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Effects of clozapine on adipokine secretions/productions and lipid droplets in 3T3-L1 adipocytes

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ABSTRACT

Clozapine, a second-generation antipsychotic (SGA), is a cause of side effects related to metabolic syndrome. The participation of serotonin 5-HT_{2C} and histamine H_1 receptors in the central nervous system has been reported as a mechanism of the weight gain caused by clozapine. In the present study, we investigated the direct pharmacological action of clozapine on the 3T3-L1 adipocytes and compared it to that of blonanserin, an SGA with low affinity for both receptors. Short-term exposure to clozapine decreased secretion and mRNA expression of leptin. Long-term exposure decreased leptin as well as adiponectin secretion, and further increased lipid droplets accumulation. However, short- and long-term exposures to blonanserin did not affect these parameters. A selective serotonin 5-HT_{2C}, but not a histamine H_1, receptor antagonist enhanced the decreased secretion of leptin induced by short-term exposure to clozapine, but did not affect the increased accumulation of lipid droplets. Our findings indicate that clozapine, but not blonanserin, strongly and directly affected the secretion of adipokines, such as leptin, in adipocytes and caused adipocyte enlargement.

1. Introduction

Second-generation antipsychotics (SGAs) cause significantly more changes than first generation antipsychotics in metabolic parameters, including obesity or abnormal glucose and lipid metabolism, increasing the chances of developing metabolic syndrome and associated disorders such as type 2 diabetes and cerebrovascular accidents (1,2). Among SGAs, clozapine and olanzapine have the maximum potential to cause metabolic syndrome (1,3,4). The weight gain associated with SGAs can be caused by appetite stimulation, lipid accumulation, or reduced energy expenditure (5). Recently, it has been suggested that histamine H_1 and serotonin 5-HT_{2C} receptors in the hypothalamus are involved in the adjustment of eating behavior and energy expenditure (6–8), and receptor antagonisms cause increased appetite and weight gain (5,8–10). There is a positive correlation between the affinity for histamine H_1 receptor and weight gain induced by antipsychotics (11), and antagonisms of SGA for both histamine H_1 and serotonin 5-HT_{2C} receptors are strongly associated with weight gain (7). Clozapine, which is likely to easily cause weight gain, has a high affinity for histamine H_1 and serotonin 5-HT_{2C} receptors (7), whereas other SGAs, such as blonanserin and aripiprazole, with low affinities for both receptors, are less likely to do so (12,13).

Many kinds of bioactive proteins, such as adipokines including leptin, adiponectin, monocyte chemoattractant protein-1 (MCP-1), interleukin-1 (IL-1), and interleukin-6 (IL-6), are secreted from adipocytes. Leptin is secreted from mature adipocytes, and then induces appetite suppression or energy expenditure through a leptin receptor in the hypothalamus (14). Decreased production and secretion of leptin attenuate the appetite-suppressing effect and subsequently cause the excessive eating that primarily affects weight gain. Adiponectin levels are reduced by weight gain and are related to the onset and exacerbation of metabolic syndrome (15). Adiponectin also inhibits the production of inflammatory

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cytokines, such as MCP-1 and IL-6 (16). MCP-1 is released by enlarged adipocytes and induces production of IL-1 and IL-6 (17), that have pivotal roles in regulating energy expenditure, body composition, and peripheral lipid metabolism (18, 19). Serum levels of leptin and lipids in patients with schizophrenia taking SGAs are high compared to those of healthy control subjects (20, 21). Patients with schizophrenia treated with clozapine or olanzapine had adiponectin levels significantly lower than those of control subjects (22). Abnormal cytokine production and abnormal levels of cytokines and cytokine receptors were observed in the blood and cerebrospinal fluid of patients with schizophrenia (23). Although weight gain due to increased body fat content causes secretion abnormality of adipokines, including leptin, adiponectin, MCP-1, and IL-6 (15–17, 24), it is unknown how clozapine affects their production and secretion.

In the present study, we examined the expression of adipokines and accumulation of lipid droplets in mature 3T3-L1 adipocytes cultured with clozapine, or blonanserin, and investigated a causal role for histamine H_1/serotonin 5-HT_{2C} receptors.

2. Materials and methods

2.1. Cell culture

3T3-L1 preadipocytes were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, 25 mM glucose; Sigma–Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin-streptomycin (Sigma–Aldrich) at 37 °C in a humidified 5% CO_2/95% air atmosphere. Two days after reaching full confluency, the preadipocytes were differentiated into mature adipocytes by culturing with DMEM supplemented with 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma–Aldrich), 0.25 μM dexamethasone (Sigma–Aldrich), and 5 μg/mL insulin (Sigma–Aldrich) for 2 days, and subsequently with a medium containing insulin for 2 days. The 3T3-L1 adipocytes were used at approximately day 8 after differentiation.

2.2. Drugs

The atypical antipsychotics clozapine (Sigma–Aldrich) and blonanserin (supplied by Sumitomo Dainippon Pharma, Osaka, Japan), a selective histamine H_1 receptor antagonist, diphenhydramine (Tocris Cookson, Bristol, UK), a selective serotonin 5-HT_{2C} receptor antagonist, SB 242084 (Sigma–Aldrich), a histamine receptor agonist, histamine (Sigma–Aldrich), and a serotonin receptor agonist, serotonin (Sigma–Aldrich) were used.

Clozapine and blonanserin were initially dissolved in a minimal amount of polyethylene glycol (PEG) and diluted with culture medium. Diphenhydramine, SB 242084, histamine, and serotonin were dissolved in a minimal amount of phosphate buffered saline (PBS) and diluted with culture medium.

2.3. Drug exposure

On day 8 after differentiation, mature 3T3-L1 adipocytes were cultured with clozapine (10, 20, 30, or 50 μM) or blonanserin (0.01, 0.05, or 0.1 μM) for 2 days (short-term exposure). Alternatively, 3T3-L1 preadipocytes were cultured with the drugs for 8 days at every medium exchange until differentiation, and then cells were continuously cultured for 2 days (10-days exposure: long-term exposure). After 2- or 10-days exposure, the secretion of adipokines, including leptin, adiponectin, MCP-1, and IL-6, in culture medium and mRNA expression of their genes in 3T3-L1 adipocytes were measured. Intracellular lipid droplets were also analyzed in drug-treated cells. The concentrations of clozapine and blonanserin used in the present study were determined in previous experiments (drug information written in Japanese: Interview form for CLOZARIL Tablets http://www.info.pmda.go.jp/go/pack/1179049F1021_1_13/ [accessed 16.09.30], for LONASEN http://www.info.pmda.go.jp/go/pack/1179048B1025_1_13/ [accessed 16.09.30]). Expected clinical plasma levels are also given in these links.

Mature 3T3-L1 adipocytes were cultured with histamine (10 μM), serotonin (10 μM), diphenhydramine (10 μM), and SB 242084 (10 μM) alone or in combination with clozapine.

2.4. Cell viability assay (MTS assay)

The 3T3-L1 cells were cultured for short- and long-term exposures periods with clozapine (10, 20, 30, or 50 μM) and blonanserin (10 or 20 μM), and control cells were exposed to vehicle medium without compounds. After culture medium was discarded, 200 μL new culture medium and 40 μL 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) solution (CellTiter 96 AQueous One Solution Cell Proliferation Assay; Promega Corp., Madison, WI, USA) were added to each well. After incubation for 1 h, absorbance was measured at 490 nm using a microplate reader (Bio-Rad, Richmond, CA, USA). In addition, we evaluated the cell viability by MTS assay and confirmed that each well has equal activity level under our culture condition.

2.5. Oil Red-O staining

After short- and long-term compound exposures, 3T3-L1 adipocytes were fixed with 10% formalin and stained with Oil Red-O (Sigma–Aldrich). The cells were photographed using a phase-contrast microscope (Olympus CKX41, Tokyo, Japan) fitted with a digital camera, at 200× magnification. The lipid droplets were dissolved in isopropanol (Wako, Tokyo, Japan) and measured at 490 nm.

2.6. Quantification of adipokines

The quantitative measurement of adipokines in culture medium was performed using commercial ELISA kits (all R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. Leptin (Mouse/Rat Leptin Quantikine ELISA kit), adiponectin (Mouse/Rat Adiponectin Quantikine ELISA kit.), MCP-1 (Mouse/Rat CCL2/JE/MCP-1 Quantikine ELISA kit), and IL-6 (Mouse IL-6 Quantikine ELISA kit) secretions were measured after short- and long-term compound exposures. The absorbance of each sample was measured using a spectrophotometric microplate reader at wavelengths of 450 nm and 570 nm.

2.7. Real-time PCR

Total RNA was extracted from 3T3-L1 preadipocytes using a High Pure RNA Isolation Kit (Roche, Mannheim, Germany) and cDNA was synthesized from the total RNA using a Prime Script RT reagent Kit (Perfect Real Time; Takara Bio Inc., Otsu, Japan) under the conditions recommended by the manufacturer. Real-time PCR analysis was performed using SYBR Premix Ex Taq (Takara Bio Inc.). Data were collected using a Step One Plus System (Applied Biosystems, Foster City, CA, USA). The real-time PCR conditions were as follows: initial amplification step (95 °C for 30 s), 40 cycles of denaturation for 5 s at 95 °C, and annealing for 30 s at 60 °C. The expression levels of adipokines were quantified by comparison with a standard curve and normalized using the expression levels

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