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Concerted Trafficking Regulation of Kv2.1 and K_{ATP} Channels by Leptin in Pancreatic β-Cells*◆

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Background: Leptin recruits K_{ATP} channels to the pancreatic β-cell membrane.
Results: Leptin causes a parallel increase in Kv2.1 channel density that is dependent on AMPK, PKA, and actin depolymerization.
Conclusion: Leptin signaling leads to simultaneous increases in K_{ATP} and Kv2.1 channel densities.
Significance: Concerted K_{ATP} and Kv2.1 channel trafficking regulation by leptin may result in coordinated inhibition of β-cell excitability.

In pancreatic β-cells, voltage-gated potassium 2.1 (Kv2.1) channels are the dominant delayed rectifier potassium channels responsible for action potential repolarization. Here, we report that leptin, a hormone secreted by adipocytes known to inhibit insulin secretion, causes a transient increase in surface expression of Kv2.1 channels in rodent and human β-cells. The effect of leptin on Kv2.1 surface expression is mediated by the AMP-activated protein kinase (AMPK). Activation of AMPK mimics whereas inhibition of AMPK occludes the effect of leptin. Inhibition of Ca^{2+}/calmodulin-dependent protein kinase kinase β, a known upstream kinase of AMPK, also blocks the effect of leptin. In addition, the cAMP-dependent protein kinase (PKA) is involved in Kv2.1 channel trafficking regulation. Inhibition of PKA prevents leptin or AMPK activators from increasing Kv2.1 channel density, whereas stimulation of PKA is sufficient to promote Kv2.1 channel surface expression. The increased Kv2.1 surface expression by leptin is dependent on actin depolymerization, and pharmacologically induced actin depolymerization is sufficient to enhance Kv2.1 surface expression. The signaling and cellular mechanisms underlying Kv2.1 channel trafficking regulation by leptin mirror those reported recently for ATP-sensitive potassium (K_{ATP}) channels, which are critical for coupling glucose stimulation with membrane depolarization. We show that the leptin-induced increase in surface K_{ATP} channels results in more hyperpolarized membrane potentials than control cells at stimulating glucose concentrations, and the increase in Kv2.1 channels leads to a more rapid repolarization of membrane potential in cells firing action potentials. This study supports a model in which leptin exerts concerted trafficking regulation of K_{ATP} and Kv2.1 channels to coordinately inhibit insulin secretion.

Pancreatic β-cells secrete insulin to maintain glucose homeostasis. Glucose-stimulated insulin secretion occurs as a consequence of Ca^{2+} influx through voltage-gated calcium channels following membrane depolarization. The β-cell membrane potential is under the control of a constellation of ion channels and transporters (1–3). A key player that couples glucose stimulation to membrane depolarization is the ATP-sensitive potassium (K_{ATP}) channel (4–6). Abnormal gating or expression of the channel in the β-cell membrane that results in a gain or a loss of channel function is now well recognized to underlie neonatal diabetes or congenital hyperinsulinism, respectively (7). The recovery of β-cell membrane potential to a resting hyperpolarized state is due to outward potassium currents carried largely by the voltage-gated delayed rectifier potassium channel Kv2.1 (1, 3, 8, 9). Pharmacological inhibition or genetic ablation of Kv2.1 results in prolonged glucose-evoked action potential duration in β-cells, elevated serum insulin, and increased glucose tolerance (10).

In addition to glucose and other nutrient signals, neuronal and hormonal signals also play important roles in regulating insulin secretion (11). Among them, leptin, a peptide hormone predominantly secreted by white adipocytes, has been shown to inhibit insulin secretion (12). Two recent studies demonstrated that leptin increases the density of K_{ATP} channels in the β-cell membrane by regulating channel trafficking (13, 14), a mechanism that likely contributes to the inhibitory effect of leptin on insulin secretion. A study by Park *et al.* (14) showed that leptin activates the AMP-activated protein kinase (AMPK)[3] through phosphorylation by the Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) to increase K_{ATP} channel trafficking

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[3] The abbreviations used are: AMPK, AMP-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; TEA, tetraethylammonium; CAMKKβ, Ca^{2+}/calmodulin-dependent protein kinase kinase β; pF, picofarad; ANOVA, analysis of variance; 8-Br-cAMP, 8-bromo-cAMP; AP, action potential.

to the cell surface. Another paper by our group (13) reported a similar finding that leptin up-regulates K_{ATP} channel density in the β-cell membrane by activating AMPK. Furthermore, we found that the cAMP-dependent protein kinase (PKA) also has a role in leptin-induced K_{ATP} channel trafficking to the plasma membrane and that signaling through leptin, AMPK, and PKA all result in actin depolymerization, which is both necessary and sufficient to promote channel trafficking to the cell surface.

In this study, we show that leptin not only up-regulates surface expression of K_{ATP} channels but also Kv2.1 channels. The signaling mechanism for Kv2.1 surface expression regulation involves CaMKKβ, AMPK, PKA, and actin depolymerization, similar to that reported recently for K_{ATP} channels. In INS-1 cells, the increase in K_{ATP} channel density resulted in a more hyperpolarized membrane potential, and the increase in Kv2.1 channel density shortened the duration of action potentials and facilitated recovery of membrane potentials back to a hyperpolarized resting state. Our findings suggest that leptin regulates the trafficking and surface abundance of K_{ATP} and Kv2.1 channels in β-cells in a concerted manner to achieve coordinated inhibition of β-cell excitability and insulin secretion.

Materials and Methods

Cell Culture, Transfection, and Viral Transduction—INS-1 cell clone 832/13 was cultured in RPMI 1640 medium with 11.1 mM D-glucose (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol (15). Cells at ~70% confluency were transfected with a Kv2.1 tagged with the fluorescent protein mCherry at the C terminus in pcDNA3 (Kv2.1-mCherry; a generous gift from Dr. H. Gaisano) using Lipofectamine 2000 according to the manufacturer's instruction.

Drug Treatments—All drugs were purchased from Sigma. For stimulation with leptin, AICAR, or 8-bromo-cAMP (8-Br-cAMP), INS-1 cells grown in 6-well plates were exposed to regular RPMI 1640 medium without serum for 30 min before treatment with leptin, AICAR, or 8-Br-cAMP for the indicated time or 30 min (unless specified otherwise). Pharmacological inhibitors, including the AMPK inhibitor compound C or the PKA inhibitor fragment 14–22 (PKI), were added 30 min before leptin, AICAR, or 8-Br-cAMP treatment. For manipulating actin, the actin-stabilizing agent jasplakinolide or the actin-destabilizing drug latrunculin B was added 10 min prior to treatment with or without leptin, AICAR, or 8-Br-cAMP.

Electrophysiology—Whole-cell patch clamp recording was used to measure Kv2.1 current density in INS-1 cells and in β-cells dissociated from human islets obtained through the Integrated Islets Distribution Program as described previously (13). Identification of human β-cells was aided by brief staining with 100 μg/ml dithizone (2–3 min) followed by quick washout and confirmed by glucose-induced membrane depolarization. The bath solution contained (in mM) the following: 140 NaCl, 5 KCl, 4 $MgCl_2$, 11 glucose, 10 HEPES, pH 7.3. Calcium was excluded from the bath solution to eliminate calcium channel currents. Micropipettes were pulled from non-heparinized Kimble glass on a horizontal puller (Sutter Instrument, Novato, CA) and had typical resistance of 2–4 megohms when filled with an internal solution containing (in mM) the following: 140 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 ATP, 10 glucose and 10 HEPES, pH 7.3. All recordings were performed using an Axon 200B amplifier and Digidata 1322A and controlled using Clampex 8.1 (Molecular Devices). Data were digitized at 10 kHz and filtered at 2 kHz. P/4 leak subtraction was used to compensate for linear leak currents. Series resistance and capacitance were compensated. Cells with a series resistance of >30 megohms were not included in the analysis.

A 30-ms prepulse to −10 mV was used to inactivate transient potassium channel currents and voltage-dependent Na^+ currents. The sustained current at +80 mV was divided by cell capacitance for current density calculation. Currents were also recorded in the presence of 10 mM tetraethylammonium (TEA). Kv2.1 currents were estimated by subtracting currents observed in the presence of TEA from those in the absence of TEA.

For conductance-voltage (*G-V*) curves, cells were held at −90 mV and stepped from −100 mV to +80 mV in +10-mV steps using a 200-ms test pulse. A 30-ms prepulse to −10 mV was again used to inactivate transient potassium channel currents and voltage-dependent Na^+ currents. The Nernst potential for K^+ using the solutions above was calculated to be −84 mV, and this value was used to convert the ionic currents to conductance (*G*) using Ohm's law. To generate steady-state *G-V* curves, the normalized conductance was plotted as a function of the test potential and fitted with Boltzmann Equation 1,

$$G_v = G_{min} + \frac{G_{max} - G_{min}}{1 + e^{-\left(\frac{V - V_{1/2}}{K}\right)}} \quad \text{(Eq. 1)}$$

where G_{max} is the maximal conductance; G_{min} is the minimal conductance after subtracting TEA-insensitive currents. $V_{½}$ is the midpoint potential for activation, and *k* is a slope factor.

For whole-cell K_{ATP} current density measurements, INS-1 cells were held at −70 mV, and K_{ATP} currents were recorded at two voltage steps (−50 and −90 mV) applied every 2 s as described previously (13). The pipette was filled with K-INT solution containing (in mM) 140 KCl, 10 K-HEPES, 1 K-EGTA, pH 7.3, and the cells were bathed in Tyrode's solution containing (in mM) 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5 Na-HEPES, 3 $NaHCO_3$, 0.16 NaH_2PO_4, pH 7.2. Diazoxide (200 μM) was applied to the bath solution immediately after break-in to maximally stimulate K_{ATP} channels. After the current had plateaued, 300 μM tolbutamide (a K_{ATP} channel antagonist) was applied to ascertain the specificity of the K_{ATP} currents.

For whole-cell Na^+ current density measurements, INS-1 cells were held at −80 mV, and voltage was stepped from −80 mV to +50 mV in +10-mV increments using a 20-ms test pulse following a 100-ms hyperpolarizing pre-pulse to −150 mV to exclude the interference of Na^+ channel inactivation. The pipette was filled with an internal solution containing (in mM) the following: 130 cesium methanesulfonate (CsMeS), 10 CsCl, 6 EGTA, 10 HEPES, 5 ATP, 2 $MgCl_2$, 10 TEA-Cl, pH 7.2. The bath solution contained (in mM) the following: 137 NaCl, 5.4 KCl, 5 HEPES, 5 $MgCl_2$, 1 $CdCl_2$, pH 7.2. The sweep with the largest current was analyzed. P/4 leak subtraction was used to compensate for linear leak currents.

For whole-cell Ca^{2+} current density measurements, INS-1 cells were held at −90 mV and voltage stepped from −80 mV to +80 mV in +10 mV increments using a 200 ms test pulse. The pipette was filled with an internal solution containing (in mM): 130 CsMeS, 10 CsCl, 6 EGTA, 10 HEPES, 5 ATP, 1 $MgCl_2$, 0.2 $CaCl_2$, 10 TEA-Cl, pH 7.2. The bath solution contained (in mM) the following: 137 NaCl, 5.4 KCl, 5 HEPES, 2 $MgCl_2$, 5 $CaCl_2$, pH 7.2. $CdCl_2$ (100 μM) was added to identify the current. The sweep with the largest current over the last 100 ms of the test pulse was analyzed. P/4 leak subtraction was used to compensate for linear leak currents.

For monitoring membrane potential, whole-cell current clamp recording was used. The extracellular solution contained (in mM) the following: 137 NaCl, 5.4 KCl, 1.8 $CaCl_2 2H_2O$, 0.5 $MgCl_2$, 5 HEPES, 3 $NaHCO_3$, 0.16 NaH_2PO_4 and 11 glucose, pH 7.2. The internal solution contained 140 potassium gluconate, 10 KCl, 6 EGTA, 5 HEPES, 5 Na_2ATP, 1 $MgCl_2$, 0.1 $CaCl_2$, pH 7.2. The membrane potential was recorded in the absence of current injection ($I = 0$). The amplifier was periodically switched to voltage clamp mode to verify seal resistance, and the recording was discontinued if the seal resistance changed significantly. The measurement was started immediately after the rupture of the cell (<70 ms). To assess the effects of drugs, the membrane potential was measured at least 2 min after drug application.

Action potentials (APs) were analyzed using Clampfit. The AP repolarization duration was measured from the peak to baseline, and the AP amplitude was taken as the difference between baseline and the peak. Only events with a complete repolarization phase were selected for analysis. For statistical analysis, the AP amplitude and repolarization duration for each cell were obtained from the average of multiple action potentials. The mean AP amplitude and repolarization duration for each experimental group were the average values from four to six cells.

Surface Biotinylation—INS-1 cells were washed twice with cold phosphate-buffered saline (PBS). Biotinylation of surface protein was carried out by incubating cells with 1 mg/ml of the membrane-impermeant, thiol-cleavable, amine-reactive biotinylation reagent EZ-Link Sulfo-NHS-SS-Biotin (Pierce) in PBS for 30 min on ice. The reaction was terminated by incubating cells for 5 min with PBS containing 20 mM glycine, followed by three washes with cold PBS. Cells were then lysed in 300 μl of lysis buffer (50 mM Tris-HCl, 2 mM EDTA, 2 mM EGTA, 100 mM NaCl, 1% Triton X-100, pH 7.4, with complete protease inhibitor) for 30 min at 4 °C. Cell lysate was cleared by centrifugation at 21,000 × *g* for 10 min at 4 °C, and 500 μg of total lysate was incubated with 100 μl of ~50% slurry of NeutrAvidin-agarose (Pierce) overnight at 4 °C. Biotinylated proteins were eluted with 2× protein loading buffer for 15 min at room temperature. Both eluent and input samples (50 μg of total cell lysate) were analyzed by immunoblotting using a mouse monoclonal anti-Kv2.1 antibody (University of California at Davis/National Institutes of Health NeuroMab Facility; see below).

Immunoblotting—INS-1 cells were washed twice with ice-cold PBS and lysed in the lysis buffer described above at 4 °C with rotation for 30 min. Cell lysate was cleared by centrifugation at 21,000 × *g* for 10 min at 4 °C. Small aliquots of the lysates were used for protein determination by the Lowry method (Pierce) with bovine serum albumin as the standard. Proteins were separated by SDS-PAGE (7.5–12.5%) and transferred onto PVDF membranes (Millipore, Bedford, MA). Membranes were incubated overnight at 4 °C with a primary antibody diluted in the Tris-buffered saline plus 0.1% Tween 20 (TBST). Antibodies against Kv2.1 (from NeuroMab clone K89/34, no cross-reactivity with Kv2.2, which was recently reported to also be expressed in β-cells (16)), GIRK1 (Kir3.1) (Alomone Labs), SUR1, and pAMPK (13) were used at 1:1000 dilution. After three 10-min washes in TBST buffer, blots were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibodies in TBST buffer. The blots were washed three times for 10 min in TBST and developed using the enhanced chemiluminescence detection kit (Super Signal West Femto, Pierce). The signals were imaged by AlphaView® (Cell Biosciences). Blots were stripped and re-probed with anti-tubulin as a loading control. The blots were quantified with ImageJ (National Institutes of Health) and normalized to the corresponding controls.

Fluorescence Microscopy—To follow Kv2.1 channel trafficking in response to leptin, INS-1 cells were transfected with the cDNA for Kv2.1-mCherry and plated onto 18-mm, number 1.5 glass coverslips (Warner Instruments) 24 h post-transfection. Cells were treated with leptin or vehicle control for 30 min, fixed with 4% paraformaldehyde, and processed for confocal microscopy on a Zeiss LSM710 three-channel spectral confocal microscope with a 63 × 1.4 numerical aperture (NA) objective (Carl Zeiss) under identical conditions with randomly selected regions of each coverslip.

Statistical Analysis—All data were analyzed with the program GraphPad Prism™. Results are expressed as mean ± S.E. Differences were tested using one-way analysis of variance (ANOVA) followed by the post hoc Dunnett's test for multiple comparisons. When only two groups were compared, the unpaired Student's *t* test was used. The level of statistical significance was set at $p < 0.05$.

Results

Leptin Increases Surface Kv2.1 Channels in Pancreatic β-Cells—We have recently shown that leptin increases surface expression of K_{ATP} channels in pancreatic β-cells. To test whether this regulation is specific to K_{ATP} channels, we examined two other potassium channels, the G-protein-coupled inwardly rectifying potassium channel GIRK1 (Kir3.1) and the voltage-gated delayed rectifier potassium channel Kv2.1. Interestingly, surface biotinylation experiments using INS-1 cells showed that treatment with 10 nM leptin over a period of 0–180 min led to a transient increase in surface expression of endogenous Kv2.1 channels, identified by a Kv2.1-specific antibody (Fig. 1*A*). The maximal effect, an ~2.5-fold increase, was observed at 30 min of treatment. This time course is reminiscent of that observed for K_{ATP} channels (13). By contrast, the same leptin treatment had no effect on surface expression of GIRK1 (Fig. 1*B*).

To determine whether leptin enhances surface expression of Kv2.1 channels by promoting channel trafficking to the cell

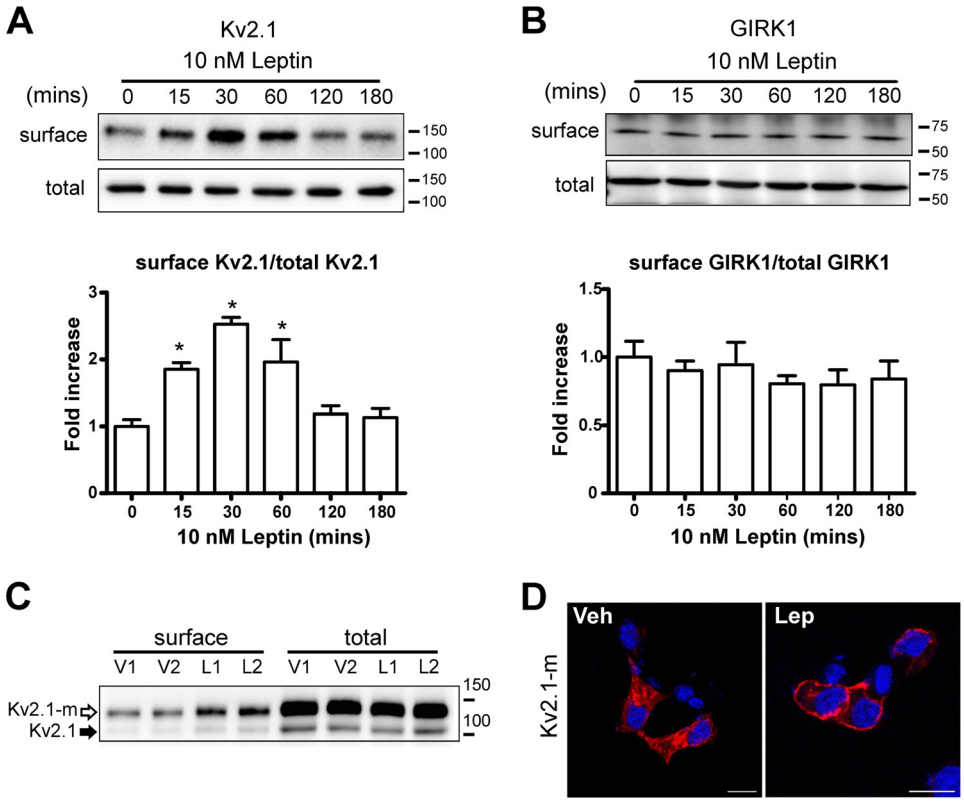

FIGURE 1. **Leptin recruits Kv2.1 channels to the cell surface in INS-1 cells.** *A,* INS-1 cells were treated with 10 nM leptin for the times indicated and subjected to surface biotinylation. *Top,* representative Western blots showing surface-biotinylated Kv2.1 pulled down with NeutrAvidin beads (*upper blot*) and total Kv2.1 in the cell lysate (*lower blot*). Molecular mass markers in this and subsequent figures are given in kDa. *Bottom, bar graph* showing the fold increase in surface Kv2.1 relative to total Kv2.1 and normalized to time 0 ($n = 4$, *, $p < 0.05$ by one-way ANOVA and Dunnett's post hoc test). Leptin caused a gradual and transient increase in surface abundance of Kv2.1 channels. *B,* same as *A* except the blots were probed with an anti-GIRK1 (Kir3.1) antibody. *Top,* representative blots. *Bottom, bar graph* showing the fold change in surface GIRK1 relative to total GIRK1, normalized to time 0 ($n = 3$). No statistically significant changes were found. *C,* Kv2.1 tagged with mCherry (*Kv2.1-m*) was used to monitor the relative distribution of channels following leptin treatment. To first confirm that Kv2.1-mCherry is regulated by leptin similar to endogenous Kv2.1 channels, INS-1 cells were transfected with Kv2.1-mCherry and treated with or without 10 nM leptin for 30 min, followed by surface biotinylation to assess surface expression of both exogenous Kv2.1-mCherry and endogenous Kv2.1. Blots shown are duplicates of vehicle-treated (*V1* and *V2*) and leptin-treated (*L1* and *L2*) cells. Leptin increased surface expression of exogenously expressed Kv2.1-mCherry like endogenous Kv2.1 channels without altering total protein levels. *D,* INS-1 cells transfected with Kv2.1-mCherry and treated with or without 10 nM leptin for 30 min (*scale bar,* 10 μm). Images shown were obtained by integrating signals from a z-stack confocal images. In control cells, a significant amount of fluorescence signal was observed intracellularly. In leptin-treated cells, there was a marked shift in the distribution of the mCherry fluorescence with most signal associated with the cell periphery, consistent with increased plasma membrane localization demonstrated by surface biotinylation.

surface, we utilized a Kv2.1 variant with a fluorescent protein mCherry fused to the channel. Surface biotinylation experiments showed that exogenously expressed Kv2.1-mCherry exhibited increased surface expression parallel to endogenous Kv2.1 upon leptin treatment (Fig. 1*C*). Confocal imaging of cells fixed after treatment with vehicle or 10 nM leptin for 30 min revealed that in vehicle-treated cells most of the fluorescence signal was located intracellularly, but in leptin-treated cells the fluorescence signal was found mostly in the cell periphery (Fig. 1*D*). This redistribution is consistent with the notion that leptin stimulates translocation of intracellular Kv2.1 channels to the plasma membrane.

Leptin-induced Kv2.1 Channel Trafficking to the Cell Surface Corresponds to an Increase in Kv2.1 Currents—To determine whether the leptin-induced increase in Kv2.1 surface expression observed using surface biotinylation results in an increase in Kv2.1 current density, whole-cell patch clamp recordings were made in control and leptin-treated INS-1 cells. In these experiments, cells were held at −90 mV. A brief (30 ms) depolarizing pulse (to −10 mV) was applied to minimize the rapidly inactivating A-type Kv currents and sodium currents. The membrane potential was then stepped from −100 mV to +80 mV to activate Kv2.1 channels as described under "Materials and Methods." The Kv2.1 currents were identified by the slowly inactivating (or non-inactivating within 250 ms) characteristic and sensitivity to inhibition by TEA (Fig. 2*A*) (17); at 10 mM, TEA blocked the maximal currents at + 80 mV by 80.35 ± 5.56% in control cells and 95.84 ± 5.97% in leptin-treated cells (Fig. 2*B*). The increased percentage of TEA-sensitive currents is consistent with an increase in Kv2.1 channels. The currents were also blocked by stromatoxin-1, a spider toxin known to block Kv2.1 (18). At 100 nM, stromatoxin-1 blocked the currents by 51 ± 0.08% (traces not shown); the extent of inhibition is consistent with that observed on Kv2.1 channels expressed in COS cells reported previously by others (18). Because inhibition of the non-inactivating potassium currents by 10 mM TEA is more complete than by 100 nM stromatoxin, we took the TEA-sensitive currents as an estimate of Kv2.1 currents. After subtracting TEA-resistant currents, the average current density in leptin-treated cells was ~2-fold (288.74 ± 52.09 pA/pF) that seen in control cells (150.08 ± 16.84 pA/pF) (Fig. 2*C*; $n = 16$, $p < 0.05$). The extent of increase was comparable with that

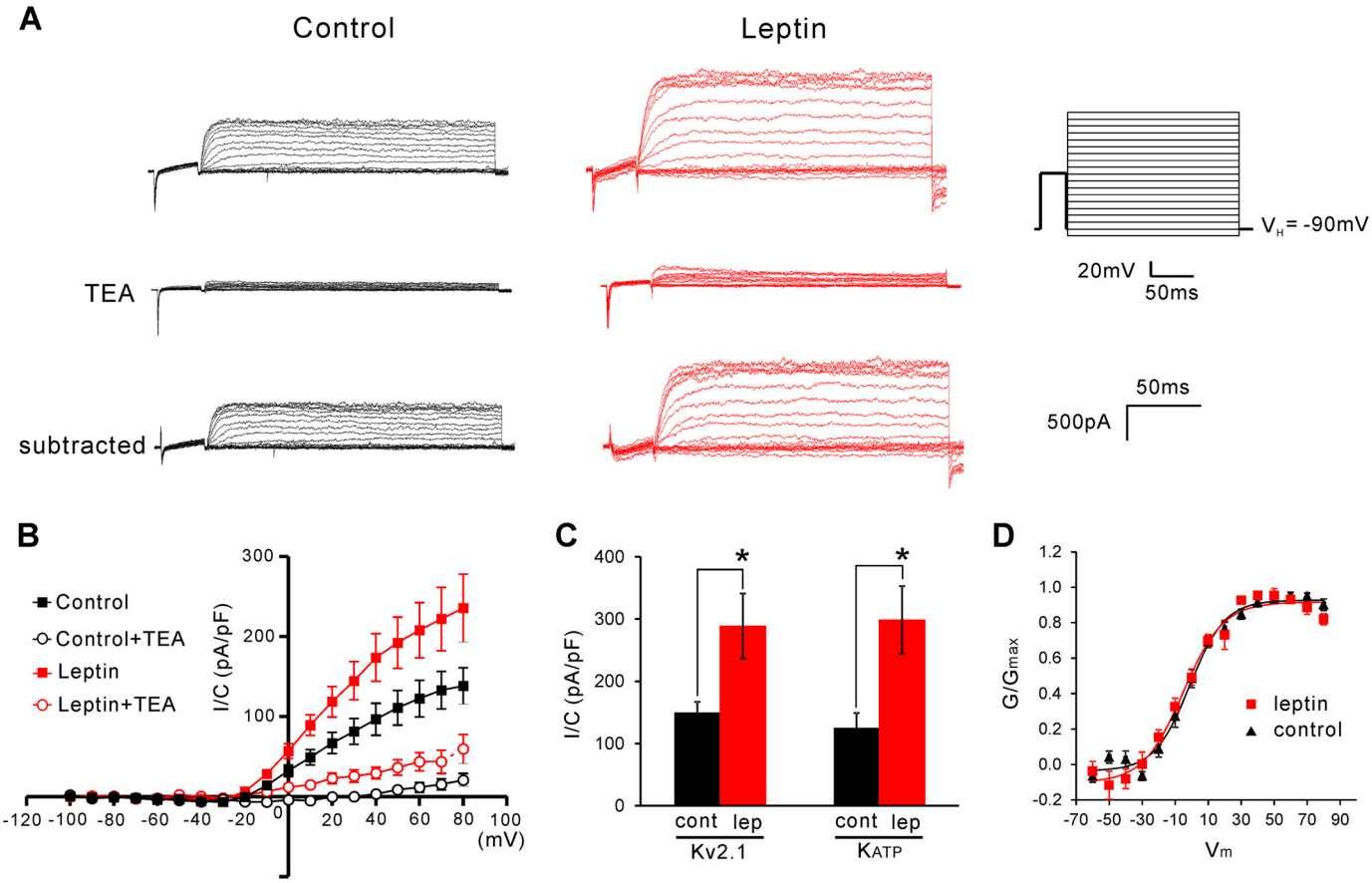

FIGURE 2. **Leptin increases Kv2.1 current density in INS-1 cells without changing voltage dependence of the channel.** *A,* whole-cell recordings of INS-1 cells were performed as described under "Materials and Methods." *Left,* representative whole-cell recordings from a control cell (*black*) and a cell pre-treated with 10 nM leptin for 30 min (*red*). The voltage step protocol and scales are shown on the *right*. *B,* averaged current density in the absence or presence of 10 mM TEA in control and leptin-treated cells plotted against the membrane voltage. Each data point represents mean ± S.E. of eight cells. *C, bar graph* showing averaged Kv2.1 current densities, calculated by subtracting TEA-insensitive potassium currents from total potassium currents, in control and leptin-treated cells ($n = 8$). K_{ATP} current densities measured as described under "Materials and Methods" are shown for comparison ($n = 5$). *, $p < 0.05$, Student's *t* test. *D, G-V* curve of potassium currents (after subtraction of TEA-insensitive component) in control and leptin-treated cells. *G* is normalized to maximal conductance observed over the entire voltage range. The *G-V* curves were fitted using a modified Boltzmann equation as described under "Materials and Methods." Each data point represents the mean ± S.E. of eight cells.

observed for K_{ATP} channels where the average current density for control cells was 125.19 ± 24.07 pA/pF, and for leptin-treated cells it was 298.45 ± 54.23 pA/pF (fold increase = 2.4; Fig. 2*C*; $n = 5$, $p < 0.05$).

A recent study found that leptin (at 100 nM) causes a significant hyperpolarizing shift in the voltage dependence of Kv2.1 channels in HEK293 cells co-expressing Kv2.1 and the long isoform of the leptin receptor LepRb (19). We therefore tested whether leptin at the 10 nM concentration we used affects the voltage dependence of Kv2.1 currents in INS-1 cells (Fig. 2*D*). The half-maximal activation voltage ($V_{½}$) for control cells is −1.11 ± 1.34 mV, with the slope factor *k* of 10.64 ± 1.20 mV. For cells treated with 10 nM leptin for 30 min, the $V_{½}$ is −5.17 ± 1.91 mV, and *k* is 12.04 ± 1.72 mV (see under "Materials and Methods"). The values are not significantly different between the control ($n = 7$) and leptin-treated cells ($n = 8$) ($p > 0.05$). Taken together, our results show that leptin at 10 nM, which is closer to the physiological concentration of the hormone, increases surface expression of Kv2.1 channels and Kv2.1 current amplitude without altering the voltage dependence of these channels.

Leptin Has No Effects on Na^+ or Ca^{2+} Current Density in INS-1 Cells—Because both K_{ATP} and Kv2.1 channels are up-regulated by leptin, we sought to determine whether other ion channels involved in β-cell excitability and insulin secretion are affected. In particular, we examined Na^+ and Ca^{2+} currents as they are major contributors of β-cell action potentials. Whole-cell Na^+ currents were recorded using the voltage step protocol and the 10 mM TEA-containing internal, Ca^{2+}-free external solutions described under "Materials and Methods." Upon voltage stimulation, transient and rapidly inactivating currents characteristic of Na^+ currents were observed (Fig. 3*A*). Analysis of peak currents showed no difference in the current density between control cells and cells pretreated with 10 nM leptin for 30 min (Fig. 3*C*). For Ca^{2+} currents, we focused on the high voltage-activated and slowly inactivating component that has been shown to be dominant in most β-cells (20–22). Using the voltage-step protocol and recording solutions described under "Materials and Methods," we observed large Na^+ currents that inactivated quickly and also smaller currents that persisted throughout the 200-ms test pulse (Fig. 3*B, panel i*). Removal of external Ca^{2+} eliminated the non-inactivating currents but not

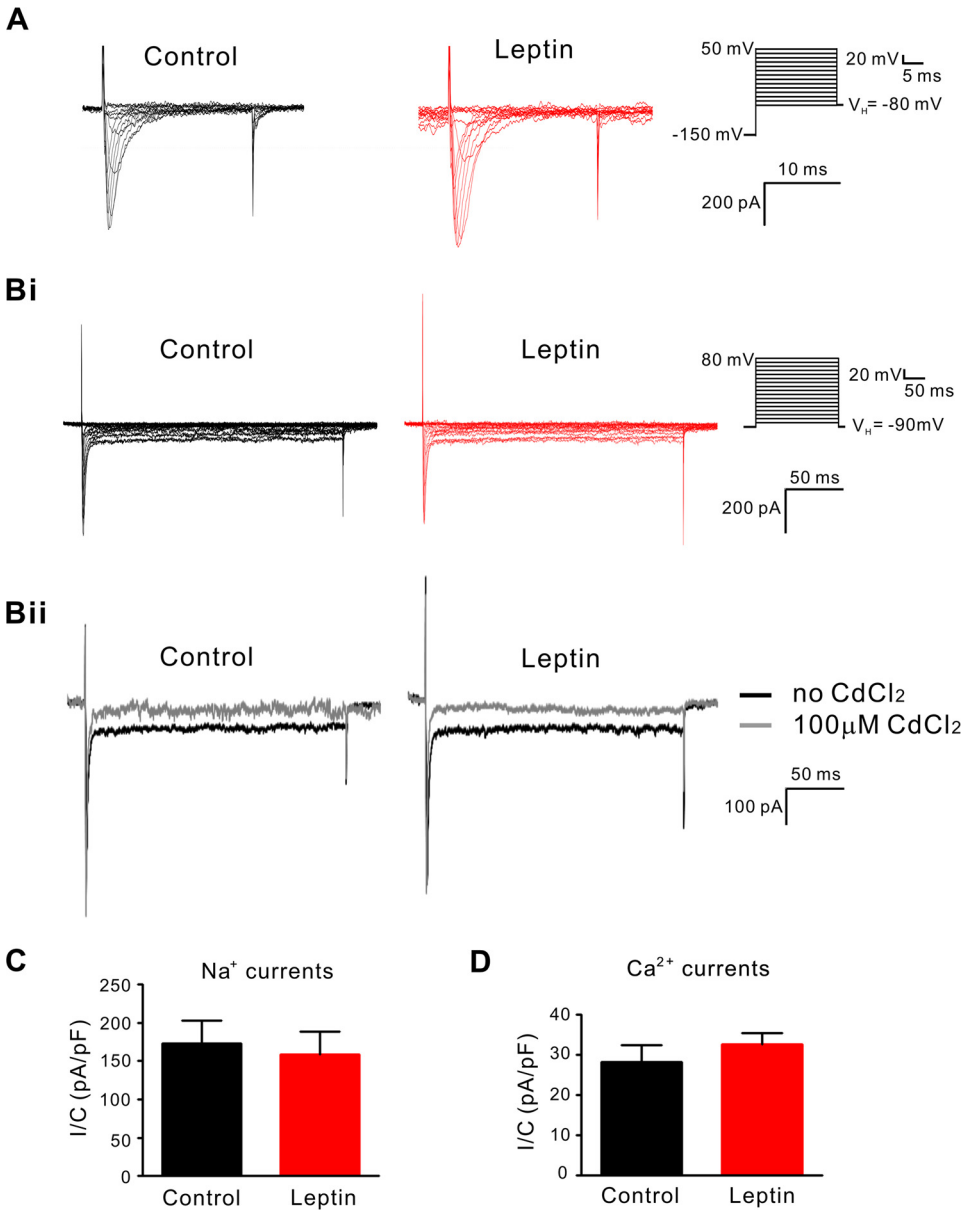

FIGURE 3. **Leptin does not affect Na^+ or Ca^{2+} current density in INS-1 cells.** *A,* whole-cell recordings of Na^+ currents in INS-1 cells. Representative recordings are from a control cell and a cell pre-treated with 10 nM leptin for 30 min. The voltage step protocol and scales are shown on the *right*. *B,* whole-cell recordings of INS-1 cells to measure Ca^{2+} currents. *B, panel i,* representative recordings from a control cell and a cell pre-treated with 10 nM leptin for 30 min. The voltage step protocol and scales are shown on the *right*. *B, panel ii,* comparison of individual Ca^{2+} current traces at voltage sweep of +30 mV in the absence or presence of 100 μM of the Ca^{2+} channel blocker $CdCl_2$. *C, bar graph* showing the average Na^+ current density in control ($n = 7$) and leptin-treated cells ($n = 9$). *D, bar graph* showing the average Ca^{2+} current density in control and leptin-treated cells ($n = 9$). There is no statistically significant difference between control and leptin-treated cells in both (*C* and *D*) ($p > 0.05$, Student's *t* test).

the rapidly inactivating currents, confirming that the non-inactivating component was carried by Ca^{2+} (data not shown). Moreover, addition of 100 μM $CdCl_2$, which blocks Ca^{2+} channels, greatly diminished the non-inactivating currents (Fig. 3*B, panel ii*). Again, analysis of the non-inactivating Ca^{2+} current density as described under "Materials and Methods" revealed no significant difference between control and leptin-treated cells. Thus, in contrast to Kv2.1 or K_{ATP} currents, neither Na^+ nor Ca^{2+} current was affected by leptin. These results further indicate the selectivity of the effect of leptin on Kv2.1 and K_{ATP} channels.

AMPK and PKA Are Involved in Surface Expression Regulation of Kv2.1 Channels—Recently, we showed that AMPK and PKA are involved in the signaling mechanism underlying K_{ATP} channel trafficking regulation by leptin; activation of either kinase mimicked the effect of leptin and increased surface expression of K_{ATP} channels, whereas inhibition of either abrogated the effect of leptin (13). Moreover, AMPK likely lies upstream of PKA, as PKA inhibitors preclude the effect of AMPK activators but not vice versa (13). To test whether a similar signaling mechanism was responsible for Kv2.1 trafficking regulation, we examined how AMPK and PKA activators or inhibitors affect the surface density of Kv2.1 channels in control and leptin-treated cells. Stimulation of AMPK with AICAR or PKA with 8-Br-cAMP led to significantly increased surface Kv2.1, as observed in leptin-treated cells. Inhibition of AMPK with compound C, or PKA with the specific peptide inhibitor

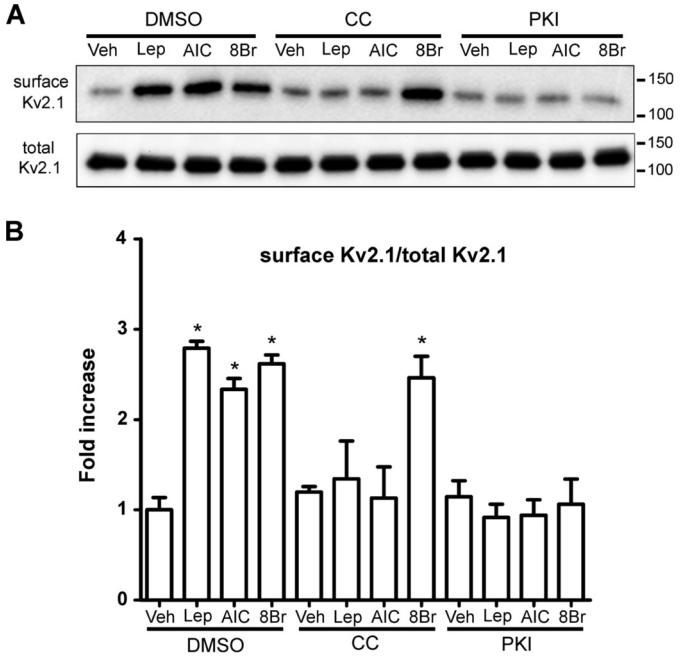

FIGURE 4. **Leptin increases surface expression of Kv2.1 channels via activation of AMPK and PKA.** *A,* surface-biotinylated and total Kv2.1 in INS-1 cells treated with various combinations of drugs. Treating cells with 10 nM leptin (*Lep*), 250 μM of the AMPK activator AICAR (*AIC*), or 10 μM of the PKA activator 8-bromo-cAMP (*8Br*) for 30 min all increased surface abundance of Kv2.1. Pre-treatment of cells with 10 μM of the AMPK inhibitor compound C (*CC*) for 30 min before treatment with the vehicle (*Veh*) DMSO (0.1%), 10 nM leptin, 250 μM AICAR, or 10 μM 8-bromo-cAMP for another 30 min precluded the ability of leptin and AICAR, but not 8-bromo-cAMP, to increase surface expression of Kv2.1. In comparison, pre-treatment of cells with 1 μM of the PKA inhibitor PKI for 30 min before treatment with 0.1% DMSO, 10 nM leptin, 250 μM AICAR, or 10 μM 8-bromo-cAMP for another 30 min precluded the ability of leptin, AICAR, as well as 8-bromo-cAMP, to increase surface expression of Kv2.1. *B, bar graph* showing the average fold change in the ratio of surface Kv2.1 to total Kv2.1 (*, $p < 0.01$ by one-way ANOVA with Dunnett's post hoc test; $n = 3$).

PKI, blocked the effects of leptin on Kv2.1 channel surface expression (Fig. 4). The PKA inhibitor PKI also prevented AICAR as well as 8-bromo-cAMP from increasing Kv2.1 surface expression; however, the AMPK inhibitor compound C did not prevent 8-bromo-cAMP from increasing Kv2.1 surface expression (Fig. 4). These results indicate that AMPK and PKA are both involved in trafficking regulation of Kv2.1 and that PKA lies downstream of AMPK in the signaling pathway. The findings lead us to conclude that Kv2.1 and K_{ATP} channels are regulated by leptin via a similar mechanism.

CaMKKβ Is Involved in Mediating the Effect of Leptin on AMPK and the Subsequent Increase in Surface Expression of Kv2.1 and K_{ATP} Channels—We have previously shown that leptin increases AMPK phosphorylation (13). Park *et al.* (14) made similar observations and further showed that leptin-induced AMPK phosphorylation is mediated by CaMKKβ, a Ca^{2+}-calmodulin-modulated upstream kinase of AMPK. To test whether CaMKKβ plays a role in mediating the effect of leptin on AMPK and the resulting trafficking regulation of Kv2.1 channels, we used surface biotinylation to evaluate the effects of CaMKKβ inhibitors on leptin-induced up-regulation of surface Kv2.1 channels. K_{ATP} channel surface expression was analyzed in parallel to serve as a positive control. Inhibition of CaMKKβ by 10 μM STO-609 blocked the ability of leptin, but not AICAR, to activate AMPK based on the level of phosphorylated AMPK recognized by an antibody against phospho-Thr-172 in the catalytic α-subunit of AMPK (Fig. 5*A*). In addition, we examined whether removing external Ca^{2+} disrupts leptin signaling as CaMKKβ is activated by increased intracellular Ca^{2+} concentrations. Inclusion of EGTA (5 mM) in the medium, which chelates external Ca^{2+}, diminished pAMPK levels upon leptin treatment but did not affect the ability of AICAR to increase pAMPK. These results suggest that leptin increases phosphorylation of AMPK by increasing Ca^{2+} influx and activating CaMKKβ, in agreement with the findings reported by Park *et al.* (14)

Next, we tested whether inhibition of CaMKKβ affects the ability of leptin to promote surface expression of K_{ATP} and Kv2.1 channels. As shown in Fig. 5, *B* and *C*, STO-609 prevented or attenuated the ability of leptin to increase surface expression of K_{ATP} and Kv2.1 channels. By contrast, inhibition of CaMKKβ did not interfere with the ability of AICAR to increase K_{ATP} or Kv2.1 channel surface expression, which was expected since AICAR increases AMPK phosphorylation and activates the kinase independent of the kinases upstream of AMPK (23). These results suggest that CaMKKβ is responsible for activating AMPK following leptin treatment.

Leptin-induced Up-regulation of Kv2.1 Channel Surface Expression Requires F-actin Depolymerization—Actin depolymerization has been reported to accompany leptin signaling (24) as well as activation of AMPK or PKA (13). We have shown that actin depolymerization is both necessary and sufficient for recruitment of K_{ATP} channels to the cell surface in INS-1 cells (13). We therefore determined whether the increased surface expression of Kv2.1 following activation of leptin receptors, AMPK, and PKA also involves actin depolymerization. In INS-1 cells pretreated for 10 min with 100 nM jasplakinolide, a cyclic peptide that binds and stabilizes filamentous actin (25), leptin failed to increase surface biotinylated Kv2.1 (Fig. 6*A*). Likewise, jasplakinolide pretreatment blocked the ability of the AMPK activator AICAR and the PKA activator 8-bromo-cAMP to increase surface abundance of Kv2.1 (Fig. 6*A*). By contrast, treating cells with latrunculin B, an F-actin-destabilizing agent, at 100 nM for 10 min in the absence of leptin, or AMPK or PKA activators, led to an increase in surface density of Kv2.1. These results indicate that F-actin depolymerization is a requisite step in leptin-induced recruitment of Kv2.1 channels to the cell surface and that actin depolymerization alone is sufficient to trigger increased trafficking of Kv2.1 channels to the plasma membrane.

Increased Surface Densities of K_{ATP} and Kv2.1 Channels Affect β-Cell Electrical Activity—Open probability of K_{ATP} channels is dictated mainly by the intracellular ATP and ADP concentrations, which are in turn linked to glucose metabolism. As such, K_{ATP} channels have a pivotal role in transitioning β-cells from a resting hyperpolarized state to an excited depolarized state during glucose stimulation. Increased surface expression of K_{ATP} channels is predicted to increase the resting state K^+ conductance to impede β-cell depolarization. Kv2.1 channels, by contrast, are important for repolarizing the β-cell membrane potential back to the resting state. An increase in Kv2.1 channel density is expected to accelerate the repolariza-

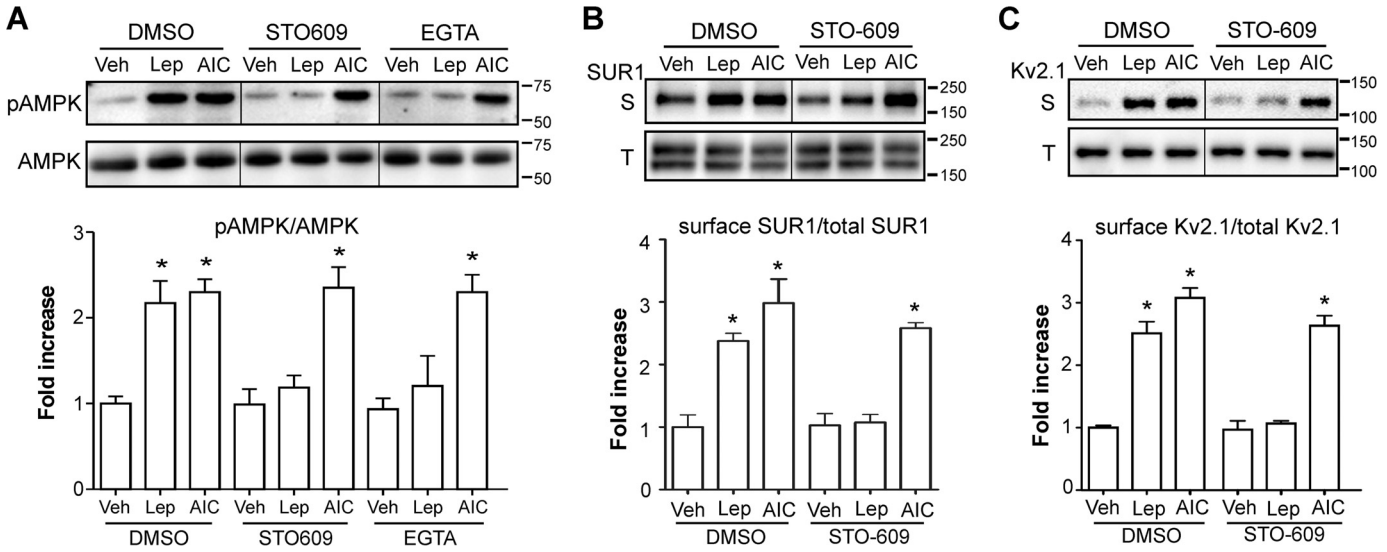

FIGURE 5. **Activation of AMPK by leptin and the consequent recruitment of K_{ATP} and Kv2.1 channels to the plasma membrane is dependent on CaMKKβ.** *A, top,* Western blots of pAMPK (AMPK phosphorylated at Thr-172) and total AMPK in INS-1 cells treated with 0.1% DMSO (*Veh*), 10 nM leptin (*Lep*) or 250 μM AICAR (*AIC*) for 30 min in the presence or absence of the CaMKKβ inhibitor STO609 (10 μM) or the calcium chelator 5 mM EGTA. *Bottom,* quantification of pAMPK from blots like those shown above. The pAMPK signal was normalized to total AMPK signal and expressed as fold increase of the value seen for the DMSO/veh control. Each *bar* represents the mean ± S.E. of four separate experiments. (n = 4/group; *, $p < 0.05$ compared with the DMSO/veh group by one-way ANOVA and Dunnett's post hoc test.) *B,* INS-1 cells were pre-treated with DMSO, 10 nM leptin. or 250 μM AICAR in the presence or absence of 10 μM STO609 for 30 min as described in *A*. Cells were subjected to surface biotinylation and analyzed for surface SUR1 and total SUR1 as described under "Materials and Methods." Representative blots are shown on *top,* and quantification of the ratio of surface SUR1 to total SUR1 upper band is shown *below* (n = 3; *, $p <$ 0.05 by one-way ANOVA and Dunnett's post hoc test). *C,* same as *B* except surface Kv2.1 and total Kv2.1 were analyzed as described under "Materials and Methods."

tion process and shorten the action potential duration. To test these predictions, whole-cell current clamp recordings were made in control and leptin-treated INS-1 cells to monitor changes of membrane potentials and action potentials.

Control INS-1 cells exposed to Tyrode's solution containing 11 mM glucose exhibited an average membrane potential of −22.04 ± 4.17 mV (n = 20), whereas cells pretreated with 10 nM leptin for 30 min had an average membrane potential of −45.4 ± 3.88 mV (n = 20), which is significantly less depolarized than that of control cells ($p < 0.0001$). To confirm that the more hyperpolarized membrane potential in leptin-treated cells is due to increased K_{ATP} conductance, tolbutamide (300 μM) was added to the bath solution to block K_{ATP} channels. Application of tolbutamide had little effect on the membrane potential in control cells (from −17.42 ± 5.53 to −16.93 ± 7.14 mV; n = 9, p = 0.83; Fig. 7), indicating that at 11 mM glucose, there was little K_{ATP} channel conductance. By contrast, in leptin-treated cells tolbutamide caused a significant depolarizing shift of the membrane potential (from −38 ± 6.42 to −14.65 ± 5.67 mV; n = 9, $p < 0.001$; Fig. 7), indicative of the presence of substantial K_{ATP} channel conductance. These results are consistent with a scenario in which leptin increases K_{ATP} channel density, hence conductance, to render β-cell membrane potential less responsive to glucose stimulation.

To evaluate the functional impact of increased Kv2.1 surface expression, we compared the electrical activity of control and leptin-treated cells. In 11 mM glucose, single action potentials as well as rapidly firing action potentials on top of depolarizing waves were observed in some but not all cells from both control and leptin-treated groups (examples shown in Fig. 8*A*). In the control group, 8 out of the 13 cells recorded (~62%) did not fire action potentials, and these cells all had initial break-in membrane potentials more positive than −15 mV. It may be that the prolonged depolarization in 11 mM glucose rendered voltage-gated calcium and sodium channels unable to recover from inactivation to generate action potentials (26–28). The five control cells that did exhibit action potentials had break-in potentials between −35 and −50 mV. In the leptin-treated group, 12 out of the 20 cells recorded (60%) did not show action potentials. Of the cells that did not fire action potentials, three had very hyperpolarized break-in membrane potentials, below −60 mV, consistent with increased K_{ATP} channel presence in the membrane; however, two cells had membrane potentials more positive than −15 mV, and the rest had membrane potential between −30 and −60 mV. The eight cells that did display action potentials had membrane potentials ranging from −23 to −60 mV. The heterogeneity in the electrical activity of single β-cells in both groups is in line with previous studies on single β-cells (29, 30).

Because Kv2.1 channels have a major role in repolarizing the membrane potential, we predicted that an increase in Kv2.1 channel density following leptin stimulation should accelerate membrane repolarization. Analysis of the repolarizing phase duration of action potentials in control and leptin-treated cells (see "Materials and Methods") showed an average repolarizing duration of 90.87 ± 15.08 ms (n = 5 cells, 13 action potentials analyzed) in control cells, which is significantly longer ($p <$ 0.05) than the 47.40 ± 6.33 ms (n = 6 cells, 24 action potentials analyzed) found in leptin-treated cells (Fig. 8*A*, *panel iii*) and is consistent with our prediction. In addition, we analyzed the action potential amplitude in both groups. Control cells had an average amplitude of 43.93 ± 12.71 mV, and leptin-treated cells had an average action potential amplitude of 59.45 ± 8.22 mV, which are not statistically different. To verify that Kv2.1 chan-

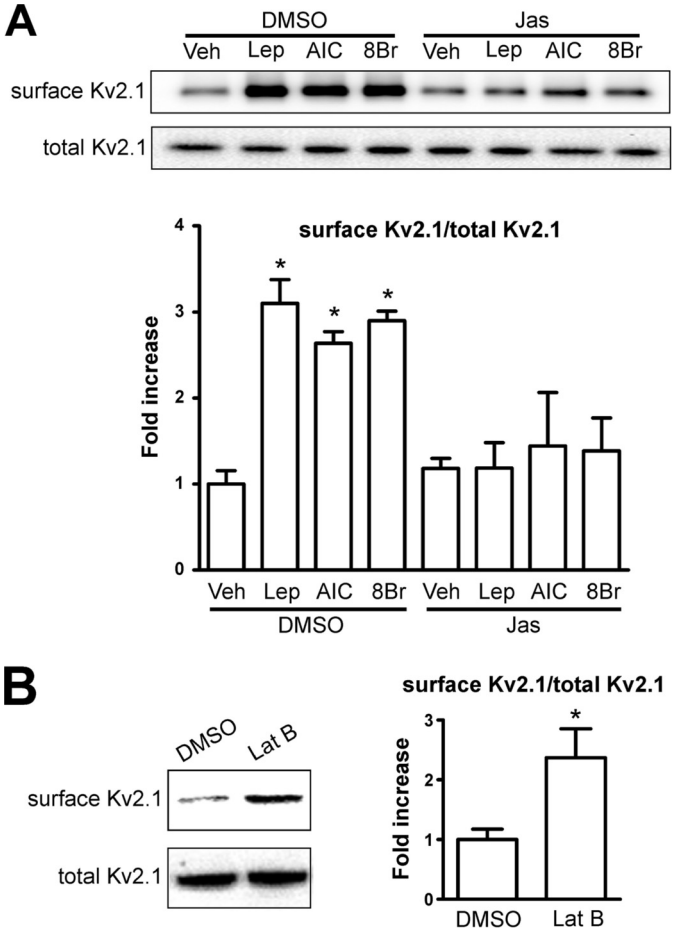

FIGURE 6. **Actin depolymerization is required for increased surface expression of Kv2.1 channels in response to leptin signaling.** *A,* INS-1 cells were treated with 100 nM of the actin-stabilizing agent jasplakinolide 10 min prior to treatment with 10 nM leptin (*Lep*), 250 μM AICAR (*AIC*) or 10 μM 8-bromo-cAMP (*8Br*) for 30 min. Surface Kv2.1 and total Kv2.1 were analyzed as described in previous figures. Stabilization of F-actin by jasplakinolide (*Jas*) prevented increase of surface SUR1 by leptin, AICAR, or 8-bromo-cAMP. *Top,* representative blots. *Bottom,* quantification of surface Kv2.1/total Kv2.1. Each *bar* is the mean ± S.E. of three independent experiments (*, $p < 0.05$ by one-way ANOVA and Dunnett's post hoc test). *B,* treating INS-1 cells with the F-actin-destabilizing drug latrunculin B (*Lat B*, 100 nM) for 10 min caused an increase in surface expression of Kv2.1 channels. *Top,* representative blots. *Bottom, bar graph* shows the ratio of surface Kv2.1 to total Kv2.1 normalized to that seen in DMSO-treated group. ($n = 4$; *, $p < 0.05$ by Student's *t* test).

nels are mainly responsible for accelerated membrane repolarization in leptin-treated cells, we applied 10 mM TEA to block Kv2.1 channels after recording the baseline action potential firing pattern in leptin-treated cells. Note we chose TEA rather than stromatoxin-1 because TEA inhibits Kv2.1 currents more completely. Although other K^+ channels, including BK (large conductance calcium- and voltage-activated potassium channels), SK (small conductance calcium-activated potassium channels), K_{ATP}, and other minor Kv channels, are also blocked by TEA to various extents, these channels are either a very small component of the overall K^+ conductance under the recording condition (such as K_{ATP} channels; data not shown) or they have been shown in previous studies to not have a significant role in determining the repolarization duration of action potentials in β-cells (31–33). TEA led to broadening of the action potential (Fig. 8*B, panel i*) and a significant increase in the duration of the repolarizing phase of an action potential (49.49 ± 8.55 ms before TEA treatment and 81.90 ± 7.25 ms after TEA treatment; $n = 4$ cells, $p < 0.05$) (Fig. 8*B, panel ii*). These results are in agreement with the notion that Kv2.1 channels contribute to the increased rate of action potential repolarization in leptin-treated cells.

Effect of Leptin on Kv2.1 Channels Is Conserved in Human β-Cells—In addition to rat INS-1 cells, Kv2.1 is known to be functionally important in human β-cells (31, 34, 35). To test whether leptin also increases Kv2.1 channel density in human β-cells, we performed whole-cell patch clamp recordings using primary β-cells dispersed from human islets. Similar to INS-1 cells, human β-cells displayed voltage-dependent outward currents with sensitivity to external TEA (10 mM) consistent with Kv2.1 channels (Fig. 9, *A* and *B*). After subtracting TEA-resistant currents, the average current density in leptin-treated cells was ~2-fold (382.93 ± 77.41 pA/pF) that seen in cells not treated with leptin (161.14 ± 39.44 pA/pF) ($n = 10$ cells from two batches of human islets, $p < 0.05$) (Fig. 9*C*). Notably, although the TEA-sensitive currents represent 56.13 ± 5.50% of total currents in control cells, they represent 79.70 ± 3.99% of total currents in leptin-treated cells. Again, the increased percentage of TEA-sensitive currents is consistent with an increase in Kv2.1 channels and echoes our findings in INS-1 cells. To further confirm that the signaling mechanism in human β-cells is also similar, we determined whether the PKA activator 8-bromo-cAMP, which mimics the effect of leptin and AICAR in INS-1 cells, was able to increase Kv2.1 current density. Results show that treating dissociated human β-cells (from another two batches of human islets) with 10 μM 8-bromo-cAMP for 30 min increased Kv2.1 current density by 2-fold as observed in INS-1 cells, without altering voltage dependence of the current (Fig. 9*D*). The average TEA-sensitive current density was 135.5 ± 24.8 pA/pF for control cells ($n = 7$) and 270.5 ± 49.4 pA/pF for 8-bromo-cAMP treated cells ($n = 8$). The difference between the two groups is statistically significant ($p < 0.05$; Fig. 9*E*). Taken together, our results indicate that the regulatory effect of leptin on Kv2.1 channels and the underlying signaling mechanism we found in the rodent INS-1 cells are conserved in human β-cells.

Discussion

In this study, we demonstrate that surface abundance of Kv2.1 channels is subject to regulation by the adipocyte-released hormone leptin. Leptin leads to a transient increase in the density of Kv2.1 channels in the β-cell membrane by a mechanism that involves CaMKKβ, AMPK, PKA, and actin depolymerization. This regulation is similar to that recently reported for K_{ATP} channels (13, 14). Indeed, this serendipitous finding was made while we tested several potassium channels as potential controls to show that the effect of leptin on K_{ATP} channels was specific. Although leptin regulates Kv2.1 and K_{ATP} channel trafficking in a strikingly similar manner, it has no effect on other membrane proteins and ion channels, including IGFR-1β (13), Kir2.1 (36), Kir3.1 (Fig. 1), Na^+ channels, and Ca^{2+} channels (Fig. 3). This selectivity suggests that the similar response of K_{ATP} and Kv2.1 channels to leptin signaling is not simply a coincidence but has biological significance. Importantly, the effects of leptin on Kv2.1 channels, and K_{ATP} chan-

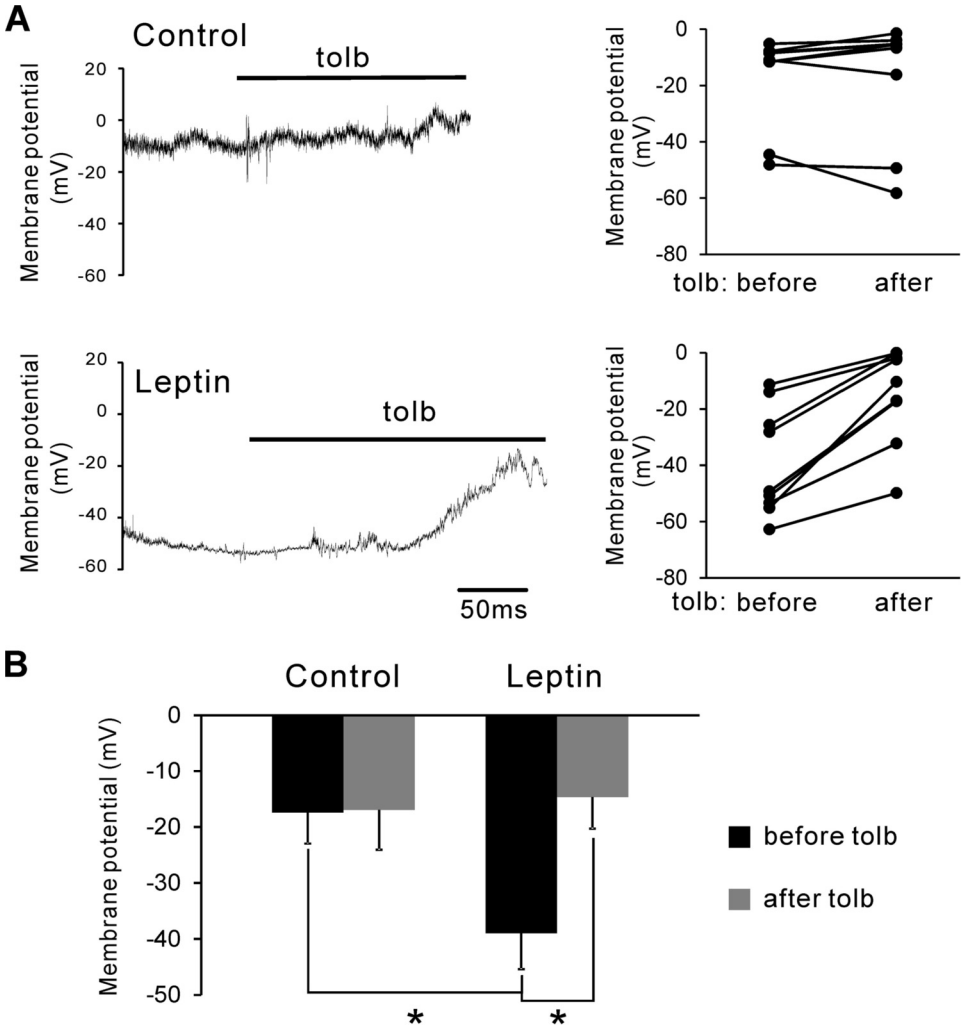

FIGURE 7. **Leptin causes a hyperpolarizing shift in INS-1 cell membrane potential in 11 mM glucose that is reversible by tolbutamide.** *A,* whole-cell recording of membrane potential from control cells and cells pre-treated with 10 nM leptin for 30 min. After the membrane potential stabilized, 300 μM tolbutamide (*tolb*) was added to block K_{ATP} currents. *Left,* representative recordings. *Right,* plots showing changes in the membrane potential after tolbutamide application in all cells recorded in control and leptin-treated groups. *B,* average membrane potentials in control and leptin-treated cells before and after the application of the K_{ATP} channel blocker tolbutamide (300 μM). Values were taken after the membrane potential stabilized. The leptin-treated cells showed a more negative average membrane potential than control cells (*, $p < 0.05$; $n = 9$, unpaired *t* test). Tolbutamide significantly depolarized the membrane potential in leptin-treated ($p < 0.001$; $n = 9$, paired *t* test) but not control ($p > 0.05$; $n = 9$, paired *t* test) cells to levels comparable with those seen in control cells either before or after tolbutamide exposure ($p > 0.05$).

nels, are observed not only in the rat insulinoma INS-1 cells but also in human β-cells, indicating that this regulatory mechanism is conserved and could be important for glucose homeostasis in humans.

Leptin has been known to inhibit glucose-stimulated insulin secretion by directly acting on β-cells since the late 1990s (12, 37). As the early studies found that leptin increases K_{ATP} conductance (12), subsequent studies have largely focused on K_{ATP} channel regulation (13, 14, 36, 38–41). To our knowledge, the study we present here is the first to demonstrate an effect of leptin on Kv2.1 channels in β-cells. In a recent study of AgRp/NPY neurons in the hypothalamus, leptin at 100 nM was shown to modulate neuronal excitability; this was attributed to an effect of leptin on the voltage sensitivity of Kv2.1 channels, as Kv2.1 channels exogenously expressed in HEK293 cells showed a hyperpolarizing shift in their voltage dependence in response to leptin signaling (19). However, we did not observe a change in the voltage dependence of endogenously expressed Kv2.1 channels in INS-1 cells upon leptin treatment, at least at the 10 nM concentration used in our study. Rather, the increase in Kv2.1 currents in β-cells we saw after leptin treatment was due to increased surface abundance of Kv2.1 channels.

Genetic and pharmacological studies have shown that Kv2.1 plays a major role in membrane repolarization and insulin secretion in both rodent and human β-cells (10, 42–44). Increased expression of Kv2.1 is expected to facilitate membrane repolarization and dampen the excitatory effect of glucose. In agreement, we observed an increased rate of membrane repolarization in action potentials in leptin-treated cells compared with control cells (Fig. 8). As leptin also increases K_{ATP} channel surface expression, which exerts a hyperpolarizing force on the β-cell membrane potential, we propose that the concerted increase in K_{ATP} and Kv2.1 expression in the β-cell membrane provides a mechanism to efficiently reduce glucose-stimulated insulin secretion. Of note, our recordings were

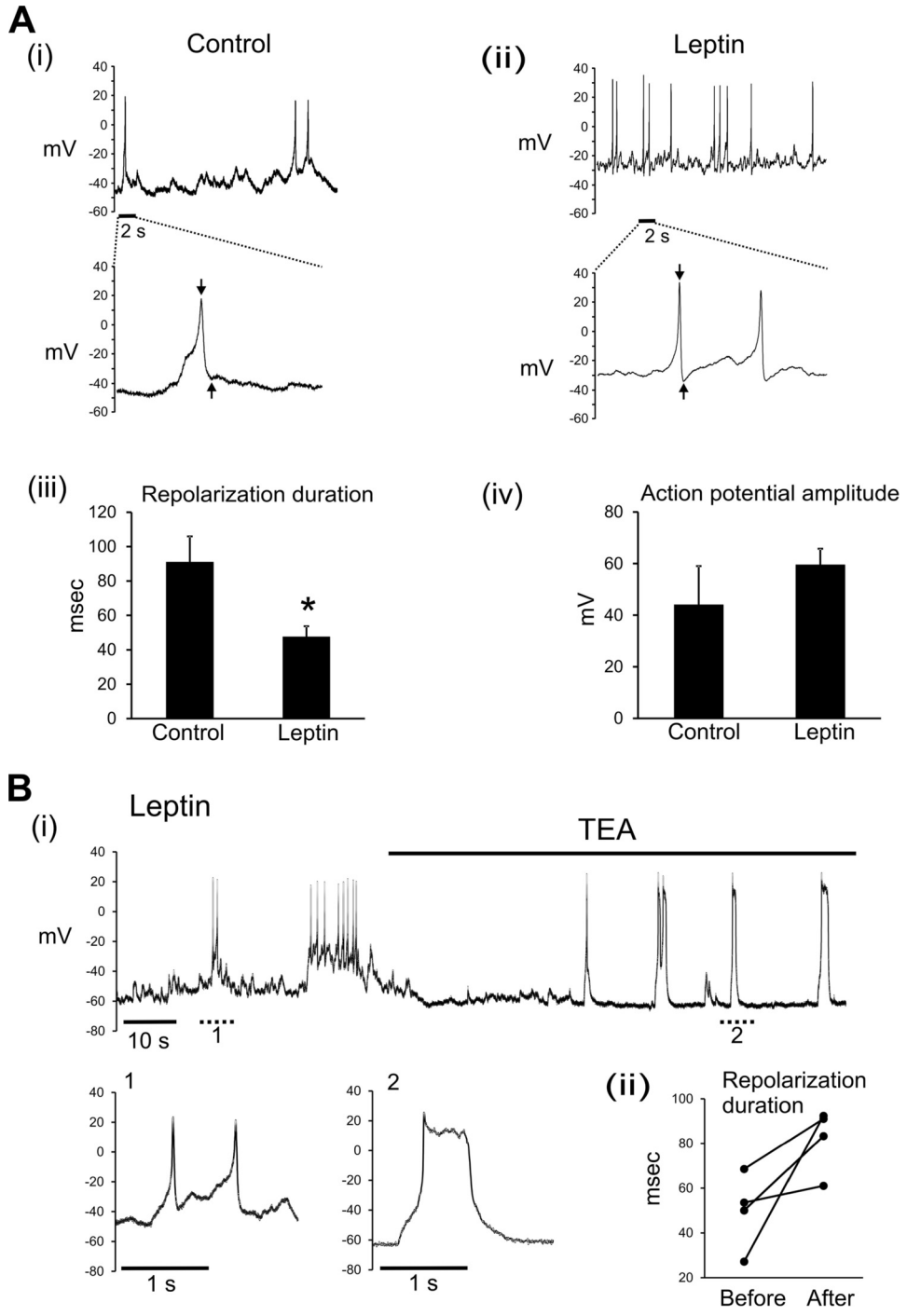

FIGURE 8. **Action potential analysis in control and leptin-treated cells.** *A,* whole-cell current clamp recording of a control cell (*panel i*) and a cell pre-treated with 10 nM leptin for 30 min prior to the recording (*panel ii*). Below each recording is the expanded view of individual action potentials. *Panel iii,* repolarization durations (peak of AP to repolarized membrane potential as indicated by the *down* and *up arrows* in *panels i* and *ii*) from control and leptin-treated cells are compared in the *bar graph*. Each *bar* represents the mean ± S.E. of 5–6 cells, and the value of each cell is the average of 3–5 well defined action potentials. *, $p < 0.05$. *Panel iv,* same as *panel iii* except that action potential amplitudes are compared between control and leptin-treated groups. *B, panel i* a representative recording from a leptin-treated cell before and after application of 10 mM TEA. Expanded views of the two sections of the recording marked by the *dotted lines* and *labeled 1* and *2* are shown on the *lower left side* of the recording. *Panel ii,* a plot of the repolarization duration before and after TEA treatment from four leptin-treated cells showing an increase in the repolarization duration after TEA treatment.

made in dispersed INS-1 cells that exhibit significant variation in their electrical behavior. Such variable electrical activity is also well documented for isolated primary β-cells (29, 30). In intact islets where β-cells are electrically coupled through gap junctions (45, 46), the electrical silencing effects of leptin expected from the increased K_{ATP} and Kv2.1 conductance would be greatly amplified, based on experimental and modeling studies (47–49).

In addition to leptin, glucose starvation (50) and glucose stimulation (51) have also been reported to increase K_{ATP} channel trafficking to the plasma membrane. In the case of glucose starvation, a gradual buildup on AMPK activity is linked to

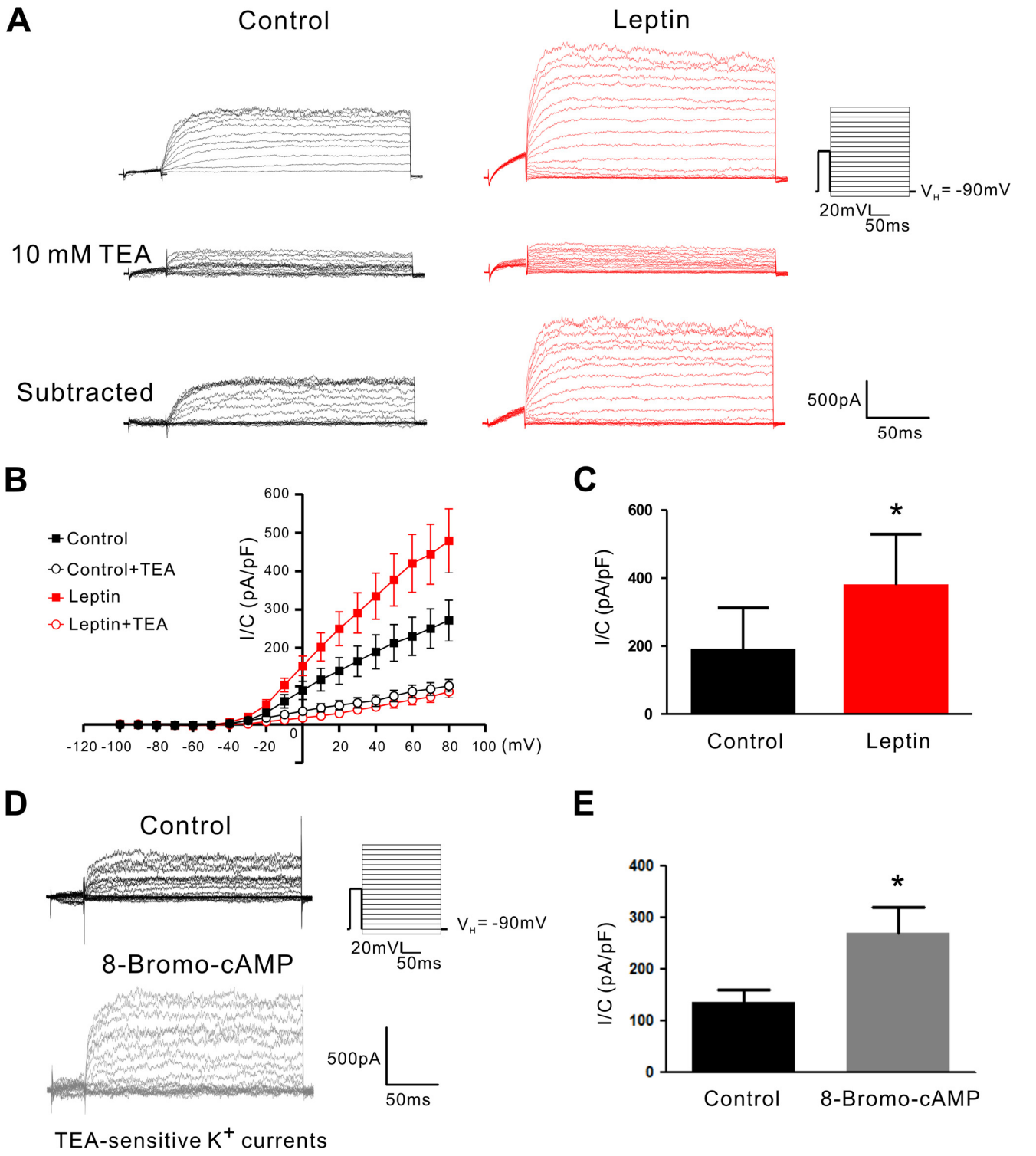

FIGURE 9. **Leptin increases Kv2.1 current density in human β-cells.** *A,* whole-cell recordings of dispersed human β-cells were performed as described under "Materials and Methods." *Left,* representative whole-cell recordings from a control cell (*black*) and a cell pre-treated with 10 nM leptin for 30 min (*red*). The voltage step protocol and scales are shown on the *right*. *B,* averaged current density in the absence or presence of 10 mM TEA in control and leptin-treated cells plotted against the membrane voltage. Each data point represents mean ± S.E. of 10 cells. *C, bar graph* showing averaged Kv2.1 current densities, calculated by subtracting TEA-insensitive currents from total currents, in control and leptin-treated cells ($n = 10$). *, $p < 0.05$, Student's *t* test. *D,* representative current traces from a control and a cell pre-treated with 10 μM 8-bromo-cAMP for 30 min using the stimulation protocol shown on the right. *E, bar graph* showing the average Kv2.1 current density calculated by subtracting TEA (10 mM)-resistant currents from total currents. The average current density of 8-bromo-cAMP-treated cells ($n = 8$) is significantly higher than that observed in control cells ($n = 7$), *, $p < 0.05$ by Student's *t* test.

increased K_{ATP} channel surface expression (14, 50). In the glucose stimulation study, calcium and PKA were found to be required for K_{ATP} channel translocation to the plasma membrane (51). Considering our previous (13) and present findings that both AMPK and PKA have a role in leptin-induced trafficking of K_{ATP} and Kv2.1 channels, it will be important to

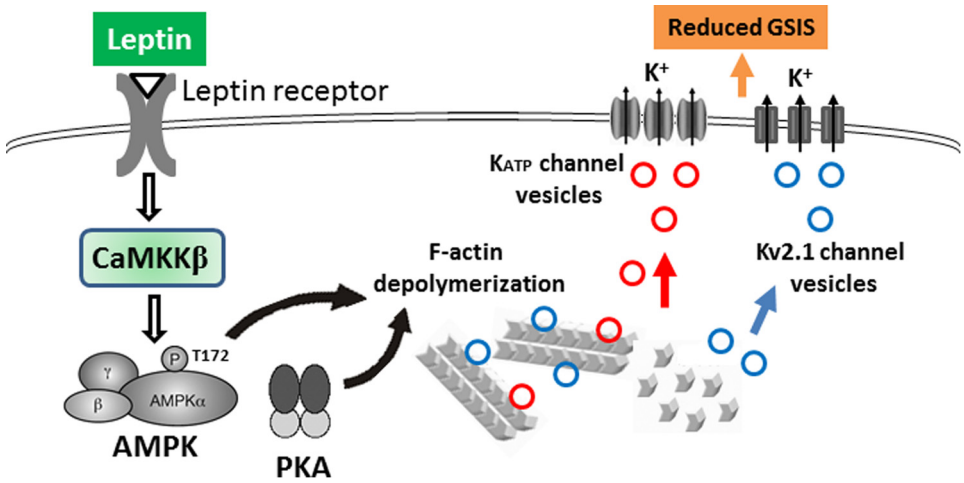

FIGURE 10. **Schematic diagram of concerted trafficking regulation of K_{ATP} and Kv2.1 channels by leptin in pancreatic β-cells.** Binding of leptin to its receptor induces a signaling cascade that involves activation of CaMKKβ and AMPK, as well as PKA either sequentially or in parallel. Activation of AMPK and PKA leads to filamentous actin (F-actin) depolymerization to promote K_{ATP} and Kv2.1 channel trafficking to the plasma membrane. The increased K^+ efflux due to increased surface density of K_{ATP} channels helps to keep β-cell membrane potential hyperpolarized in the presence of stimulating glucose, whereas the increased K^+ efflux due to increased Kv2.1 channel density following an action potential facilitates membrane repolarization, which together reduce glucose-stimulated insulin secretion (*GSIS*).

determine in the future whether glucose starvation and glucose stimulation also have an effect on Kv2.1 trafficking. Coordinated trafficking of K_{ATP} and Kv2.1 channels would be expected to enhance the physiological impact of the various signals.

Strikingly, the effects of leptin on both K_{ATP} and Kv2.1 channels are transient. What might be the physiological significance of such transient regulation? We speculate that the transient nature of the leptin effect is important to provide β-cells the ability to respond to and integrate other signals to control insulin secretion. In a study showing that glucose stimulation recruits K_{ATP} channels to the cell surface, Yang *et al.* (51) proposed that the mechanism may allow β-cells to transition more efficiently when blood glucose falls by facilitating membrane potential repolarization and recovery of voltage-gated calcium channels from inactivation such that β-cells can respond better upon subsequent glucose stimulation. In our cells grown in 11 mM glucose, leptin-induced trafficking of K_{ATP} and Kv2.1 channels was associated with a more hyperpolarized membrane potential and a more rapid return to resting membrane potential in those cells that did fire action potentials. Transient up-regulation of K_{ATP} and Kv2.1 channel density by leptin may thus also serve to prime β-cells to efficiently respond to subsequent glucose stimulation. In this regard, it is worth noting that plasma leptin levels are modulated by physiological factors such as circadian rhythm, nutritional state, insulin levels, and neuronal regulation (52–54) and that acute changes in leptin levels within the physiological range have been shown to markedly inhibit insulin secretion *in vivo* (55). Moreover, leptin concentrations in humans have been shown to oscillate following a pulsatile pattern with a pulse duration of ~33 min (56). Future studies elucidating the temporal relationship between signaling through leptin and other physiological cues in β-cells will be needed to fully understand the role of the regulatory mechanism we uncovered here in glucose homeostasis.

Another question arising from our study yet to be addressed is whether K_{ATP} and Kv2.1 channels are localized to the same secretory vesicle population in β-cells and whether such colocalization underlies the coordinated trafficking regulation by leptin. Presently, the subcellular localization of K_{ATP} channels remains unresolved, with some studies suggesting localization in insulin granules (57, 58) and others suggesting localization in chromogranin-positive but insulin-negative dense-core granules (51). Even less is known about the subcellular localization of Kv2.1 channels. An interesting hypothesis to test in the future is that K_{ATP} and Kv2.1 channels may be sorted into the same secretory vesicles such that both channels are simultaneously regulated by leptin signaling.

It is worth noting that Kv2.1 channels in β-cells have been reported to have other functional roles unrelated to repolarizing membrane potential. For example, Dai *et al.* (59) recently reported that Kv2.1 regulates insulin secretion independent of its electrical function by interacting with syntaxin 1A-binding domain via the channel's C terminus. Moreover, increased expression of Kv2.1 has been linked to apoptosis in neurons (60) and β-cells (61) following apoptotic signals. It remains to be determined whether leptin regulation of Kv2.1 channel surface expression also affects the insulin secretion machinery or β-cell survival. Nevertheless, our study here further underscores the complex role and regulation of Kv2.1 in pancreatic β-cells.

In summary, our study reveals concerted regulation of two potassium channels critically important for β-cell electrical activity by the satiety hormone leptin to modulate β-cell electrical response to glucose (Fig. 10). In addition to K_{ATP} and Kv2.1, many other ion channels and transporters contribute to shaping the electrical activity of β-cells. Although leptin has little effect on surface expression of Na^+ or Ca^{2+} currents in INS-1 cells (Fig. 3), it has been shown or implicated to regulate several other ion channels in other cell types, including AMPA receptors (62) and TrpC channels (63) in hippocampal neurons. It will be important to determine in future studies whether leptin may regulate additional channels/transporters in β-cells to modulate insulin secretion or other functions.

Author Contributions—P. C. C. and S. L. S. conceived and coordinated the study and wrote the paper. Y. W. and S. L. S. designed, performed, and analyzed the experiments shown in Figs. 2, 3, and 7–9. S. L. S. and P. C. C. designed, performed, and analyzed the experiments shown in Figs. 1, 4–6, and 10. All authors reviewed the results and approved the final version of the manuscript.

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