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## Modulation of the Pancreatic Islet $\beta$ -Cell-delayed Rectifier Potassium Channel Kv2.1 by the Polyunsaturated Fatty Acid Arachidonate<sup>\*,§</sup>

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### Abstract

Glucose stimulates both insulin secretion and hydrolysis of arachidonic acid (AA) esterified in membrane phospholipids of pancreatic islet  $\beta$ -cells, and these processes are amplified by muscarinic agonists. Here we demonstrate that nonesterified AA regulates the biophysical activity of the pancreatic islet  $\beta$ -cell-delayed rectifier channel, Kv2.1. Recordings of Kv2.1 currents from INS-1 insulinoma cells incubated with AA (5  $\mu$ M) and subjected to graded degrees of depolarization exhibit a significantly shorter time-to-peak current interval than do control cells. AA causes a rapid decay and reduced peak conductance of delayed rectifier currents from INS-1 cells and from primary  $\beta$ -cells isolated from mouse, rat, and human pancreatic islets. Stimulating mouse islets with AA results in a significant increase in the frequency of glucose-induced  $[Ca^{2+}]$  oscillations, which is an expected effect of Kv2.1 channel blockade. Stimulation with concentrations of glucose and carbachol that accelerate hydrolysis of endogenous AA from islet phospholipids also results in accelerated Kv2.1 inactivation and a shorter time-to-peak current interval. Group VIA phospholipase A<sub>2</sub> (iPLA<sub>2</sub> $\beta$ ) hydrolyzes  $\beta$ -cell membrane phospholipids to release nonesterified fatty acids, including AA, and inhibiting iPLA<sub>2</sub> $\beta$  prevents the muscarinic agonist-induced accelerated Kv2.1 inactivation. Furthermore, glucose and carbachol do not significantly affect Kv2.1 inactivation in  $\beta$ -cells from iPLA<sub>2</sub> $\beta^{-/-}$  mice. Stably transfected INS-1 cells that overexpress iPLA<sub>2</sub> $\beta$  hydrolyze phospholipids more rapidly than control INS-1 cells and also exhibit an increase in the inactivation rate of the delayed rectifier currents. These results suggest that Kv2.1 currents could be dynamically modulated in the pancreatic islet  $\beta$ -cell by phospholipase-catalyzed hydrolysis of membrane phospholipids to yield non-esterified fatty acids, such as AA, that facilitate Ca<sup>2+</sup> entry and insulin secretion.

Glucose metabolism within the pancreatic islet  $\beta$ -cell generates a multitude of signals that regulate insulin secretion. Changes in the relative concentrations of the metabolites ATP and ADP cause  $\beta$ -cell depolarization via closure of ATP-sensitive potassium channels (K<sub>ATP</sub>)<sup>3</sup>, which results in activation of voltage-operated Ca<sup>2+</sup> channels, Ca<sup>2+</sup> influx, and insulin secretion (1). The extent of  $\beta$ -cell depolarization and insulin release are regulated in part by

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the activation of repolarizing ion channels, including the voltage-gated potassium channel, Kv2.1 (2–4). One mechanism employed by pancreatic  $\beta$ -cells to regulate the biophysical activity of the ion channels involved in insulin release involves hydrolysis of membrane phospholipids to yield mediators that include inositol triphosphates and free fatty acids (5–8).

Pharmacologic, biochemical, and genetic evidence suggests that glucose-stimulated hydrolysis of esterified arachidonic acid from  $\beta$ -cell membrane phospholipids is required for physiological insulin secretion (7–25). Pancreatic islet  $\beta$ -cells contain high levels of arachidonic acid compared with other tissues (7,9–12,24,25), and about two-thirds of islet  $\beta$ -cell glycerophospholipids contain arachidonic acid as the *sn*-2 substituent (23–25). Islet phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzyme(s) activated by stimulation with glucose and/or by G-protein signaling mechanisms catalyze hydrolysis of arachidonic acid from  $\beta$ -cell membrane phospholipids and cause levels of free arachidonic acid to rise by up to 3-fold to the mid-micromolar range (7,13,14,27,28). Such concentrations of arachidonic acid amplify both the rise in  $\beta$ -cell [Ca<sup>2+</sup>] and insulin secretion induced by glucose (7,15–17,23,25,27,28).

Pharmacologic inhibition or molecular biologic suppression of expression of the pancreatic islet  $\beta$ -cell Group VIA phospholipase A<sub>2</sub> (iPLA<sub>2</sub> $\beta$ ) enzyme (24,29,30) results in attenuation of insulin secretory responses induced by glucose and other secretagogues (18–22,24). The products of iPLA<sub>2</sub> $\beta$  action on its phospholipid substrates include a nonesterified fatty acid, such as AA, and a 2-lysphospholipid, such as lysophosphatidylcholine (LPC), and  $\beta$ -cell levels of both products correlate with iPLA<sub>2</sub> $\beta$  expression level (21,22,31,32). Modulation of iPLA<sub>2</sub> $\beta$  activity and the rate of secretagogue-induced hydrolysis of arachidonic acid from  $\beta$ -cell membrane phospholipids might thus represent a tunable signal to amplify or attenuate islet insulin secretion in various (patho)physiologic settings (19,24).

The products of iPLA<sub>2</sub> $\beta$  action, including physiologically attainable concentrations of nonesterified AA, amplify secretagogue-induced rises in  $\beta$ -cell [Ca<sup>2+</sup>] (7,17,18,27,33), and several mechanisms have been proposed to explain these effects. These include facilitating Ca<sup>2+</sup> entry (7,17), perhaps by effects of AA on voltage-operated Ca<sup>2+</sup> channels (34), effects of AA and LPC on K<sub>ATP</sub> (35), effects of AA 12-lipoxygenase product(s) on an electrogenic plasma membrane Na<sup>+</sup>/K<sup>+</sup>-ATPase (36), and effects of LPC (37) on plasma membrane store-operated cation channels (38). Arachidonic acid modulates the activity of a number of ion channels, including K<sub>ATP</sub> channels and delayed rectifier K<sup>+</sup> channels (29–41). The effects of AA on  $\beta$ -cell intracellular [Ca<sup>2+</sup>] dynamics are likely to reflect the combined actions on such ion channels, possibly in concert with effects on G-protein-coupled receptor 40 signaling.

In this study, we investigated the effects of arachidonic acid and iPLA<sub>2</sub> $\beta$  on the Kv2.1-delayed rectifier channel of the pancreatic  $\beta$ -cell and on islet [Ca<sup>2+</sup>] dynamics. We find that incubating islet  $\beta$ -cells with exogenous arachidonic acid results in significant reduction of total Kv activity and increased glucose-stimulated islet [Ca<sup>2+</sup>] oscillations. Concentrations of glucose and carbachol that stimulate islet PLA<sub>2</sub> activity (13,14,18) are also demonstrated here to reduce Kv currents and thereby decrease repolarization. We observe similar reductions in Kv currents with a genetically modified INS-1 insulinoma cell line that overexpresses iPLA<sub>2</sub> $\beta$ . As far as we are aware, genetically modified cell lines with stably altered expression of iPLA<sub>2</sub> $\beta$  have not heretofore been used to examine the role of the enzyme in modulating the activity of specific  $\beta$ -cell ion channels involved in regulating [Ca<sup>2+</sup>] dynamics.

<sup>3</sup>The abbreviations used are: K<sub>ATP</sub>, ATP-sensitive potassium channels; Kv2.1, voltage-dependent delayed rectifier potassium channel; AA, arachidonic acid; iPLA<sub>2</sub> $\beta$ , Group VIA Phospholipase A<sub>2</sub>; INS-1, rat insulinoma cell line; HEK, human embryonic kidney cell line; LPC, lysophosphatidylcholine; GFP, green fluorescent protein; BEL, bromenol lactone; KRB, Krebs-Ringer buffer.

## EXPERIMENTAL PROCEDURES

### Isolation and Culture of Mouse, Rat, and Human Islets of Langerhans, Insulinoma Cells, and HEK Cells

Islets were isolated from pancreata of 1- to 5-month-old C57BL/J6 wild-type mice (Jackson Laboratories); of  $iPLA_2\beta^{-/-}$  mice, which were generated as described elsewhere (24); or of rats using collagenase digestion and Ficoll gradients as described previously (2). Human cadaveric islets were a generous gift from Dr. M. Garfinkel, University of Chicago. Islets were dissociated in 0.005% trypsin, placed on glass coverslips, and cultured for 16 h in RPMI 1640 medium supplemented with 10% fetal calf serum, concentrations of glucose specified in the figure legends, 100 IU/ml penicillin, and 100  $\mu$ g/ml streptomycin. INS-1 cells and HEK cells expressing Kv2.1-GFP (a generous gift from Michael Tamkun (42)) were cultured for 16 h in RPMI 1640 medium containing 11.1 mM glucose and supplemented with 10% fetal calf serum, 100 IU/ml penicillin, and 100  $\mu$ g/ml streptomycin. Cells and islets were maintained in a humidified incubator at 37 °C under an atmosphere of 95% air/5% CO<sub>2</sub>.

### Electrophysiological Recordings

Voltage-activated currents were recorded using whole-cell ruptured patch clamps with an Axopatch 200B amplifier and pCLAMP software (Molecular Devices, Sunnyvale, CA). Patch electrodes (2–4 micro-ohms) were loaded with intracellular solution containing (in mmol/liter) KCl, 140; MgCl<sub>2</sub>·6H<sub>2</sub>O, 1; EGTA, 10; Hepes, 10; MgATP, 5; pH 7.25 with KOH. Cells were perfused with a Krebs-Ringer buffer (KRB) containing (in mmol/liter) NaCl, 119; CaCl<sub>2</sub>·[(H<sub>2</sub>O)<sub>6</sub>], 2; KCl, 4.7; Hepes, 10; MgSO<sub>4</sub>, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; glucose, 14.4; adjusted to pH 7.3 with NaOH. When indicated, cells were incubated with arachidonic acid (Sigma) in ethanol (<0.3%). Ethanol (0.03%) treatment of cells alone had no effect on their KV currents. When indicated, cells were pretreated with a bromenol lactone (BEL)  $iPLA_2\beta$  inhibitor (43) obtained from Sigma and/or treated with carbachol (Sigma) in KRB with 20 mM glucose. Cells were also pretreated with the S or R enantiomers of BEL obtained from Cayman Chemical Co. (Ann Arbor, MI) in 20 mM glucose and then treated with carbachol. S-BEL inactivates  $iPLA_2\beta$  but not  $iPLA_2\gamma$ , and R-BEL inactivates  $iPLA_2\gamma$  but not  $iPLA_2\beta$  (71). The inactivation curves were fitted to the waveform with a single exponential function using Excel software and plotted with the standard error of the mean as a dashed line.

### Confocal Immunofluorescence Microscopy

An inverted microscope (Olympus, Tokyo, Japan) with a dual rotating-disk confocal scanner (CSU10; Yokogawa Electric, Tokyo, Japan) was used for confocal imaging of HEK cells expressing KV2.1-GFP. The cells were illuminated at 488 nm, and emitted light was filtered through a 530/30 nm filter (Omega Filters, Brattleboro, VT).

### Measurement of Cytoplasmic [Ca<sup>2+</sup>]

Mouse islets were incubated (20 min, 37 °C) in KRB supplemented with 5  $\mu$ mol/liter of Fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR). Fluorescence imaging was performed using a Nikon Eclipse TE2000-U microscope equipped with an epifluorescence illuminator and a 20 $\times$  fluorescence objective (Fryer Company Inc., Huntley, IL) and MetaFluor software (Molecular Devices, Sunnyvale, CA). Cells were perfused at 37 °C at a flow of 1 ml/min with appropriate KRB-based solutions that contained glucose concentrations specified in the figures with or without the indicated concentrations of arachidonic acid or tolbutamide. The ratios of emitted fluorescence intensities at excitation wavelengths of 340 and 380 nm ( $F_{340}/F_{380}$ ) were determined every 5 s with background subtraction.

## RESULTS

### Effects of Arachidonic Acid on $\beta$ -Cell Kv2.1 Channel Activity

Stimulation of pancreatic islet  $\beta$ -cells with glucose induces both the secretion of insulin and hydrolysis of arachidonic acid from membrane phospholipids, and the latter event is thought to facilitate  $\text{Ca}^{2+}$  entry and thereby to amplify insulin secretion (7,15,17,18,27,33,36,44). One mechanism whereby arachidonic acid might affect glucose-induced  $\text{Ca}^{2+}$  entry into  $\beta$ -cells is by modulating activity of delayed rectifier  $\text{K}^+$  channels that limit glucose-induced depolarization of the  $\beta$ -cell plasma membrane (2–4,40–42,45–51), and we therefore examined the effects of arachidonic acid on  $\beta$ -cell Kv2.1 channel activity.

As illustrated in Fig. 1A, whole cell recordings from rat INS-1 insulinoma cells show a typical Kv2.1-like delayed rectifier current when stimulated with depolarizing voltage steps. Maximal INS-1 Kv current amplitude at a depolarization step of 60 mV was significantly reduced by 33.4% upon incubation with 5  $\mu\text{M}$  arachidonic acid ( $n = 7$ ). INS-1 Kv currents also exhibited significantly accelerated current decline from their respective peak current amplitudes (Fig. 1, C and D) upon incubation with 5  $\mu\text{M}$  arachidonic acid. Arachidonic acid also significantly shortened by a mean of 8 ms the (“time-to-peak”) interval required to achieve peak INS-1 Kv current amplitude during a 30 mV depolarization (Fig. 2C). These effects of arachidonic acid are not use-dependent because Kv currents elicited after a 5-min incubation of cells with 5  $\mu\text{M}$  arachidonic acid when held at  $-80$  mV show effects similar those observed (20) with cells subjected to depolarizing steps during the 5 min period of incubation with arachidonate (Fig. 2B). There were also no significant changes in Kv current inactivation with continued depolarizing steps applied after a 5 min preincubation with 5  $\mu\text{M}$  arachidonic acid. The effects of arachidonate on Kv2.1 were rapid and occurred within 45 s.

To examine further effects of arachidonic acid on the activity of the Kv2.1 channel, which is the predominant delayed rectifier of  $\beta$ -cells (4,46), HEK cells expressing a Kv2.1-GFP construct (42) were studied (Fig. 1E). HEK cells alone have no Kv-like currents whereas HEK cells expressing Kv2.1-GFP show Kv currents similar to INS-1 cells. Like the INS-1 cells Kv2.1-GFP expressing HEK cells developed significantly reduced Kv current amplitudes with increases in their tau's of inactivation when incubated with arachidonic acid (Fig. 1E). The rate of Kv2.1 activation is not accelerated as significantly in HEK cells as in INS-1 cells. These results demonstrate that arachidonic acid has direct effects on Kv2.1 current amplitude and inactivation kinetics.

### Arachidonic Acid Regulates Delayed Rectifier Currents in Both Rodent and Human Pancreatic Islet $\beta$ -Cells

Because arachidonic acid affects the activity of Kv2.1 channels expressed in immortalized cell lines, we examined the possibility that similar effects would be observed with Kv currents in rodent and human primary  $\beta$ -cells. When incubated with 5  $\mu\text{M}$  arachidonate, both mouse and rat primary  $\beta$ -cells exhibited decreased Kv-like current amplitudes, increased rates of current inactivation, and slightly shorter time-to-peak current intervals than did cells incubated without arachidonic acid (Fig. 3, A and B). Human  $\beta$ -cells also displayed significantly reduced Kv-like current amplitudes and time-to-peak intervals when incubated with arachidonic acid, although the human Kv-like currents exhibited only a slight acceleration in decay rates upon incubation with arachidonic acid (Fig. 3C). These results suggest that arachidonic acid can modulate the activity of the pancreatic islet  $\beta$ -cell repolarizing delayed rectifier Kv currents that are activated upon depolarization of the  $\beta$ -cell plasma membrane.

## Arachidonic Acid Increases $[Ca^{2+}]$ Fluctuations of Rodent Islets of Langerhans

Because insulin secretion kinetics closely parallel islet  $\beta$ -cell  $[Ca^{2+}]$  fluctuations (1) and Kv2.1 participates in regulating  $\beta$ -cell  $[Ca^{2+}]$  (2), effects of arachidonate on  $\beta$ -cell  $[Ca^{2+}]$  were examined. Stimulation of mouse islets with 14.4 mM glucose induced typical  $[Ca^{2+}]$  oscillations, and their frequency was minimally increased upon incubation with 5  $\mu$ M arachidonic acid and returned to basal frequency after washout (Fig. 4A). Incubating islets with 10  $\mu$ M arachidonic acid induced a significant increase in  $[Ca^{2+}]$  oscillation frequency that returned to prestimulation levels after washout (Fig. 4B). Because arachidonic acid has also been found to modulate  $\beta$ -cell  $K_{ATP}$  channel activity as well as the sodium potassium ATPase ( $Na^+/K^+$ -ATPase; Refs. 36 and 39), we next examined effects of AA on islet  $[Ca^{2+}]$  fluctuations when  $K_{ATP}$  activity was blocked with tolbutamide alone or in combination with ouabain to block  $Na^+/K^+$ -ATPase activity. When islets were induced to undergo  $[Ca^{2+}]$  oscillations by stimulation with glucose, adding 250  $\mu$ M tolbutamide or 50  $\mu$ M ouabain resulted in an immediate and persistent rise in  $[Ca^{2+}]$  (Fig. 4C and supplemental Fig. 1). Subsequent addition of 10  $\mu$ M AA induced fast oscillations in  $[Ca^{2+}]$  (10 out of 15 islets, supplemental Fig. 1), which is an effect typical of Kv2.1 channel inhibition (47). These results suggest that AA modulates the activity of ion channels in addition to the  $K_{ATP}$  channel or the  $Na^+/K^+$ -ATPase that affect  $\beta$ -cell  $[Ca^{2+}]$ , such as Kv2.1.

## The Muscarinic Receptor Agonist Carbachol Modulates Kv2.1 Currents in $\beta$ -Cells

The muscarinic agonist carbachol amplifies both arachidonic acid hydrolysis from pancreatic islet  $\beta$ -cell membrane phospholipids and insulin secretion (14,18,44), and we examined the effects of carbachol on  $\beta$ -cell Kv currents. As illustrated in Fig. 5, B and C, Kv currents recorded from INS-1 cells stimulated with carbachol (500  $\mu$ M) displayed a significantly shorter time-to-peak current interval and an increased rate of inactivation compared with currents recorded before stimulation. This suggests that muscarinic receptor occupancy modulates  $\beta$ -cell Kv channel activity. The previously suggested possibility that a  $PLA_2$  participates in muscarinic agonist effects on  $\beta$ -cells (14,18) is consistent with our observation that pretreatment of INS-1 cells with the  $iPLA_2\beta$  inhibitor BEL (10  $\mu$ M; Ref. 43) resulted in a significant reduction in the carbachol-induced increase in the rate of Kv inactivation (Fig. 5, D and F), although the BEL-treated cells still exhibited a slight reduction in the time-to-peak Kv current interval upon stimulation with carbachol (Fig. 5D). Pretreatment of cells with the  $iPLA_2\gamma$ -selective (71) enantiomer of BEL (R-BEL), which has little effect on  $iPLA_2\beta$  activity, did not influence carbachol-induced inactivation of INS-1 Kv currents ( $n = 7$ ; supplemental Fig. 2, A and C), whereas the  $iPLA_2\beta$ -selective enantiomer (S-BEL) (71) still prevented Cch induced Kv inactivation ( $n = 6$ ; supplemental Fig. 2, B and D).

To examine the physiological effects of  $iPLA_2\beta$  on  $\beta$ -cell Kv currents, studies were performed with islet  $\beta$ -cells isolated from  $iPLA_2\beta$ -null mice, which were prepared as described elsewhere (24). Because phospholipase C is also activated by Cch, these experiments utilized intracellular calcium buffering to prevent phospholipase C activation. Carbachol and glucose were applied to control and  $iPLA_2\beta^{-/-}$  mouse pancreatic islet  $\beta$ -cells, and Kv currents were then recorded (20). The control  $\beta$ -cell Kv currents exhibit a significant left shift in their time to peak response to 20 mM glucose, and this response is maintained upon addition of carbachol (500  $\mu$ M; Fig. 6A, inset). A left shift in Kv activation in response to glucose is also observed with  $iPLA_2\beta^{-/-}$   $\beta$ -cells (Fig. 6B, inset). Interestingly, 20 mM glucose causes a significant increase in inactivation in the control  $\beta$ -cells, and this effect is significantly augmented upon addition of carbachol (500  $\mu$ M; Fig. 6, A and C, thin black line and thick gray lines, respectively). The  $iPLA_2\beta^{-/-}$   $\beta$ -cells, however, failed to exhibit significant changes in Kv inactivation with glucose (20 mM) in the presence or absence of carbachol (500  $\mu$ M; Fig. 6, B and D, thin black line and thick gray lines, respectively). These results suggest that muscarinic receptor



occupancy increases  $\beta$ -cell Kv current inactivation by iPLA<sub>2</sub> $\beta$ -regulated mechanism(s), and this results in a shorter time to peak Kv current response.

### Phospholipase A<sub>2</sub> Regulates Delayed Rectifier Current Activity

iPLA<sub>2</sub> $\beta$  is expressed by islet  $\beta$ -cells from rats (29), humans (47), and mice (48), and by several insulinoma cell lines (31,38,39). Upon activation, iPLA<sub>2</sub> $\beta$  hydrolyzes the *sn*-2 substituent from phospholipid substrates, such as phosphatidylcholine, to yield a free fatty acid and a lysophospholipid, such as LPC (44,54,55). Because arachidonate is the most abundant *sn*-2 substituent in  $\beta$ -cell membrane phospholipids (23,25,26), activation of iPLA<sub>2</sub> $\beta$  upon stimulation of islets with glucose results in liberation of arachidonate as the preponderant free fatty acid (7). Stably transfected INS-1 cells that overexpress iPLA<sub>2</sub> $\beta$  (INS1-iPLA<sub>2</sub> $\beta$ ) exhibit amplified insulin secretory responses to glucose and other secretagogues (21,56). We therefore compared Kv activity in control INS-1 cells and those that overexpress iPLA<sub>2</sub> $\beta$  ("INS-1-iPLA<sub>2</sub> $\beta$  cells"), both treated with 20 mM glucose. As illustrated in Fig. 7, Kv currents from INS-1-iPLA<sub>2</sub> $\beta$  cells exhibit accelerated activation rates and a significant increase in decay rates, although the peak Kv-like current amplitudes do not differ significantly between the two cell lines. These results demonstrate that iPLA<sub>2</sub> $\beta$  expression level in  $\beta$ -cell lines correlates with their Kv-like channel activity.

## DISCUSSION

Concentrations of glucose that stimulate insulin secretion also induce phospholipid hydrolysis in pancreatic islet  $\beta$ -cells, and the resultant accumulation of phospholipid-derived mediators including AA is thought to amplify insulin secretion (11–20,23–25,27,28,31–34,36,57,58). The results presented here demonstrate that AA modulates Kv2.1-delayed rectifier K<sup>+</sup> channel currents in  $\beta$ -cells, and these channels are thought to modulate glucose-induced fluctuations in  $\beta$ -cell [Ca<sup>2+</sup>] and insulin secretion (2–4,40–42,45–51). Whole cell patch clamp recordings of delayed rectifier currents elicited by application of graded degrees of depolarization to  $\beta$ -cells incubated with AA display a shorter time-to-peak current interval, a decrease in peak current amplitude, and accelerated inactivation compared with control cells. The iPLA<sub>2</sub> $\beta$  expressed in pancreatic islet  $\beta$ -cells hydrolyzes AA esterified in membrane phospholipids (18–22,24,31,32,36), and we find that overexpression of iPLA<sub>2</sub> $\beta$  in  $\beta$ -cells mimics the effects of exogenous AA on Kv-like currents. Furthermore,  $\beta$ -cells that do not express iPLA<sub>2</sub> $\beta$  or  $\beta$ -cells treated with pharmacological inhibitors of iPLA<sub>2</sub> $\beta$  have attenuated muscarinic agonist-induced Kv channel inactivation. These findings suggest that iPLA<sub>2</sub> $\beta$  might participate in dynamic regulation of the repolarizing effects of Kv2.1 channel activation in  $\beta$ -cells.

Arachidonic acid causes other slowly inactivating delayed rectifier K<sup>+</sup> channels, such as Kv3.4, to resemble fast inactivating A-type K<sup>+</sup> currents (41). The phospholipid content of esterified AA is higher in pancreatic islets than in other tissues (23,25,26,29), and arachidonate is hydrolyzed from phospholipids upon stimulation of islets with secretagogues (13,14,27,31,36,44) and accumulates to micromolar concentrations (7,27,28) that are sufficient to modulate delayed rectifier Kv channel activity (41). We find that incubating rodent and human pancreatic islet  $\beta$ -cells with low micromolar concentrations of AA results in accelerated inactivation of their delayed rectifier Kv-like currents (Figs. 1 and 2). Kv2.1 is the primary  $\beta$ -cell-delayed rectifier potassium channel (4,46), and  $\beta$ -cells incubated with AA secrete more insulin when stimulated than do cells incubated in the absence of AA (7). This response mimics the effects of Kv2.1 blockade under similar conditions (28,29). These results suggest that AA might modulate the duration of stimulus-induced depolarization in  $\beta$ -cells through reduction in the repolarizing function of Kv2.1.

The kinetics of insulin secretion from islets closely parallels  $\beta$ -cell [Ca<sup>2+</sup>] fluctuations (1), and Kv2.1 participates in regulating [Ca<sup>2+</sup>] in  $\beta$ -cells stimulated with glucose (2). Secretagogue-

induced hydrolysis of AA from  $\beta$ -cell phospholipids (7,13,14,27,28) is also thought to facilitate  $\text{Ca}^{2+}$  entry (15–17,18,33) and thereby to amplify insulin secretion (44). The frequency of glucose-induced  $[\text{Ca}^{2+}]$  oscillations in islets increases when Kv2.1 channels are inhibited (47). This results in a reduction in the rate of  $\beta$ -cell repolarization, increased duration of stimulated depolarization, and amplification of insulin secretion (47). Similarly, incubating islets with AA results in an increased frequency of glucose-induced  $[\text{Ca}^{2+}]$  fluctuations (Fig. 4B).  $\text{K}_{\text{ATP}}$  channels are partially inhibited by AA (39), and this could contribute to the increase in  $[\text{Ca}^{2+}]$  fluctuations when islets are incubated with AA (39). AA causes increased islet  $[\text{Ca}^{2+}]$  fluctuations even when  $\text{K}_{\text{ATP}}$  channels are inhibited with tolbutamide, however, which supports our findings that modulation of Kv2.1 activity could also participate in effects of AA on  $\beta$ -cell  $[\text{Ca}^{2+}]$  fluctuations.

Arachidonate is the most abundant *sn*-2 substituent of islet phospholipids from rodents and humans (23,25,26) and accumulates to greater levels than do other fatty acids in secretagogue-stimulated islets (7,27). The  $\text{iPLA}_2\beta$  enzyme is expressed by islets from rats, mice, and humans (20,24–26) and by several insulinoma cell lines (31,52,53), and  $\text{iPLA}_2\beta$  catalyzes the hydrolysis of the *sn*-2 ester bond of phospholipids to yield a free fatty acid, such as arachidonic acid, and a 2-lysophospholipid, such as LPC (54,55). Secretagogue-stimulated hydrolysis of AA from  $\beta$ -cell phospholipids is suppressed by the  $\text{iPLA}_2\beta$  inhibitor BEL (18,52,53), and overexpression of  $\text{iPLA}_2\beta$  in insulinoma cells causes a rise in  $\text{PLA}_2$  product levels and is associated with amplification of insulin secretory responses (21,56). Changes in local levels of nonesterified AA within the phospholipid bilayer could affect the activities of proteins in membranes. AA facilitates  $\text{Ca}^{2+}$  influx into  $\beta$ -cells (17,18,33) and has been suggested to influence ion movement through pumps and channels (34–36). Our observations suggest that Kv2.1 might represent one such channel. The  $\text{PLA}_2$  product AA blocks the activity of these channels, and overexpression of  $\text{iPLA}_2\beta$  in insulinoma cells is associated with amplification of insulin secretion and suppression of Kv2.1 channel activity, which is consistent with previous observations that blockade of Kv2.1 activity by other means also results in increased insulin secretory responses (48,49).

The muscarinic agonist carbachol amplifies both glucose-induced insulin secretion and eicosanoid release that reflects hydrolysis of arachidonate from islet membrane phospholipids (14,18,44), and the  $\text{iPLA}_2\beta$  inhibitor BEL blunts both responses (18). Carbachol also induces accumulation of inositol trisphosphates (18) and diacylglycerol (14) in islets by a BEL-insensitive mechanism that probably involves coupling of the muscarinic receptor with a G-protein that activates a phospholipase C. Accumulation of diacylglycerol in phospholipid bilayers facilitates  $\text{PLA}_2$  activation (63,64) and phospholipids hydrolysis to yield free fatty acids, such as arachidonic acid or linoleic acid (50,51), near Kv2.1 channels might affect channel activity. We observe that carbachol shortens the depolarization-induced time-to-peak Kv2.1 current and accelerates current inactivation in INS-1 cells and primary mouse  $\beta$ -cells; the latter effect is prevented by the  $\text{iPLA}_2\beta$  inhibitor BEL or in cells from genetically modified mice that do not express  $\text{iPLA}_2\beta$ . These findings suggest that  $\text{iPLA}_2\beta$  catalyzes formation of product(s) that negatively regulate the repolarizing effects of  $\beta$ -cell Kv2.1 channels.

Kv1.1 is a delayed rectifier K channel related to Kv2.1 that is expressed at high levels in the central nervous system, and its activity is affected in a manner similar to that reported here by the  $\text{iPLA}_2\beta$  inhibitor BEL (65). This raises the possibility that regulation of delayed rectifier, voltage-gated K channels by  $\text{iPLA}_2\beta$ , or products of its action might be a phenomenon that is not confined to  $\beta$ -cells. Kv1.1 channel activity (66) and subcellular location (67) are also affected by free fatty acids, and it is possible that colocalization or direct interaction of  $\text{iPLA}_2\beta$  and voltage-gated K channels, such as Kv1.1 and Kv2.1, occur.

$\beta$ -Cell iPLA<sub>2</sub> $\beta$  subcellular redistribution is known to occur upon stimulation (21), and iPLA<sub>2</sub> $\beta$  undergoes some established interactions with other signaling proteins (68). Interactions with additional proteins are suspected (69), and iPLA<sub>2</sub> $\beta$  has an ankyrin-repeat domain (29) and a Smad-4-like protein interaction domain (30) that might mediate interaction with a variety of signaling partners or the assembly of multimeric protein aggregates (70) that allow local, direct delivery of the products of iPLA<sub>2</sub> $\beta$  action to effector molecules regulated by them. In  $\beta$ -cells, such molecules could include ion channels like Kv2.1.

The evolution of type II diabetes is associated with changes in blood free fatty acids that have been linked to insulin resistance, including a rise in concentrations of AA and a fall in linoleic acid levels (59–61). Prolonged exposure of  $\beta$ -cells to AA stimulates their proliferation and amplifies secretagogue-induced insulin release (62), both of which also occur during the evolution of type II diabetes. Similarly, overexpression of iPLA<sub>2</sub> $\beta$  in insulinoma cell lines is associated with increased proliferation rates and amplified secretory responses (21,56), and suppression of iPLA<sub>2</sub> $\beta$  expression is associated with reduced proliferation rates and insulin secretion (22). The progressive rise in insulin resistance during the evolution of type II diabetes requires development of a compensatory hypersecretion of insulin to maintain glucose homeostasis, and a rise in AA levels might stimulate such a response. The deterioration of glucose tolerance induced in mice fed a high fat diet is exacerbated in iPLA<sub>2</sub> $\beta$ -null mice, and pancreatic islets isolated from these mice also fail to develop the exaggerated insulin secretory responses observed with islets isolated from wild-type mice (20). This suggests that iPLA<sub>2</sub> $\beta$  participates in  $\beta$ -cell compensatory responses to increasing insulin resistance, and our results suggest that modulation of Kv2.1 channel activity by fatty acids could be one of the steps involved in such  $\beta$ -cell compensation.

In conclusion, our results indicate that AA negatively regulates the activity of the delayed rectifier potassium channel Kv2.1 in  $\beta$ -cells. Reducing Kv current prolongs stimulus-induced depolarization of the  $\beta$ -cell plasma membrane and thereby amplifies insulin secretion (46, 48). This could account for the observations that overexpression of iPLA<sub>2</sub> $\beta$  in insulinoma cells is associated with both amplified insulin secretion (21) and reduced Kv2.1 channel activity (Fig. 5) and for the facts that pharmacologic suppression of iPLA<sub>2</sub> $\beta$  activity is associated with reduced insulin secretory responses (19) and increased Kv2.1 channel activity (Fig. 6). This series of observations suggests that modulation of  $\beta$ -cell Kv2.1 channel activity by product(s) of iPLA<sub>2</sub> $\beta$  action might be an important signaling event in the regulation of insulin secretion and in  $\beta$ -cell compensatory responses to the progression of insulin resistance.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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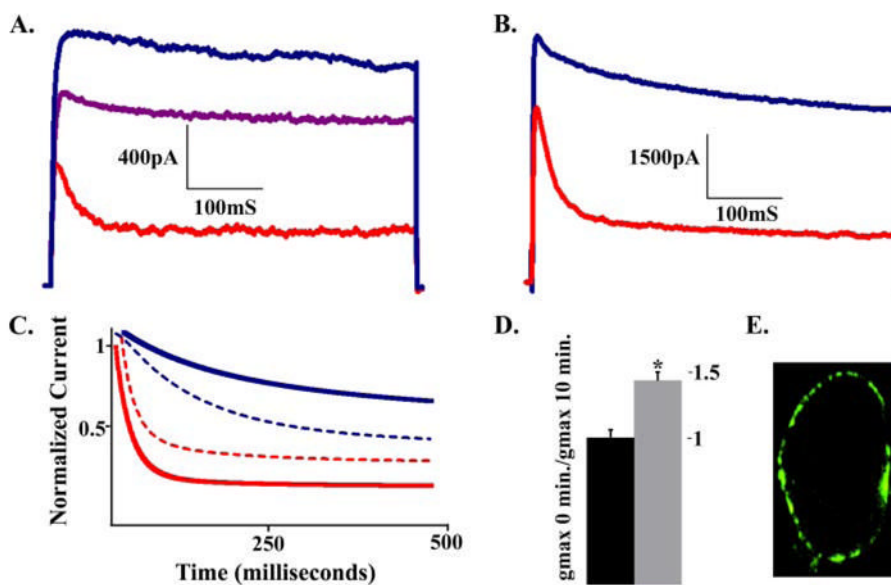
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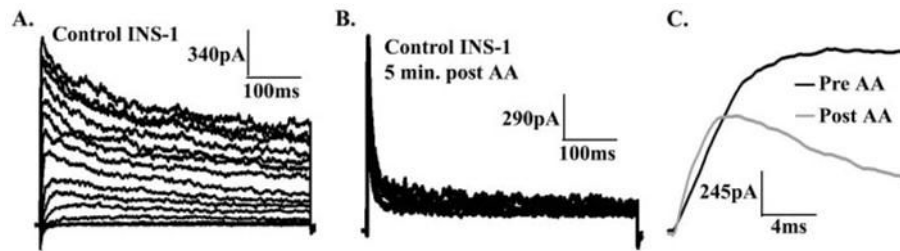
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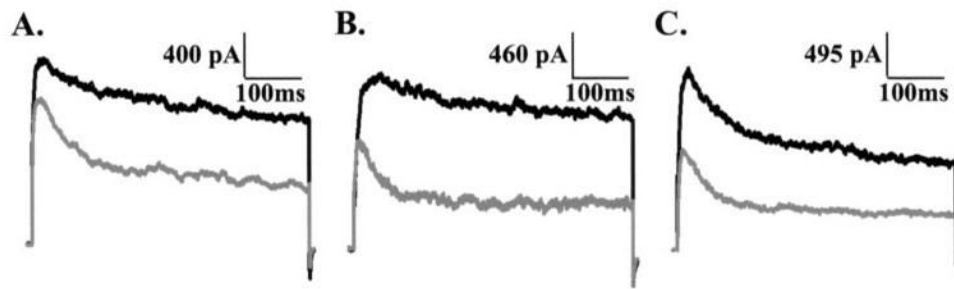
**FIGURE 1. Arachidonic acid negatively regulates  $\beta$ -cell Kv2.1 channel activity**

A, Kv currents from an INS-1 cell recorded at +60 mV before (*blue trace*) or after (*red trace*) 10 min of incubation with 5  $\mu$ M arachidonic acid or 15 min (*purple trace*) after washout of arachidonic acid. B, Kv currents recorded from a HEK cell expressing Kv2.1 at +60 mV before (*blue trace*) and 10 min after (*red trace*) incubation with 10  $\mu$ M arachidonic acid. C, decay curves of Kv current traces from INS-1 cells at +60 mV before (*blue line*) or after (*red line*) incubation with 5  $\mu$ M arachidonic acid. *Dashed lines* represent standard errors of the mean ( $n = 7$ ). D,  $G_{max}$  changes before and after INS-1 incubation with vehicle (*black bar*) or arachidonic acid (*gray bar*); displayed values are the mean  $\pm$  S.E. ( $n = 7$ ,  $p < 0.05$ ). E, immunofluorescent confocal-microscopic localization of a Kv2.1-GFP construct expressed in HEK cells.



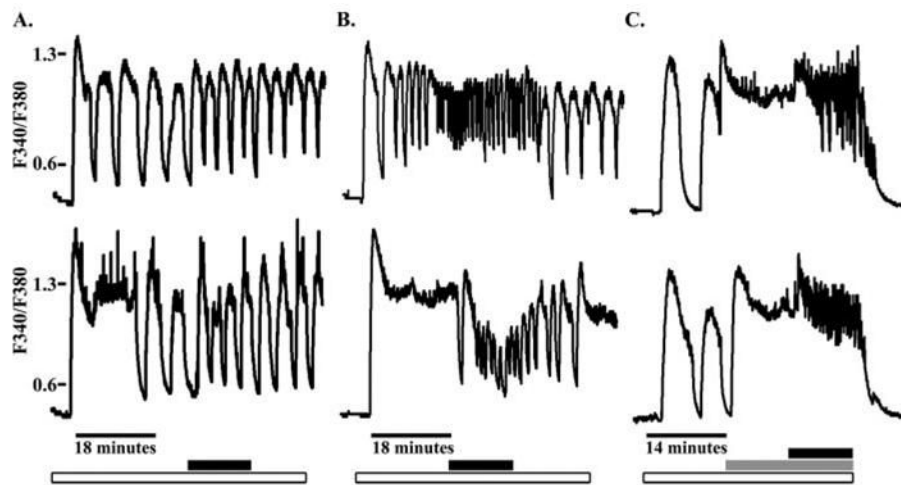
**FIGURE 2. Arachidonic acid affects  $\beta$ -cell-delayed rectifier currents in a use-independent manner**  
A, control INS-1 cell-delayed rectifier currents elicited by depolarization for 500 ms in 10-mV steps over the range from  $-70$  to  $+80$  mV. B, current recordings from the same INS-1 cell in response to depolarization for 500 ms in 10 mV increments over the range from  $+30$  to  $+80$  mV after 5 min of incubation with  $5 \mu\text{M}$  AA. C, INS-1 KV current traces recorded  $+30$  mV before (*black line*) or 10 min after (*gray line*) incubation with  $5 \mu\text{M}$  arachidonic acid.



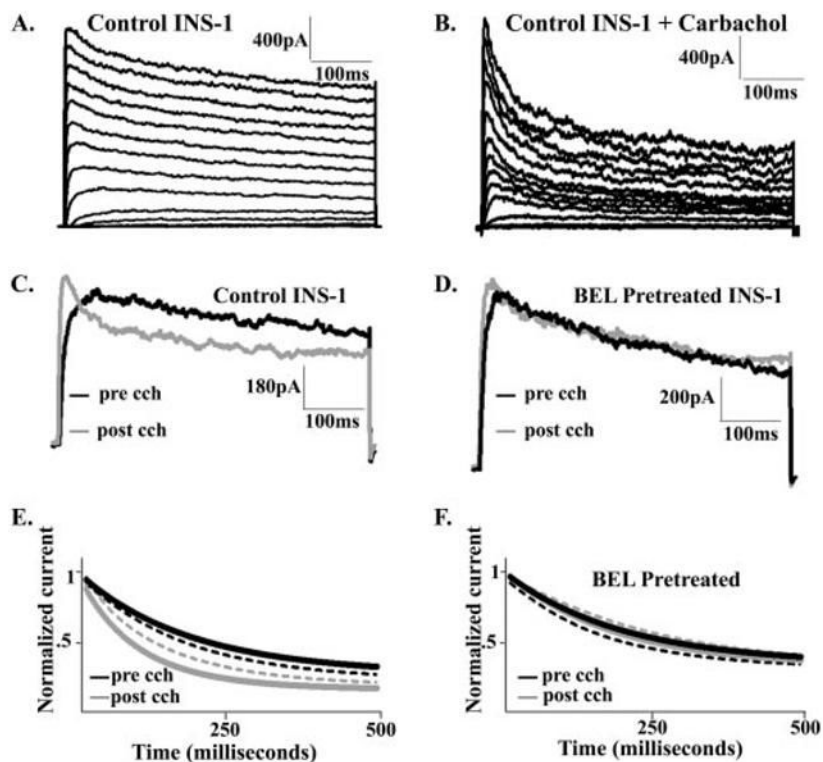


**FIGURE 3. Arachidonic acid affects delayed rectifier currents of primary  $\beta$ -cells prepared from rodent and human pancreatic islets**

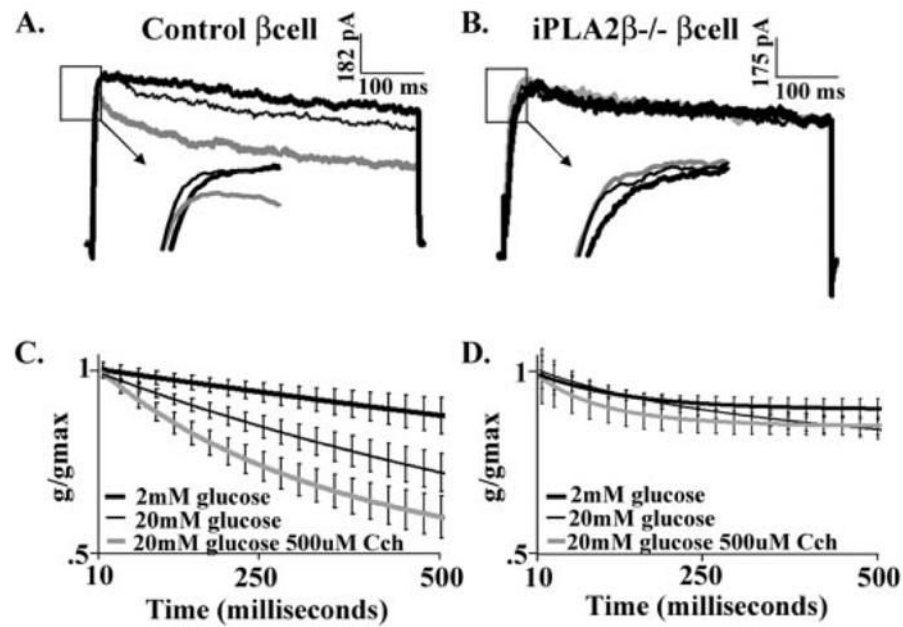
Delayed rectifier currents recorded during a 500 ms depolarization step at +60 mV with primary  $\beta$ -cells isolated from rat (A), mouse (B), or human (C) islets before (*black traces*) or 10 min after (*gray traces*) incubation with 5  $\mu$ M arachidonic acid.



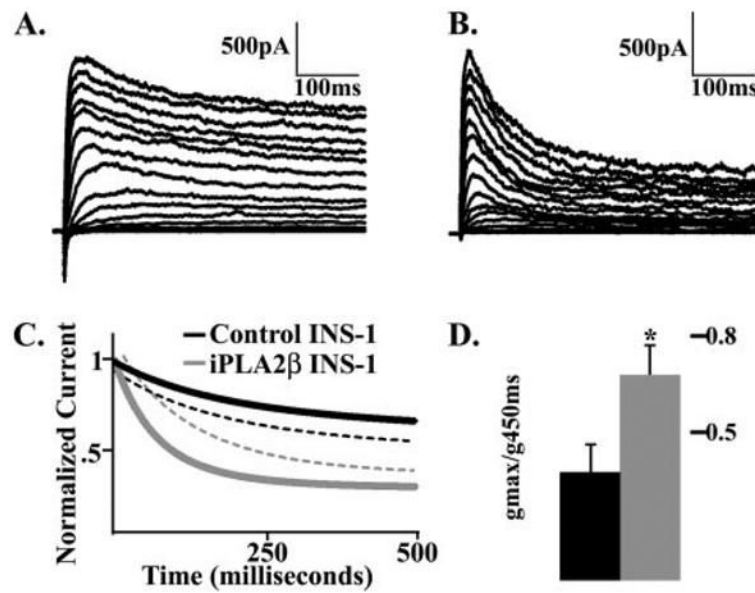
**FIGURE 4. Arachidonic acid affects glucose-induced  $[Ca^{2+}]$  oscillations in mouse pancreatic islets**  
*A*, representative calcium traces recorded with mouse islets loaded with Fura-2 and incubated with 14.4 mM glucose (*white bar*) in the presence (*black bar*) or absence of 5  $\mu$ M arachidonic acid. *B* and *C*, calcium traces recorded with mouse islets incubated with 14.4 mM glucose alone (*white bar*) or in combination with 250  $\mu$ M tolbutamide (*gray bar*) and 10  $\mu$ M arachidonic acid (*black bar*). The intervals in the recordings with no bars represent calcium from islets incubated with 2 mM glucose and no tolbutamide or arachidonic acid.



**FIGURE 5. Effects of the muscarinic agonist carbachol on INS-1 cell-delayed rectifier currents**  
**A**, traces recorded from an INS-1 cell subjected to 500-ms depolarization in 10-mV increments from  $-70$  to  $+80$  mV. **B**, traces recorded from the same INS-1 cell as in **A** 10 min after incubation with carbachol ( $500 \mu\text{M}$ ). **C**, traces from an INS-1 cell subjected to a 500 ms depolarization step from  $-80$  to  $-5$  mV. **D**, traces from an INS-1 cell pretreated with  $10 \mu\text{M}$  BEL and then subjected to a 500-ms step from  $-80$  to  $-5$  mV before (*black trace*) and 10 min after incubation with  $500 \mu\text{M}$  carbachol (*gray bar*). **E**, decay curves of Kv current traces from an INS-1 cell subjected to 500-ms depolarization at  $+60$  mV before (*black line*) and after (*gray line*) incubation with  $500 \mu\text{M}$  carbachol. *Dashed lines* represent standard errors of the mean ( $n = 7$ ). **F**, Kv current decay curves from INS-1 cells that had been pretreated with BEL as in **D** and then subjected to 500-ms depolarization at  $+60$  mV before (*black line*) and after (*gray line*) incubation with  $500 \mu\text{M}$  carbachol. *Dashed lines* represent standard errors of the mean ( $n = 6$ ).



**FIGURE 6. Delayed rectifier currents from  $iPLA_2\beta^{-/-}$  and control mouse  $\beta$ -cells**  
 A, Kv currents recorded from a control mouse  $\beta$ -cells treated with 2 mM glucose (*thick black line*), 20 mM glucose (*thin black line*), and 20 mM glucose and 500  $\mu$ M carbachol (*thick gray line*) during a 500-ms step from  $-80$  mV to  $-5$  mV. B, Kv currents recorded from  $iPLA_2\beta$  null mouse  $\beta$ -cell treated with 2 mM glucose (*thick black line*), 20 mM glucose (*thin black line*), and 20 mM glucose and 500  $\mu$ M carbachol (*thick gray line*) during a 500-ms step from  $-80$  mV to  $-5$  mV. *Insets* of A and B depict the expanded current traces from their respective *boxed* areas. C and D, Kv current decay curves from control (C;  $n = 8$ ) and  $iPLA_2\beta$  null (D;  $n = 7$ )  $\beta$ -cells cells treated with 2 mM glucose (*thick black line*), 20 mM glucose (*thin black line*), and 20 mM glucose and 500  $\mu$ M carbachol (*thick gray line*),  $\pm$  S.E. black bars.



**FIGURE 7. Delayed rectifier currents from INS-1 cells that express different levels of iPLA<sub>2</sub>β.** *A*, delayed rectifier currents recorded from control INS-1 cells subjected to 500 ms of depolarization at  $-70$  to  $+80$  mV in 10-mV increments. *B*, delayed rectifier currents recorded from stably transfected INS-1 cells that overexpress iPLA<sub>2</sub>β and treated as in *A*. *C*, Kv current decay curves recorded from control INS-1 cells (*black trace*) or from stably transfected INS-1 cells that overexpress PLA<sub>2</sub>β (*gray trace*) subjected to a 500-ms depolarization from  $-80$  to  $+60$  mV. *Dashed lines* represent standard errors of the mean ( $n = 6$ ). *D*, change in  $G_{\max}$  observed with control INS-1 cells (*gray bar*) or with stably transfected cells that overexpress iPLA<sub>2</sub>β (*black bar*) subjected to a 500-ms depolarization step at  $+60$  mV. Mean values ( $\pm$ S.E.,  $n = 6$ ) are displayed ( $p < 0.05$ ).