Glucagon-Like Peptide-1 Receptor Activation Antagonizes Voltage-Dependent Repolarizing K^+ Currents in β-Cells

A Possible Glucose-Dependent Insulinotropic Mechanism

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Glucagon-like peptide-1 (GLP-1) acts through its G-protein–coupled receptor to enhance glucose-stimulated insulin secretion from pancreatic β-cells. This is believed to result from modulation of at least two ion channels: ATP-sensitive K^+ (K_{ATP}) channels and voltage-dependent Ca^{2+} channels. Here, we report that GLP-1 receptor signaling also regulates the activity of β-cell voltage-dependent K^+ (K_V) channels, themselves potent glucose-dependent regulators of insulin secretion. GLP-1 receptor activation with exendin 4 (10^{-8} mol/l) in rat β-cells antagonized K_V currents by 43.3 ± 6.3%, whereas the GLP-1 receptor antagonist exendin 9-39 had no effect. The effect of GLP-1 receptor activation on K_V currents could be replicated (current reduction of 55.7 ± 6.0%) by G-protein activation with GMP-PNP (10 nmol/l). The cAMP pathway antagonist Rp-cAMPS (100 μmol/l) prevented current inhibition by exendin 4, implicating cAMP signaling in GLP-1 receptor modulation of β-cell K_V currents. Finally, exendin 4 (10^{-8} mol/l) increased the amplitude (130 ± 5.7%) and duration (285 ± 15.9%) of the β-cell depolarization response to current injection, independent of any effect on K_{ATP} or Ca^{2+} channels. The present results demonstrate that GLP-1 receptor signaling can antagonize β-cell repolarization by reducing voltage-dependent K^+ currents, an effect likely to contribute to GLP-1's glucose-dependent insulinotropic effect. *Diabetes* 51 (Suppl. 3):S443–S447, 2002

The glucoincretin hormone glucagon-like peptide-1 (GLP-1) and its analogs have been widely studied because of their glucose-dependent insulinotropic effect (1,2) and because of a recently reported ability to promote β-cell mass expansion (3–5). Currently, GLP-1 is thought to potentiate glucose-stimulated insulin secretion (GSIS) through several mechanisms, including antagonism of ATP-sensitive K^+ (K_{ATP}) channels, potentiation of voltage-dependent Ca^{2+} channels (VDCCs), release of intracellular Ca^{2+} stores, and activation of nonspecific cation channels (NSCCs), in addition to direct effects on exocytosis (1). The most well-characterized signal transduction pathway of GLP-1 involves G-protein–mediated elevations of cAMP and activation of protein kinase A (1), although other potential signaling mechanisms have been identified, including the phospholipase C (6), mitogen-activated protein kinase (7), and cAMP-regulated guanine nucleotide exchange factor II (8,9) pathways.

Recently, we have shown that dominant-negative antagonism of the voltage-dependent K^+ (K_V) channels expressed in β-cells enhances GSIS (10). Briefly, K_V channels, notably Kv2.1, are thought to open in response to membrane depolarization caused by glucose-induced K_{ATP} channel closure (11). The resulting outward K^+ current would effectively repolarize the β-cell, closing VDCCs and limiting Ca^{2+} influx and insulin secretion. Because we have shown that K_V channels are important regulators of insulin secretion, we postulate that physiological secretagogues, such as GLP-1, may enhance β-cell excitability in part through antagonism of repolarizing K^+ currents.

We now show that GLP-1 receptor activation antagonizes voltage-dependent outward K^+ currents in rat β-cells. Intracellular signaling through cAMP is required for this effect. Additionally, GLP-1 receptor activation enhanced the amplitude and duration of the depolarization response to current injection independent of any effect on K_{ATP} or Ca^{2+} channels. The present data indicate that the insulinotropic effect of GLP-1 may in part be mediated by an inhibition of β-cell K_V channels dependent on cAMP signaling, leading to enhanced action potential amplitude and duration.

RESEARCH DESIGN AND METHODS

Western blotting. A volume of 50 μg of protein from each sample was loaded and separated on a 10% polyacrylamide gel. Protein was transferred to a PVDF-Plus membrane (Fisher Scientific, Nepean, Ontario, Canada) and incubated with primary antibody (Kv1.4, -1.6, -2.1, and -4.2; Alomone Labs, Jerusalem, Israel) or antibody-antigen solutions (diluted as per suppliers' instructions) for 1.5 h, followed by a subsequent incubation with secondary antibodies (donkey anti-rabbit, 1:7,500; Amersham Pharmacia Biotech, Baie

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C-clamp, current-clamp; GLP-1, glucagon-like peptide-1; GMP-PNP, guanosine 5′-[β,γ-imido]triphosphate; GSIS, glucose-stimulated insulin secretion; K_{ATP} channel, ATP-sensitive K^+ channel; K_{Ca} channel, Ca^{2+}-sensitive K^+ channel; K_V channel, voltage-dependent K^+ channel; NSCC, nonspecific cation channel; Rp-cAMPS, the Rp-diastereomer of adenosine-3′,5′-cyclic monophosphothioate; TEA, tetraethylammonium; VDCC, voltage-dependent Ca^{2+} channel.

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d'Urfe, Quebec, Canada) for 1 h at room temperature. Visualization was by chemiluminescence (ECL; Amersham Pharmacia Biotech) and exposure to Kodak film (Eastman Kodak, Rochester, NY) for 5 s to 10 min.

Islet isolation and cell culture. Islets of Langerhans were isolated from male Wistar rats (250–300 g) by collagenase digestion as described previously (10). After isolation, islets were dispersed to single cells by treatment with 0.015% trypsin (Invitrogen Canada, Burlington, Ontario, Canada) in Ca^{2+}- and Mg^{2+}-free PBS at 37°C and 5% CO_2 for 10 min. Islet cells were plated on glass coverslips in 35-mm dishes in RMPI media supplemented with 2.5 mmol/l glucose, 0.25% HEPES, 7.5% fetal bovine serum, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate (penicillin-streptomycin from Invitrogen Canada) and cultured for 1–3 days before electrophysiological recordings.

Electrophysiological studies. Cells were patch-clamped in the whole-cell configuration using an EPC-9 amplifier and Pulse software from HEKA Electronik (Lambrecht, Germany). Patch pipettes were prepared from 1.5-mm thin-walled borosilicate glass tubes using a two-stage Narishige (Tokyo) micropipette puller, and the pipettes had typical resistances of 3–6 megaohm (MΩ) when fire-polished and filled with intracellular solution. Intracellular solutions contained (in mmol/l): 140 KCl, 1 $MgCl_2 \cdot 6H_20$, 1 EGTA, 10 HEPES, and 5 MgATP (pH 7.25) with KOH. Extracellular solutions contained (in mmol/l): 140 NaCl, 2 or 0 $CaCl_2$ (current-clamp [C-clamp] recordings), 4 KCl, 1 $MgCl_2 \cdot 6H_2O$, and 10 HEPES (pH 7.3) with NaOH. Outward currents were elicited with a series of 500-ms depolarizing pulses in 20-mV increments, to +70 mV from a holding potential of −70 mV. Sustained outward current was taken as the mean current during the final 25 ms of the depolarization. Membrane potential measurements were performed in the C-clamp mode of the EPC-9 amplifier/Pulse software. Cells were transiently depolarized with a current injection of 100 pA for 5 ms. All electrophysiological recordings were performed at 32–35°C and normalized to cell capacitance. Results were confirmed in rat β-cells identified by their electrical responsiveness to 11.1 mmol/l glucose in the amphotericin B (100 μg/ml) perforated-patch configuration. Pharmacological compounds were applied via addition to either the intracellular solution or the bath solution by perfusion for at least 10 min before final current recording. Exendin 4 and exendin 9-39 were from Bachem (Torrance, CA). Guanosine 5′-[β,γ-imido]triphosphate (GMP-PNP) and the Rp-diastereomer of adenosine-3′,5′-cyclic monophosphothioate (Rp-cAMPS) were from Sigma-Aldrich Canada (Oakville, Ontario, Canada). Sustained outward currents elicited with the maximal depolarization (to +70 mV) were compared using the Student's unpaired *t* test ($P < 0.05$ considered significant). Time courses of current amplitudes were analyzed by one-way ANOVA and Dunnett's posttest. Repolarization waveforms were fitted to an exponential function to give a single time constant using Pulsefit software (HEKA Electronik).

RESULTS

K_V channel expression in rat islets. We have previously demonstrated protein expression of Kv1.4, -1.6, and -2.1 in insulin-secreting cells (10). Here, we confirm expression of these channels in rat islet protein lysates (50 μg) by Western blotting and further demonstrate expression of Kv4.2 (Fig. 1*A*). Rat brain protein lysates (50 μg) were used as a positive control, and specificity of the bands was demonstrated by competition with control antigen (not shown). Specific bands were not observed in rat islet lysates probed for Kv1.1, -1.3, -3.1, -3.3, and -3.4, whereas only a very weak band was observed for Kv1.2 (not shown). We have previously been unable to demonstrate mRNA expression of Kv1.5, -1.7, and -2.2 in purified rat islet total RNA by RT-PCR (10).

GLP-1 receptor activation antagonizes β-cell voltage-dependent outward K^+ currents, an effect dependent on cAMP signaling. Voltage-dependent outward K^+ currents were recorded from rat islet cells patch-clamped in the whole-cell configuration. Currents were similar to those described previously (10), but inactivated to a greater extent over 500 ms because of a higher bath temperature (33–35°C) than used previously (Figs. 1*B* and 2*A*). The majority of these outward K^+ currents (82.3 ± 1.8%, $n = 10$, $P < 0.001$) were sensitive to the general K_V and Ca^{2+}-sensitive K^+ (K_{Ca}) channel antagonist tetraethylammonium (TEA; 15 mmol/l) (Fig. 1). The GLP-1 receptor agonist exendin 4 (10^{-8} mol/l) decreased voltage-dependent outward K^+ currents from rat islet cells by 43.3 ± 6.3% ($n = 6$, $P < 0.01$) without appreciably altering current kinetics (Fig. 1). Similarly, but not shown, rat GLP-1 (10^{-8} mol/l) also reduced the observed currents by 40.8 ± 4.1% ($n = 9$, $P < 0.001$). These results were not replicated by the GLP-1 receptor antagonist exendin 9-39 (10^{-8} mol/l), which had no significant effect on outward K^+ currents (Fig. 1, $n = 9$).

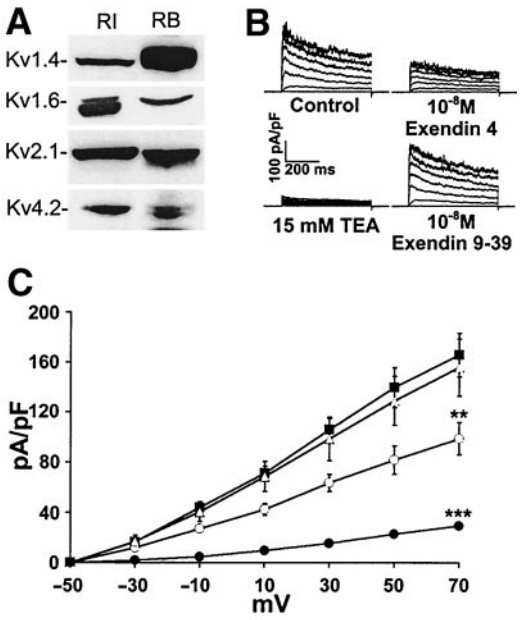

FIG. 1. GLP-1 receptor activation antagonizes voltage-dependent outward K^+ currents in β-cells. *A*: Kv1.4, -1.6, -2.1, and -4.2 protein expression was detected in rat pancreatic islet (RI) and rat brain (RB) protein lysates by Western blot. Specificity of detected bands was determined by competition with control antigen (not shown). Rat β-cells were voltage-clamped in the whole-cell configuration, and outward currents were elicited with a series of depolarizing pulses from a holding potential of −70 mV in 20-mV increments, to +70 mV. *B*: Representative traces are shown for control cells and those treated with exendin 4 (10^{-8} mol/l), exendin 9-39 (10^{-8} mol/l), and TEA (15 mmol/l). *C*: Current-voltage relationships are shown for control (■) cells and those treated with exendin 4 (10^{-8} mol/l; ○), exendin 9-39 (10^{-8} mol/l; △), and TEA (15 mmol/l; ●). **$P < 0.01$, ***$P < 0.001$ compared with control.

The inhibitory effect of exendin 4 or GLP-1 could be replicated by inclusion in the intracellular pipette solution of the nonhydrolyzable GTP analog GMP-PNP (10 nmol/l), a G-protein activator (current reduction of 55.7 ± 6.0%; $n = 8$, $P < 0.01$) (Fig. 2*A*). The time course for current reduction by GMP-PNP (Fig. 2*B*) reflects the time necessary for intracellular dialysis of the compound and subsequent G-protein signaling. Also shown for comparison is the time course for current reduction by exendin 4 (10^{-8} mol/l) (Fig. 2*B*), which reflects the time necessary for exendin 4 to equilibrate in the bath solution by perfusion in addition to receptor activation and signaling. Antagonism of the cAMP signaling pathway by preincubation of cells with Rp-cAMPS (100 μmol/l, 30 min) and inclusion of Rp-cAMPS (100 μmol/l) in the bath solution blocked the inhibitory effect of exendin 4 (10^{-8} mol/l) on outward K^+ currents ($n = 5$) (Fig. 2*C*). The above results indicate that G-protein signaling is sufficient to antagonize voltage-

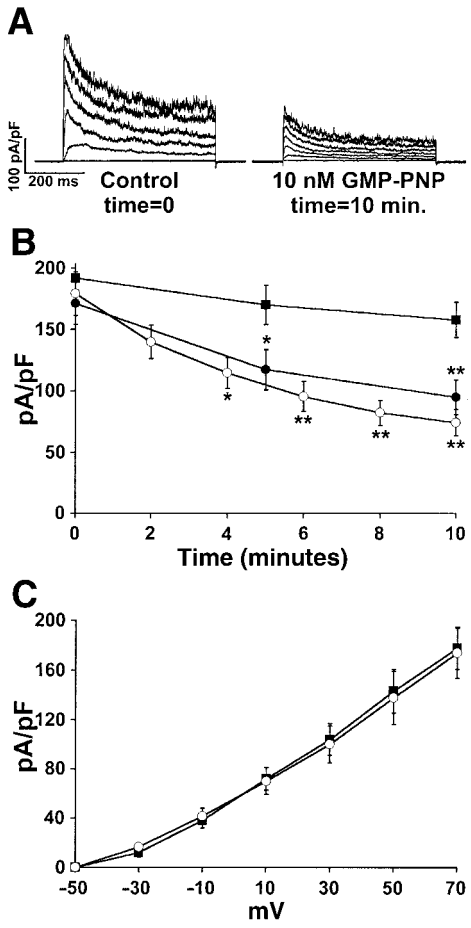

FIG. 2. The inhibitory effect of GLP-1 receptor activation is replicated by direct G-protein activation and is blocked by antagonism of cAMP signaling. Intracellular dialysis of the nonhydrolyzable GTP analog GMP-PNP (10 nmol/l) antagonized voltage-dependent outward K^+ currents in β-cells, similar to the effect of exendin 4. Representative traces are shown in *A*. *B*: Maximum outward K^+ current is plotted against time after intracellular access. Perfusion of exendin 4 (10^{-8} mol/l; ○) or intracellular dialysis of GMP-PNP (10 nmol/l; ●) resulted in a time-dependent decrease in maximum currents compared with control (■). *C*: Cells were pretreated with the cAMP pathway antagonist Rp-cAMPS (100 μmol/l). In the continued presence of 100 μmol/l Rp-cAMPS, exendin 4 (10^{-8} mol/l; ○) was no longer able to antagonize outward K^+ currents compared with control (■). *$P < 0.05$, **$P < 0.01$ compared with time = 0.

dependent outward K^+ currents in rat β-cells and that cAMP signaling is required for the effect of GLP-1 receptor activation.

GLP-1 receptor activation antagonizes β-cell repolarization. GLP-1 receptor activation has been implicated in the regulation of β-cell membrane potential through its effects on K_{ATP} channels and VDCCs (1). We therefore wanted to determine whether regulation of voltage-dependent outward K^+ currents by GLP-1 receptor activation affects membrane repolarization separately from the modulation of K_{ATP} or Ca^{2+} channels. To accomplish this, C-clamp experiments were performed in the presence of high intracellular ATP (5 mmol/l) to close the K_{ATP} channels and in the absence of extracellular Ca^{2+} to eliminate the Ca^{2+} currents. In the absence of current (I = 0), resting membrane potentials were -56.9 ± 3.3 mV ($n = 11$), reflecting the closure of K_{ATP} channels. Because β-cell action potentials do not occur in the absence of Ca^{2+} (12,13), a transient depolarization was generated by a 5-ms current injection (100 pA). As expected under Ca^{2+}-free conditions, transient depolarizations generated in this manner resulted only from current injection and not from activation of voltage-dependent channels, since no evoked action potentials could be elicited at intermediate levels of current injection (10–200 pA) or by a current injection ramp from 0 to 200 pA (not shown). Current injection (100 pA for 5 ms) depolarized cells to -0.5 ± 3.3 mV ($n = 11$), and repolarization occurred with a time constant of 4.6 ± 1.0 ms ($n = 11$) (Fig. 3). In the presence of the general K_V channel antagonist TEA (15 mmol/l), which blocks rat β-cell–repolarizing K^+ currents by >80%, the level of depolarization increased slightly (7.9 ± 7.03 mV, $n = 6$, $P = 0.12$), and the repolarization time constant increased significantly (16.54 ± 3.21 ms, $n = 6$, $P < 0.001$) (Fig. 3). This effect could be completely washed out (not shown). Similarly, GLP-1 receptor activation with exendin 4 (10^{-8} mol/l) increased the level of depolarization (16.6 ± 7.8 mV, $n = 6$, $P < 0.05$) and prolonged the repolarization time constant significantly (13.1 ± 2.08 ms, $n = 6$, $P < 0.001$). Neither treatment with TEA nor treatment with exendin 4 significantly affected the resting membrane potential in the absence of current injection. These results suggest that GLP-1 receptor–mediated reductions in repolarizing K^+ currents may lead to enhanced (i.e., larger and prolonged) action potentials.

DISCUSSION

The ability of GLP-1 to directly enhance GSIS from pancreatic β-cells has been attributed to GLP-1 receptor activation leading to enhanced depolarization and increases in the intracellular concentration of Ca^{2+} as well as direct effects on insulin exocytosis (1). The electrogenic effects of GLP-1 have in the past been attributed to its ability to inhibit K_{ATP} channels and to augment Ca^{2+} influx through VDCCs (1,14). In the present study, we provide two novel observations. First, GLP-1 receptor activation antagonizes rat β-cell K_V channels, an effect dependent on cAMP signaling. Second, antagonism of K_V channels by GLP-1 leads to a slowing of membrane repolarization after a depolarizing stimulus independent of K_{ATP} channels or VDCCs, suggesting a contribution by K_V channels to the electrogenic effects of GLP-1.

The GLP-1 receptor is expressed in the islets of several species, including rat, from which it was originally cloned (15). The current reduction observed in the present study is unlikely to be a nonspecific effect because both GLP-1 and the GLP-1 receptor agonist exendin 4 antagonized outward K^+ currents in β-cells, but the truncated peptide (exendin 9-39) had no effect (Fig. 1). Furthermore, the ability of the G-protein activator GMP-PNP to replicate the effect of exendin 4 implicates G-proteins in the intracellular signal transduction machinery leading to the reduction in voltage-dependent K^+ current.

Because the majority of reports demonstrate GLP-1 modulation of K_{ATP} channels or VDCCs to be cAMP dependent (1), we studied whether β-cell voltage-dependent K^+ current modulation by GLP-1 receptor activation was also dependent on cAMP. Indeed, antagonism of the cAMP signaling pathway with Rp-cAMPS was sufficient to abolish the effect of GLP-1 receptor activation on voltage-dependent outward K^+ currents. Hormone-mediated re-

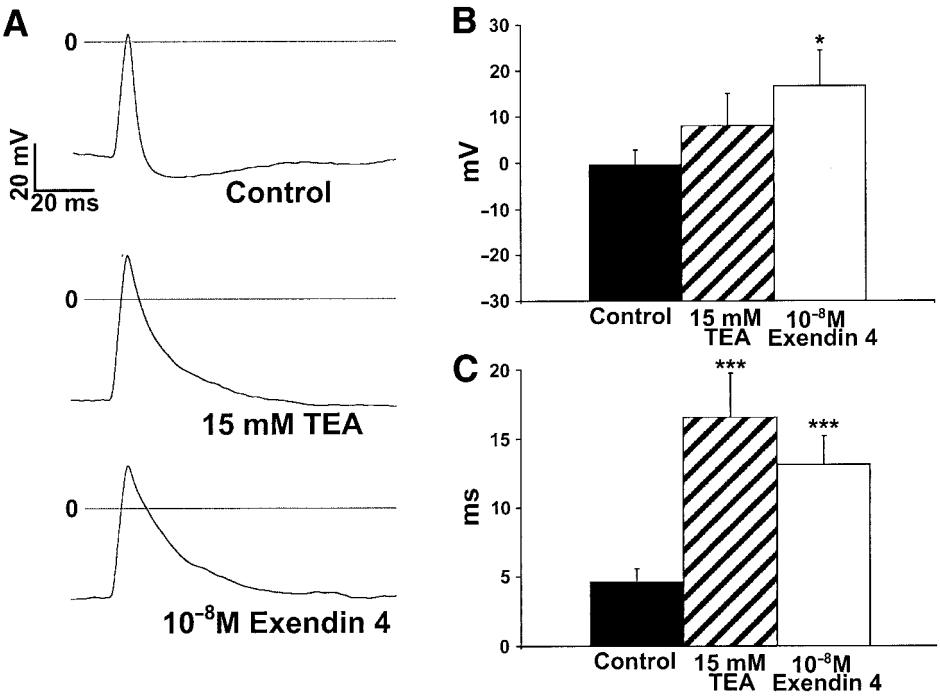

FIG. 3. Amplitude and duration of transient depolarization generated by current injection are increased in β-cells by GLP-1 receptor activation. C-clamp experiments were performed in cells by whole-cell patch-clamp with high intracellular ATP (5 mmol/l) and no Ca^{2+} to eliminate the contribution of K_{ATP} and Ca^{2+} currents to membrane potential. Treatment of cells with the general K_V channel antagonist TEA (15 mmol/l) or with exendin 4 (10^{-8} mol/l) increased the amplitude and repolarization time constant of transient depolarizations generated by injection of a 100-pA current for 5 ms. Representative waveforms are shown in *A*. Maximum depolarization (*B*) and repolarization time constants (*C*) are plotted for control (■) cells and those treated with TEA (15 mmol/l; ▨) and exendin 4 (10^{-8} mol/l; □). *$P < 0.05$, ***$P < 0.001$ compared with control.

ductions in voltage-dependent K^+ currents have been previously demonstrated in arterial smooth muscle (16), human megakaryocytes (17), human acromegalic somatotropes (18), and rat sensory neurons (19). In two of these studies, cAMP signaling was implicated in the effect (16,19). One recent study has demonstrated that the K_VLQT K_V channel association with a neuronal A kinase anchoring protein is essential for cAMP modulation of channel current in a heterologous system (20). It remains to be determined whether the GLP-1 receptor or β-cell K_V channels interact directly (or are colocalized on lipid-rich membrane patches) with the signal transduction or excitatory machinery.

K_V channels open in response to membrane depolarization (whether generated by current injection or glucose) and are believed to participate in repolarization of the β-cell (11). Reduction in K_V channel activity by GLP-1 may be expected to result in prolonged action potential duration and enhanced electrical excitability (21). Because alterations in K_{ATP} channel and VDCC activity are expected to contribute to the electrogenic effects of GLP-1, we sought to determine the contribution of GLP-1 receptor modulation of K_V channels to membrane repolarization separate from the contribution of other channels. K_{ATP} channels were blocked by high intracellular ATP, whereas VDCCs and K_{Ca} channels were neutralized by the absence of extracellular Ca^{2+}. Under these conditions, action potentials will not be elicited by small current injections to bring the cell to a "threshold" potential. Indeed, using a multiple current pulse or current ramp protocol, we were unable to elicit evoked action potentials in rat islet cells in the absence of Ca^{2+} (not shown). Therefore, the depolarization phase of the waveforms shown (Fig. 3) result solely from current injection and not from opening of VDCCs or voltage-dependent Na^+ channels, whereas the extent and speed of repolarization is determined largely by the activation of voltage-dependent K^+ currents. In the absence of GLP-1 receptor activation, the β-cells repolarized quickly upon current injection (time constant = 4.5 ms) and an after-hyperpolarization was observed, reflecting a lag in the inactivation of K_V channels. After GLP-1 receptor activation with exendin 4, resting membrane potential was unchanged, whereas upon current injection, the depolarization amplitude and repolarization time constant was increased and the after-hyperpolarization disappeared.

These results suggest that reduction of K_V channel activity by GLP-1 receptor activation will enhance β-cell action potentials. K_V channels are strict depolarization-dependent (and therefore nutrient-dependent) regulators of secretion. These channels, which are closed under basal (i.e., low-glucose) conditions, open in response to nutrient-induced depolarization and contribute to β-cell repolarization, limiting Ca^{2+} influx and insulin secretion. Therefore, the nutrient dependence of GLP-1 may in part be explained by its ability to antagonize K_V channels. In addition to nonelectrogenic targets, the insulinotropic effect of GLP-1 may presently be attributed to a number of electrogenic effectors, such as K_{ATP} channels, VDCCs, NSCCs, and now K_V channels. Important future experiments must distinguish the contribution of each of these targets to GLP-1–stimulated electrical activity and insulin secretion.

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