK (pAMPK: activated AMPK) as well as its directly downstream phospho-Acetyl-CoA carboxylase (pACC) was significantly increased. The pAMPK/AMPK ratio, an indicator of AMPK activity, was significantly positively correlated with feeding efficiency and weight gain. As treatment was prolonged (16 and 36-day olanzapine treatment), the rats were no longer hyperphagic, and there were no longer any changes in DVC AMPK signaling. Although the DVC H1R protein expression was not significantly altered by olanzapine, the pAMPK expression was significantly positively correlated with the H1R level after the 8, 16, and 36-day olanzapine treatments. Moreover, we showed that an H1R agonist, 2-(3-trifluoromethylphenyl) histamine, significantly inhibited the olanzapine-induced hyperphagia and DVC AMPK activation in a dose-dependent manner. These results suggest a time-dependent role of DVC AMPK in olanzapine-induced obesity. Thus, olanzapine-induced DVC AMPK activation may be at least partially related to olanzapine's antagonistic effect on the H1R.

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Olanzapine-Activated AMPK Signaling in the Dorsal Vagal Complex Is Attenuated by Histamine H1 Receptor Agonist in Female Rats

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Weight gain and its related metabolic disorders are major side effects associated with second generation antipsychotic drug treatment. The dorsal vagal complex (DVC) and AMP-activated protein kinase (AMPK) are implicated in the regulation of food intake and body weight. Blocking the histamine H1 receptor contributes to antipsychotic-induced weight gain. The present study investigated the time-dependent effect of olanzapine treatment (8, 16, and 36 d) on DVC AMPK signaling in olanzapine-induced weight gain and whether these changes are associated with olanzapine-induced H1 receptor antagonism. During the 8-day olanzapine treatment, the rats were hyperphagic and rapidly gained weight. The phosphorylation of AMPK (pAMPK) (activated AMPK) as well as its directly downstream phospho-acetyl-coenzyme A carboxylase was significantly increased. The pAMPK/AMPK ratio, an indicator of AMPK activity, was significantly positively correlated with feeding efficiency and weight gain. As treatment was prolonged (16 and 36 d of olanzapine treatment), the rats were no longer hyperphagic, and there were no longer any changes in DVC AMPK signaling. Although the DVC H1 receptor protein expression was not significantly altered by olanzapine, the pAMPK expression was significantly positively correlated with the H1 receptor level after the 8-, 16-, and 36-day olanzapine treatments. Moreover, we showed that an H1 receptor agonist, 2-(3-trifluoromethylphenyl) histamine, significantly inhibited the olanzapine-induced hyperphagia and DVC AMPK activation in a dose-dependent manner. These results suggest a time-dependent role of DVC AMPK in olanzapine-induced obesity. Thus, olanzapine-induced DVC AMPK activation may be at least partially related to olanzapine’s antagonistic effect on the H1 receptor. (Endocrinology 155: 4895–4904, 2014)

Second generation antipsychotic drug (SGA) treatment, in particular olanzapine and clozapine, can cause serious weight gain and other metabolic problems (1). In recent years, multiple contributors have been identified to be involved in antipsychotic-induced weight gain, such as the histaminergic H1 and H3 receptors, serotonin 2C, dopaminergic D2, muscarinic M3, and the adrenergic-α receptors (reviewed in Refs. 2–4). Moreover, neuropeptide Y (NPY), proopiomelanocortin (POMC) (5, 6), and central ghrelin signaling (7) have also been reported to contribute to olanzapine-induced obesity. However, the exact cellular mechanisms that promote SGA-induced weight gain are not fully understood.

The dorsal vagal complex (DVC), comprising the nucleus of the tractus solitarius (NTS), dorsal motor nucleus of the vagus, and area postrema, is well known for mediating appetite by responding to satiety signals. The NTS integrates the signals between the hypothalamus and the gut to regulate food intake (8). The dorsal motor nucleus of the vagus, located ventral to the NTS and containing brown adipose tissue; DVC, dorsal vagal complex; FMPH, 2-(3-trifluoromethylphenyl) histamine; icv, intracerebroventricular; NPY, neuropeptide Y; NTS, nucleus of the tractus solitarius; O/F(H), olanzapine/FMPH 200 nmol; pAMPK, phospho-AMPK; POMC, proopiomelanocortin; SD, Sprague-Dawley; SGA, second generation antipsychotic drug; WS, vehicle/saline.
primary efferents to regulate the gut, is involved in mediating food intake (9). Therefore, an imbalance of the systems in the DVC that mediate food intake can lead to obesity and other metabolic disorders.

AMP-activated protein kinase (AMPK), is a sensor of cellular energy status, which is activated by the cellular AMP/ATP ratio and upstream kinases (10). AMPK in the central nervous system plays an important role in the regulation of energy balance, food intake, and body weight in mammals (11, 12). AMPK in the NTS has been reported to mediate food intake (13, 14). AMPK activity in the NTS increases in food-deprived rats and is abolished by refeeding (14). The injection of the AMPK inhibitor, Compound C, into the fourth ventricle and NTS reduces food intake (14). Previous studies in rats have reported that the DVC is involved in mediating olanzapine-induced weight gain (15, 16). However, whether DVC AMPK signaling plays a role in mediating olanzapine-induced food intake and weight gain is unknown.

Clinical evidence has indicated that SGA induces weight gain (in particular olanzapine and clozapine) in a time-dependent manner. In the first 3 months, patients experienced a rapidly increased body weight (early stage); during 3–18 months, the rate of weight gain decreased (middle stage); and finally, after 18 months, weight plateaued at a heavy level and was maintained with continuing antipsychotic treatment (late stage) (17, 18). Similar effects have been found in the animal model, although each period is shorter (7, 19). The evidence suggests that the mechanisms that promote olanzapine-induced weight gain at different stages may vary. Does the DVC AMPK signaling play a different role in the different stages of olanzapine-induced obesity?

Olanzapine and clozapine have been reported to activate hypothalamic AMPK (20–23). Clozapine’s activation of AMPK is significantly linked to its antagonistic effect on the H1 receptors (23). However, it is not known whether the effect of olanzapine on the DVC AMPK signaling is also linked to the antagonism of the central H1 receptors. The present study provides evidence for the first time that olanzapine treatment activates AMPK signaling in the DVC in a time-dependent manner and that this effect is related to olanzapine-induced weight gain. Furthermore, we tested whether DVC AMPK activation could be modulated by central H1 receptor activation and its relationship with olanzapine-induced hyperphagia.

**Materials and Methods**

**Animals, drugs, and chemicals**

Female Sprague-Dawley (SD) rats (weight 200–225 g) were obtained from the Animal Resources Centre. The rats were housed under environmentally controlled conditions (22°C on a 12-h light, 12-h dark cycle; lights on 7 AM). Rats were allowed ad libitum access to food and water throughout the studies. All of the animal experiments were approved by the Animal Ethics Committee, University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Olanzapine (Zyprexa) was obtained from Eli Lilly. 2-(3-trifluoromethylphenyl) histamine (FMPH) and pyrilamine were obtained from Sigma-Aldrich (Sigma).

**Experiment 1**

To investigate the role of DVC AMPK signaling and H1 receptors at different stages of olanzapine-induced weight gain, we conducted this experiment to detect changes in AMPK signaling and the H1 receptors under olanzapine treatment for 8, 16, and 36 days. Briefly, SD rats were randomly divided into 3 groups (n = 12/group) and fed with sweet cookie dough pellets (62% carbohydrate, 22% protein, 6% fiber, and 10% vitamins and minerals) mixed with either olanzapine (1 mg/kg) or placebo 3 times daily at 8-hour intervals at 7 AM, 3 PM, and 11 PM (7). We measured food intake and body weight every 48 hours. At the end of treatment, all rats were euthanized between 9 AM and 11 AM by CO2 asphyxiation 2 hours after the last drug treatment. The DVC were quickly dissected on ice, frozen in liquid nitrogen, and then stored at −80°C. Postmortem white adipose tissue (perirenal, perioryv, inguinal, and omental fat) and liver were dissected and individually weighed.

**Experiment 2**

To determine the effect of energy intake restriction on olanzapine-induced weight gain and whether changes in the DVC AMPK signaling were a secondary effect of elevated food intake and body weight gain induced by olanzapine, we conducted a pair-feeding experiment for 8 days similar to experiment 1 (8-d treatment cohort). Rats were randomly divided into 2 groups (n = 12/group) and treated with olanzapine or vehicle as described in experiment 1. All of the rats in both groups (olanzapine and control) received restricted food intake (80% of the control group based on previous measurements) (7). Body weight was measured every 2 days. Rats were killed, and the DVC was collected using the same procedure as experiment 1.

**Experiment 3**

To further investigate whether DVC AMPK signaling is linked to olanzapine’s antagonistic effect on the central H1 receptors and olanzapine-induced hyperphagia, the olanzapine-treated rats received an intracerebroventricular (icv) injection of an H1 receptor agonist, FMPH. Briefly, after acclimatization for 1 week, SD rats were implanted with a 24-gauge guide cannula into the lateral ventricle under isoflurane anesthesia (1.0 mm posterior to the bregma, 1.5 mm lateral to the midline, and 3.5 mm below the top skull) (24). After recovery for 1 week, the rats were orally treated with olanzapine or vehicle (same as in experiment 1) for 4 days. On day 5, the rats were given an icv 100 or 200 nmol injection of the H1 receptor agonist, FMPH, or saline as a control (in 5 μL, at a rate of 5 μL/min) (25) (n = 5–8/group: group 1, vehicle/saline [V/S]; group 2, vehicle/FMPH 200 nmol; group 3, olanzapine/saline; group 4, olanzapine/ FMPH 100 nmol; group 5, olanzapine/FMPH 200 nmol [O/ F(H)]). Thirty minutes later, the rats were orally treated with...
olanzapine or vehicle. We measured the food intake of the rats at 1, 2, 4, 16, and 24 hours after the olanzapine or vehicle were given. Before measuring food intake, rats were allowed ad libitum access to food and water. To check whether the effect of FMPH on food intake could be attenuated by the H1 receptor antagonism, another group was injected with pyrilamine, an H1 receptor antagonist, by icv 30 minutes before the FMPH was given (group 6, O/F(H)/pyrilamine 800 nmol). The same treatment was repeated after a 3-day drug washout period, and the rats were killed 1 hour after their last treatment (between 12 PM and 2 PM) using CO2 asphyxiation. The DVC nuclei were immediately dissected on ice, frozen in liquid nitrogen, and then stored at −80°C.

Western blotting

Western blotting was conducted following the procedure in Ref. 26. The DVC tissues were homogenized using Nonidet P40 cell lysis buffer (Life Technologies) containing β-glycerophosphate, a protease inhibitor cocktail, and phenylmethanesulfonyl fluoride (Sigma). The protein concentration was detected by DC protein assay (Bio-Rad Laboratories). A total of 15- to 25-μg proteins were loaded onto 4%-to 12% Bis-Tris gels (Bio-Rad Laboratories) and run for 50 minutes at 200 V. The proteins were transferred to polyvinylidene difluoride (Bio-Rad Laboratories) membranes at 100 V for 1 hour. The membranes were then blocked for 1 hour in 5% BSA in Tris-buffered saline with 0.1% Tween 20 at room temperature. The membranes were incubated with primary antibodies in 1% BSA at 1:1000 at 4°C overnight; primary antibodies: AMPKα, acetyl-coenzyme A carboxylase (ACC), and phospho-AMPK (pAMPKα) (Cell Signaling Technology) and pACCα (Millipore), and the histamine H1 receptor (Santa Cruz Biotechnologies, Inc). The membranes were then washed in Tris-buffered saline with 0.1% Tween 20 (3 × 5 min) and incubated with goat antirabbit (1:5000; Santa Cruz Biotechnologies, Inc) and horseradish peroxidase conjugated secondary antibody at room temperature for 1 hour. Proteins were detected with the enhanced chemiluminescence kit (GE Healthcare). Bio-Rad Quantity One software was used to quantify the results. The levels of protein expression were normalized to those of actin (27). The densities of bands among the different blots were normalized to the average band density in the control group. Data are presented as the average density relative to the control group (27).

Statistical analysis

Statistical analysis was performed with the SPSS 19.0 program. In experiments 1 and 2, the statistical differences of relative food intake and body weight between olanzapine and the control group every 2 days were analyzed using the two-way ANOVA (olanzapine × time as repeated measure) followed by an independent unpaired Student’s t test (two-tailed). The differences in the H1 receptor, AMPKα, ACC, pAMPKα, and pACCα protein expression were analyzed using an independent unpaired Student’s t test (two-tailed). In the icv experiment (experiment 3), the Kruskal-Wallis H test and then the Mann-Whitney U test were used to analyze the difference in food intake. The statistical differences of pAMPKα and pACCα expression were analyzed by one-way ANOVA followed by post hoc Tukey’s multiple comparison in the DVC. Correlations were identified using Pearson’s correlation. All data were presented as mean ± SEM. Statistical significance was defined as P < .05.

Figure 1. Effects of olanzapine (3 mg/kg/d, three times daily on (A) relative food intake (compared with control), (B) body weight, (C) feeding efficiency, and (D) water intake, during the 36-day treatment. Rats were trained to eat cookie dough containing olanzapine or vehicle as indicated for 36 days (see Materials and Methods) (n = 12/group). All data are presented as mean ± SEM. Statistical significance was defined as P < .05 (*, P < .05; **, P < .01 vs control). FI, food intake; WG, weight gain.
Results

Food intake, body weight, water intake, and peripheral tissues weight

To investigate the effect of olanzapine on DVC AMPK signaling in olanzapine-induced weight gain, rats were treated with olanzapine or vehicle for 8, 16, and 36 days to represent the 3 stages (26). Compared with the control group, the olanzapine-treated group had a significantly elevated food intake during day 4–12 (up to 40% vs control, \( P < .05 \)) (Figure 1A) during the 36-day treatment. The olanzapine treatment significantly increased body weight from day 4 (by up to 7%, \( P < .05 \)) (Figure 1B). Feeding efficiency was significantly increased by all of the olanzapine treatments (\( P < .05 \)) (Figure 1C). Like previous studies, water intake was not altered by olanzapine throughout the treatment period (Figure 1D) (28, 29). There was a significant increase of sc periovary (\( P \leq .011 \)), perirenal (\( P \leq .032 \)), inguinal (\( P \leq .017 \)), and total fat mass (periovary+perirenal+inguinal+omentum, \( P \leq .010 \)) but not omental fat mass (\( P \leq .435 \)) after 8-, 16-, and 36-day olanzapine treatments.

Olanzapine activated DVC AMPK signaling after only 8 days of olanzapine treatment

Because the DVC AMPK plays an important role in feeding and body weight regulation (13, 14), we investigated the time-dependent effects of olanzapine on DVC AMPK signaling by treating rats with olanzapine for 8, 16, and 36 days. After the 8-day olanzapine treatment when rats were hyperphagic, the protein expression of the pAMPK\(\alpha\) and its directly downstream pACC\(\alpha\) were significantly increased (120\%\(\pm\)6%, \( P = .030 \); 121\%\(\pm\)5%, \( P = .022 \) vs control, respectively) (Figure 2, A, F, and H) without changes been seen in total AMPK\(\alpha\) and ACC expression.
pression (P > .05) (Figure 2, A, E, and G) compared with the control. Pearson’s correlation revealed that the ratio of pAMPK/AMPK (an indicator of AMPK activity) was significantly correlated with cumulative food intake \((r = 0.714, P = 0.014)\) (Figure 2A), weight gain \((r = 0.685, P = 0.020)\) (Figure 2B), and feeding efficiency \((r = 0.615, P = 0.044)\) (Figure 2C). These findings suggest that DVC AMPK signaling may be associated with olanzapine-induced hyperphagia. After 16 or 36 days of olanzapine treatment, the protein expression of AMPKα, pAMPKα, ACC, and pACCα in the DVC was not significantly altered (Figure 2, B–H). Pearson’s correlation revealed that the pAMPKα expression was positively correlated with the last 48 hours of food intake (16 days, \(r = 0.639 \text{ and } P = 0.034\) [Figure 3D] and 36 days, \(r = 0.655 \text{ and } P = 0.040\) [Figure 3E]), suggesting that during long-term olanzapine treatment (16 and 36 d), loss of AMPK activation may partially explain why olanzapine did not increase food intake during chronic treatment.

In the central nervous system, studies have shown that the antipsychotic-induced activation of AMPK is related to its antagonistic effect on the histamine H1 receptors, in particular clozapine and olanzapine (23, 26). Olanzapine has affinity for many receptors, including the H1 receptor, which is significantly involved in olanzapine-induced weight gain (30), via the regulation of AMPK signaling (23, 26). Therefore, we examined the time-dependent effect of olanzapine on the expression of the DVC H1 receptor. We performed Western blotting to detect the protein expression of the H1 receptors. The DVC H1 receptor’s expression was not significantly altered by the 8-, 16-, and 36-day olanzapine treatments (Figure 2, A–D). However, the DVC H1 receptor expression was significantly positively correlated with DVC pAMPKα expression in all 3 experiments (8 days, \(r = 0.760 \text{ and } P = 0.011\); 16 days, \(r = 0.671 \text{ and } P = 0.034\); and 36 days, \(r = 0.685 \text{ and } P = 0.020\)). These correlations suggest that although the H1 receptor in the DVC was not significantly altered, it may be related to DVC AMPK signaling.

Olanzapine treatment activated the DVC AMPK signaling in pair-fed rats

The data from the pair-feeding experiment show that when rats consumed the same amount of food, the olanzapine-treated group had a similar weight gain compared with the control group (all \(P > .05\), \(n = 12\)/group, two-way ANOVA [olanzapine \(
\times\text{time as a repeated measure}\) [Figure 4, A and B] followed by an independent unpaired Student’s t test). The total expression of AMPK in the DVC was not altered by olanzapine \((P > .05)\). Olanzapine treatment significantly increased pAMPKα expression \((137 \pm 11\%, P = 0.010\) vs control, \(n = 12\)/group) and pACCα

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**Figure 3.** Correlations within DVC pAMPK, food intake, and weight gain after 8, 16, and 36 days of olanzapine treatment. A–C, Correlations within the DVC pAMPK/AMPK activity, food intake, weight gain, and feeding efficiency after 8 days of treatment of olanzapine or vehicle. D and E, Correlations within DVC pAMPK and the last 48 hours of food intake after 16 and 36 days of olanzapine treatment. All data are presented as mean ± SEM. Statistical significance was defined as \(P < .05\). pAMPKα, AMPK phosphorylated Thr172 at AMPKα-subunits.
expression (169 ± 19%, P = .004 vs control) as well as total ACC expression (131 ± 12%, P = .049 vs control) (Figure 4, C and D). Although the H1 receptor expression was not significantly altered (P > .05), it was significantly correlated with the expression of pAMPKα (r = 0.537, P = .007, Pearson’s correlation). This evidence suggests that the activation of DVC AMPK signaling is related to olanzapine’s antagonistic effect on the H1 receptor.

Olanzapine-induced hyperphagia and activated DVC AMPK were inhibited by the H1 receptor agonist in a dose-dependent manner

We further investigated whether olanzapine’s effect on DVC AMPK is associated with its antagonistic effect on the H1 receptor and whether this effect is related to olanzapine-induced hyperphagia. Olanzapine-treated rats were given a central injection of different doses of the H1 receptor agonist, FMHP. Figure 5A shows that the olanzapine-treated rats receiving the saline injection (as a control) had a significantly increased food intake from 0–1 hour (by 46%, P = .020, Mann-Whitney U test), 1–2 hours (by 66%, P = .083), and 2–4 hours (by 63%, P = .007) after the last drug treatment. FMHP 100 nmol inhibited the olanzapine-induced hyperphagia at 2–4 hours (by 60%, P = .004).

In terms of DVC AMPK signaling, our data revealed significantly increased pAMPKα (135 ± 5% vs V/S) and pACCα (134 ± 7% vs V/S) expression after 5 days of olanzapine treatment (ANOVA, F(5,31) = 11.057, P = .000; F(5,28) = 5.988, P = .001, respectively, n = 5–7/group, Tukey post hoc tests) (Figure 5, B and C). This stimulatory effect of olanzapine on DVC pAMPKα and pACCα expression was inhibited by the 200 nmol FMHP (109 ± 5%, P = .013; 109 ± 4%, P = .078, respectively, Tukey post hoc tests) but not by the 100 nmol FMHP compared with saline (124 ± 3%, P > .05; 117 ± 4%, P > .05, respectively, Tukey post hoc tests). Pyrilamine treatment reversed the inhibitory effect of the 200 nmol FMHP on pAMPKα and pACCα expression after the olanzapine treatment (141 ± 12%, P = .000; 136 ± 7%, P = .037, respectively, Tukey post hoc tests). An ivc injection of FMHP in the vehicle-treated rats did not significantly change the DVC pAMPKα and pACCα levels. Moreover, the DVC pAMPKα expression was also positively correlated with food intake at 1 hour (Figure 5D). These findings indicate that the DVC AMPK appears to regulate olanzapine-induced hyperphagia and weight gain and that this effect is linked to olanzapine’s antagonistic effect on the central H1 receptors.

Discussion

The present study provides the first evidence that olanzapine treatment time dependently affects DVC AMPK signaling. Rats treated with olanzapine for 8 days had significantly activated DVC AMPK signaling. However, the DVC AMPK activation vanished with prolonged olanzapine treatment (16 and 36 d). During short term-olanzapine treatment (<12 d), the rats had an elevated food intake and rapid weight gain, consistent with previous studies (19, 31–33). The significant correlation between DVC AMPK activity and food intake, weight gain, and feeding efficiency suggests that DVC AMPK signaling
may significantly contribute to the olanzapine-induced hyperphagia during this period. Because treatment was prolonged, the olanzapine treatment no longer led to an elevated food intake (3–5 wk). However, a higher body weight was maintained compared with the control animals both in our study and data from other groups (19, 31, 33). Consistently, with food intake, long-term olanzapine treatment did not significantly activate DVC AMPK signaling, and pAMPK expression was positively correlated with the last 48 hours of food intake. These data suggest that during long-term olanzapine treatment, the disappearance of DVC AMPK activation may at least partly explain why long-term olanzapine treatment (>2 wk) no longer induces hyperphagia.

In addition, diet should also be considered as a factor that influences the development of olanzapine-induced obesity. In humans, a calorie-restricted diet has been identified to be effective for patients on olanzapine treatment (34). Exercise and diet have improved the aerobic capacity and body composition of obese patients treated with olanzapine (35). In this study, we investigated whether diet restriction reduces olanzapine-induced weight gain and its effect on DVC AMPK signaling. Our data show that when energy intake is restricted, olanzapine-treated rats had a similar weight gain compared with vehicle-treated rats during short-term treatment. These data suggest that increased energy intake may play a major role in short-term olanzapine-induced weight gain. Restricting energy intake may be effective for controlling olanzapine-induced weight gain, at least during short-term treatment. Although weight gain was not significantly increased, the olanzapine treatment significantly activated DVC AMPK signaling. These findings suggest that the olanzapine-induced activation of DVC AMPK signaling is likely to be directly caused by olanzapine treatment rather than being a secondary effect of food intake. Moreover, it has also been reported that male rats on a cafeteria diet (hypercaloric) treated with olanzapine for 4 months showed increased body weight and fat pads compared with standard diet groups (standard diet vehicle, standard diet with olanzapine, and cafeteria diet vehicle groups) (36). It is possible that different diet types may also modify the effect of olanzapine on obesity development and other metabolic side effects. Further studies are needed in this area.
In addition to regulating food intake, the fact that the rats gain weight without hyperphagia suggests that other factors are involved in olanzapine-induced weight gain. In the clinic, for example, decreased energy expenditure has been reported to be involved in the weight gain induced by olanzapine and clozapine treatment (37–39). Previous studies in animal models also show that SGAs, including olanzapine and clozapine, decrease energy expenditure by inhibiting sympathetic activity in brown adipose tissue (BAT) (32, 40, 41). Moreover, data from our group has reported that chronic olanzapine (36 d) treatment is associated with decreased temperature in BAT, accompanied by decreased BAT uncoupling protein 1 expression and peroxisome proliferator activated coactivator 1α expression, which are important markers of BAT thermogenesis (42). DVC AMPK signaling has also been shown to be involved in regulating energy expenditure via its regulation of heart rate (14). However, the role of DVC AMPK in BAT thermogenesis has not been fully studied. In this study, DVC AMPK was activated by olanzapine after the 8-day treatment but not the 16- or 36-day treatments. These changes in DVC AMPK do not match the changes in BAT temperature and uncoupling protein 1 expression and peroxisome proliferator activated coactivator 1α expression, which are important markers of BAT thermogenesis (42). DVC AMPK signaling has also been shown to be involved in regulating energy expenditure via its regulation of heart rate (14). However, the role of DVC AMPK in BAT thermogenesis has not been fully studied. In this study, DVC AMPK was activated by olanzapine after the 8-day treatment but not the 16- or 36-day treatments. These changes in DVC AMPK do not match the changes in BAT temperature and uncoupling protein 1 and peroxisome proliferator activated coactivator 1α expression. This suggests that the decreased BAT temperature after chronic olanzapine treatment could be due to reduced sympathetic activity on BAT. This effect can occur independently of olanzapine’s effect on DVC AMPK signaling. Therefore, there is no association between AMPK phosphorylation and energy expenditure in the DVC. Mechanisms besides AMPK may be involved in olanzapine-induced weight gain, for example hypothalamic NPY and POMC (5–7).

We also investigated the mechanism by which olanzapine activates DVC AMPK signaling. Olanzapine has a high affinity for the H1 receptors, one of the major contributors to olanzapine-induced weight gain. Previous evidence has demonstrated that the NTS within the DVC is heavily innervated by hypothalamic histaminergic projections (43, 44). The NTS contains abundant H1 receptors, which are predominantly located on neurons (44). It has been reported in rats that the activation of the NTS H1 receptor increases arterial pressure and heart rate (44). However, the role of the DVC H1 receptors in food intake regulation is unknown. Our study demonstrated that central H1 receptor activation inhibited the hyperphagia and DVC AMPK activation induced by olanzapine. We show that the pAMPK expression was correlated with food intake. Therefore, it is possible that the antagonism of the DVC H1 receptors by olanzapine may contribute to AMPK activation in the DVC and thus increase food intake. It would be helpful to identify the neurochemical phenotype of neurons in the DVC that express AMPK. In addition, it is desirable to test whether the direct activation of the DVC H1 receptors blocks olanzapine-induced food intake and AMPK activation.

The cross-communication of the DVC and hypothalamic pathways in regulating food intake and energy balance has already been studied (45). The DVC receives projections from the hypothalamus to regulate food intake (45). In this study, the H1 receptor agonist was injected into the lateral ventricle (easy access to the hypothalamus). Thus, the inhibitory effect of the H1 receptor agonist on DVC AMPK signaling could also be an indirect effect caused by the hypothalamus. In our previous study, we reported that olanzapine (3 mg/kg) significantly activated hypothalamic AMPK after an 8-day treatment (26). This change is consistent with the change in DVC AMPK signaling. However, we have performed correlation analyses and found that pAMPK expression in the hypothalamus and the DVC are not correlated. With prolonged olanzapine treatment, the hypothalamic AMPK was not activated after the 16-day treatment and was actually inhibited after the 36-day treatment. These changes in DVC AMPK signaling are not consistent with the changes in AMPK signaling in the hypothalamus. This inconsistency suggests that the hypothalamic and DVC AMPK may be mediated via independent mechanisms. In fact, previous research has reported a difference in controlling meal size between the hypothalamus and DVC (46). The activation of DVC AMPK signaling after the 8-day olanzapine treatment may be directly caused by olanzapine’s action on the DVC H1 receptors rather than being a secondary effect of the hypothalamus. DVC neurons integrate multiple neuropeptides and signals from the forebrain, integrate gut and adiposity signals for long-term energy storage, and determine food intake (8). Because DVC AMPK is an energy sensor that responds to energy status (14), the inactivation of AMPK during long-term olanzapine treatment may be caused by a negative response to energy overload. Moreover, it has been reported that chronic olanzapine (≥14 d) treatment in rats did not change ghrelin (7), DVC NPY, or POMC expression (6), which are all important regulators of AMPK. It is possible that the lack of DVC AMPK activation after chronic olanzapine treatment may be caused by multiple parameters besides the H1 receptors, such as ghrelin, NPY, or POMC. Further studies are needed.

Both hypothalamic and DVC AMPK phosphorylation is associated with food intake in the early stages of olanzapine treatments. At the middle and late stages of olanzapine treatments, DVC but not hypothalamic AMPK phosphorylation was still associated with food intake. Previous studies showed that after a long-term energy
overload, hypothalamic AMPK signaling does not play a key role in controlling food intake (5, 47). DVC AMPK signaling may still regulate food intake in the long-term treatment of olanzapine. In addition, other recent studies have shown that hypothalamic NPY, Agouti-related peptide, orexin, and forkhead box protein O1 could also contribute to olanzapine-induced food intake, weight gain, and adiposity (6, 7, 48, 49). On the other hand, it is noted that the NPY and POMC expression in the DVC was not changed by olanzapine administration in rats (6).

Previous studies have also reported that G protein-coupled receptors, including the H1 receptor, can be desensitized after a continuous activation by a receptor agonist (50–52). There are some exceptions to this, because the serotonin 2A receptor can be desensitized by both agonist and antagonist exposure. Chronic olanzapine treatment causes the desensitization of serotonin 2A receptor signaling and the down-regulation of serotonin 2A receptor expression (53, 54). Olanzapine is an H1 receptor antagonist. However, there is no evidence to suggest that either olanzapine treatment or other H1 receptor antagonists leads to the desensitization of H1 receptor signaling. In the present study, H1 receptor expression is not changed by chronic olanzapine treatment, indicating that a desensitization effect is unlikely.

In conclusion, the present study demonstrates that the effect of olanzapine on DVC AMPK signaling varies during different treatment periods. After short-term treatment (8 d), olanzapine activated AMPK signaling, which may contribute to olanzapine-induced hyperphagia. With prolonged treatment, DVC AMPK was not significantly activated, and the rats gained weight even without excessive food intake, suggesting that other parameters besides AMPK contribute to increased body weight. Central H1 receptor activation blocks DVC AMPK activation during short-term treatment, suggesting that the antagonistic effects of olanzapine on the H1 receptor may be responsible for the activation of DVC AMPK.

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