Mechanistic Investigation of N,N-Diethyl-4-(phenyl-piperidin-4-ylidenemethyl)-benzamide–Induced Insulin Depletion in the Rat and RINm5F Cells

Monicah A. Otieno,*† Nicole Bavuso,*† Joseph Milano,* Linda Foster-Brown,* Khanh-Hui Bui,† Yan Li,† Thomas Hudzik,‡ Debra Wescott,* Calvert Louden,* Martin Dyroff,* and François Pognan*†

*Safety Assessment US; †DMPK; and ‡Neuroscience, AstraZeneca Pharmaceuticals, Wilmington, Delaware 19850

These studies describe the effect of N,N-diethyl-4-(phenyl-piperidin-4-ylidenemethyl)-benzamide (AR-M100390), a delta-opioid agonist, on the pancreas and its mechanisms for pancreatic toxicity. Rats were treated with 5, 100, and 600 µmol/kg of AR-M100390 for 3 and/or 7 days; another group of rats treated with 600 µmol/kg of compound were allowed to recover for 14 days. AR-M100390 (600 µmol/kg) caused vacuolation in the β-cell of the rat pancreas that was associated with depletion of insulin and hyperglycemia after 7 days of dosing. The loss of insulin by AR-M100390 was due to specific inhibition of rat insulin2 mRNA transcription in vivo. Insulin depletion and hyperglycemia were reversible. The effects of AR-M100390 in rats were reproduced in the rat pancreatic β-cell line RINm5F, where it inhibited intracellular insulin content and secretion without affecting cell survival. Loss of insulin in vitro was also a result of specific inhibition of insulin2 mRNA transcription and was reversible. Pretreatment of cells with the δ-opioid antagonist naltrindole or pertussis toxin did not reverse loss of insulin in AR-M100390-treated cells suggesting that the effects were not mediated by the δ-opioid receptor. AR-M100390 inhibited KCl-mediated calcium mobilization in RINm5F cells, suggesting that L-type calcium channels found in these cells and in pancreatic β-cells may partially play a role in the inhibition of insulin secretion by this compound. In summary, the in vitro and in vivo studies suggest that inhibition of insulin by AR-M100390 is due to a combination of inhibition of insulin synthesis and/or release.

Key Words: AR-M100390; cyclizine; pancreas; insulin; rat; RINm5F.

†To whom correspondence should be addressed at Bristol Myers-Squibb, Discovery Toxicology Lawrenceville, Mail Stop F14-01, Route 206 & Provinceline Road, Princeton, NJ 08543. Fax: (609) 252-7156. E-mail: monicah.otieno@bms.com.

‡Present address: UMDNJ—Robert Wood Johnson Medical School, Piscataway, NJ 08854.

§Present address: Bristol Myers-Squibb, Discovery Toxicology Lawrenceville, Mail Stop F14-01, Route 206 & Provinceline Road, Princeton, NJ 08543.

## References

We refer to the literature for further details on the methods and results. The key references include:


2. Cusi, K., and Defronzo, R.A. (2001). Insulin secretion (Cusi and Defronzo, 2001; Qi and Rodrigues, 2007). In a minority of patients with T2DM the initial defect is drug-induced insulin resistance in man has been previously described; examples include the antipsychotics clozapine and olanzapine (Tschorner et al., 2007; Wofford et al., 2006), which cause a dramatic increase in weight gain that may be a trigger for insulin resistance. Other drugs, such as phenytoin impair insulin secretion by affecting calcium flux into β-cells of the pancreas (Pace and Livingston, 1979; Siegel et al., 1982), whereas glucocorticoids induce insulin resistance by increasing glucose production from the liver (Qi and Rodrigues, 2007).

Examples of molecules that cause pancreatic toxicity in animals include cyclizine, chlorcyclizine, and cyproheptadine, which induce islet cell toxicity in the rat (Hanai, 1984; Hruban et al., 1972; Wold et al., 1971).

The aims of the following studies were (1) to characterize pancreatic toxicity mediated by AR-M100390 in the rat, (2) evaluate the utility of the rat pancreatic β-cell line RINm5F as an in vitro surrogate to study pancreatic toxicity by AR-M100390, (3) examine reversibility of pancreatic toxicity by AR-M100390 in vivo and in vitro, and (4) investigate the mechanisms by which AR-M100390 inhibits intracellular and secreted insulin in vitro and in vivo.

The in vitro and/or in vivo effects of AR-M100390 were studied by measuring insulin protein and mRNA. Whereas most species have a single insulin gene, the rat and mouse have...
two nonallelic insulin genes. These genes are coordinately expressed (Melloul et al., 2002) resulting in two isomeric insulin proteins that differ by two amino acids (Toriumi and Imai, 2003). This study investigated the effect of AR-M100390 on insulin1 or 2 mRNA in RINm5F cells or in the rat pancreas.

Glucose-dependent depolarization of the pancreatic β-cell resulting in opening of calcium channels is a well-recognized mechanism for calcium-mediated insulin secretion in pancreatic β-cells (Dunbar, 1994; Dunne et al., 2001; Mears, 2004; Navarro-Tableros et al., 2007; Satin et al., 1995). The calcium channel blocking drugs darodipine, nicardipine, diltiazem, verapamil, and cyproheptadine block glucose-induced insulin secretion in vitro (Donatsch et al., 1980; Semple et al., 1988). Inhibition of intracellular calcium flux as a mechanism for inhibition of insulin secretion in vitro by AR-M100390 was also examined.

This study expands our understanding of drug-induced pancreatic toxicity and provides support for the use of RINm5F cells as a suitable in vitro cell line to study compounds that cause pancreatic lesions similar to that described for AR-M100390.

**MATERIALS AND METHODS**

**Materials.** Cyclizine, cyproheptadine, and other reagents were purchased from Sigma (St Louis, MO). AR-M100390 was synthesized as previously described (Wei et al., 2000). Rat Insulin ELISA kits (1-2-3 high range and normal) were obtained from ALPCO Diagnostics (Windham, NH). Protease inhibitor Cocktail I was purchased from Calbiochem (San Diego, CA). The Calcium-3 assay kit was obtained from Molecular Devices (Sunnyvale, CA).

**Cell culture.** RINm5F cells were obtained from ATCC (Manassas, VA) and cultured in Roswell Park Memorial Institute media containing 11mM glucose supplemented with 10% fetal bovine serum, 2mM glutamine, 100 IU/ml penicillin, and 100µg/ml streptomycin in a humidified atmosphere (5% CO2, 95% air).

**In vivo treatment.** Animals were handled and treated according to the Institutional Animal Care and Use Committee guidelines within AstraZeneca (Wilmington, DE). Han Wistar rats (six per treatment group) were treated with vehicle (saline) or 5, 100, and 600 µmol/kg/day of AR-M100390 for 7 days. A separate group of rats were treated with 600 µmol/kg/day for 7 days followed by a 1-4-day recovery period. Another group was treated with 600 µmol/kg/day for 3 days. Blood sampling for glucose, lipids, and insulin measurements were taken on days 2, 4, 8, and 22. Blood sampling for AR-M100390 concentration measurements were collected on days 4 and 8. The animals were euthanized with CO2 on days 4, 8, and 22 and the pancreas isolated and processed for histopathology, insulin immunohistochemistry, and insulin mRNA analyses.

**Immunohistochemistry.** The pancreatic tissue was trimmed and fixed for 24 h in 10% neutral buffered formalin. The paraffin blocks were sectioned at 5 µm, dried overnight, and heated. The slides were deparaffinized, washed, blocked, and stained on the Ventana Discovery System (Tucson, AZ) according to the manufacturer’s instructions. Slides were incubated for 24 min in rabbit anti-guinea pig insulin antibody (Dako, Carpintera, CA) at a 1:100 dilution. After washing, the slides were incubated with a biotinylated goat-anti rabbit secondary antibody (Jackson Immunoresearch, Westgrove, PA) for 14 min. Insulin immunoreactivity was detected by diaminobenzamide detection kit (Ventana Discovery System, Tucson, AZ) according to the manufacturer’s directions. The slides were counterstained with hematoxylin.

**Insulin measurements: in vivo.** Blood was collected in serum tubes and stored at −80°C until analysis for insulin with the 1-2-3 normal range insulin ELISA kit (ALPCO Diagnostics) according to the manufacturer’s directions.

**Insulin measurements: in vitro.** Cells (1 × 10^5) were seeded in 24-well plates and grown for 3 days. Growth media was replaced with serum-free media supplemented with 5mM glucose, and cells were treated with test compounds overnight (16–24 h). Insulin secreted in the media was determined directly by measurement of an aliquot (10 µl) of the media. For insulin content determinations, cells were rinsed with phosphate-buffered saline, lysed in 500 µl of buffer containing 20mM Tris-HCl, pH 7.5, 1mM MgCl2, 1mM ethylenediaminetetraacetic acid dipotassium salt (K2EDTA), 1mM ethylene glycol bis-(2-aminoethoxy)-tetraacetic acid, 1× protease inhibitors, 0.1% Triton X-100, and an aliquot of the lysate (10 µl) was analyzed for insulin with the 1-2-3 High Range Rat Insulin ELISA kit (ALPCO Diagnostics) according to the manufacturer’s directions.

**Glucose, cholesterol, and triglyceride measurements.** Blood was collected from control and treated animals into tubes coated with K2EDTA. Plasma from the samples was analyzed for glucose, cholesterol, and triglycerides levels with the Roche clinical chemistry assays on the Hitachi 912 (Roche Diagnostics Systems, Indianapolis, IN).

**Measurement of blood concentrations of AR-M100390.** Plasma (25 µl) was precipitated with 200 µl of cold acetonitrile. The samples were centrifuged and the supernatant analyzed by reverse-phase gradient elution by liquid chromatography/mass spectrometry. The method had a lower limit of quantitation of 2nM AR-M100390 and an upper limit of 20µM. Analysis was done on a Reverse-Phase High Performance Liquid Chromatography System (HP1100, Hewlett Packard, Wilmington, DE) interfaced to a Hewlett Packard MSD single quadruple mass spectrometer. Separations were obtained at a flow rate of 1 ml/min on a Zorbax SB-C8 (4.6 mm × 30 cm, 5 µm) column in a mobile phase consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). AR-M100390 was eluted and quantitated on a linear gradient starting with 20% A to 80% B over 1.5 min with electrospray ionization in the positive ion mode for mass m/z 349 (parent ion of AR-M100390).

**RNA isolation: in vivo.** RNA from the pancreas was isolated with the Qiagen Midi-Preps according to the manufacturer’s directions (Qiagen, Valencia, CA).

**RNA isolation: in vitro.** RINm5F cells were seeded in 24-well plates and treated with vehicle (DMSO), 10µM AR-M100390, and 10µM cyclizine in serum-free medium under similar conditions as those described above for insulin protein determinations; cells were rinsed with phosphate-buffered saline and stored at -80°C until analysis. RNA was isolated with the RNeasy purification system with DNase treatment according to the manufacturer’s protocol (Qiagen, Valencia, CA).

**Insulin TaqMan reverse transcription PCR.** RNA concentration was determined spectrophotometrically (260/280 nm) and RNA integrity assessed with the Agilent Bioanalyzer 2100 (Agilent, Wilmington, DE). Total RNA (2.5 µg) was reverse transcribed using random hexamer oligonucleotides and the Superscript II reverse transcription system from Invitrogen (Carlsbad, CA). The cDNA product was diluted to 25 ng/µl and increasing amounts (0.154, 0.91, 2.76, 8.33, 25, and 50 ng) were used as templates to create a standard curve for insulin1 or insulin2 transcripts by quantitative real-time PCR. The Quantitech SYBR Green real-time PCR reagent (Qiagen, Valencia, CA) was used to assay for the two insulin transcripts with primers (300nM) as previously described (Toriumi and Imai, 2003). Thermo cycling was performed on the DNA Engine Opticon 2 (MJ Research, Waltham, MA) using the following conditions: hot start at 95°C for 15 min followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The relative amounts of insulin1 or insulin2 transcripts from cDNA isolated from control or treated cells was determined from the standard curve and this was normalized to the amount of 18S mRNA also quantitated from the same samples.

**Intracellular calcium measurements.** Intracellular calcium mobilization in response to depolarization with 10mM KCl was recorded on the Flexstation II (Molecular Devices, Sunnyvale, CA). RINm5F cells (2 × 10^3) were seeded in black-walled 96-well plates and allowed to grow with the growth media. Growth media was removed and cells were loaded with the Calcium-3 dye for 1 h in
Results

Characterization of AR-M100390-Mediated Pancreatic Toxicity In Vivo

Treatment of rats with 600 μmol/kg of AR-M100390 resulted in vacuolation of the β-cell of the rat pancreas that was similar to that reported for cyclizine and cyproheptadine (Hanai, 1984; Hruban et al., 1972), whose structures are shown in Figure 1. Pancreatic toxicity was not detected in rats treated with 5 or 100 μmol/kg of AR-M100390 as determined by histology (data not shown) and lack of effects on insulin or glucose levels (Table 1). Treatment with 600 μmol/kg of AR-M100390 for 3 days resulted in a significant decrease in insulin immunostaining in the insulin-producing β-cells (Fig. 2B) that was not accompanied by vacuoles in the pancreas. Treatment for 7 days resulted in a near complete loss of insulin staining accompanied by large vacuoles in the β-cells (Fig. 2C). The qualitative decrease in insulin staining after 3 days of treatment was associated with a twofold increase in glucose levels compared with controls (Table 1) without an effect on circulating insulin levels. The near complete loss in staining after 7 days of treatment was associated with an approximate 85% decrease in serum insulin and a threefold increase in serum glucose (Table 1).

To evaluate whether inhibition of insulin and elevation of glucose were reversible, rats were treated with AR-M100390 for 7 days and allowed to recover for 14 days before analysis of pancreas for vacuoles and insulin immunostaining; blood was also collected for measurement of insulin and glucose levels after 7 and 14 days of recovery. Insulin staining was detected in the pancreas during the recovery period, however, vacuoles were still present in the β-cells of the pancreas during this time (Fig. 2D). The circulating insulin and glucose levels normalized to control values after 7 days of recovery and were maintained through 14 days of recovery (Table 1).

Temporal measurements of insulin and glucose observed after 7 days of dosing was accompanied by significant decreases in serum cholesterol and triglyceride levels (Fig. 3B).

Blood concentrations in rats treated with 600 μmol/kg AR-M100390 were 7.81 and 10.6μM on days 3 and 7, respectively (Table 1); a comparison of the concentrations on day 7 to the binding activity of the compound at its receptor of 11.3nM (Plobeck et al., 2000) revealed a 1000-fold safety margin. The compound was also detected at pharmacologically relevant concentrations of 20nM in rats treated with 5 μmol/kg of AR-M100390 without any evidence of pancreatic toxicity (Table 1).

Effect of AR-M100390 on Insulin mRNA In Vivo

In order to understand the mechanism for insulin loss by AR-M100390, insulin1 and insulin2 mRNA levels were measured from pancreas isolated from control and treated animals. There was a selective decrease in insulin2 mRNA after treatment with AR-M100390 for 3 or 7 days and an increase in insulin1 mRNA (Fig. 4). Removal of drug during the recovery period restored insulin2 mRNA levels.

Characterization of AR-M100390 and Cyclizine In Vitro

To determine whether RINm5F cells was a suitable in vitro system to study compounds that have AR-M100390-like effects in vivo, the cells were treated with AR-M100390 and cyclizine for 16–24 h before measurement of intracellular and secreted insulin levels. AR-M100390 mediated a dose-dependent decrease in insulin content with a maximal inhibition of ~90% at the highest concentration tested (10μM) (Fig. 5A, closed...
circles). Interestingly, blood levels for AR-M100390 that resulted in loss of insulin was \(~10\mu M\), suggesting a reasonable correlation between in vitro and in vivo findings. Cyclizine, known to induce a similar pancreatic lesion as AR-M100390, but has not been tested in RINm5F cells, also mediated a similar dose-dependent decrease in insulin protein up to 10\mu M (Fig. 5B, closed circles). Cells treated with both compounds were 100\% viable at all concentrations tested as determined by Trypan Blue exclusion (Data not shown). Cyproheptadine, previously shown to deplete insulin in these cells (Miller et al., 1993) was used as a positive control (Fig. 5C).

The effect of AR-M100390 and cyclizine on insulin secretion was also examined in vitro. Both compounds were less potent in inhibiting insulin secretion compared with intracellular insulin protein (Figs. 5A and 5B, open circles) and they mediated a biphasic response with an increase in insulin at low doses followed by a decline at high doses. Cyproheptadine, on the other hand, was more potent in its inhibition of insulin secretion compared with cellular insulin content.

Effect of Antagonist and Reversibility In Vitro

Pretreatment of RINm5F with the \(\delta\)-opioid antagonist naltrindole, followed by addition of AR-M100390 did not reverse inhibition of insulin content in vitro (Fig. 6A); this was confirmed by negative findings in studies with pertussis toxin, which uncouples \(G_{i}\)-specific signaling pathways to the \(\delta\)-opioid receptor (data not shown). Inhibition of intracellular insulin levels by AR-M100390 in RINm5F cells was reversible after removal of the compound (Fig. 6B).

Effect of AR-M100390 and Cyclizine on Insulin mRNA In Vitro

The mechanism for insulin inhibition in RINm5F cells was determined by examining the effect of AR-M100390, cyclizine, and cyproheptadine on insulin1 and insulin2 mRNA transcription. Treatment of RINm5F cells with these compounds resulted in a 50–70\% decrease in insulin2 mRNA levels without an effect on insulin1 mRNA (Fig. 7).

Mechanisms of Insulin Secretion by AR-M100390 and Cyclizine In Vitro

Insulin secretion in pancreatic \(\beta\)-cells is triggered by calcium as a result of membrane depolarization due to activation of \(K_{ATP}\) channels resulting in opening of L-type calcium channels (Dunne et al., 2001). The following studies examined the effect of AR-M100390 and cyclizine on calcium mobilization after membrane depolarization of RINm5F with 10mM KCl, a known activator of L-type calcium channels. Addition of KCl to RINm5F cells in the absence of compound resulted in mobilization of calcium into the cells that was detected with
a preloaded calcium-sensitive fluorescent dye and quantitated as fluorescent units (Fig. 8, red closed circles). Treatment with AR-M100390 or cyclizine followed by depolarization with 10mM KCl resulted in slight increases in calcium mobilization at low concentrations followed by a 50% decrease at 10 μM (Figs. 8A and 8B); insulin secretion is inhibited by 50% by both compounds at this concentration (Figs. 5A and 5B). Cyproheptadine, a potent calcium channel antagonist (Dohmoto et al., 2003) and positive control, inhibited KCl-mediated calcium mobilization into the cells (Fig. 8C) that correlated well with inhibition of insulin secretion (Fig. 5C).

DISCUSSION

AR-M100390 caused vacuolation of the pancreatic β-cells in the rat that was associated with loss of intracellular and serum insulin. Pancreatic toxicity mediated by AR-M100390 is similar to that described for cyclizine and cyproheptadine (Hanai, 1984; Hruban et al., 1972; Wold et al., 1971). Changes in serum insulin and glucose preceded vacuolation in AR-M100390-treated animals, identifying these biochemical endpoint as potential biomarkers for the pancreatic lesion. Loss of insulin in animals treated with AR-M100390 was accompanied by decreases in circulating triglycerides and cholesterol, implying an inhibition in their synthesis because insulin is required for triglyceride and cholesterol synthesis in the liver and/or adipose tissue (Genuth, 1998).

The origin of vacuoles in β-cells of pancreas after drug treatment was not investigated, but staining of the vacuole content for protein or fat was negative (data not shown). Electron microscopy studies identified vacuoles in cyclizine-treated animals as dilated Golgi apparatus (Hanai, 1984), the site of preproinsulin processing (Molinete et al., 2000). Pioneering studies tracking translocation of secretory proteins with radiolabel found that treatment with the protein synthesis inhibitor cycloheximide resulted in empty vacuoles as existing proteins migrated through the Golgi apparatus in the cell, but was not replaced by newly synthesized proteins (Jamieson, 1988). This may be a mechanism for formation of vacuoles in pancreatic β-cells after treatment with AR-M100390.

A compensatory hyperinsulinemia and immediate normalization of glucose was observed in the recovery period following termination of treatment with AR-M100390, suggesting that the pancreas was not permanently damaged by AR-M100390. Absolute normal functioning of the pancreas could only be confirmed by a glucose tolerance test, which was not conducted in this study. Loss of insulin content by AR-M100390 was also reversed in vitro, confirming the nontoxic property of the compound.

Characterization of an in vitro cell line that predicts AR-M100390-mediated pancreatic toxicity was deemed useful not only for studying mechanisms of toxicity, but also as a potential...
screening tool. Treatment of RINm5F cells, a rat pancreatic β-cell line, with AR-M100390 and cyclizine resulted in an almost complete depletion of insulin content that was very similar to in vivo findings. The decrease in potency in inhibition of secreted insulin by AR-M100390 and cyclizine compared with inhibition of cellular insulin content could be due to a long half-life of the secreted protein in the media, although this was not determined in the present experiments. These cells have previously been used to study the effects of cyproheptadine (Miller et al., 1993), which also produces a similar pancreatic lesion, suggesting that they can be used to study compounds that cause these types of lesions.

**FIG. 5.** Intracellular and secreted insulin measurements in RINm5F cells. RINm5F cells were treated with (A) AR-M100390, (B) cyclizine, and (C) cyproheptadine for 16–24 h before measurement of intracellular (closed circles) and secreted insulin (open circles) in cell lysates and media as described in “Materials and Methods.” Data are represented as mean ± SD of triplicate measurements.

**FIG. 6.** The effect of the δ-opioid antagonist naltrindole on AR-M100390-mediated inhibition of intracellular insulin (A) RINm5F cells were pretreated with 100nM naltrindole for 2 h before addition of 10μM AR-M100390 for 16–24 h and intracellular insulin concentrations was determined as described in “Materials and Methods.” The effect of AR-M100390 or naltrindole only on insulin concentrations was also determined. (B) Reversibility of insulin inhibition by AR-M100390 in vitro. RINm5F cells were treated with 10μM AR-M100390 for 16–24 h and intracellular insulin concentrations were measured; in parallel experiments, with matched controls, the compound was removed, replaced with fresh media, and the cells allowed to grow for another 48 h before measurement of intracellular insulin. Data are represented as mean ± SD of triplicate measurements. Statistical significance in a Student’s t-test comparison of control and treated values is indicated (*p < 0.01).
The inability to reverse the depletion of insulin with naltrindole in vitro, suggests that the effects of AR-M100390 are not mediated by the δ-opioid receptor; in addition, pancreatic toxicity was not detected in the rat at pharmacological concentrations of AR-M100390.

Both in vivo and in vitro investigations found that loss of insulin by AR-M100390 was a result of specific inhibition of insulin2 mRNA and upregulation of this insulin gene directly correlated with reappearance of insulin after compound withdrawal. Paradoxically there was an increase in insulin1 mRNA with treatment, suggesting that its upregulation was not sufficient to overcome loss of insulin by AR-M100390. The present studies find that cyclizine also selectively inhibits insulin2 mRNA in RINm5F cells that is correlated with its inhibition of insulin content in these cells. The effect of cyclizine on insulin mRNA in vivo has not been reported, but the profile would most likely be similar to that of AR-M100390.

Whereas preproinsulin exists as a single gene in most species, the rat and mouse have two nonallelic preproinsulin genes (or insulin genes) that are found on the same chromosome (rat) or different chromosomes (mouse) (Dumonteil and Philippe, 1996; Melloul et al., 2002). The insulin1 gene has a single intron, whereas the insulin2 gene, which resembles the insulin genes found in other species, has two introns (Dumonteil and Philippe, 1996; Melloul et al., 2002). The rodent insulin1 and insulin2 genes have highly conserved C1, E1, and CRE regulatory sequences that are critical for insulin transcription (Furukawa et al., 2002), therefore selective inhibition of insulin2 mRNA by cyclizine and AR-M100390 was surprising.

Previous studies examining the effect of cyproheptadine in RINm5F cells reported an inhibition of insulin synthesis that was not associated with a change in preproinsulin mRNA (Hawkins and Fischer, 2004). We however find that cyproheptadine selectively inhibits insulin2 mRNA in RINm5F cells, which is in agreement with published in vivo studies where the compound inhibited insulin2 mRNA to a greater extent than insulin1 (Giddings et al., 1991). Selective inhibition of insulin2 mRNA has also been described in studies of dexamethasone-induced diabetes in rats, where a marked inhibition of insulin2 mRNA compared with insulin1 was observed (Toriumi and...
Imai, 2003). The explanation for the differences in transcriptional regulation of insulin by AR-M100390 is not evident, but directed studies at the promoters for both genes may provide insight into the cis and/or trans factors involved in insulin gene regulation by this compound. A comparative analysis of the insulin gene promoters from 14 species, including chimpanzee, dog, rat, mouse, and zebrafish find that the rodent promoters are consistently dissimilar to humans and caution should be taken in extrapolating rodent findings to humans (Hay and Docherty, 2006). Indeed, cyproheptadine and cyclizine have been used clinically without adverse reports suggestive of pancreatic toxicity in man.

Membrane depolarization of the pancreatic β-cell resulting in opening of L-type calcium channels allowing entry of calcium into cells is a known mechanism for mediating insulin release. Pancreatic β-cells are electrically active and in the presence of stimulatory concentrations of glucose (> 5mM) will depolarize (Dunne et al., 2001); dihydropyridine-sensitive or L-type calcium channels have previously been characterized in RINm5F (Schmidt et al., 1991). Depolarization of these cells with KCl resulted in mobilization of calcium into the cells that was partially inhibited by cyclizine and AR-M100390, suggesting that these compounds may be weak L-type calcium channel antagonists at high concentrations. There may therefore be a dual mechanism by which these compounds regulate insulin, an effect on insulin2 gene that contributes to the loss of intracellular insulin and an inhibition of calcium channels on pancreatic cells that may partially contribute to inhibition of secreted insulin.

In conclusion, this study found that (1) AR-M100390 depletes insulin content in vivo and in vitro by inhibiting insulin2 mRNA transcription, (2) the effects on insulin are reversible after removal of compound, (3) confirms the use of RINm5F cells as an in vitro model to study pancreatic toxicity mediated by compounds such as AR-M100390, and (4) inhibition of calcium channels may partially contribute to inhibition of insulin secretion by AR-M100390.

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REFERENCES


