Blocks of the Delayed-Rectifier Potassium Current in Pancreatic β-Cells Enhance Glucose-Dependent Insulin Secretion

James Herrington,1 Yun-Ping Zhou,2 Randal M. Bugianesi,1 Paula M. Dulski,1 Yue Feng,2 Vivien A. Warren,1 McHardy M. Smith,1 Martin G. Kohler,1 Victor M. Garsky,3 Manuel Sanchez,4 Michael Wagner,1 Kristin Raphaeli,1 Priya Banerjee,1 Chinweze Ahaghotu,1 Denise Wunderler,1 Birgit T. Priest,1 John T. Mehl,5 Maria L. Garcia,1 Owen B. McManus,1 Gregory J. Kaczorowski,1 and Robert S. Slaughter1

Delayed-rectifier K⁺ currents (I_{DR}) in pancreatic β-cells are thought to contribute to action potential repolarization and thereby modulate insulin secretion. The voltage-gated K⁺ channel, Kᵥ2.1, is expressed in β-cells, and the biophysical characteristics of heterologously expressed channels are similar to those of I_{DR} in rodent β-cells. A novel peptidyl inhibitor of Kᵥ2.1/Kᵥ2.2 channels, guangxitoxin (GxTX)-1 (half-maximal concentration ~1 nmol/l), has been purified, characterized, and used to probe the contribution of these channels to β-cell physiology. In mouse β-cells, GxTX-1 inhibits 90% of I_{DR} and, as for Kᵥ2.1, shifts the voltage dependence of channel activation to more depolarized potentials, a characteristic of gating-modifier peptides. GxTX-1 broadens the β-cell action potential, enhances glucose-stimulated intracellular calcium oscillations, and enhances insulin secretion from mouse pancreatic islets in a glucose-dependent manner. These data point to a mechanism for specific enhancement of glucose-dependent insulin secretion by applying blockers of the β-cell I_{DR}, which may provide advantages over currently used therapies for the treatment of type 2 diabetes. Diabetes 55: 1034–1042, 2006

Insulin secretion from pancreatic β-cells in response to glucose is regulated by ATP-sensitive K⁺ channel (KᵥATP channel) and by KᵥATP channel-independent pathways (1). In the KᵥATP channel–dependent pathway, changes in cellular ATP/ADP levels brought about by the metabolism of glucose cause closure of KᵥATP channels, which sets the resting membrane potential of these cells. The closure of KᵥATP channels leads to depolarization of the plasma membrane, opening of voltage-gated calcium channels, and an increase in cytosolic free calcium, [Ca²⁺]ᵢ, to trigger insulin secretion (2). Sulfonylureas, the current first-line treatment in type 2 diabetes, block KᵥATP channels to induce insulin secretion, but because the action of sulfonylureas is not glucose dependent, patients often exhibit episodes of hypoglycemia (3).

In addition to KᵥATP channels, several other types of K⁺ channels are present in β-cells, such as large-conductance calcium-activated K⁺ channel (4), a voltage-independent calcium-activated K⁺ channel (5,6), and a rapidly inactivating voltage-gated K⁺ channel (7,8). The delayed-rectifier K⁺ current (I_{DR}) is prominent in β-cells from several species, including humans (9), and is thought to contribute to repolarization of action potentials (10). Inhibition of I_{DR} should broaden action potentials, raise intracellular calcium levels, and enhance insulin secretion in a glucose-dependent manner (8,11,12). For these reasons, the β-cell I_{DR} has been considered a potential target for the development of novel agents for treatment of type 2 diabetes (13), although its molecular identity remains to be defined.

Of the Kᵥ channel subtypes that have been reported to be expressed in islet tissue, Kᵥ2.1 is prominent in β-cells and exhibits biophysical properties similar to the β-cell I_{DR} (13). A Kᵥ2.1 dominant-negative construct reduced β-cell I_{DR} and enhanced glucose-stimulated insulin secretion (GSIS) (8). In addition, hanatoxin (HaTX), a peptidyl Kᵥ2.1 inhibitor, has been reported to enhance GSIS (14) and to induce calcium oscillations in mouse and human islets (15). Lack of commercial availability of HaTX has hindered further studies on the mechanism of reported effects on GSIS.

To probe the role of I_{DR} in β-cells, a novel peptide inhibitor, guangxitoxin (GxTX)-1, was purified to homogeneity and synthetically produced. This peptide is a potent inhibitor of Kᵥ2.1/Kᵥ2.2 channels and inhibits most of I_{DR} in mouse β-cells. GxTX-1 broadens the β-cell action potential, enhances calcium oscillations, and augments GSIS but has no effect on secretion under low-glucose conditions. These data suggest that Kᵥ2.1 is a component of I_{DR}.

© 2006 by the American Diabetes Association.
in β-cells and that I_Dr represents an attractive target for the treatment of type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**GxTX-1 purification.** Aliquots of *Plesiophrictus guangxiensis* sp. nov. (16) venom (Spider Pharm, Yarnell, AZ) up to 300 μl original venom volume, were reconstituted with 10 vol of 20 mmol/l ammonium acetate, pH 6.2, and loaded onto a Brownlee CX-300 column pre-equilibrated with the same buffer at 1 ml/min. The column was developed using distilled deionized water as solvent A and 1 mol/l ammonium acetate at pH 6.2 as solvent B, using a gradient of 1.73% B/min. Absorbance at 280 nm was monitored, and peak fractions were collected, lyophilized, reconstituted in 140 mmol/l NaCl and 5 mmol/l HEPES-K, pH 7.4, and assayed for inhibition of K_V2.1 channel activity (see below). Selected fractions were pooled and subjected to reverse-phase high-performance liquid chromatography (HPLC) (C8, 4.6 × 250 mm; Vydac, Hesperia, CA) using 0.1% heptafluorobutyric acid in water (solvent A) and 0.1% heptafluorobutyric acid in 95% isopropanol:5% water (solvent B). Active fractions were pooled, injected onto a Brownlee CX-300 column pre-equilibrated with the same buffer at 1 ml/min, and absorbance at 210 nm was monitored. Active material eluting at 1% B, and eluted with a gradient of 10–50% B over 51 min at 1.0 ml/min, was pooled and loaded onto a C18 column (4.6 × 250 mm; Vydac) with 10 mmol/l trifluoroacetic acid (TFA) in water (solvent A) and 9 mmol/l TFA in 95% acetonitrile:water as solvent B. Material was eluted with a gradient of 10–90% B over 51 min at 1.0 ml/min. Absorbance was monitored at 210 nm. After lyophilization, active fractions were pooled and loaded onto a C18 column (4.6 × 250 mm; Vydac) with 10 mmol/l TFA in water as solvent A and 9 mmol/l TFA in 95% acetonitrile/water as solvent B. Material was eluted with a gradient of 20–50% B over 56 min at 1.0 ml/min, and absorbance at 210 nm was monitored. Active material eluting from this column was subjected to amino acid sequencing and mass spectrometry (17).

**Peptide synthesis.** GxTX-1E and GxTX-1D were synthesized (−0.5 mmol) by solid-phase methodology using a Boc protection strategy (18). Refolding of HPLC-purified hexahydro-peptides was achieved by air oxidation (0.1 mg/ml) in 2 mol/l urea, 1 mol/l Tris, pH 8.0, 0.15 mmol/l reduced glutathione, and 0.30 mmol/l oxidized glutathione. Folding and overall yields were 30 and 3.8% and folded with a gradient of 10–50% B over 51 min at 1.9 ml/min. Absorbance was monitored at 220 nm. The third-stage C4 column (4.6 × 250 mm; Vydac) was developed with 10 mmol/l trifluoroacetic acid (TFA) in water (solvent A) and 9 mmol/l TFA in 95% acetonitrile:water as solvent B. Material was eluted with a gradient of 10–90% B over 51 min at 1.0 ml/min. Absorbance was monitored at 210 nm. After lyophilization, active fractions were pooled and loaded onto a C18 column (4.6 × 250 mm; Vydac) with 10 mmol/l TFA in water as solvent A and 9 mmol/l TFA in 95% acetonitrile/water as solvent B. Material was eluted with a gradient of 20–50% B over 56 min at 1.0 ml/min, and absorbance at 210 nm was monitored. Active material eluting from this column was subjected to amino acid sequencing and mass spectrometry (17).

**Residues that are identical to GxTX-1E are shaded in gray. Cysteines are shown in dark gray.** DRG, dorsal root ganglion; TTXr, tetrodotoxin resistant.

**TABLE 1**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Amino acid identity to GxTX-1E</th>
<th>Channel activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GxTX-1E</td>
<td>DFGGYSQVPPK</td>
<td>36 K_2.2 K_2.2 K_4.3</td>
<td></td>
</tr>
<tr>
<td>GxTX-1D</td>
<td>DFGGYSQVPPK</td>
<td>35 K_2.2</td>
<td></td>
</tr>
<tr>
<td>Jingzhaoxin-III</td>
<td>DFGGYSQVPPK</td>
<td>22 Cardiac TTXr</td>
<td></td>
</tr>
<tr>
<td>GxMTx-4</td>
<td>KAGGGGGGPPK</td>
<td>16 Stretch channel</td>
<td></td>
</tr>
<tr>
<td>VSTX1</td>
<td>KAGGGGGGPPK</td>
<td>15 K_2.1</td>
<td></td>
</tr>
<tr>
<td>Hainanxin-III</td>
<td>KAGGGGGGPPK</td>
<td>14 DRG Na_2, 1 mol/l</td>
<td></td>
</tr>
<tr>
<td>Huwenvoentin-1</td>
<td>KAGGGGGGPPK</td>
<td>14 CAv_2.2 100 mmol/l</td>
<td></td>
</tr>
<tr>
<td>Hainanxin-IV</td>
<td>KAGGGGGGPPK</td>
<td>12 Nav_2.2 TTXs not DRG Na_2</td>
<td></td>
</tr>
<tr>
<td>ω-GsTx SIA</td>
<td>KAGGGGGGPPK</td>
<td>12 DRG Na_2, 45 mol/l</td>
<td></td>
</tr>
<tr>
<td>GxTx-2</td>
<td>KAGGGGGGPPK</td>
<td>9 K_2.1</td>
<td></td>
</tr>
<tr>
<td>ScTx1</td>
<td>KAGGGGGGPPK</td>
<td>8 K_2.1, K_2.2 K_2.1/9,3 K_4.2</td>
<td></td>
</tr>
<tr>
<td>Han toxin</td>
<td>KAGGGGGGPPK</td>
<td>7 K_2.1</td>
<td></td>
</tr>
<tr>
<td>SGTx</td>
<td>KAGGGGGGPPK</td>
<td>6 K_2.1</td>
<td></td>
</tr>
</tbody>
</table>

Residues that are identical to GxTX-1E are shaded in gray. Cysteines are shown in dark gray. DRG, dorsal root ganglion; TTXr, tetrodotoxin resistant.
**RESULTS**

To assess the contribution of K_{v,2.1} to the β-cell I_{DB}, 85 venoms were screened in a functional ^{86}Rb⁺ efflux assay using a stable CHO.K_{v,2.1} cell line. Venom from the tarantula, *Plesiophrictus guangxiensis* sp. nov., was found to display the highest inhibitory potency and was fractionated by HPLC. The most active purified fraction, GxTX-1, was sequenced and found to consist of two variants, 65% GxTX-1E and 35% GxTX-1D, that only differ at the NH₂-terminal residue, glutamate or aspartate, respectively (Table 1). A small inactive peak that elutes separately from GxTX-1 is present in the native purified sample (Fig. 1A).

Such behavior has also been observed in the separation of HaTX (28). A second less-active fraction, GxTX-2, was also sequenced. GxTX-2 is related to the K_{v,2.1} gating-modifier peptides, HaTX, ScTx1, and SGTx (28–30). GxTX-1 exhibits its very little sequence identity with any of these peptides but is related to the gating-modifier peptides, Jingzhao-toxin-III (31), GsMTx-4 (32), and VSTX1 (33), peptide-dazed tarantula venom inhibitors of sodium, nonspecific stretch, and the bacterial K⁺ channel KvAP, respectively (Table 1). To confirm the identity of the purified peptides and because of their low abundance in the native venom, both GxTX-1E and GxTX-1D were produced by solid-phase synthesis. Folded GxTX-1E coelutes with native peptide in reverse-phase HPLC (Fig. 1). GxTX-1D shows identical chromatographic behavior (not shown). In the ^{86}Rb⁺ efflux assay, GxTX-1E is a slightly more potent K_{v,2.1} inhibitor than the purified native mixture and GxTX-1D (half-maximal concentration [IC₅₀] values of 0.71, 1.5, and 1.8 nmol/l, respectively; n = 2) (Fig. 1B), suggesting that synthetic GxTX-1 has similar characteristics to native peptide. Because of the advantage of being able to produce large quantities of biologically active peptide, synthetic GxTX-1E was further characterized and used to probe the role of I_{DB} in β-cells.

**GxTX-1E is a gating-modifier peptide.** Inhibition of K_{v,2.1} by GxTX-1E was characterized by whole-cell voltage-clamp electrophysiology. GxTX-1E (43 nmol/l) inhibited the current in CHO.hK_{v,2.1} cells at both +20 mV and +80 mV (Fig. 2A–C). At +20 mV, 43 nmol/l GxTX-1E inhibited CHO.hK_{v,2.1} current 98 ± 1% (n = 5). The current-voltage relation shows that GxTX-1E shifts the voltage-dependence of hK_{v,2.1} channel activation, a hallmark feature of gating-modifier peptides (Fig. 2D). Using IonWorks automated electrophysiology, K_D values for inhibition of K_{v,2.1} by GxTX-1E were estimated from fits to a single (2.6 nmol/l; Fig. 2E, dashed line; average values of 2.0 ± 0.4 nmol/l [n = 4]) or a four-equivalent-site model (15.1 nmol/l; Fig. 2E, solid line; average 12.0 ± 1.4 nmol/l [n = 4]) (25). The potency of GxTX-1E on hK_{v,2.1} channels expressed in *Xenopus* oocytes was 5.1 ± 0.4 nmol/l (n = 3) using a single-site model (not shown). GxTX-1E inhibited K_{v,2.2} channels with similar potency (K_D of 2.6 ± 0.4 nmol/l, n = 3) as K_{v,2.1} when tested by IonWorks automated electrophysiology (Fig. 2F) and also shifted the voltage dependence of K_{v,2.2} channel opening (not shown).

**GxTX-1E selectivity for K_{v,2.1}/K_{v,2.2} channels.** GxTX-1E was tested at 4 μmol/l, 1,000-fold above its IC₅₀ for K_{v,2.1}, against a variety of ion channels. Using Ion-
Works automated electrophysiology, GxTX-1E had no significant effect on KV1.2, KV1.3, KV1.5, or KV3.2 channels. However, as observed with HaTX (28) and ScTx1 (29), GxTX-1E inhibited KV4.3 channels with IC_{50}s of 24 (Fig. 2F) and 54 nmol/l in two experiments. Thus, GxTX-1E is at least eightfold weaker on KV4.3 as compared with KV2 channels. Because KV4 channels produce a rapidly inactivating A-type current, they are not expected to be related to the slowly inactivating IDR. In other assays, GxTX-1E had no significant activity against the high-conductance, calcium-activated K{\text{H}1001} channel, the calcium channels Cav1.2 and Cav2.2, or the sodium channels Na_v1.5, Na_v1.7, and Na_v1.8. Therefore, GxTX-1E is an appropriate probe for studying the contribution of KV2 channels to the /H9252-cell IDR.

GxTX-1E inhibits the delayed rectifier of mouse /H9252-cells. Similar to hKV2.1 channels, I_{DR} of mouse β-cells inactivates slowly and is inhibited by GxTX-1E (Fig. 3A–C). When measured at +20 mV, 43 nmol/l GxTX-1E inhibited I_{DR} of mouse β-cells by 89 ± 3% (n = 11). As seen with hKV2.1, the inhibition of I_{DR} by GxTX-1E is less prominent at more positive voltages (Fig. 3D). However, at greater depolarizations, significant differences were seen between the interaction of GxTX-1E with the β-cell current and hKV2.1. At +80 mV, the fraction of mouse β-cell I_{DR} blocked by 43 nmol/l GxTX-1E was 57 ± 3% (n = 11) compared with 93 ± 2% for hKV2.1 channels (Fig. 3E). In addition, the potency of the nonselective K{\text{H}1001} channel blocker TEA is greater on the β-cell I_{DR} (IC_{50} 2.2 nmol/l) than on hKV2.1 expressed in Xenopus oocytes (IC_{50} 8.2 nmol/l) (Fig. 3F). The potency of TEA block of hKV2.1 channels was confirmed with CHO.KV2.1 cells (IC_{50} 9.3 nmol/l; not shown).

Mouse β-cell action potentials are broadened by application of GxTX-1E. Consistent with the idea that the β-cell I_{DR} is involved in action potential repolarization (10), GxTX-1E broadened glucose-induced action potentials in mouse β-cells (Fig. 4A–C). In contrast to previous reports where a nonselective K{\text{H}1001} channel inhibitor, TEA, was used (34), the selectivity of GxTX-1E confirms that action potential broadening in the presence of high glucose occurs through inhibition of I_{DR} alone. To measure the effect of GxTX-1E on action potential repolarization more systematically, we evoked action potentials by depolarizing current injection (Fig. 4D). GxTX-1E slowed the rate (dV/dt) of action potential repolarization by 53 ± 7% (n = 4) and increased action potential duration by 30 ± 6% (n = 4; measured at half height). GxTX-1E had no effect on the resting membrane potential of /H9252-cells in low glucose (not shown), unlike inhibitors of K_{ATP} channels.

GxTX-1E enhances glucose-stimulated [Ca{\text{H}1001}^{2+}] oscillations. Prolongation of the action potential by inhibition of I_{DR} should result in increased [Ca{\text{H}1001}^{2+}] in response to elevated glucose. We tested this idea by measuring changes in [Ca{\text{H}1001}^{2+}] oscillations in dissociated β-cells using fura-2 imaging. Glucose at 8 mmol/l was optimal for

FIG. 2. Inhibition of CHO.hKV2.1 channels by GxTX-1E. A: Membrane currents before and after application of 43 nmol/l GxTX-1E. B: Current amplitude at +20 (○) and +80 (●) mV plotted versus time. C: Membrane current activated by 10 s steps for the same cell as in A. D: Plot of relative current versus voltage before and after the addition of 43 nmol/l GxTX-1E. E: GxTX-1E inhibition of hK{\text{H}2.1} measured by IonWorks. Solid and dashed lines represent the fit of a four-equivalent and single-site model, respectively. F: GxTX-1E inhibition of hK{\text{H}2.2} and hK{\text{H}2.4.3} measured by IonWorks. Lines are fits of a single-site model.
FIG. 3. Inhibition of $I_{\text{KATP}}$ of mouse β-cells by GxTX-1E. A–D: Experimental procedures were the same as in Fig. 2. E: Fraction of unblocked current in 43 nmol/l GxTX-1E at +20 (○) and +80 (●) mV for CHO.hKv2.1 channels and mouse β-cell $I_{\text{KATP}}$. F: Dose-dependence of TEA block of the mouse β-cell $I_{\text{KATP}}$ and hKv2.1 expressed in Xenopus oocytes measured at +20 mV.

FIG. 4. GxTX-1E broadens the β-cell action potential. A and B: Action potentials induced by 10 mmol/l glucose were recorded before (A) and after (B) the addition of 43 mmol/l GxTX-1E. C: Representative action potentials from A and B are aligned at the peak to illustrate action potential broadening by GxTX-1E. D: Action potentials triggered by injection of positive current (100 pA) before and after the addition of 100 nmol/l GxTX-1E.
inducing \([\text{Ca}^{2+}]_i\) oscillations that were stable for nearly 1 h (Fig. 5A and B). For example, when the area under the curve is measured from 15 to 30 min and from 30 to 45 min for the cells in Fig. 5A and B, \([\text{Ca}^{2+}]_i\) rose only 3 and 10\%, respectively, between these time periods. On average, \([\text{Ca}^{2+}]_i\) increased 9\% (\(n = 27\)) over this time. We then asked if inhibition of I_{\text{DR}} by GxTX-1E would enhance \([\text{Ca}^{2+}]_i\). GxTX-1E (43 nmol/l) was added to the bath after 15 min of stable \([\text{Ca}^{2+}]_i\) oscillations. Figure 5C–F shows examples of individual cells from a single experiment and demonstrates the types of responses seen after exposure to GxTX-1E. In some cells, the oscillations became broader (Fig. 5C), while in others the frequency increased (Fig. 5D). In some cells, GxTX-1E restored oscillations that had stopped (Fig. 5E), while in others GxTX-1E had only a modest effect (Fig. 5F). On average, GxTX-1E produced a 38\% increase in \([\text{Ca}^{2+}]_i\), relative to the period before GxTX-1E addition. Importantly, GxTX-1E had no effect on \([\text{Ca}^{2+}]_i\) when applied in low (3 mmol/l) glucose (not shown), and the enhanced \([\text{Ca}^{2+}]_i\) oscillations produced by GxTX-1E in 8 mmol/l glucose were rapidly terminated upon lowering of glucose (Fig. 5C–F).

**GSIS is enhanced by GxTX-1E.** The findings that GxTX-1E is an effective inhibitor of the \(\beta\)-cell I_{\text{DR}} and enhances glucose-dependent \([\text{Ca}^{2+}]_i\) oscillations suggest that it should augment GSIS. As expected, insulin secretion at 16 mmol/l glucose was enhanced 3.5-fold by 2 \(\mu\text{mol/l GxTX-1E when tested in a static assay (Fig. 6A; } P < 0.001\). TEA (10 nmol/l) enhanced GSIS twofold (Fig. 6A; \(P = 0.01\)). These effects on insulin secretion were glucose dependent, since neither GxTX-1E nor TEA had an effect at 2 mmol/l glucose. As a positive control, 10 nmol/l GLP-1 enhanced GSIS by fivefold (\(P < 0.001\)). The enhancement of GSIS by GxTX-1E was also observed in perfusion studies of mouse islets. The addition of GxTX (1 \(\mu\text{mol/l}) to the perfusate with 16 mmol/l glucose doubled the rate of insulin secretion (average insulin concentration 2.1 \(\pm\) 0.5 ng/ml) compared with perfusion in glucose alone (1.1 \(\pm\) 0.2 ng/ml) (Fig. 6B).

Addition of TEA after a 20-min wash of GxTX resulted in additional enhancement over the GxTX-enhanced signal (Fig. 6B, \(P = 0.060\)). Since recovery from GxTX-1E inhibition of hKV2.1 is slow (off time constant \([\tau_{\text{off}}}] \sim 28\) min; not shown), the effect of TEA in these experiments may be due to both TEA and the residual effects of GxTX-1E. The additional enhancement produced by TEA may also arise from the action of TEA on other channels besides KV channels.

In separate static assays of intact islets, GxTX-1E was found to have an \(EC_{50}\) of 400 nmol/l for the enhancement of GSIS (\(n = 2\), not shown). However, in dispersed islet cells, GxTX-1E was more potent (Fig. 6C) than it is in intact islets. Both 50 nmol/l and 1 \(\mu\text{mol/l GxTX-1E exhibited a significant enhancement over 16 mmol/l glucose alone (average [10–20 min] respective % insulin content/ min 0.54 \(\pm\) 0.07, 0.48 \(\pm\) 0.05, and 0.31 \(\pm\) 0.04, } P < 0.05\), and were not significantly different from each other.
The enhancement of GSIS by GxTX-1E was not due to effects on the K\(_{ATP}\) channel–independent pathway, as shown in Fig. 6D. In the presence of elevated KCl (30 mmol/l) to depolarize the plasma membrane and diazoxide (250 \(\mu\)mol/l) to maintain K\(_{ATP}\) channels in the open state, insulin secretion was significantly higher at 16 than at 2 mmol/l glucose (10.2 vs. 6.1 ng \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\), \(n = 4\), \(P = 0.01\)), but neither 1 \(\mu\)mol/l GxTX-1E nor 5 mmol/l TEA caused additional insulin release. Note that in the controls, GxTX-1E and TEA repeated their enhancement of insulin secretion over that at 16 mmol/l glucose (\(P < 0.05\)). Taken together, these data point to a mechanism for specific enhancement of insulin secretion at elevated glucose levels by blocking the \(\beta\)-cell I\(_{DR}\).

**DISCUSSION**

In this study, we report the identification, purification, primary sequence determination, synthesis, and use of GxTX-1, a potent inhibitor of the \(\beta\)-cell I\(_{DR}\). GxTX-1 broadens \(\beta\)-cell action potentials, increases calcium oscillations, and enhances GSIS. GxTX-1 inhibits 90% of the \(\beta\)-cell I\(_{DR}\), making it a suitable probe for the physiological role of the I\(_{DR}\). GxTX-1 broadens the glucose-induced action potential but has no effect on the resting potential in low glucose. Similarly, GxTX-1 augments glucose-dependent calcium oscillations without affecting resting [Ca\(_{\text{2+}}\)]\(_i\). Further, the stimulation of insulin secretion by GxTX-1 is strictly glucose dependent, as expected, since I\(_{DR}\) is only active at membrane potentials above –20 mV, a depolarization level only seen in elevated glucose. This property, coupled with slow opening kinetics (Fig. 3A), makes the I\(_{DR}\) ideal for contributing to the repolarization phase of the \(\beta\)-cell action potential. The observation that GxTX-1 has no effect on the K\(_{ATP}\) channel–independent pathways suggests that the observed effects of GxTX-1 on insulin secretion are limited to inhibition of I\(_{DR}\).

The concentration (43 nmol/l) at which GxTX-1 broadens action potentials and enhances calcium oscillations and the concentration (50 nmol/l) at which GxTX-1 enhances insulin secretion from dispersed islet cells are consistent with inhibition of the mouse \(\beta\)-cell I\(_{DR}\). However, higher concentrations (EC\(_{50}\) ~ 400 nmol/l) of GxTX-1 were required to enhance GSIS in intact islets, suggesting a more difficult access of the peptide to the core of the islets as compared with dispersed single \(\beta\)-cells. A similar scenario has been suggested to explain the effects of HaTX on calcium oscillations in whole islets (15). Nonetheless, the effects of GxTX-1 taken together are consistent with
the hypothesis that GxTX-1 enhances insulin secretion from mouse islets through inhibition of the β-cell I_{DR}.

Identity of the delayed-rectifier channels in mouse β-cells. Selectivity studies with GxTX-1 suggest that the major, GxTX-1-sensitive component of the β-cell I_{DR} contains K_{2,2} subunits. The only other channel we identified that is blocked by GxTX-1 is K_{4,3}, which is not likely to contribute significantly to the β-cell I_{DR}, since it is neither expressed in mouse β-cells (13) nor found in human islets by PCR (35). In addition, K_{2,4} channels normally produce a rapidly inactivating current, distinct from the slowly inactivating I_{DR} of β-cells.

Of the two known K_{2,2} family members, K_{2,1} is the best candidate to encode I_{DR}. K_{2,1} is expressed at high levels in islets from various species (8,12), and immunohistochemical analysis indicates that expression of K_{2,1} in primate islets is restricted to β-cells (35). The other member of the K_{2,2} family, K_{2,2}, appears to be specifically located in δ-cells of primate islets (35) and is not found in rat islets (8).

Although block of the β-cell I_{DR} suggests the presence of K_{2,1}, clear differences between the mouse β-cell I_{DR} and hK_{2,1} were observed. The GxTX-1–induced shift in voltage-dependent channel opening was greater for hK_{2,1} than for mouse β-cell I_{DR}, and mouse β-cell I_{DR} is more sensitive to TEA than hK_{2,1} (Fig. 3F). These pharmacological differences are not likely due to species differences. The sequences of mouse and human K_{2,1} are nearly identical, and sequence identity is 100% in the regions where gating modifiers and TEA are thought to interact. Indeed, the I_{DR} of human β-cells is also more sensitive to TEA (IC_{50} 0.54 mM) than for hK_{2,1} (9) than for hK_{2,1}. The pharmacological differences between native and heterologously expressed channels may be due to posttranslational modification, association of unknown accessory subunits, or heterotetramerization of α subunits. Gating modifier peptides, unlike pore blockers, bind to the channel with a stoichiometry of 4:1, and the number of inhibitor molecules bound determines the amplitude of the shift in channel gating (25). Coassembly of K_{2,1} subunits with GxTX-1–insensitive, TEA-sensitive subunits, might result in a pharmacological profile similar to that of the I_{DR} of β-cells.

In this study, we have shown that GxTX-1 is a novel and suitable tool to probe the role of the β-cell I_{DR} in insulin secretion and to investigate the molecular composition of the β-cell I_{DR}. The glucose dependence of the effects of GxTX-1 on both [Ca^{++}]_{i} oscillations and insulin release predict that a blocker of I_{DR}, unlike K_{ATP} channel blockers, should not induce hypoglycemia and may represent an improved approach for the treatment of type 2 diabetes. Lastly, the availability of biologically active synthetic GxTX-1 should facilitate studies of the mechanisms that control insulin secretion.

ACKNOWLEDGMENTS

The authors thank John P. Felix, Kevin Ratliff, William Schmalhofer, Brande Williams, and Dr. Lizhen Yan for assistance in technical aspects and/or discussion of this manuscript.

REFERENCES


15. Tanarina NA, Kuznetsov A, Dukes I, Philipson LH: Delayed rectifier potassium channel K_{2,1} role in β-cell physiology and insulin secretion (Abstract). Diabetes 53 (Suppl. 2):A572, 2002


