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

Role of Muscarinic Antagonism in Antipsychotic-Induced Diabetes and Hyperglycemia

David E. Johnson,[1] Hanae Yamazaki,[2] Karen M. Ward,[1] Anne W. Schmidt,[1] Wesley S. Lebel,[3] Judith L. Treadway,[4] E. Michael Gibbs,[4] Walter S. Zawalich,[2] and Hans Rollema[1]

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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 M_3 antagonists. These findings demonstrate that low concentrations of olanzapine and clozapine can markedly and selectively impair cholinergic-stimulated insulin secretion by blocking muscarinic M_3 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

From the [1]Department of Neuroscience, Pfizer Global Research and Development, Groton, Connecticut; the [2]Yale School of Nursing, Yale University, New Haven, Connecticut; the [3]Department of General Pharmacology, Pfizer Global Research and Development, Groton, Connecticut; and the [4]Department of Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, Connecticut.

Address correspondence and reprint requests to Hans Rollema, Pfizer Global Research and Development, Department of Neuroscience, Groton Laboratories, MS 8220-4159, Eastern Point Road, Groton, CT 06340. E-mail: hans_rollema@groton.pfizer.com.

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D.E.J., K.M.W., A.W.S., W.S.L., J.L.T., E.M.G., and H.R. are employed by and hold stock in Pfizer. W.S.Z. has received consulting fees and research support from Pfizer.

KPSS, Krebs-Henseleit physiological salt solution; KRBB, Krebs-Ringer bicarbonate buffer; SGA, second-generation antipsychotic.

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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 M_3 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 M_3 receptors in isolated rat urinary bladder (17) and at human M_3 muscarinic receptors expressed in Chinese hamster ovary (CHO) cells.

RESEARCH DESIGN AND METHODS

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.

Fluo-4AM was purchased from Molecular Probes (Eugene, OR), [^{3}H]-*N*-methyl-scopolamine from NEN (Boston, MA), ^{125}I-labeled insulin and myo-[2-

TABLE 1
Antipsychotic effects on carbachol-induced inositol phosphate accumulation in isolated rat islets

Experimental conditions (glucose 7 mmol/l, 10 μmol/l carbachol plus drug)	Inositol phosphate accumulation (cpm/40 islets)	Percent of control
G7 controls	6,290 ± 731*	100
G7 + carbachol	16,620 ± 1,073	264
G7 + carbachol + 10 μmol/l atropine	5,766 ± 190*	92
G7 + carbachol + 100 nmol/l clozapine	12,772 ± 963	203
G7 + carbachol + 10 μmol/l clozapine	7,668 ± 1,357*	122
G7 + carbachol + 10 nmol/l olanzapine	12,141 ± 935*	193
G7 + carbachol + 100 nmol/l olanzapine	10,305 ± 1,139*	164
G7 + carbachol + 10 μmol/l olanzapine	6,940 ± 799*	110
G7 + carbachol + 10 μmol/l ziprasidone	16,359 ± 162	260
G7 + carbachol + 10 μmol/l risperidone	15,235 ± 1,492	242

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.

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^{3}H]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-001) was the generous gift of Dr. Gerald Gold (Eli Lilly, Indianapolis, IN). Clozapine, haloperidol, risperidone, atropine, 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).

Insulin secretion studies. Insulin output from isolated islets was determined as previously described (18). Briefly, islets from male Sprague-Dawley rats (325–425 g) were perifused at a flow rate of 1 ml/min with Krebs- Ringer bicarbonate buffer (KRBB; 115 mmol/l NaCl, 5 mmol/l KCl, 2.2 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 24 mmol/l $NaHCO_3$, and 170 mg/dl BSA). The KRBB was maintained at 37°C and aerated with 95% O_2/5% CO_2. Three perifusion protocols were used. In initial studies, islets were perifused with 7 mmol/l glucose to establish stable rates of secretion and then stimulated with 7 mmol/l glucose plus 10 μmol/l carbachol to evoke carbachol-induced potentiation of insulin release. Test compounds (olanzapine, clozapine, haloperidol, atropine, ziprasidone, and risperidone) dissolved in DMSO were included during the stimulatory period. Similar amounts of DMSO (1 μl/1 ml) were added to control perifusions. In subsequent studies on the effects of lower concentrations (10–100 nmol/l), test compounds were also included during the 30-min stabilization period with 7 mmol/l glucose. Finally, islets were perifused for 30 min with 3 mmol/l glucose and stimulated with 8 mmol/l glucose, approximately equipotent in increasing insulin secretion to the combination of 7 mmol/l glucose plus 10 μmol/l carbachol. Details are included in the figure legends. Insulin released into the medium was measured by radioimmunoassay using rat insulin as a standard. Data presented in the figures and RESULTS are expressed as picograms of insulin release per islet and represent the means ± SE of at least three observations.

Inositol phosphate studies. Inositol phosphate accumulation in islets was measured as previously described (18). Briefly, groups of 18–22 islets were incubated for 3 h at 37°C in an oxygenated KRBB solution containing 5.0 mmol/l glucose and 10 μCi of myo-2-^{3}H-inositol (specific activity 18.5 Ci/mmol). After labeling, the islets were washed with 5 ml fresh KRBB and placed in small glass vials, to which 400 μl of 10 mmol/l LiCl in KRBB was added to prevent inositol phosphate degradation. The vials were capped and incubated for 30 min, inositol phosphate generation was stopped by adding 400 μl 20% perchloric acid, and total inositol phosphates were measured as previously described (19,20). In those experiments where the impact of 10–100 nmol/l olanzapine and clozapine on inositol phosphate accumulation was assessed, the compounds were included during the 3-h labeling period and the subsequent 30-min incubation. Results are expressed as counts per minute of inositol phosphates/40 islets, and data in Table 1 represent the means ± SE of 3–6 (drug treatments) or 5–11 (carbachol controls) observations.

Muscarinic binding affinities and functional antagonist activities

In vitro binding affinities. Radioligand binding assays in INS-1 cell lines and in cell membranes from CHO cells expressing human muscarinic M_3 receptors were performed essentially as previously described (21). Briefly, membranes were incubated for 60 min at room temperature with [^{3}H]-*N*-methyl-scopolamine and various concentrations of test compounds. Incubations were terminated by rapid filtration onto GF/B filters presoaked in 0.5% polyethylenimine. Nonspecific binding was determined using a saturating concentration of atropine, a potent nonselective muscarinic inhibitor, and radioactivity was quantified by liquid scintillation counting. IC_{50} values were determined by linear regression of the concentration-response data, and K_i values were calculated according to the Cheng Prusoff equation: $K_i = IC_{50}/[1 + (L/K_d)]$, where L is the concentration of the radioligand used in the experiment and the K_d value is the dissociation constant for the radioligand (determined previously by saturation analysis). Reported K_i values are the means ± SE of at least three separate experiments performed in duplicate.

In vitro functional activities in CHO cells expressing hM_3 receptors. Functional antagonist activities were determined in CHO cell lines transfected with human muscarinic M_3 receptors by measuring effects on intracellular calcium flux using a Fluorimetric Imaging Plate Reader ($FLIPR_{384}$; Molecular Devices). Cells were plated at 12,500 cells/well in clear-bottom, 384-well, collagen-l–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% CO_2). 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% CO_2. Cells were washed four times with assay buffer (145 mmol/l NaCl, 10 mmol/l glucose, 5 mmol/l KCl, 1 mmol/l $MgSO_4$, 10 mmol/l HEPES, and 2 mmol/l $CaCl_2$, adjusted to pH 7.4) and maintained 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 nmol/l of the muscarinic agonist carbachol (EC_{50} = 6.8 ± 2.3 nmol/l) to test for antagonist activity. IC_{50} values were estimated by nonlinear regression of concentration-response data and K_b values were calculated using the Cheng Prusoff equation. K_b values are the means of four separate experiments, each performed in triplicate.

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 mm wide and 6 mm long. The strips were suspended in 4-ml tissue baths, containing Krebs-Henseleit physiological salt solution (KPSS) or high K^+, Ca^{2+}-free KPSS, maintained at 37°C and aerated with 95% O_2/5% CO_2. The force of isometric contraction was recorded using Grass FT03C force displacement strain gauges interfaced with a Yew or Allen servorecorder. 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 nmol/l to 10 μmol/l) of the muscarinic agonist carbachol (EC_{50} = 1.6 ± 0.2 μmol/l). In some experiments, possible effects on Ca channels were examined by stimulating tissue with Ca^{2+} (range 10 μmol/l to 30 mmol/l). When a maximum response to carbachol or a final concentration of 30 mmol/l Ca^{2+} was obtained, tissues were washed with KPSS or K^+, Ca^{2+}-free KPSS, 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 Ca^{2+}. These results were averaged and compared with tissues receiving solvent alone. Antagonist affinities were estimated according to the following formula: $pK_b = -\log([\text{antagonist}]/[\text{concentration ratio} - 1])$.

Statistics. Statistical significance was determined using the Student's t test or ANOVA with Newman-Keuls test for unpaired data. A P value ≤0.05 was considered significant.

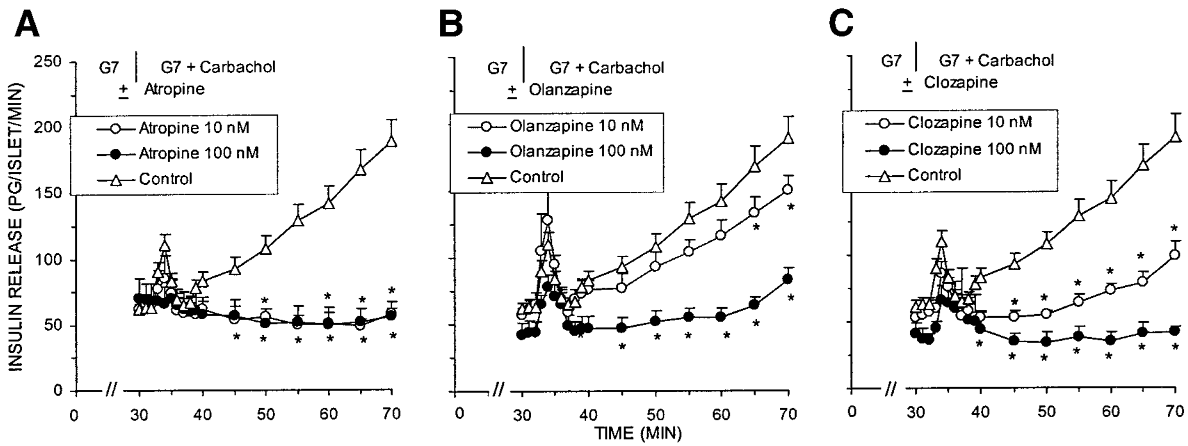

FIG. 1. Atropine, olanzapine, and clozapine potently inhibit carbachol-potentiated insulin secretion in rat islets. Groups of 14–16 islets were isolated and perifused for 30 min in the presence of 7 mmol/l glucose to establish stable rates of insulin secretion. Control islets (*n* = 20) were stimulated with 10 μmol/l carbachol for an additional 40 min. Other islets were treated as described above except that atropine (*A*), olanzapine (*B*), or clozapine (*C*) at 10–100 nmol/l were included during the entire perifusion. Control perifusions contained vehicle (0.1% DMSO). Data represent means ± SE of at least four experiments. **P* < 0.05 vs. control.

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 $pg \cdot islet^{-1} \cdot min^{-1}$ ($n = 20$; Figs. 1 and 2). Addition of 10 μmol/l carbachol produced an immediate short-lasting response that peaked at 110 ± 8 $pg \cdot islet^{-1} \cdot min^{-1}$, followed by a gradual increase in release rates that averaged 189 ± 17 $pg \cdot islet^{-1} \cdot min^{-1}$ 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 $pg \cdot islet^{-1} \cdot min^{-1}$, $n = 17$, 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 M_3 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-

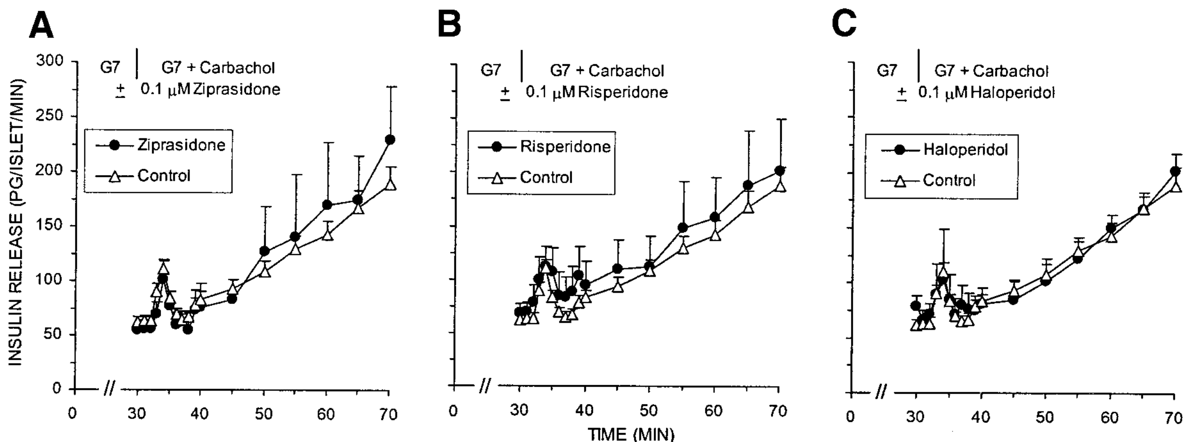

FIG. 2. Lack of effect of ziprasidone, risperidone, or haloperidol on carbachol-potentiated insulin secretion. Groups of islets were perifused as described in the legend of Fig. 1. After the 30-min stabilization period in 7 mmol/l glucose with or without ziprasidone, risperidone, or haloperidol (all at 100 nmol/l), islets were stimulated with carbachol for 40 min in the continued presence of the drug. Control responses are the same as shown in Fig. 1. Data represent means ± SE of at least three experiments.

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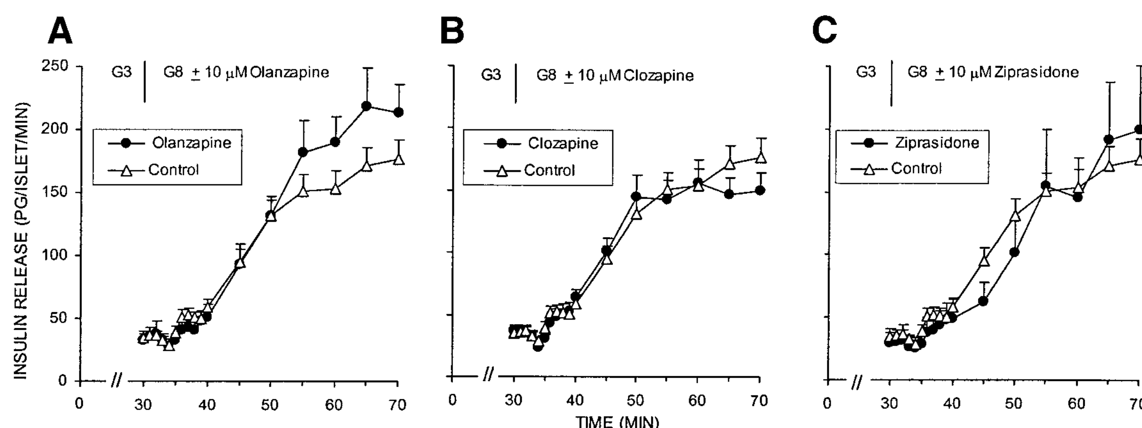

FIG. 3. Olanzapine, clozapine, or ziprasidone do not affect 8 mmol/l glucose–induced insulin secretion in rat islets. Groups of islets were isolated and perifused for 30 min with 3 mmol/l glucose to establish stable basal rates of insulin secretion and stimulated for 40 min with 8 mmol/l glucose alone or in the additional presence of olanzapine, clozapine, or ziprasidone (all at 10 μmol/l). Control perifusions contained vehicle (0.1% DMSO). At least six experiments were conducted under each condition, and data represent means ± SE.

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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 hM_3 receptors expressed in CHO cells (olanzapine K_b = 36 nmol/l, clozapine K_b = 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 hM_3 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. 4*A* 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. 4*C*). The K_b values of the test compounds for antagonism at the rat M_3 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 hM_3 (CHO cells) and rM_3 (rat urinary bladder) receptors

	M_3 binding affinity	M_3 functional activity	
Drug	INS-1 cells K_i (nmol/l)*	CHO cells K_b (nmol/l)†	Urinary bladder K_b (nmol/l)‡
Clozapine	25 ± 3	59 ± 30	32
Olanzapine	38 ± 6	36 ± 10	84
Risperidone	>1,600	>2,800	>1,000
Ziprasidone	>1,600	>2,800	>1,000
Haloperidol	—	>2,800	>1,000

Data are means ± SE. *Displacement of [^{3}H]-*N*-methylscopolamine binding to INS-1 cells; †inhibition of carbachol-induced calcium flux in hM_3 receptors expressed in CHO cells; ‡inhibition of carbachol-induced contractions of rat urinary bladder.

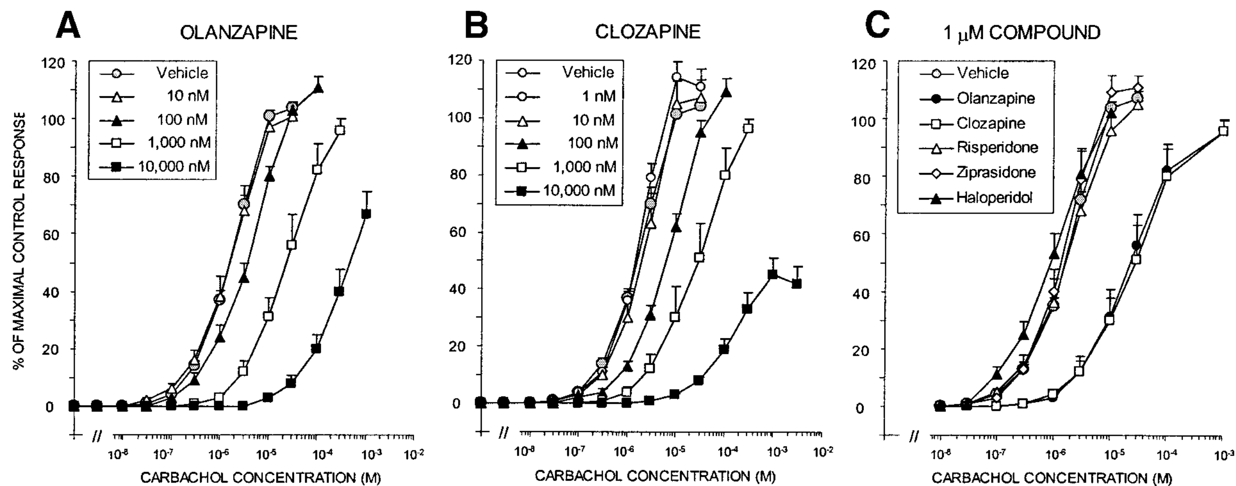

FIG. 4. Cumulative concentration-response curves for carbachol-induced contractions of rat urinary bladder in the absence (controls, 0.5% DMSO) and presence of increasing concentrations of olanzapine (*A*), clozapine (*B*) or 1 μmol/l of the test compounds (*C*). Each data point represents means ± SE percent inhibition of the maximum control response at the applied carbachol concentration.

leads to a full or partial reversal of the hyperglycemia (4,7,8,10). While it is accepted that these SGAs harbor an increased risk of disrupting glucose regulation in patients, the pharmacological basis for the differences in diabetic liability that exist within the SGA class is not known. One possible mechanism for hyperglycemia is the impairment of cholinergic-regulated insulin secretion. Clozapine and olanzapine are potent anticholinergics and could interfere with these processes, but their effects on cholinergic activation of the β-cell have not been investigated in detail. In this study, we have explored how a number of antipsychotics impact both fuel and neurohumorally mediated insulin secretion from isolated perifused rat islets. The results suggest that inhibition of cholinergic-stimulated insulin secretion is a possible contributing factor in the disruption of glucose homeostasis by olanzapine and clozapine.

The regulation of insulin secretion from pancreatic β-cells is a complex physiological process controlled by a number of interacting signaling components, including acetylcholine (13). Released from the vagus nerve, acetylcholine stimulates insulin secretion through the M_3 muscarinic receptor subtype coupled to phospholipase-C activation (14,15,22). Under our experimental conditions, 10 μmol/l carbachol produced a biphasic insulin secretory response in the presence of physiological glucose levels (7 mmol/l), with increases in second-phase release rates of three- to fivefold above prestimulatory rates. The addition of 10 μmol/l atropine, olanzapine, or clozapine completely blocked carbachol-stimulated insulin secretion, whereas ziprasidone, risperidone, or haloperidol had no effect. Subsequent studies demonstrated that olanzapine or clozapine concentrations as low as 10–100 nmol/l significantly impaired cholinergic-potentiated secretion when added during the prestimulatory period. These concentrations are near the therapeutic free plasma levels of olanzapine and clozapine in patients (23,24), although tissue levels may be higher (25). Notably, insulin secretion in response to glucose alone (8 mmol/l) was unaffected by all test compounds, providing evidence that olanzapine and clozapine inhibit insulin secretion mediated via muscarinic receptor activation. The selective nature of the anticholinergic effects exhibited by olanzapine and clozapine is supported by the data showing potent muscarinic M_3 receptor binding and functional antagonism of olanzapine and clozapine. Both SGAs bind with high affinity to muscarinic receptors in the rat pancreatic INS-1 cell line and are potent antagonists at human M_3 receptors expressed in CHO cells. In addition, olanzapine and clozapine are competitive antagonists at the native rat muscarinic M_3 receptor, as demonstrated by the parallel shifts of carbachol concentration-response curves in the isolated urinary bladder, a functional M_3 selective assay. At high concentrations, clozapine inhibited calcium-induced contractions in this preparation (data not shown), which suggests that the blunting of the maximum carbachol response by 10 μmol/l 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 M_3 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-

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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 M_3 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-HT_{2A} 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-HT_{2A} receptors (46). Since most antipsychotics are high-affinity antagonists at this receptor, the combination of 5-HT_{2A} 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. M_3 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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