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 Gprotein-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⁺ (KATP) channels and voltage-dependent Ca²⁺ channels. Here, we report that GLP-1 receptor signaling also regulates the activity of β-cell voltage-dependent K⁺ (Kᵥ) channels, themselves potent glucose-dependent regulators of insulin secretion. GLP-1 receptor activation with exendin 4 (10⁻⁵ mol/l) in rat β-cells antagonized Kᵥ 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 Kᵥ 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 Kᵥ currents. Finally, exendin 4 (10⁻⁵ 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 KATP or Ca²⁺ 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

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Currently, GLP-1 is thought to potentiate glucose-stimulated insulin secretion (GSIS) through several mechanisms, including antagonism of ATP-sensitive K⁺ (KATP) channels, potentiation of voltage-dependent Ca²⁺ channels (VDCCs), release of intracellular Ca²⁺ 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⁺ (Kᵥ) channels expressed in β-cells enhances GSIS (10). Briefly, Kᵥ channels, notably Kv2.1, are thought to open in response to membrane depolarization caused by glucose-induced KATP channel closure (11). The resulting outward K⁺ current would effectively repolarize the β-cell, closing VDCCs and limiting Ca²⁺ influx and insulin secretion. Because we have shown that Kᵥ 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 KATP or Ca²⁺ channels. The present data indicate that the insulinotropic effect of GLP-1 may in part be mediated by an inhibition of β-cell Kᵥ 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-dUrfé, Canada) and processed through an alkaline phosphatase reaction (incubation with substrate solution for 30 min).
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**RESULTS**

**K+ 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. 1A). 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 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 cell 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. 1B and 2A). The majority of these outward K+ currents (82.3 ± 1.8%, n = 10, P < 0.001) were sensitive to the general K+ channel antagonist tetraethylammonium (TEA; 15 mM) (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 ± 6.8% (n = 5, P < 0.01) without appreciably altering current kinetics (Fig. 1). Similarly, but not shown, rat GLP-1 (10–7 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 = 5).

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 mM), a G-protein activator (current reduction of 55.7 ± 6.0%, n = 8, P < 0.01) (Fig. 2A). The time course for current reduction by GMP-PNP (Fig. 2B) 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. 2B), 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. 2C). The above results indicate that G-protein signaling is sufficient to antagonize voltage-
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<sub>ATP</sub> channels and VDCCs (1). We therefore wanted to determine whether regulation of voltage-dependent outward K<sup>+</sup> currents by GLP-1 receptor activation affects membrane repolarization separately from the modulation of K<sub>ATP</sub> or Ca<sup>2+</sup> channels. To accomplish this, C-clamp experiments were performed in the presence of high intracellular ATP (5 mmol/l) to close the K<sub>ATP</sub> channels and in the absence of extracellular Ca<sup>2+</sup> to eliminate the Ca<sup>2+</sup> currents. In the absence of current (I = 0), resting membrane potentials were −56.9 ± 3.3 mV (n = 11), reflecting the closure of K<sub>ATP</sub> channels. Because β-cell action potentials do not occur in the absence of Ca<sup>2+</sup> (12,13), a transient depolarization was generated by a 5-ms current injection (100 pA). As expected under Ca<sup>2+</sup>-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 Kv channel antagonist TEA (15 mmol/l), which blocks rat β-cell-repolarizing K<sup>+</sup> 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<sup>−8</sup> 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<sup>+</sup> 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<sup>2+</sup> 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<sub>ATP</sub> channels and to augment Ca<sup>2+</sup> influx through VDCCs (1,14). In the present study, we provide two novel observations. First, GLP-1 receptor activation antagonizes rat β-cell Kv channels, an effect dependent on cAMP signaling. Second, antagonism of Kv channels by GLP-1 leads to a slowing of membrane repolarization after a depolarizing stimulus independent of K<sub>ATP</sub> channels or VDCCs, suggesting a contribution by Kv channels to the electrogenic effects of GLP-1.
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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 Ca²⁺ to eliminate the contribution of KATP and Ca²⁺ currents to membrane potential. Treatment of cells with the general Kᵥ 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 KᵥLQT Kᵥ 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 Kᵥ channels interact directly (or are colocalized on lipid-rich membrane patches) with the signal transduction or excitatory machinery.

Kᵥ 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 Kᵥ channel activity by GLP-1 may be expected to result in prolonged action potential duration and enhanced electrical excitability (21). Because alterations in KᵥATP channel and VDCC activity are expected to contribute to the electrogentic effects of GLP-1, we sought to determine the contribution of GLP-1 receptor modulation of Kᵥ channels to membrane repolarization separate from the contribution of other channels. KᵥATP channels were blocked by high intracellular ATP, whereas VDCCs and Kᵥca channels were neutralized by the absence of extracellular Ca²⁺. 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 Ca²⁺ (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 Kᵥ 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 Kᵥ channel activity by GLP-1 receptor activation will enhance β-cell action potentials. Kᵥ 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 Ca²⁺ influx and insulin secretion. Therefore, the nutrient dependence of GLP-1 may in part be explained by its ability to antagonize Kᵥ channels. In addition to nonelectrogenic targets, the insulinotropic effect of GLP-1 may presently be attributed to a number of electrogentic effectors, such as KᵥATP channels, VDCCs, NSCCs, and now Kᵥ 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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