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Dietary Fructose and GLUT5 Transporter Activity Contribute to Antipsychotic-Induced Weight Gain

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Receptors for antipsychotics in the hypothalamus contribute to antipsychotics-induced weight gain; however, many of these receptors are also expressed in the intestine. The role of these intestinally-expressed receptors, and their potential modulation of nutrient absorption, have not been investigated in the context of antipsychotics-induced weight gain. Here we tested the effect of dietary fructose and intestinal fructose uptake on clozapine-induced weight gain in mice. Weight gain was determined in wild type mice and mice lacking the GLUT5 fructose transporter that were "orally-administered" 20 mg/kg clozapine for 28 days. To assess the role of dietary fructose, clozapine-treated mice were fed controlled diets with different levels of fructose. Effect of clozapine treatment on intestinal fructose transport activity and expression levels of various receptors that bind clozapine, as well as several genes involved in gluconeogenesis and lipogenesis were measured using real-time RT-PCR and western blotting. Oral administration of clozapine significantly increased body weight in wild type C57BL/6 mice but not in GLUT5 null mice. The clozapine-induced weight gain was proportional to the percentage of fructose in the diet. Clozapine-treated mice increased intestinal fructose uptake without changing the intestinal expression level of GLUT5. Clozapine-treated mice expressed significantly higher levels of intestinal H1 histamine receptor in the wild type but not GLUT5 null mice. Clozapine also increased the intestinal expression of fructokinase and several genes involved in gluconeogenesis and lipogenesis. Our results suggest that increased intestinal absorption and metabolism of fructose contributes to clozapine-induced weight gain. Eliminating dietary fructose might prevent antipsychotics-induced weight gain.

Key words: clozapine/histamine/gluconeogenesis/lipogenesis/intestine/gene expression

Introduction

Atypical antipsychotics, such as clozapine, olanzapine, and risperidone have all been reported to induce weight gain which can increase the risk for cardiovascular disease, type II diabetes, metabolic syndrome, and dyslipidemia.[1–4] Weight gain is a common side effect of antipsychotic treatment in adults and children.[1,5–8] Studies have also shown that antipsychotics induce weight gain in rodents.[9,10] Interestingly, many clinical studies have shown that, independent of weight gain, atypical antipsychotics such as clozapine olanzapine and risperidone can increase insulin resistance and dyslipidemia.[11–19] The mechanisms that mediate antipsychotics induced weight gain and metabolic dysfunction are not well understood.

Fructose is a major ingredient found in soft drinks, pastries, desserts and is present in most processed foods.[20–23] During the past few decades, consumption of high fructose corn syrup (HFCS) has been correlated with the development of obesity.[24,25] Given the relatively high percentage of fructose in modern diet, and its ability to contribute to weight gain, we postulated that antipsychotics-mediated modulation of fructose absorption and metabolism might contribute to weight gain. Intestinal fructose absorption occurs in the duodenum and proximal jejunum on the luminal membrane of enterocytes through a facilitative transport via the fructose transporter, GLUT5.[26–28] Here we directly tested the hypothesis that antipsychotics might modulate intestinal GLUT5 function.

Although the central nervous system is a well-studied site of action of antipsychotics, the receptors targeted by antipsychotics are also expressed in peripheral areas such as in the enteric system.[29] In general, all antipsychotics are ligands at G-protein coupled receptors (GPCRs) such as D2-like (D2, D3, and D4) dopamine receptors with varying affinity for serotonin and histamine receptors.[30,31] Recent studies have shown that H1 histamine receptors,

serotonin receptors and dopamine D3 and D4 receptors are expressed in the intestinal mucosa, the region where the GLUT5 transporter is also expressed.[29,32–35] Interestingly, H1 histamine receptors in the brain have been implicated in antipsychotics-induced weight gain.[36,37] Here we assessed the effect of chronic antipsychotic treatment on intestinal expression of GPCRs that bind antipsychotics. We also tested the hypothesis that clozapine-induced weight gain causes changes in expression of genes involved in intestinal gluconeogenesis and lipogenesis that is dependent on fructose in the diet and mediated by the GLUT5 fructose transporter.

Methods

Animals

GLUT5 knock out (KO) or null mice on a C57BL/6 genetic background were obtained from St Jude's Research Hospital and a local breeding colony established. Eight to 10-week old female GLUT5 null mice and wild type littermates were used in this study as described previously.[9] The mice were individually housed in a climate-controlled environment (22°C –24°C) on a 12-hour dark and 12-hour light cycle. They were provided ad libitum water. Animals administered clozapine and vehicle were pair-fed to control for hyperphagia. All procedures were performed under a protocol approved by the Rutgers-New Jersey Medical School IACUC committee.

Drug Administration

Clozapine (Sigma Chemical Company) was dissolved in vehicle, dimethylsulfoxide (DMSO), and mixed in melted peanut butter (Jiffy, The J.M. Smucker Company), and placed into silicone molds (Ted Pella, Inc) to generate 100 mg pellets. The molds with peanut butter pellets were stored at −80°C until treatment time. The amount of clozapine in each 100 mg pellet was calculated to deliver a dose of 10 mg/kg. The mice were fed 100 mg pellets containing vehicle or clozapine twice a day in the morning and evening. Each mouse quickly consumed the peanut butter pellets as described previously.[9]

Fructose Diets

For experiments in which the fructose content of the diet was changed, we used custom isocaloric chow with 0%, 10%, and 20% fructose (LabDiet). To maintain equal calories, the fructose amount was balanced with dextrose. The mice were pair fed to the 0% fructose group and vehicle control. The wild type C57BL/6 mice were acclimatized to the chow with different fructose composition for 2 weeks prior to the 28-day vehicle and clozapine treatment. For all other experiments, mice were fed regular chow that included ground corn, dried sugar beet pulp and cane molasses, all of which contain significant amount of fructose.

Body Weight, Food Intake, and Blood Glucose Measurements

Body weights were measured every 3 days during the 4 weeks of treatment. During the course of the treatment, animals administered clozapine and vehicle were pair-fed to control for hyperphagia. Food intake was measured by subtracting the amount of food remaining at the end of each week from the amount of food that was originally placed in the cages. Blood glucose levels were measured, before the vehicle/clozapine treatment of the day, once a week beginning day 1 from a drop of blood obtained from tail tip laceration. Glucose levels were measured using ACCU-CHEK glucose monitoring kit (Roche).

In vitro Intestinal Nutrient Uptake Assay

The uptake assay was performed as described previously.[27] After the mice were anesthetized, an incision was made in the animal's abdomen and the intestine was exteriorized. A catheter was inserted into the lumen of the proximal region, flushed out with ice-cold Krebs-Ringer-bicarbonate (KRB) solution and immediately after, the duodenum and proximal jejunum region was excised, everted, cut into 4 segments, and mounted on steel rods. For each mouse, four 1-cm everted segments were pre-incubated at 37°C for 5 minutes in KRB solution bubbled with 95% O_2 – 5% CO_2. These everted segments were then incubated in 12 mL of 50 mM glucose or fructose solutions at 37°C in an oxygenated stirred (1200 rpm) solution, containing either [^{14}C] *D*-glucose or [^{14}C] *D*-fructose (both 1 μCi/mL), respectively. Segments were rinsed in 30 mL of ice-cold KRB Ringer solution for 20 seconds immediately after incubation. *L*-[^{3}H]glucose was used to correct for adherent fluid and passive diffusion of glucose and fructose. For *L*-proline uptake, the segment was incubated in 50 mM *L*-proline, 1 μCi/mL *L*-[3H]proline and 0.5 μCi/mL ^{14}C]inulin-carboxyl (3.9 μCi/mL). The inulin was used to correct for adherent fluid in estimating proline uptake rate. The radioactivity was quantitated using a liquid scintillation counter as described previously.[27]

Total RNA Extraction and Real-time Reverse Transcriptase-Polymerase Chain Reaction

Sections of the jejunum were cut and placed in RNALater (Life Technologies) then stored in −80°C freezer for subsequent RNA extraction. Total RNA was extracted from 100 mg of intestinal tissue using 1 mL of TRIzol Reagent (Life Technologies). The RNA was treated with Turbo-free DNAse (Life Technologies) and cDNA generated using SuperScript III reverse transcriptase (Life Technologies) as described previously.[38] Real-Time PCR was performed using the Applied Biosystems 7500 Real-time PCR System (Life Technologies) and gene-specific TaqMan probes (Life Technologies). The PCR conditions were 95°C for 10 minutes, 40 cycles at 95°C for 15

seconds, 60°C–62°C (primer specific, optimal annealing temperature) for 1 minute. The relative amount of cDNA in the samples for each target gene measured was normalized to levels of internal control β-actin.

Protein Analysis

After the jejunum was removed from each animal, it was rinsed with ice-cold KRB Ringer solution and the mucosa scraped off and immediately placed into liquid nitrogen. Collected mucosal tissue was then stored at −80°C until protein extraction. For total protein extraction from intestinal tissue samples, ~1 g of tissue sample was homogenized per 20 mL of T-Per Tissue Protein Extraction Reagent (Thermo Fisher Scientific). Protein concentration was quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Proteins were resolved by SDS-PAGE, transferred overnight to nitrocellulose membrane. The target proteins were detected using primary antibodies: anti-GLUT5 (1:1000) (Millipore); anti-fructokinase (KHK) (1:500) (Abcam, Inc); anti-fructose 1,6-bisphosphatase (FBPase1) (1:1000) (Abcam, Inc); anti-histamine H1 receptor (HR1) (1:1000) (Millipore, Inc). The protein samples were immunodetected by Immun-Star WesternC Chemiluminescence Kit (Bio-Rad Laboratories, Inc). Membranes were visualized on a ChemiDocXRS (Bio-Rad Laboratories, Inc), image system and analyzed using Quantity One 1-D analysis software (Bio-Rad Laboratories, Inc). The intensity of the protein band of interest was divided by the intensity of the housekeeping protein β-actin.

Statistical Data Analysis

All statistical analyses were performed using SPSS statistical software (PASW Version 18.0) and graphs were created using SigmaPlot (Version 11; Systat Software, Inc). Statistical analyses included independent samples *t* tests or ANOVA (1-way, 2-way or 2-way repeated measures), followed by Student–Newman–Keuls (SNK) test post hoc analysis where appropriate. Differences were considered statistically significant when the *P* value was less than .05.

Results

Clozapine-Induced Weight Gain is Dependent on Dietary Fructose Levels and Expression of GLUT5 Fructose Transporter

To determine if the level of fructose in the diet affected clozapine-induced weight gain, wild type C57BL/6 mice were maintained on a diet containing 0%, 10%, and 20% fructose and orally-administered vehicle or 20 mg/kg clozapine for 28 days. The mice were pair-fed to the 0% fructose group and vehicle control. Body weight was measured every 3 days. Mice on diets with different levels of fructose administered vehicle did not show any significant difference in body weight gain over the 28-day treatment period (supplementary figure 1); however, the results in figure 1A show that mice administered clozapine exhibited a statistically significant interaction between the percentage of fructose in the diet and day of treatment (2-way, repeated measure ANOVA, $P < .001$, $F_{18, 162} = 4.686$). Post hoc SNK multiple comparison test showed that mice fed diets with 20% fructose exhibited significant clozapine-induced weight gain compared to mice fed diets with no fructose or 10% fructose by day 16 ($P < .001$, $q = 5.392$; $P < .001$, $q = 5.061$, respectively). Mice fed diets with 10% fructose exhibited significant clozapine-induced weight gain compared to mice fed diets with no fructose by day 25 ($P = .006$, $q = 3.982$). These results suggest that there is a positive correlation between dietary fructose levels and clozapine-induced weight gain.

Dietary fructose absorption in the intestine occurs via the luminaly-expressed GLUT5 intestinal fructose transporter. To determine if clozapine-induced weight gain was mediated by the GLUT5 intestinal fructose transporter, GLUT5 null mice and wild type litter mates, on regular chow, were orally administered vehicle or 20 mg/kg clozapine for 28 consecutive days. As expected both wild type (figure 1B) and GLUT5 null mice (figure 1C) administered vehicle and clozapine gained weight after 28 days. The difference in the mean values among the different days is statistically significant after allowing for effects of differences in treatment ($P < .001$, 2-way repeated measure ANOVA, $F_{9, 108} = 25.242$). The weight gain in wild type mice that were treated with clozapine was significantly greater than vehicle-treated wild type mice beginning day 25 (2-way repeated measure ANOVA, post hoc SNK test, $P = .01$, $q = 3.709$; figure 1B). In GLUT5 null mice, the same statistical analysis showed that there was no significant difference in weight gain between vehicle- and clozapine-treated mice ($P > .05$; figure 1C). These results suggest that the ability of mice lacking the GLUT5 fructose transporter to gain weight is similar to wild type littermates; however, the GLUT5 null mice do not exhibit the clozapine-induced weight gain observed in the wild type littermates. This strongly suggests that GLUT5 fructose transporter is essential for clozapine-induced weight gain.

Clozapine Treatment Selectively Increases Intestinal Fructose Uptake Activity but not GLUT5 Expression in Wild Type Mice

We next determined if clozapine treatment affected intestinal fructose uptake and expression of GLUT5. The jejunum was isolated from wild type and GLUT5 null mice that were orally administered vehicle or clozapine (20 mg/kg) for 28 days and transport of fructose, proline and glucose measured across the luminal (apical) membrane of the enterocytes. The results in figure 2A shows that fructose uptake was significantly increased by ~3.9-fold in wild type mice administered clozapine ($P = .017$, Student's *t* test). There was no clozapine-induced increase

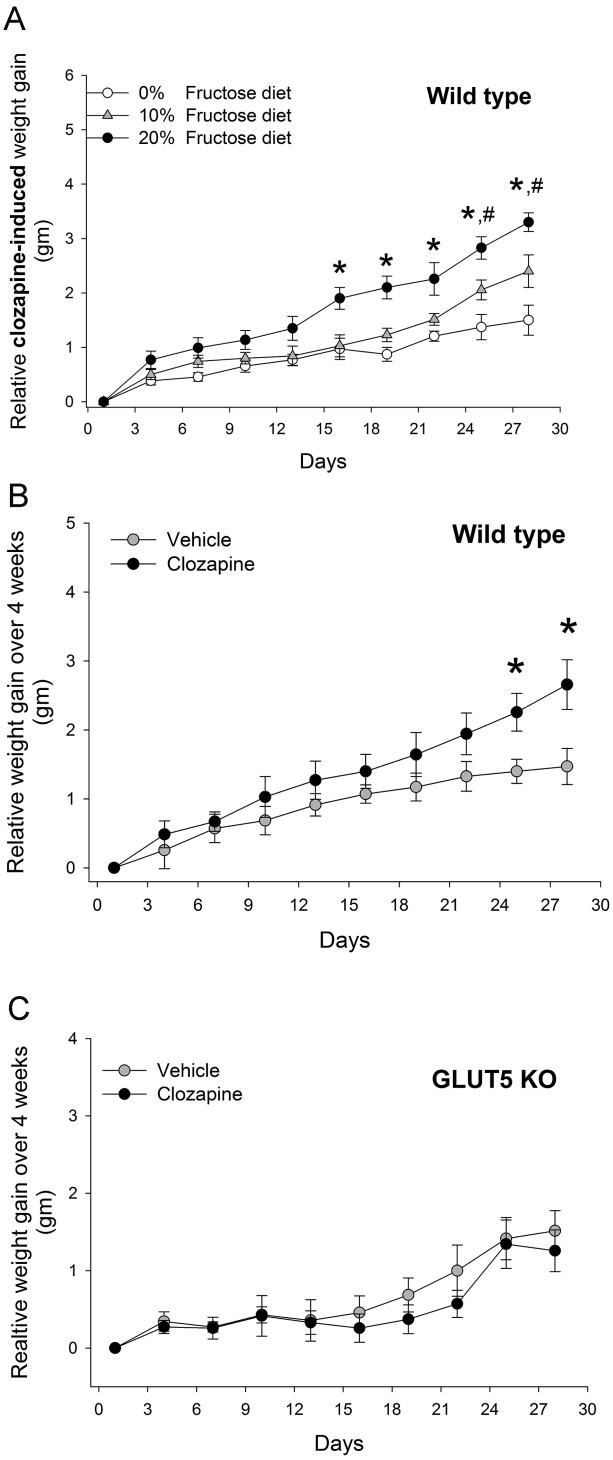

Fig. 1. **(A)** Wild type C57BL/6 female mice were provided diets with 0% (open circle), 10% (gray triangle) and 20% (filled circle) fructose for 6 weeks. Two weeks after being switched to the fructose-controlled diets, mice were orally administered 100-mg peanut butter pellets with or without 20 mg/kg clozapine a day for 28 days. Body weights were measured every third day beginning day 1. Clozapine-induced weight gain was calculated for mice fed diets with 0%, 10%, and 20% fructose. Statistically significant differences in clozapine-induced weight gain were found after day 16 between mice on 0% or 10% and 20% fructose (*, $P < .001$) and after day 25 between, 0% and 10%, or 20% fructose (#, $P = .006$), 2-Way repeated measures ANOVA, post hoc Student–Newman–Keuls (SNK) test. $N = 7$ mice in each of the treatment group. Female wild type (**B**) and female GLUT5 null mice (**C**) were maintained on "regular" chow (which contains fructose) and orally administered 100-mg peanut butter pellets with vehicle or 20 mg/kg clozapine a day for 28 days. (**B**) Wild type clozapine-treated mice gained statistically significant weight compared to wild type vehicle-treated mice after day 25 (*, $P < .01$, 2-Way repeated measures ANOVA, post hoc SNK test, $n = 10$). (**C**) In contrast, over the same treatment period, the weight gain in GLUT5 knock out (KO) clozapine- and vehicle-treated mice was not statistically different ($P > .05$, 2-Way repeated measures ANOVA, post hoc SNK test, $n = 9$). Error bars represent ± standard error of the mean. Data were normally distributed and passed the equal variance test.

in proline ($P = .918$, Student's t test) or glucose transport ($P = .991$, Student's t test) in the wild type mice. No detectable basal intestinal fructose uptake was observed in jejunum isolated from GLUT5 null mice (figure 2B) suggesting that fructose uptake in jejunum is mediated exclusively via GLUT5 transporters and not GLUT2, GLUT7, or GLUT8. In addition, clozapine-treated GLUT5 null mice did not exhibit significant changes in fructose, proline, or glucose uptake (figure 2B).

To determine if increase in fructose uptake was due to clozapine-induced increase in intestinal GLUT5 expression, we next measured the levels of GLUT5 mRNA and protein in the jejunum of wild type mice treated with vehicle or clozapine (20 mg/kg) for 28 days. The results show that clozapine treatment does not significantly alter the expression of GLUT5 mRNA (figure 2C) or protein (figure 2D) in the jejunum of wild type mice. Consistent with the results from the uptake studies, no difference in GLUT2 expression was observed between the treatment groups (data not shown). Together, these results suggest that fructose transport in the jejunum is mediated by GLUT5 transporter and clozapine treatment selectively increases apical fructose transport. Furthermore, the results suggest that the clozapine-induced increase in intestinal fructose uptake via GLUT5 is not due to an increase in GLUT5 expression but rather by an increase in its transport activity.

Clozapine Treatment Selectively Increases Intestinal Histamine H1 Receptor Expression via GLUT5-Dependent Mechanism

Clozapine is a selective antagonist with high affinity at D2-like (D2, D3, and D4) dopamine receptors, 5-HT2 serotonin receptors, as well as H1 and H2 histamine receptors receptor. Recent studies have shown that these receptors are expressed in the intestinal mucosa region of the jejunum, the region where GLUT5 fructose transporter is expressed.[36,37] Results in figure 3 shows that 28-day clozapine treatment did not significantly change the expression of intestinal dopamine D3 or D4 receptors (figure 3A) or serotonin 5-HT2A or 5-HT2C receptors (figure 3B). Clozapine significantly increased intestinal

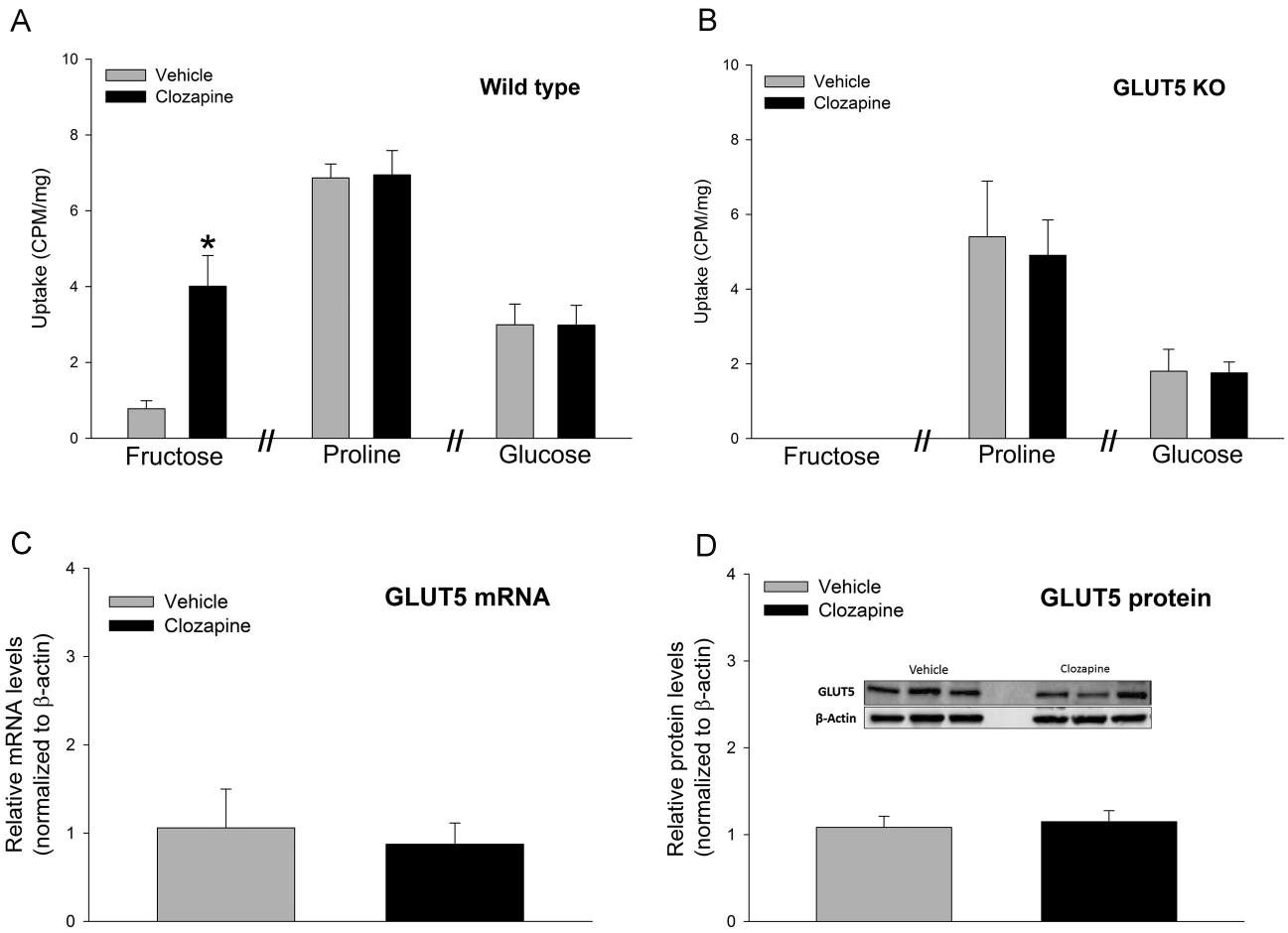

Fig. 2. Uptake of radiolabeled fructose, proline and glucose in jejunum tissue obtained from wild type mice (**A**) and GLUT5 null (knock out [KO]) mice (**B**) that were maintained on regular chow and orally administered peanut butter pellets containing vehicle (gray bars) or 20 mg/kg clozapine (black bar) twice a day for 28 days. On day 29, clozapine-treated wild type mice exhibited significant increase in only intestinal fructose uptake (*, $P = .017$, Mann-Whitney Rank Sum Test, $n = 7$). No fructose uptake was detectable in the jejunum tissue isolated from GLUT5 KO mice. On day 29, the expression of GLUT5 mRNA (**C**) and protein (**D**) in the jejunum of wild type mice was measured using reverse transcriptase-polymerase chain reaction (RT-PCR) (**C**) and western blot analysis (**D**). The levels of GLUT5 was normalized to internal control β-actin. A representative western blot is shown in the inset in panel **D**. Samples were run in triplicate. Error bars represent ± standard error of the mean.

histamine H1 receptor mRNA levels ($P = .002$, Mann-Whitney rank sum test) but not the histamine H2 receptor mRNA levels (figure 3C). The clozapine-induced increase in intestinal histamine H1 receptor was also observed at the protein level ($P = .022$, Student's t test). Interestingly, the clozapine-induced increase in intestinal histamine H1 receptor protein was not observed in GLUT5 null mice (figure 3D). Together these results suggest that clozapine treatment selectively increases histamine H1 receptor expression and this increase is dependent on intestinal fructose uptake via the GLUT5 transporter.

Clozapine Treatment Increases Intestinal Expression of Select Genes in the Gluconeogenesis Pathway

The key enzymes in the fructose-driven gluconeogenic pathway are fructokinase (KHK), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1, 6 bisphosphatase (FBPase-1), and glucose-6-phosphatase (G6Pase). To determine if the intestinal expression of these enzymes are affected by clozapine treatments and fructose transport, we measured the expression levels of the enzymes in GLUT5 null mice and wild type littermates following a 28-day oral administration of vehicle or clozapine (20 mg/kg). The results in figure 4 show that the expression of KHK (figure 4A) and FBPase-1 (figure 4C) mRNA and protein are significantly increased. There was no significant change in expression of PEPCK or G6Pase (data not shown). The clozapine-induced increase in expression of KHK was observed in GLUT5 null mice (figure 4B); however, there was no clozapine-induced increase in FBPase-1expression in the GLUT5 null mice (figure 4D). These results suggest that clozapine increases intestinal expression of KHK independent of fructose uptake; however, the increase of intestinal FBPase-1 expression requires both clozapine treatment and GLUT5 function. To determine

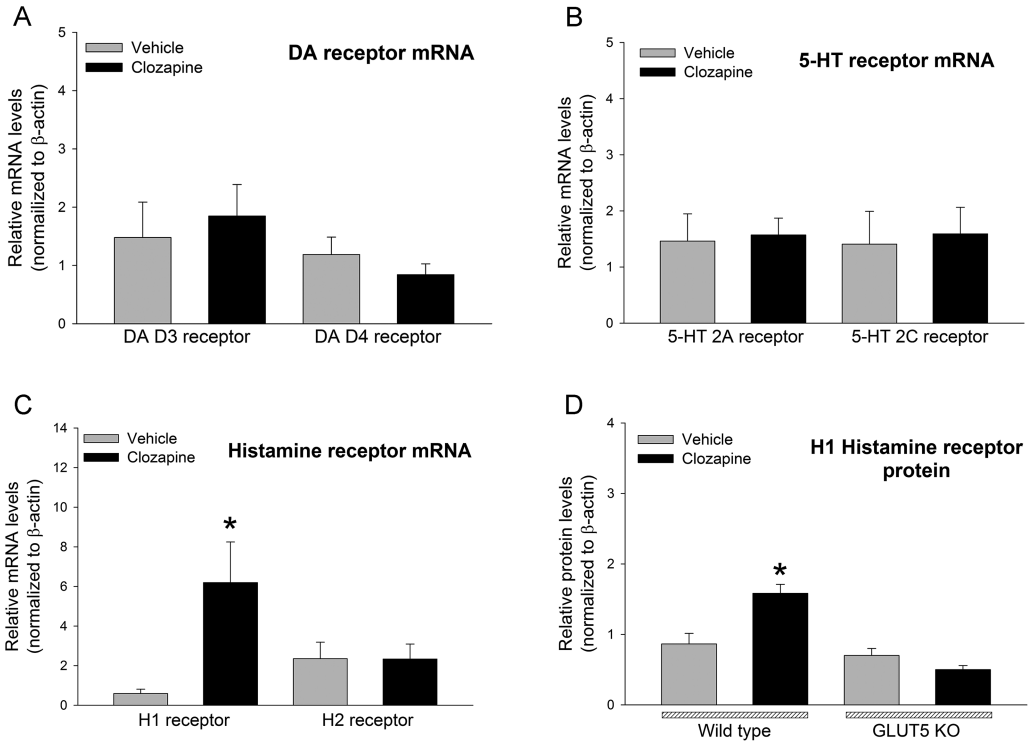

Fig. 3. Female wild type mice were maintained on regular chow and orally administered 100-mg peanut butter pellets with vehicle (gray bar; $n = 10$) or 20 mg/kg clozapine (black bar; $n = 10$) 2 times a day. On day 29, the expression of dopamine (DA) D3 and D4 receptor mRNA (**A**), serotonin (5-HT) 5-HT2A and 5-HT2C mRNA (**B**) and histamine H1 and H2 receptor mRNA (**C**) in the jejunum was measured using reverse transcriptase-polymerase chain reaction (RT-PCR) (**A–C**). Clozapine induced significant increase in only H1 histamine receptor mRNA in wild type mice (*, $P = .002$, Mann-Whitney Rank Sum Test, $n = 10$). The clozapine-induced increase in H1 histamine receptor mRNA resulted in a corresponding increase in H1 receptor protein level in wild type but not GLUT5 null (knock out [KO]) mice (**D**). There was a statistically significant clozapine-induced increase in H1 histamine receptor protein levels in wild type mice (*, $P = .022$, Student's t test). Error bars represent ± standard error of the mean. The expression levels of receptor mRNA and protein was normalized to internal control β-actin. Samples were run in triplicate.

potential effect of clozapine treatment on steady-state blood glucose level in wild type and GLUT5 null mice fed regular chow, we measured steady-state blood glucose levels, weekly during treatment. GLUT5 null mice had lower steady-state plasma glucose level compared to wild type mice (supplementary figure 2). Clozapine treatment significantly decreased blood glucose level by day 28 in wild type mice but by day 15 in GLUT5 null mice (supplementary figure 2). These results suggest that GLUT5-mediated fructose uptake attenuates the blood glucose lowering ability of clozapine in our treatment model.

Clozapine Treatment Increases Intestinal Expression of Select Genes in the Lipogenic Pathway

Antipsychotic drugs are known to induce dyslipidemia and perturbation in lipogenesis, independent of, or prior to, weight gain. Studies have shown that the intestine is also a site of lipid synthesis in the body, and studies have shown that a high-fructose diet stimulates de novo lipogenesis and causes hypertriglyceridemia and insulin resistance in rodents.[39] The key enzymes in the lipogenic pathway are, fasting-induced adipocyte factor (FIAF), fatty acid synthase (FAS), sterol regulatory element binding protein-1c (SREBP-1c), protein tyrosine phosphatase-1b (PTP1b), acetyl-CoA carboxylase (ACC), and peroxisome proliferator-activated receptor gamma (PPARγ). To determine if the intestinal expression of these enzymes are affected by clozapine treatments and fructose transport, we measured the expression levels of the enzymes in GLUT5 null mice and wild type littermates following a 28-day oral administration of vehicle or clozapine (20 mg/kg). Clozapine significantly increased expression of FIAF mRNA in both wild type and GLUT5 null mice (figure 5A). The levels of FAS (figure 5B) and SREBP-1c (figure 5C) mRNA were significantly increased by clozapine treatment in wild type but not GLUT5 null mice. Levels of other relevant intestinal lipogenic proteins (PTP1b, ACC, and PPARγ) were not altered by clozapine treatment in wild type or GLUT5 null mice (data not shown). These results suggest the clozapine-induced increase of FAS and SREBP-1c, but not FIAF, is dependent on GLUT5 function.

Discussion

Our novel results suggest that atypical antipsychotics such as clozapine, in addition to their effects on the CNS, can modulate nutrient uptake in the enteric system and induce

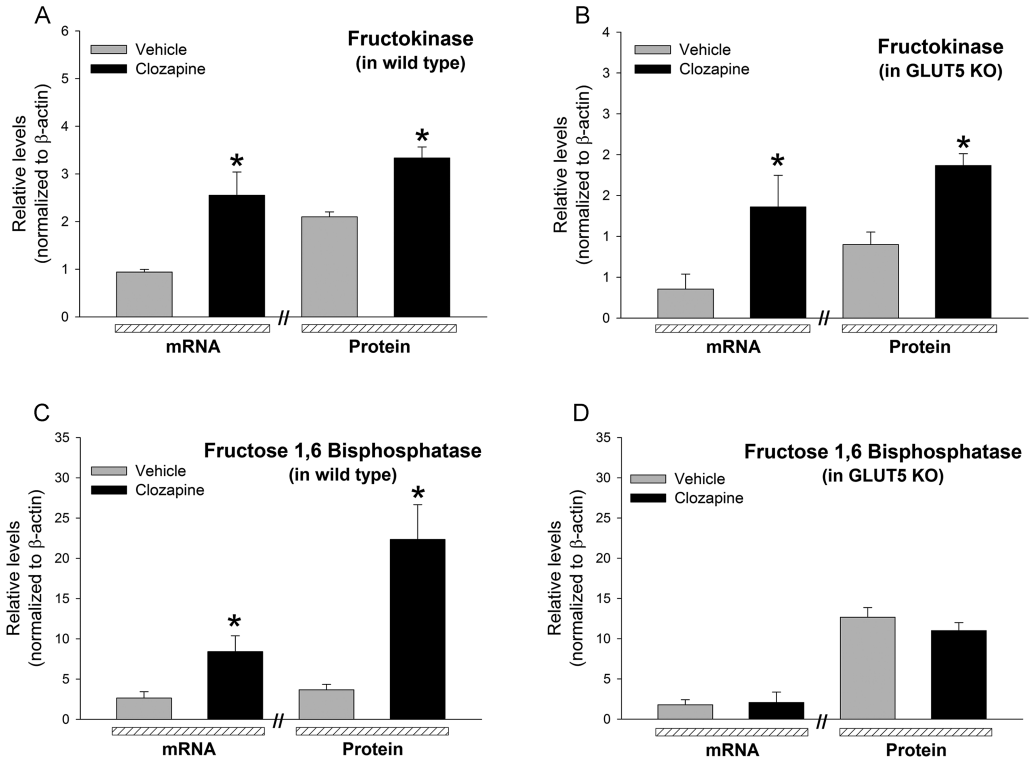

Fig. 4. Female wild type mice (**A** and **C**) or GLUT5 null (knock out [KO]) mice (**B** and **D**) were maintained on regular chow and orally administered 100-mg peanut butter pellets with vehicle (gray bar) or 20 mg/kg clozapine (black bar) 2 times a day. On day 29, the expression of fructokinase (**A** and **B**) and fructose 1,6 bisphosphatase (**C** and **D**) mRNA and protein were measured. Clozapine induced significant increase in fructokinase mRNA (*, $P = .007$, Mann-Whitney Rank Sum Test, $n = 16$) and protein (*, $P = .008$, Student's t test, $n = 6$) in wild type mice (**A**). Clozapine also induced significant increase in fructokinase mRNA (*, $P = .028$, Mann-Whitney Rank Sum Test, $n = 16$) and protein (*, $P = .01$, Student's t test, $n = 6$) in GLUT5 null (KO) mice (**B**). Clozapine induced significant increase in fructose 1,6 bisphosphatase mRNA (*, $P = .037$, Mann-Whitney Rank Sum Test, $n = 20$) and protein (*, $P = .03$, Student's t test, $n = 6$) in wild type mice (**C**). There was no significant clozapine-induced change in fructose 1,6 bisphosphatase mRNA and protein n GLUT5 null (KO) mice (**D**). Error bars represent ± standard error of the mean. The expression levels of receptor mRNA and protein was normalized to internal control β-actin. Samples were run in triplicate.

weight gain. One limitation of our current study was that we used only female mice to be consistent with previous studies which used female rodent models for studying antipsychotics-induced weight gain.[9] Our working model suggests that clozapine acting via target receptors, such as the histamine H1 receptor, post-translationally increases the function of intestinal GLUT5 fructose transporter. Clozapine-induced increased absorption of dietary fructose leads to increased expression of key genes involved in gluconeogenesis and lipogenesis; by comparing the effects of clozapine in wild type and GLUT5 null mice, we determined that clozapine-induced increase in expression of histamine H1 receptor (figures 3C and 3D), FBPase1 (figures 4C and 4D), FAS (figure 5B), and SREBP-1c (figure 5C) is dependent on expression of the GLUT fructose transporter. In contrast, expression of fructokinase (figures 4A and figures 4B) and FIAF (figure 5A) is increased in clozapine-treated mice but is not dependent on the expression of GLUT5. Upregulated gene expression in the enterocytes of clozapine-treated mice could increase metabolism of fructose which might contribute to the increased clozapine-induced fructose uptake activity observed in our study. Our results suggest that increased expression of genes involved in gluconeogenesis and lipogenesis might be mediated by metabolites of fructose. While not tested here, signaling proteins such as AMP kinase that sense the levels of AMP and ATP might mediate the fructose-induced regulation of these genes. Previous studies have shown that the expression of FBPase1, FAS, and SREBP-1c is regulated by AMP kinase.[40–43]

Atypical antipsychotics, including clozapine, are high affinity antagonists at H1 histamine receptor.[31] Studies have shown that H1 histamine receptor null mice exhibit weight gain.[44] H1 histamine receptor in the hypothalamus has been implicated in antipsychotics induced weight gain.[36,37] Given the upregulation of H1 receptor mRNA and its co-localization with GLUT5 in the intestinal mucosa, the modulation of fructose uptake by antipsychotics may also be mediated by intestinal H1 receptors. H1 receptors couple to Gq/11 G-proteins and modulates intracellular levels of calcium and subsequently protein kinase C and AMP kinase (reviewed in Haas et al[45]) which as described previously regulate key genes involved in gluconeogenesis and lipogenesis. Clozapine directly

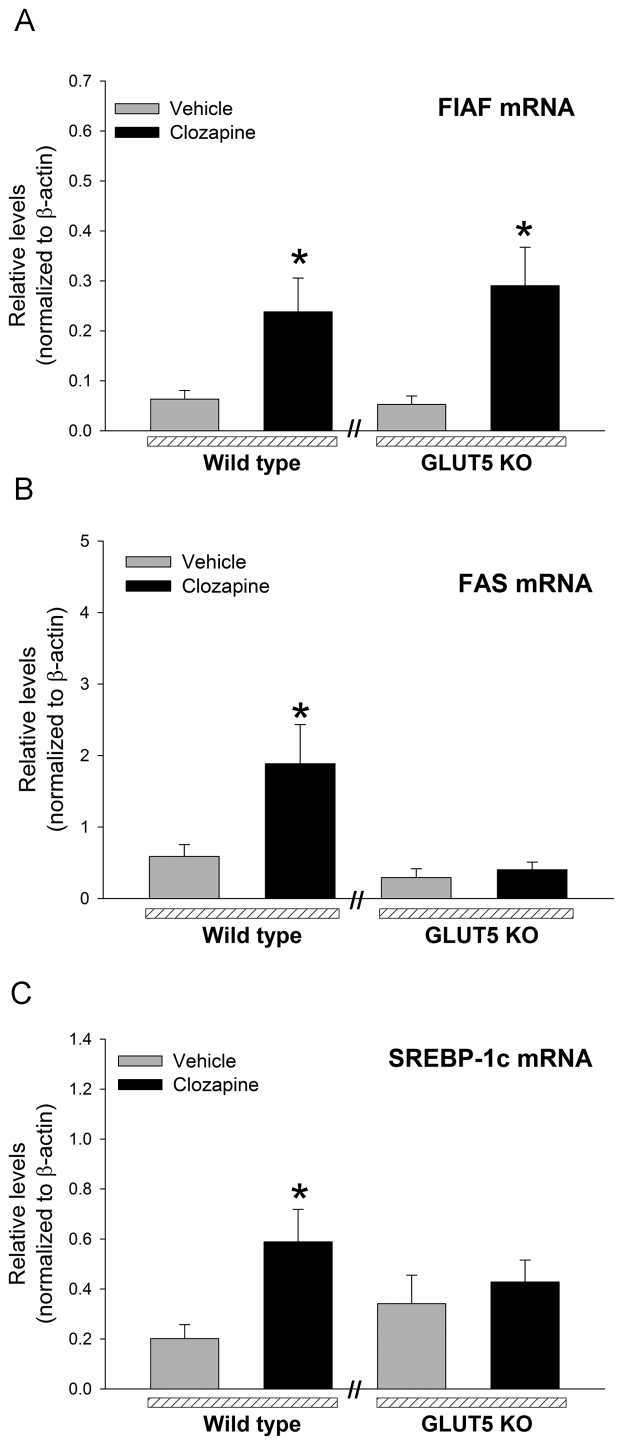

Fig. 5. Female wild type mice and GLUT5 null (knock out [KO]) mice were maintained on regular chow and orally administered 100-mg peanut butter pellets with vehicle (gray bar) or 20 mg/kg clozapine (black bar) 2 times a day. On day 29, the expression of fasting-induced adipose factor (FIAF) mRNA (**A**), fatty acid synthase (FAS) mRNA (**B**) and sterol regulatory element binding protein 1c (SREBP-1c) mRNA (**C**) in the jejunum of wild type and GLUT5 KO mice was measured using reverse transcriptase-polymerase chain reaction (RT-PCR). Clozapine-induced significant increase in FIAF mRNA in wild type mice (*, $P = .04$, Student's t test, $n = 18$) and GLUT5 KO mice (*, $P = .017$, Mann-Whitney Rank Sum Test, $n = 18$). Clozapine-induced significant increase in FAS mRNA in wild type mice (*, $P = .031$, Mann-Whitney Rank Sum Test, $n = 20$) but not in GLUT5 KO mice. Clozapine-induced significant increase in SREBP-1c mRNA in wild type mice (*, $P = .014$, Mann-Whitney Rank Sum Test, $n = 20$) but not in GLUT5 KO mice. Error bars represent ± standard error of the mean. The expression levels of receptor mRNA was normalized to internal control β-actin.

modulated the expression of KHK (figure 4A) and FIAF (figure 5A) genes independent of GLUT5-mediated fructose uptake, potentially via H1 receptor mediated signaling.

Given the high fructose content of the average modern diets,[21,23,46] and its ability to induce obesity in rodents[24] and humans,[25,47] we suggest that enhanced fructose absorption and metabolism in the gut may contribute to antipsychotics-induced weight gain. Clozapine induced an increase in expression of lipogenic genes such as FAS, FIAF, and SREBP-1c which contribute to hypertriglyceridemia and insulin resistance. Based on Human Equivalent Dose (HED) formulas provided by the FDA, a 20 mg/kg/d dose of clozapine in mice would correspond to ~120 mg/d in humans, well below the typical 300 to 450 mg/d target dosage for clozapine in the clinic. Our results have direct clinical implications as reducing or eliminating fructose from the diets of patients on antipsychotics could attenuate antipsychotics-induced weight gain and prevent the development of antipsychotics-induced hypertriglyceridemia and insulin resistance. The results also identify the GLUT5 fructose transporter as a novel therapeutic target for the treatment of antipsychotics-induced metabolic disorders.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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