Inhibitory Effects of Antipsychotics on Carbachol-Enhanced Insulin Secretion From Perifused Rat Islets

Role of Muscarinic Antagonism in Antipsychotic-Induced Diabetes and Hyperglycemia

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Treatment with the atypical antipsychotics olanzapine and clozapine has been associated with an increased risk for deterioration of glucose homeostasis, leading to hyperglycemia, ketoacidosis, and diabetes, in some cases independent of weight gain. Because these events may be a consequence of their ability to directly alter insulin secretion from pancreatic β-cells, we determined the effects of several antipsychotics on cholinergic- and glucose-stimulated insulin secretion from isolated rat islets. At concentrations encompassing therapeutically relevant levels, olanzapine and clozapine reduced insulin secretion stimulated by 10 µmol/l carbachol plus 7 mmol/l glucose. This inhibition of insulin secretion was paralleled by significant reductions in carbachol-potentiated inositol phosphate accumulation. In contrast, risperidone or ziprasidone had no adverse effect on cholinergic-induced insulin secretion or inositol phosphate accumulation. None of the compounds tested impaired the islet secretory responses to 8 mmol/l glucose alone. Finally, in vitro binding and functional data show that olanzapine and clozapine (unlike risperidone, ziprasidone, and haloperidol) are potent muscarinic M3 antagonists. These findings demonstrate that low concentrations of olanzapine and clozapine can markedly and selectively impair cholinergic-stimulated insulin secretion by blocking muscarinic M3 receptors, which could be one of the contributing factors to their higher risk for producing hyperglycemia and diabetes in humans. Diabetes 54:1552–1558, 2005

RESEARCH DESIGN AND METHODS

Recent reviews of clinical databases have revealed that olanzapine and clozapine carry a higher risk for producing hyperglycemia, ketoacidosis, and new-onset type 2 diabetes than other second-generation antipsychotics (SGAs) or haloperidol, a first-generation antipsychotic (1–6). The use of olanzapine and clozapine is often associated with notable weight gain and dyslipidemia, which are known risk factors in the development of diabetes. However, several reports have described cases of hyperglycemia following olanzapine and clozapine treatment that were not associated with weight gain (7,8). Furthermore, cases exist where switching to other SGAs, such as ziprasidone or risperidone, resulted in the reversal of olanzapine- or clozapine-associated hyperglycemia, suggesting that fundamental differences exist among the SGAs (9–11).

The mechanisms responsible for the increased diabetes risk of olanzapine and clozapine are not known, but in contrast to other SGAs, both compounds are potent muscarinic receptor antagonists (12). This led us to consider the possibility that disruption of the cholinergic processes regulating insulin secretion is one of the underlying mechanisms for impaired glucose regulation. Therefore, we investigated the effects of several antipsychotics on cholinergic-stimulated insulin secretion and the activation of phospholipase C using isolated rat pancreatic islets. Since the cholinergic activation of insulin release is mediated through muscarinic M3 receptors on β-cells (13–15), we also determined binding affinities of these agents to muscarinic receptors in the rat pancreatic INS-1 cell line (16), as well as functional antagonist activities at native rat M3 receptors in isolated rat urinary bladder (17) and at human M3 muscarinic receptors expressed in Chinese hamster ovary (CHO) cells.

Animals were treated in compliance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Institutional Animal Care and Use Committee. Flow-4AM was purchased from Molecular Probes (Eugene, OR), [3H]-N-methyl-scopalamine from NEN (Boston, MA), 125I-labeled insulin and myo-[2-
In vitro binding affinities. Muscarinic binding affinities and functional antagonist activities were determined in CHO cell lines expressing hM₂ receptors. Functional antagonist activities were determined in CHO cell lines transfected with human muscarinic M₂ receptors by measuring effects on intracellular calcium flux using a Fluorimetric Imaging Plate Reader (FLIPR; Molecular Devices). Cells were plated at 12,500 cells/well in clear-bottom, 384-well, collagen-I-coated plates 48 h before the assay and maintained in growth medium (Dulbecco’s modified Eagles medium, 500 μg/ml G418, 100 μmol/l nonessential amino acids, 10 μmol/l HEPEs buffer, 2 mmol/l l-glutamine, 10% FBS [heat inactivated] at 37°C, and 5% CO₂). On the day of the assay, growth medium was removed and cells incubated with 8 μmol/l Fluo-4AM dye and 2.5 mmol/l probenecid for 75 min at 37°C and 5% CO₂. Cells were washed four times with assay buffer (145 mmol/l NaCl, 10 mmol/l glucose, 5 mmol/l KCl, 1 mmol/l MgCl₂, 10 mmol/l HEPEs buffer, and 2 mmol/l CaCl₂ adjusted to pH 7.4) and incubated in assay buffer for 45 min at 37°C. Calcium flux was measured at excitation and emission wavelengths of 488 and 516 nm, respectively. Compounds were first added to test for agonist activity and 30 min later challenged with 10 μmol/l of the muscarinic agonist carbachol (EC₅₀ = 6.5 ± 2.3 mmol/l) to test for antagonist activity. IC₅₀ values were estimated by nonlinear regression of concentration-response data and Kᵢ values were calculated according to the Cheng Prusoff equation: Kᵢ = IC₅₀/[L/(Kᵢ)], where L is the concentration of the radioligand used in the experiment and the Kᵢ value is the dissociation constant for the radioligand (determined previously by saturation analysis). Reported Kᵢ values are the means ± SE of at least three separate experiments performed in duplicate. In vivo functional activities in CHO cells expressing hM₂ receptors. Ex vivo functional activity in urinary bladder. Male Sprague-Dawley rats (220–275 g) were killed and the urinary bladder rapidly removed and cut into three longitudinal strips (2–3 mm wide and 6 mm long). The strips were suspended in 4-ml tissue baths, containing Krebs-Henseleit physiological salt solution (KPS) or high K⁺, Ca²⁺-free KPS, maintained at 37°C and aerated with 95% O₂/5% CO₂. After a 90-min equilibration period, at a resting tension of 4 g, control cumulative concentration-response curves were generated by stimulating tissues with increasing concentrations (0.1 mmol/l to 10 μmol/l) of the muscarinic agonist carbachol (EC₅₀ = 1.6 ± 0.2 μmol/l). In some experiments, possible effects on Ca²⁺ channels were examined by stimulating tissue with Ca²⁺ (range 10 μmol/l to 30 mmol/l). When a maximum response to carbachol or a final concentration of 30 mmol/l Ca²⁺ was obtained, tissues were washed with KPS or K⁺-, Ca²⁺-free KPS, respectively, until baseline tension was restored. Tissues were then exposed to either solvent (DMSO) or one of the test compounds for 30 min, at which time a second cumulative concentration-response curve was constructed using the appropriate spasmogen. For each tissue, results were expressed as a percent of its own maximum control response to carbachol or Ca²⁺. These results were averaged and compared with tissues receiving solvent alone. Antagonist activities were estimated according to the following formula: pKᵢ = −log[antagonist]/[concentration ratio − 1]. Statistics. Statistical significance was determined using the Student’s t test or ANOVA with Newman-Keuls test for unequal data. A P value ≤0.05 was considered significant.

<table>
<thead>
<tr>
<th>Experimental conditions (glucose 7 mmol/l, 10 μmol/l carbachol plus drug)</th>
<th>Inositol phosphate accumulation (cpm/40 islets)</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>G7 controls</td>
<td>6,290 ± 731*</td>
<td>100</td>
</tr>
<tr>
<td>G7 + carbachol</td>
<td>16,620 ± 1,073</td>
<td>264</td>
</tr>
<tr>
<td>G7 + carbachol + 10 μmol/l atropine</td>
<td>5,766 ± 190*</td>
<td>92</td>
</tr>
<tr>
<td>G7 + carbachol + 100 nmol/l clozapine</td>
<td>12,772 ± 963</td>
<td>203</td>
</tr>
<tr>
<td>G7 + carbachol + 10 μmol/l clozapine</td>
<td>7,668 ± 1,357*</td>
<td>122</td>
</tr>
<tr>
<td>G7 + carbachol + 100 nmol/l olanzapine</td>
<td>12,141 ± 935*</td>
<td>193</td>
</tr>
<tr>
<td>G7 + carbachol + 10 μmol/l olanzapine</td>
<td>10,305 ± 1,130*</td>
<td>164</td>
</tr>
<tr>
<td>G7 + carbachol + 10 μmol/l ziprasidone</td>
<td>6,940 ± 799*</td>
<td>110</td>
</tr>
<tr>
<td>G7 + carbachol + 10 μmol/l risperidone</td>
<td>16,359 ± 162</td>
<td>260</td>
</tr>
<tr>
<td>G7 + carbachol + 10 μmol/l risperidone</td>
<td>15,235 ± 1,492</td>
<td>242</td>
</tr>
</tbody>
</table>

Data are means ± SE. See RESEARCH DESIGN AND METHODS for more details. *Significant difference (P < 0.05) when compared with 7 mmol/l glucose (G7) + carbachol.

2H]inositol from PerkinElmer Life and Analytical Sciences (Boston, MA), type P collagenase from Roche Diagnostics (Indianapolis, IN), and forskolin from Calbiochem (La Jolla, CA). Rat insulin standard (lot no. A52-AWK-601) was the generous gift of Dr. Gerald Gold (Eli Lilly, Indianapolis, IN). Clozapine, haloperidol, risperidone, olanzapine, and all other analytical-grade chemicals were purchased from Sigma (St. Louis, MO) or Fluka Chemika-BioChemika (Ronkonkoma, NY). Ziprasidone and olanzapine were synthesized at Pfizer (Groton, CT).
RESULTS

Carbachol- or glucose-stimulated insulin secretion from rat islets: effects of SGAs. Basal rates of insulin released in the presence of 7 mmol/l glucose were 62 ± 5 pg · islet⁻¹ · min⁻¹ (n = 20; Figs. 1 and 2). Addition of 10 μmol/l carbachol produced an immediate short-lasting response that peaked at 110 ± 8 pg · islet⁻¹ · min⁻¹, followed by a gradual increase in release rates that averaged 180 ± 17 pg · islet⁻¹ · min⁻¹ after 40 min (Figs. 1 and 2). When added together with 10 μmol/l carbachol, equimolar levels (10 μmol/l) of atropine, olanzapine, and clozapine completely abolished carbachol-potentiated secretion, but ziprasidone, risperidone, or haloperidol had no effect (results not shown). Subsequent studies using preincubation with therapeutically relevant concentrations of the antipsychotics were conducted. In these experiments, the test compounds were present during the stabilization period with 7 mmol/l glucose and during the stimulatory period with 7 mmol/l glucose plus 10 μmol/l carbachol (Figs. 1 and 2). Atropine, olanzapine, and clozapine at 100 nmol/l reduced the acute first phase and virtually abolished the sustained second phase of insulin secretion. Concentrations of olanzapine and clozapine as low as 10 nmol/l significantly inhibited insulin secretion during the last 10 min and the entire sustained second phase, respectively (Fig. 1). In contrast, ziprasidone, risperidone, or haloperidol at 100 nmol/l had no effect under these conditions (Fig. 2).

The cholinergic specificity of the inhibitory effect of atropine, olanzapine, and clozapine was investigated further using an 8-mmol/l glucose stimulus. Islet responses to 8 mmol/l glucose alone (175 ± 17 pg · islet⁻¹ · min⁻¹, n = 17, after 40 min of stimulation) were comparable to those evoked by 7 mmol/l glucose with 10 μmol/l carbachol (compare Figs. 1 and 2 with Fig. 3). Olanzapine, clozapine, ziprasidone, atropine, risperidone, or haloperidol (all at 10 μmol/l) had no inhibitory effect on 8 mmol/l glucose–induced insulin secretion (shown for olanzapine, clozapine, and ziprasidone in Fig. 3).

Inositol phosphate studies. Since cholinergic-mediated insulin secretion is a result of muscarinic M₃ receptor activation coupled to phospholipase-C activation (13–15,22), we examined the impact of the antipsychotics on carbachol-induced phospholipase-C activation by monitor-
ing labeled inositol phosphate accumulation (Table 1). The addition of 10 μmol/l carbachol increased inositol phosphate accumulation ~2.5–3.0 fold above that measured in the presence of 7 mmol/l glucose alone. The inclusion of 10 μmol/l clozapine, olanzapine, or atropine together with carbachol and 7 mmol/l glucose resulted in significant reductions in inositol phosphate accumulation (P < 0.05), while inclusion of 10 μmol/l ziprasidone or risperidone had no effect. Lower concentrations of clozapine (100 nmol/l) and olanzapine (10 and 100 nmol/l) also significantly reduced inositol phosphate accumulation when present during the 3-h labeling period and the subsequent 30-min stimulation period with carbachol. The 3-h exposure to olanzapine did not nonspecifically impair phospholipase-C activation, since glucose-induced inositol phosphate accumulation was not significantly different in islets pretreated with 100 nmol/l olanzapine and stimulated with 8 mmol/l glucose (9,942 ± 1,833 cpm/40 islets) compared with control islets stimulated with 8 mmol/l glucose alone (8,651 ± 835 cpm/40 islets).

**Receptor binding affinities and functional activities at muscarinic receptors.** In vitro binding affinities and functional activities (Table 2) show that olanzapine and clozapine potently displace the muscarinic antagonist N-methylscopolamine from rat INS-1 cell membranes (K_i = 25–38 nmol/l) and have high functional antagonist potency at hM3 receptors expressed in CHO cells (olanzapine K_i = 36 nmol/l, clozapine K_i = 59 nmol/l). Ziprasidone, risperidone, and haloperidol lack affinity for muscarinic receptors in INS-1 cell membranes (K_i >1.6 μmol/l) and antagonist activity at hM3 receptors (K_i >2.8 μmol/l).

**Carbachol-induced contractions in isolated rat urinary bladder.** Olanzapine (0.001–10 μmol/l) and clozapine (0.01–1 μmol/l) caused concentration-related parallel rightward shifts of the carbachol concentration-response curves compared with the control curves, acting as competitive full antagonists (Figs. 4A and B). At 10 μmol/l, clozapine depressed the maximum carbachol response, probably via Ca^{2+} channel inhibition, since at this concentration clozapine blocked calcium-induced contractions of the rat urinary bladder (results not shown). Ziprasidone, haloperidol, and risperidone (0.01–10 μmol/l) did not shift the carbachol concentration-response curve, exemplified by the comparison of the effects at 1 μmol/l of all test compounds (Fig. 4C). The K_i values of the test compounds for antagonism at the rat M3 muscarinic receptor are consistent with their muscarinic receptor binding affinities and functional antagonism potencies (Table 2).

**DISCUSSION**

Accumulating clinical evidence indicates that the SGAs olanzapine and clozapine have an increased risk of triggering hyperglycemic events in schizophrenic patients (1–5), which can occur independently of the weight gain associated with these compounds (4,7–9). The level of hyperglycemia is often severe (6–8) and can be directly attributed to drug treatment, as cessation of use often

**TABLE 2**

In vitro binding affinities at muscarinic receptors in rat INS-1 cells and functional antagonist activities at muscarinic hM3 (CHO cells) and rM3 (rat urinary bladder) receptors

<table>
<thead>
<tr>
<th>Drug</th>
<th>M3 binding affinity</th>
<th>M3 functional activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INS-1 cells K_i (nmol/l)*</td>
<td>CHO cells K_i (nmol/l)†</td>
</tr>
<tr>
<td>Clozapine</td>
<td>25 ± 3</td>
<td>59 ± 30</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>38 ± 6</td>
<td>36 ± 10</td>
</tr>
<tr>
<td>Risperidone</td>
<td>&gt;1,600</td>
<td>&gt;2,800</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>&gt;1,600</td>
<td>&gt;2,800</td>
</tr>
<tr>
<td>Haloperidol</td>
<td></td>
<td>&gt;2,800</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Displacement of [3H]-N-methylscopolamine binding to INS-1 cells; †inhibition of carbachol-induced calcium flux in hM3 receptors expressed in CHO cells; ‡inhibition of carbachol-induced contractions of rat urinary bladder.
represents means ± SE percent inhibition of the maximum control response at the applied carbachol concentration.

In this study, we have explored how a number of antipsychotics impact both fuel and neurohumorally mediated insulin secretion from isolated perfused rat islets. The results suggest that inhibition of cholinergic-stimulated insulin secretion is a possible contributing factor in the blunting of the maximum carbachol response by 10 μM clozapine is a consequence of calcium channel blockade, a known effect of high clozapine concentrations (26).

The important role of cholinergic stimulation in controlling insulin release is clearly demonstrated by studies in mice (27), rats (28), monkeys (29), and humans (30). While M₃ receptor blockade by itself does not cause hyperglycemia under normal conditions, it could prevent the ability of β-cells to compensate for hyperglycemia caused by compromised peripheral glucose utilization. A number of conditions (e.g., overfeeding, high-fat diet, central adiposity) that precipitate obesity and/or insulin resistance are associated with compensatory insulin secretion mediated in large part through the autonomic nervous system (31). A recent study by Teff and Townsend (32) is an elegant demonstration of the importance of cholinergic regulation of insulin secretion during hyperglycemic conditions. These authors showed that prolonged mild hyperglycemia in healthy subjects resulted in a compensatory increase in β-cell function, which was significantly attenuated by muscarinic blockade with atropine. These findings support the clinical importance of the vagal system in metabolic compensation and the potential for interference by muscarinic antagonists, consistent with the results of the present study.

Hypotheses are emerging to suggest that imbalance or dysfunction of this autonomic regulation results in pathophysiology, such as metabolic syndrome (33). In individu-
als predisposed to metabolic disorders related to autonomic dysregulation, treatment with antimuscarinic SGAs that can exacerbate insulin resistance and hyperinsulinemia (34,35) may further tip this balance by reducing islet compensation and thereby precipitating hyperglycemia or diabetes. Such drug-induced loss of β-cell function could play an important role in the development of acute ketoacidosis occasionally reported in olanzapine- or clozapine-treated patients (6–9). Our finding that these compounds do not modify glucose-stimulated insulin release is consistent with the clinical observation that olanzapine does not affect insulin secretion under hyperglycemic clamp conditions in healthy subjects (36). It is important to note that clinical tests, which use intravenous glucose infusions instead of oral glucose or a meal challenge, do not elicit vagal, i.e., cholinergic, stimulation of insulin secretion (32) and are thus inadequate to demonstrate interference by antimuscarinics such as olanzapine.

It has been reported that many pathophysiologic conditions, including schizophrenia (37), have an altered autonomic balance and are thus likely to have a changed dependency on cholinergic input. Reports on β-cell function in schizophrenics treated with olanzapine or clozapine have been limited to date, and further study is required.

Besides directly affecting β-cell function, it is possible that the antimuscarinic activities of olanzapine and clozapine might interfere with glucose utilization in other target tissues. In the liver, for example, release of acetylcholine from parasympathetic nerve endings has been shown to increase hepatic glucose uptake in preclinical models (38), while in contrast, activation of adrenergic receptors via the sympathetic nerves increases glucose output from the liver (39). The presence of a high-affinity antimuscarinic agent could block the parasympathetic pathway, resulting in a shift toward increased hepatic glucose output, mimicking the effects of parasympathetic neuropathy, which has been suggested to contribute to increased hepatic glucose production (40) and insulin resistance in obese type 2 diabetic subjects (41). In addition, it should be noted that the increased diabetes risk is not limited to the newer SGAs with antimuscarinic properties, since chlorpromazine, the first-generation antipsychotic introduced in the 1950s, also has affinity for muscarinic receptors (42), and has been associated with the development of diabetes in schizophrenic patients (43–45). Chlorpromazine is a moderately potent antagonist at human and rat muscarinic M₃ receptors (results not shown), and further studies are needed to characterize its effects on insulin release.

While we have focused on the antimuscarinic properties of olanzapine and clozapine, it is clear that additional mechanisms contribute to the increased diabetic liability of olanzapine and clozapine. One of the possible mechanisms that may work in synergy with muscarinic antagonist activity is 5-HT₂A receptor antagonism. It has been demonstrated that glucose uptake into skeletal muscle, which is responsible for a large portion of glucose clearance, can be enhanced through a recently described pathway involving agonist activation of 5-HT₂A receptors (46). Since most antipsychotics are high-affinity antagonists at this receptor, the combination of 5-HT₂A antagonism and antimuscarinic properties could increase their diabetic liability. Inhibition of glucose transport has also been implicated in antipsychotic-induced hyperglycemia and was suggested as the mechanism of acute hyperglycemia observed in mice treated with certain SGAs (47). However, effective concentrations are several orders of magnitude above therapeutic levels and inhibitor potencies do not differentiate antipsychotics with low diabetic risk from the high-risk SGAs. Finally, it should be kept in mind that while the focus of this study has been on effects on peripheral tissues, the SGAs have potent and multiple interactions with a wide variety of receptors in the central nervous system, which will impact autonomic tone and are therefore likely to play an important role in the diabetic side effects of certain antipsychotics.

In conclusion, these studies demonstrate that olanzapine and clozapine significantly impair cholinergic-potentiated insulin secretion in rat pancreatic islets. This inhibitory effect was specific, occurred near therapeutic concentrations, and could not be duplicated by other SGAs or by haloperidol. Considering the importance of acetylcholine in the physiologic regulation of insulin secretion, this effect provides a plausible mechanism that may contribute to the increased diabetic liability of olanzapine and clozapine. M₃ receptor blockade is, however, not the sole mechanism of action, since marketed drugs that selectively target muscarinic receptors do not produce diabetes. Given the complexity of glucose regulation and the number of additional receptors to which SGAs bind with high affinity, it is evident that muscarinic receptor blockade in combination with other factors is needed to impair glucose regulation. Which other factors play a role is not known, but elucidating additional mechanisms that may contribute to olanzapine- or clozapine-induced hyperglycemia will increase our understanding of the differential in risk levels among SGAs and facilitate the design and development of novel drugs without hyperglycemic liability.

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REFERENCES


DIABETES, VOL. 54, MAY 2005 1557
OLANZAPINE AND CLOZAPINE INHIBIT INSULIN SECRETION

45. Zumoff B: The effects of psychotropic drugs and diuretics on blood glucose levels in diabetes mellitus. Compr Ther 5:72–74, 1979