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Acute administration of clozapine concurrently increases blood glucose and circulating plasma ghrelin levels in rats

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Neurotransmitter;
Adrenergic receptor

Summary
Objective: Among antipsychotics, clozapine ranks highest in terms of the risk for weight gain and developing diabetes. However, the mechanism by which clozapine induces weight gain and diabetes remains unclear. The aim of this study was to determine the mechanism of clozapine-induced weight gain and hyperglycemia, and to clarify whether clozapine-induced hyperglycemia results from impairment of the system regulating appetite.
Methods: Circulatory glucose, insulin, leptin and ghrelin levels were analyzed after acute administration of clozapine in rats. Clozapine (10 mg/kg) or a vehicle was injected intraperitoneally and blood samples were collected at 0, 15, 30, and 60 min after the injection. Clozapine (5, 10 or 20 mg/kg) or the vehicle was given, and blood samples were collected at 30 min after the injection. Since clozapine has receptor affinity for multiple neurotransmitters, selective antagonists of it, including dopamine, serotonin, α-adrenergic, muscarine and histamine were administered to clarify the pathway of clozapine-induced blood glucose and changes in plasma ghrelin.
Results: Clozapine administration increased the blood glucose level at all time points ($p<0.05$) compared to controls. Plasma ghrelin was elevated at 30 min ($p=0.0124$) and 60 min ($p=0.00152$). Blood glucose was increased in rats given 5 ($p=0.0344$), 10 ($p<0.0001$), or 20 mg/kg ($p<0.0001$) clozapine, while plasma ghrelin was increased in rats treated with 10 mg/kg ($p=0.0009$) or 20 mg/kg ($p=0.0059$) clozapine. Blood glucose was increased in rats treated with a selective α_1-adrenergic receptor antagonist ($p<0.0001$), while plasma ghrelin was significantly increased in rats given a selective α_1- ($p=0.025$) or α_2-adrenergic receptor antagonist ($p=0.0003$).

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Conclusions: Clozapine impairs glucose metabolism and the appetite-regulation system. Clozapine increases blood glucose independent of insulin. The antagonistic action of α-adrenergic receptors is one of the mechanisms that induces both hyperglycemia and elevation of ghrelin.

1. Introduction

Among antipsychotics, second-generation antipsychotic (SGA) agents such as clozapine, olanzapine, quetiapine and risperidone show promise as alternatives to first-generation antipsychotics because of their low incidence of extrapyramidal effects and beneficial effects on the negative symptoms of schizophrenia (Meltzer et al., 1989). Clozapine appears to be the most effective antipsychotic drug for treatment-resistant schizophrenia (Meltzer, 1990); however, it has been reported to have the highest propensity to cause diabetes (Newcomer et al., 2002; Lean and Pajonk, 2003; American Diabetes Association et al., 2004) and weight gain (Allison et al., 1999). Each SGA has its own unique multiple receptor profile (Schotte et al., 1996). Clozapine is characterized by high affinity for histamine $(H)_1$ (inhibition constant $(K_i) = 2.1$), serotonin (5-HT) 2A ($K_i = 3.3$), and 5-HT6 ($K_i = 4.0$) receptors, and mild affinity for 5-HT2C ($K_i = 13$), α_1-adrenergic ($K_i = 23$), muscarine ($K_i = 34$), dopamine (D)2 ($K_i = 150$), and α_2-adrenergic receptors ($K_i = 160$) (Schotte et al., 1996), and acts as an antagonist. The affinity balance for neurotransmitter receptors may be related to clozapine-induced weight gain and diabetes.

It has been reported that clozapine treatment increases appetite and results in significant weight gain that causes insulin resistance, resulting in diabetes mellitus (Henderson et al., 2000). However, a minority of patients with hyperglycemia following treatment with clozapine do not gain weight (Newcomer et al., 2002), suggesting that clozapine has a direct metabolic effect rather than an indirect effect secondary to weight gain. One possible explanation for its antipsychotic effect that could be independent of changes in adiposity is a direct effect on glucose transporter function. Dwyer and colleagues have shown that certain antipsychotic agents, including clozapine, can inhibit glucose uptake via interactions with glucose transporter proteins *in vitro* (Dwyer et al., 1999; Ardizzone et al., 2001). Serotonin (5-HT) receptor activity may also have a role in glucose regulation. Both 5-HT_{1A} and $_2$ receptors have been implicated in it, although the exact roles of these receptors are complex (Cummings et al., 2002; Dwyer and Donohoe, 2003). Changes in plasma concentrations of noradrenaline and adrenaline during treatment with clozapine may also be relevant to understanding the effects of the drug on glucose metabolism that are independent of adiposity (Spivak et al., 1998; Elman et al., 1999).

It has been shown that 75% of patients treated with clozapine have an increased desire to eat, resulting in overeating and weight gain (Bromel et al., 1998). Previously, we reported that plasma ghrelin was significantly increased after 6-month administration of olanzapine, one of the SGAs, compared to the pretreatment level in schizophrenic patients (Murashita et al., 2005). This result suggested that olanzapine could directly act on secretion of ghrelin, with increased plasma ghrelin inducing appetite, resulting in weight gain since ghrelin is a circulatory hormone known to stimulate appetite and food intake (Nakazato et al., 2001; Wren et al., 2001). Ghrelin was originally discovered in the human and rat stomachs as a cognate endogenous ligand for growth hormone (GH) secretagogue receptor (Kojima et al., 1999). This 28-amino acid peptide has a posttranslational *n*-octanoyl modification indispensable for its biological activity (Kojima et al., 1999). Ghrelin is upregulated under negative energy balance conditions such as hypoglycemia and anorexia nervosa, whereas it is downregulated under positive energy conditions such as feeding and obesity (Ariyasu et al., 2001). Not only centrally but also peripherally, administration of ghrelin increases food intake, and body weight gain (Kojima et al., 1999; Wren et al., 2000; Nakazato et al., 2001; Wren et al., 2001). Ghrelin transmits starvation signals either to the nucleus of the solitary tract in the brainstem via the vagal afferent pathway and/or to the arcuate nucleus (ARC) via the bloodstream (Date et al., 2002). It activates agouti-related peptide-producing and neuropeptide Y (NPY)-producing neurons localized in the ARC of the hypothalamus, which is one of the brain regions of primary importance in the regulation of feeding (Nakazato et al., 2001; Wren et al., 2001). Meanwhile, adipocyte-derived leptin signals the state of fat stores to the hypothalamus via the circulation of blood, inhibiting food intake and further accumulation of fat (Schwartz et al., 2000). Ghrelin and leptin both act on the ARC (Schwartz et al., 2000; Nakazato et al., 2001), and may have opposite actions in the regulation of weight gain.

To understand the mechanism of clozapine-induced weight gain and hyperglycemia, and to clarify whether clozapine-induced hyperglycemia results from impairment of the appetite-regulating system, we investigated the acute effects of clozapine on blood glucose and insulin levels, as markers of glucose metabolism, and on circulatory ghrelin and leptin levels, as appetite-regulating factors. In addition to acute effects on glucose metabolism and appetite-regulating factors, various neurotransmitter receptor antagonists were also examined to clarify the pathway regulating blood glucose and ghrelin induced by clozapine.

2. Subjects, materials and methods

2.1. Animals

All procedures in animal experiments were approved by the Hokkaido University Graduate School of Medicine Committee on Animal Research. Eleven-week-old male Sprague–Dawley