

β -Cell–Specific Protein Kinase A Activation Enhances the Efficiency of Glucose Control by Increasing Acute-Phase Insulin Secretion

Kelly A. Kaihara,¹ Lorna M. Dickson,¹ David A. Jacobson,² Natalia Tamarina,¹ Michael W. Roe,³ Louis H. Philipson,¹ and Barton Wicksteed¹

Acute insulin secretion determines the efficiency of glucose clearance. Moreover, impaired acute insulin release is characteristic of reduced glucose control in the prediabetic state. Incretin hormones, which increase β -cell cAMP, restore acute-phase insulin secretion and improve glucose control. To determine the physiological role of the cAMP-dependent protein kinase (PKA), a mouse model was developed to increase PKA activity specifically in the pancreatic β -cells. In response to sustained hyperglycemia, PKA activity potentiated both acute and sustained insulin release. In contrast, a glucose bolus enhanced acute-phase insulin secretion alone. Acute-phase insulin secretion was increased 3.5-fold, reducing circulating glucose to 58% of levels in controls. Exendin-4 increased acute-phase insulin release to a similar degree as PKA activation. However, incretins did not augment the effects of PKA on acute-phase insulin secretion, consistent with incretins acting primarily via PKA to potentiate acute-phase insulin secretion. Intracellular calcium signaling was unaffected by PKA activation, suggesting that the effects of PKA on acute-phase insulin secretion are mediated by the phosphorylation of proteins involved in β -cell exocytosis. Thus, β -cell PKA activity transduces the cAMP signal to dramatically increase acute-phase insulin secretion, thereby enhancing the efficiency of insulin to control circulating glucose. *Diabetes* 62:1527–1536, 2013

Glucose-stimulated insulin release is biphasic, characterized by an initial acute burst of insulin secretion followed by a sustained suprabasal release of insulin (1,2). The duration of the initial burst of insulin release, known as acute or first-phase insulin secretion, lasts 1.5–10 min (3). The sustained or second phase of insulin secretion persists as long as blood glucose levels remain elevated. During the first phase of glucose-stimulated insulin secretion, ~0.4% of insulin granules undergo exocytosis (4). This phase of insulin release, however, is absolutely critical in determining the efficiency of blood glucose clearance and does so by increasing the transendothelial transportation of insulin

into skeletal muscle, where up to 80% of glucose uptake occurs (5). Defects in acute insulin secretion have been found in individuals with type 2 diabetes, those with impaired fasting glucose, and those with a familial risk of type 2 diabetes (6–9). The association of impaired acute insulin release with the progression to type 2 diabetes underscores the significance of acute-phase insulin secretion in metabolic regulation. However, despite the physiological importance of acute-phase insulin release, its regulation and underlying molecular mechanisms remain unresolved.

Incretin-based therapies in patients with type 2 diabetes improve glucose control and metabolic health, although these effects may not be exclusively attributable to incretin actions on β -cells because of effects on other tissues that also improve glucose control. Nevertheless, incretins potentiate insulin secretion by increasing β -cell cAMP levels and result in the restoration and enhancement of acute- and sustained-phase insulin release. An increase in cAMP concentration activates the cAMP-dependent protein kinase (PKA) and guanine-nucleotide exchange protein activated by cAMP (EPAC) (10,11). Both PKA and EPAC have been implicated as the primary transducers of the cAMP signal to potentiate the acute phase of glucose-stimulated insulin secretion. This conflict may, at least in part, lie in the use of in vitro systems, indirect measurements of insulin secretion, and the use of pharmacological agents (12–17). Moreover, interpretation of insulin secretion and glucose control in studies using incretins are complicated by the effects of these hormones on other tissues. This prevents a clear understanding of the mechanism by which β -cell cAMP signaling regulates acute-phase insulin release, and so precludes the development of therapies to restore acute-phase insulin release to regain glucose control.

To avoid the problems associated with earlier studies and to gain a physiological insight to the function of β -cell PKA activity, a mouse model was developed to genetically increase PKA activity specifically in the islet β -cells. These mice used the recently developed MIP-CreERT strain to provide highly β -cell–specific gene induction and a “knock-in”-activated PKA catalytic subunit allele that retains expression under the endogenous control elements. These mice were used to determine the physiological role of β -cell PKA activity in potentiating glucose stimulated insulin secretion. Here, it is shown that an increase in PKA activity, targeted solely to the islet β -cells, strongly potentiates acute-phase insulin release in response to a single glucose bolus and, under hyperglycemic conditions, also enhances the sustained phase.

RESEARCH DESIGN AND METHODS

Generation and maintenance of mice. Heterozygous PKA-C α R mice (18) were crossed with heterozygous MIPCreERT mice (19). Wild-type, heterozygous

From the ¹Kovler Diabetes Center, Section of Adult and Pediatric Endocrinology, Diabetes, and Metabolism, Department of Medicine, The University of Chicago, Chicago, Illinois; the ²Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee; and the ³Departments of Medicine and Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, New York.

Corresponding author: Barton Wicksteed, wicksteed@uchicago.edu.

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See accompanying commentary, p. 1389.

PKA-C α R, heterozygous MIPCreERT, and β -C α R mice were born at expected Mendelian ratios and weaned by 28 days. At 10 weeks of age, all mouse genotypes received three intraperitoneal injections of tamoxifen dissolved in corn oil at 100 mg/kg body weight. Mice were under the day-to-day care of University of Chicago Animal Resources Center Facility staff according to the guidelines of The University of Chicago IACUC, with 2–5 animals per cage on a 12:12-h light-dark cycle.

Isolation of islets. Islets were harvested from tamoxifen-treated mice at 11–13 weeks of age by collagenase digestion, Histopaque (Sigma cat. 11191) density-gradient centrifugation, and hand-picking (20).

PKA analysis. PKA activity was measured in lysates prepared from islets of 13-week-old tamoxifen-treated mice using an in vitro phosphorylation assay (21) in the absence of added cAMP (for basal PKA activity) and in the presence of 5 μ mol/L exogenous cAMP (for maximal PKA activity). PKA activity values were corrected for values obtained in the presence of added PKA inhibitor PKI. Lysates of freshly isolated islets were resolved by SDS-PAGE and immunoblotted for PKA subunits using PI3kinase p85 as a loading control.

Glucose and arginine tolerance tests. Overnight-fasted mice were administered glucose intraperitoneally (1.0 g, 3.0 g, or 0.5 g /kg body weight), oral glucose (1.0 g/kg body weight), or arginine intraperitoneally (1.0 g/kg body weight). Blood was sampled from tail veins for glucose and insulin measurements, and mice were administered 5 μ g/kg body weight extendin-4 15 min before intraperitoneal glucose 2 g/kg body weight.

Hyperglycemic clamp. Hyperglycemic clamps were conducted at the Vanderbilt University Mouse Metabolic Phenotyping Center as previously described (22), with glucose clamped to 200 mg/dL.

Measurement of β -cell mass. Pancreata were stained for insulin and glucagon to identify islets of Langerhans. Images were collected on a Leica TCS SP2 and analyzed using ImageJ software. Total pancreatic insulin content was measured by ELISA (ALPCO) in extracts of the entire pancreas sonicated in 0.1 mol/L HCl.

Calcium imaging. Islets maintained at 37°C were preincubated (30 min, Krebs-Ringer bicarbonate HEPES buffer, 2 mmol/L glucose) and loaded with fura-2-AM on the stage of a NIKON TE2000 microscope. Measurements were acquired at 10-s intervals and data were expressed as the relative change in 340/380 ratio over the course of the 60 s before stimulation.

Perforated patch electrophysiology. Patch electrodes were used as previously described (23) to record β -cells identified by a resting membrane voltage near -70 mV. Reagents are listed in Supplementary Table 2

Statistical analysis. Data, expressed as means \pm SEM, were analyzed by Student two-sample unpaired *t* tests, one-way ANOVA, and two-way ANOVA with Bonferroni post hoc tests (GraphPad Software). *P* < 0.05 was considered significant.

RESULTS

Increasing PKA activity specifically in the β -cells.

β -Cell PKA activity was induced by crossing heterozygous PKA-C α R “knock-in” mice (18) with heterozygous MIP-CreERT mice and by administering tamoxifen to the offspring at 10 weeks of age (Supplementary Fig. 1), inducing efficient Cre-mediated, β -cell-specific recombination (Supplementary Fig. 2). This breeding strategy yielded four offspring genotypes. β -caPKA mice carry both the PKA-C α R allele and the MIP-CreERT allele, and have PKA activity that can be increased in the β -cells after tamoxifen administration. The other three genotypes, which were used as littermate controls, were the parental genotypes (PKA-C α R and MIP-CreERT) and wild-type mice. To determine the level to which PKA activity was altered in the β -cells of β -caPKA mice, islets were isolated from mice of all four genotypes and assayed for PKA activity using an in vitro assay (Fig. 1A) (21). In the presence of excess exogenous cAMP (5 μ mol/L), PKA activity in the islets of β -caPKA mice was similar to that of controls, showing that maximal islet PKA activity did not differ. In the absence of exogenous cAMP, islet PKA activity in β -caPKA mice was significantly elevated relative to littermate controls. In β -caPKA islet lysates, this cAMP-dependent PKA activity was 6% of the maximal value (obtained in the presence of 5 μ mol/L exogenous cAMP) compared with 0.6% in littermate controls, demonstrating enhanced basal PKA activity. Analysis of

Cre-mediated recombination (Supplementary Fig. 2) showed most β -cells underwent recombination. Immunoblotting revealed that expression of PKA subunits (the catalytic subunit, PKA-C α , and the 3 regulatory subunits, PKA-R1 α , PKA-R2 α and PKA-R2 β) was similar in islets from β -caPKA mice to the levels in littermates, indicating that altered PKA activity was not attributable to and does not feed-back on expression of PKA subunits (Fig. 1B). Although only one active PKA-C α allele is present in the PKA-C α R mice, PKA-C α levels were similar to levels in the wild-type, MIP-CreERT, and β -caPKA mice. This is most likely attributable to the PKA-C α subunit being highly unstable when not bound to regulatory subunits (24,25). Thus, R-C subunit interaction may provide a greater impact on catalytic subunit levels than gene expression.

β -Cell PKA enhances insulin secretion. To determine the effects of increased β -cell PKA activity on both phases of insulin secretion, hyperglycemic clamp and islet perfusion studies were performed (Fig. 2). The hyperglycemic clamps were performed using standard procedures to clamp circulating glucose at 200 mg/dL. Basal plasma insulin did not differ between β -caPKA and littermate control mice (1.35 \pm 0.06 ng/mL and 1.34 \pm 0.16 ng/mL, respectively; Fig. 2A). As glucose levels increased on the commencement of glucose infusion, insulin secretion during the acute phase was enhanced 2.5-fold in the β -caPKA mice compared with littermate controls (12.0 ng/mL/min vs. 4.8 ng/mL/min; *P* < 0.03). Although a standard glucose

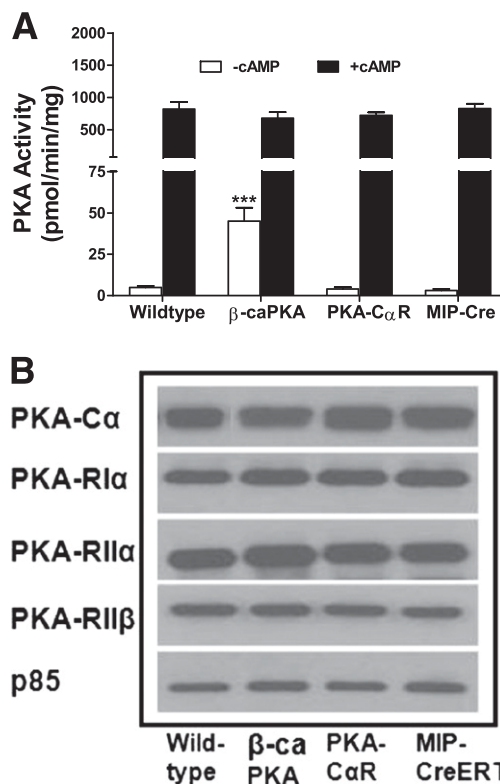


FIG. 1. Increased PKA activity in the islets of β -caPKA mice. **A**, PKA activity was measured in lysates prepared from islets of β -caPKA mice and control littermates using an in vitro assay. PKA activity was measured in the presence of 5 μ mol/L exogenous cAMP (maximal PKA activity) and in the absence of added cAMP. **B**, Lysates prepared from islets of mice of all four genotypes were analyzed by immunoblotting and probed for PKA catalytic (PKA-C α) and regulatory (PKA-R1 α , R2 α , and R2 β) subunits, and, for the loading control, PI-3 kinase p85 subunit (p85). One-way ANOVA, ****P* < 0.01.

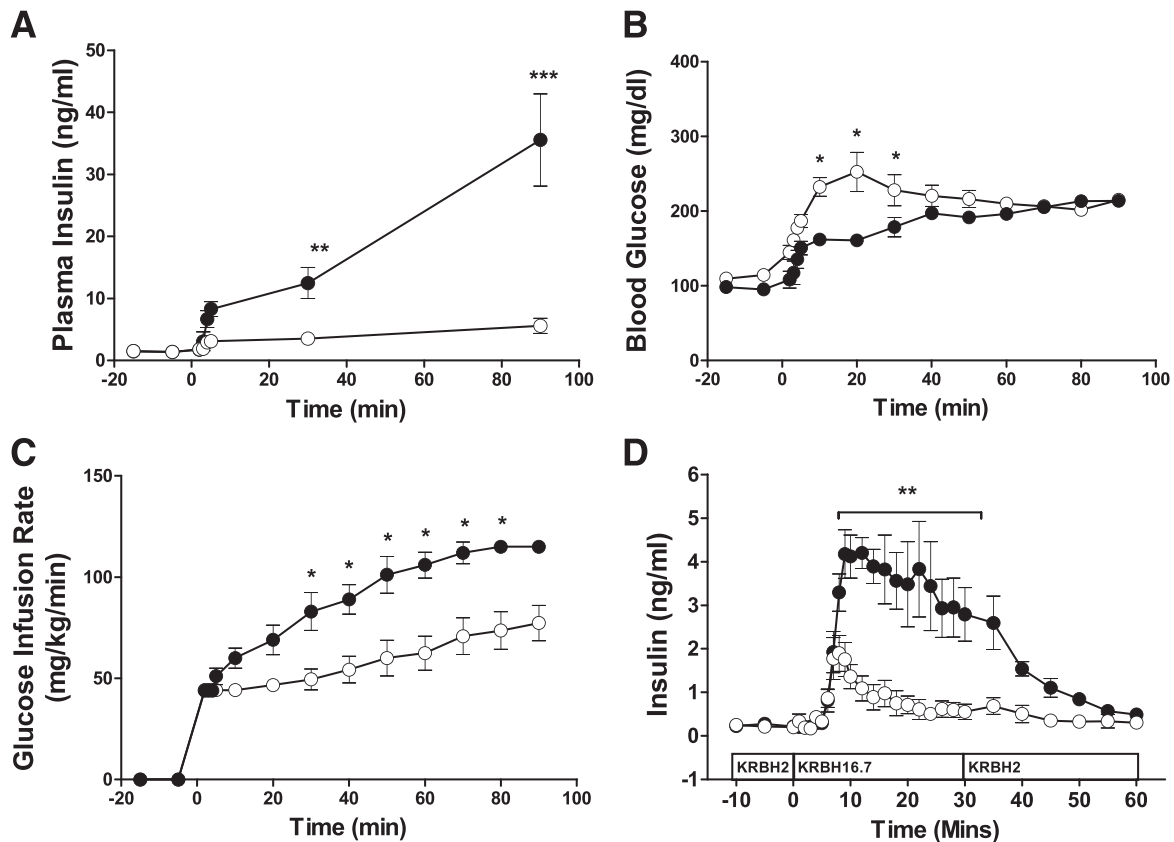


FIG. 2. Insulin secretion in response to sustained hyperglycemia. The effects of sustained elevated glucose levels were analyzed using hyperglycemic clamps and by perfusion of isolated islets of Langerhans. **A**, In the hyperglycemic clamp, glucose levels were matched between β -caPKA (●) and wild-type littermate control (○) tamoxifen-treated mice at 200 mg/dL. Plasma insulin levels were subsequently measured in blood samples. Blood glucose and glucose infusion rates (**B**, **C**) are shown. **D**, Perfusion of islets isolated from β -caPKA (●) and wild-type littermate controls (○) was performed in Krebs-Ringer bicarbonate HEPES buffered saline (KRHB) using a change from 2 mmol/L glucose to 16.7 mmol/L glucose to stimulate both acute and sustained insulin secretion. Two-way ANOVA with Bonferroni posttest analyses. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

infusion rate was used, this underestimated the dramatically enhanced insulin secretion of the β -caPKA mice (Fig. 2B, C). However, when circulating glucose in β -caPKA and littermate controls were matched at 200 mg/dL (90 min), the β -caPKA mice were shown to exhibit seven-fold higher circulating insulin than littermate controls (35.5 ± 7.4 ng/mL vs. 5.6 ± 1.2 ng/mL; $P < 0.001$; Fig. 2A). To confirm these data without the complication of insulin action on the maintenance of hyperglycemia and to determine whether these effects are islet autonomous, perfusion analyses were performed using islets isolated from tamoxifen-treated β -caPKA and littermate control mice (Fig. 2D). These perfusion analyses gave similar results as the hyperglycemic clamp study, with peak acute-phase insulin secretion elevated 2.4-fold in islets from β -caPKA mice relative to controls (4.17 ± 0.55 ng/mL vs. 1.75 ± 0.39 ng/mL; $P < 0.01$) and sustained-phase insulin increased 5.5-fold (32.54 ± 8.4 ng/mL/min vs. 5.91 ± 1.8 for the 20- to 30-min interval; $P < 0.05$). PKA activity potentiated glucose-stimulated insulin secretion but did not increase insulin secretion at substimulatory (basal) glucose concentrations, consistent with previous studies of cAMP effects on insulin secretion (26,27). These data show that the *in vivo* elevation of β -cell PKA activity strongly enhances the acute and sustained phases of insulin secretion.

PKA enhances acute-phase insulin proportionately to the glucose challenge. Acute-phase insulin release is critical for efficient glucose clearance. Here, β -caPKA mice

were used to determine the physiological effect of PKA activity on acute-phase insulin release and glucose control. At 10 weeks of age, β -caPKA and littermate controls were administered tamoxifen to induce PKA activity in β -caPKA mice. During the week before tamoxifen administration, the tolerance of the mice to a standard 1 g/kg i.p. glucose challenge was similar between β -caPKA and littermate control mice (Supplementary Fig. 3A, B). During the week after tamoxifen-mediated induction of the PKA-C α R allele, there was a specific enhancement of acute insulin release, with levels peaking 3.5-fold higher at 2 min in β -caPKA mice versus wild-type littermates ($2,318 \pm 504$ pg/mL in β -caPKA vs. 666 ± 119 pg/mL in wild-type littermates; Fig. 3C). By 10 min, insulin levels were not significantly different from those of controls. Thus, in response to the transient hyperglycemia induced by a glucose bolus, increased β -cell PKA activity specifically enhanced acute-phase insulin release. Although insulin secretion was not significantly stimulated in control mice, this was consistent with previously reported responses to a 1 g/kg i.p. glucose challenge (28–31) and is most likely attributable to a modest peak in insulin levels that is cleared by target tissues before sampling from the tail vein. Glucose tolerance in β -caPKA mice was enhanced, with glucose peaking lower and earlier (172 ± 13 mg/mL at 10 min) compared with littermate controls (257 ± 22 mg/dL at 20 min) and glucose exposure in β -caPKA mice being 58% of that in controls (Fig. 3H). Optimal glucose control requires insulin

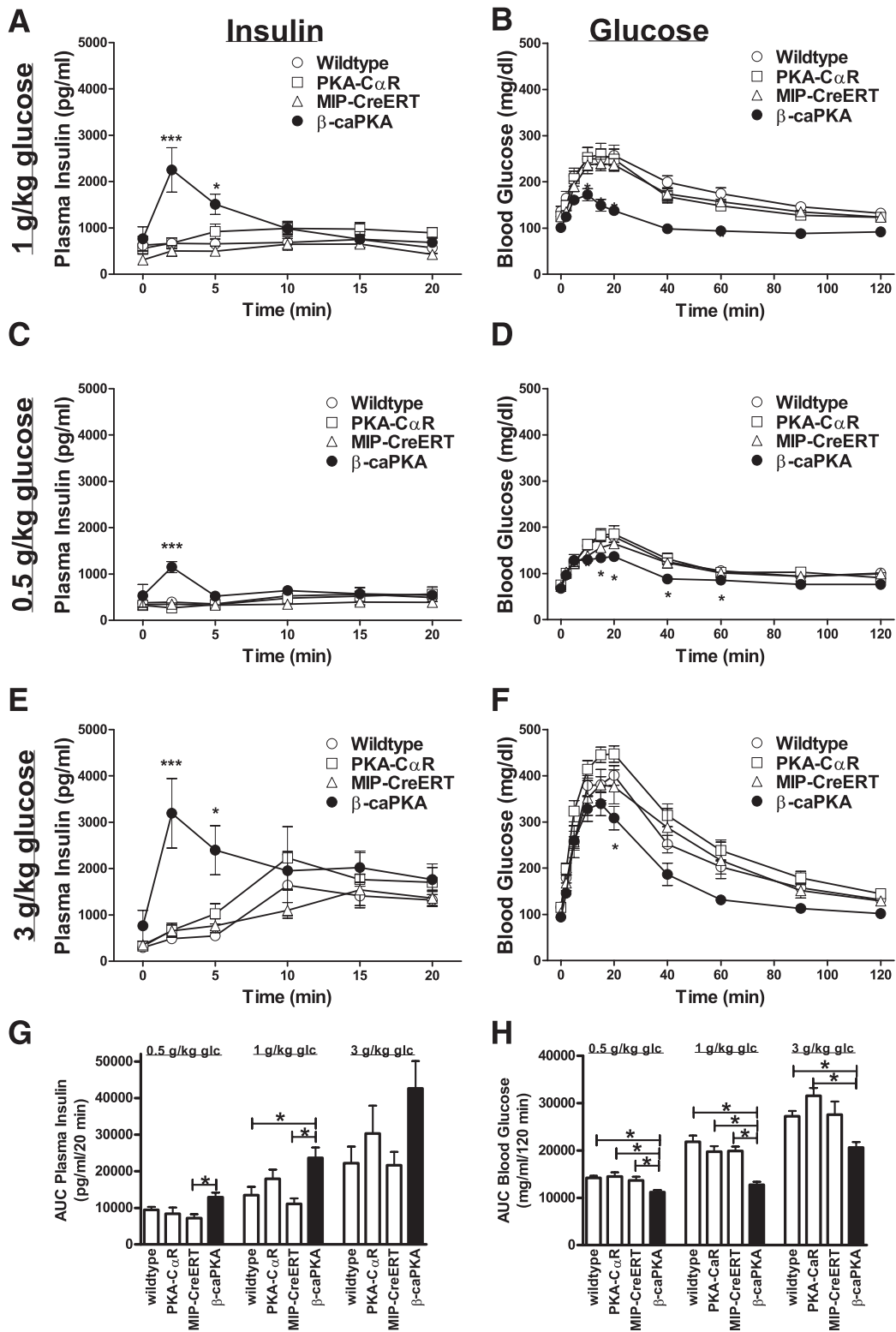


FIG. 3. Intra-peritoneal glucose tolerance tests. Circulating insulin (*A, C, E*) and glucose (*B, D, F*) were measured after intra-peritoneal glucose (1 g/kg, *A* and *B*; 0.5 g/kg, *C* and *D*; 3 g/kg, *E* and *F*) administration at 0 min. Plasma insulin was measured at 0, 2, 5, 10, 15, and 20 min and blood glucose was measured over the course of 120 min after glucose administration. β -caPKA, ●; wild-type, ○; PKA-C α R, □; MIP-CreERT, △. Area under the curve (AUC) for insulin and glucose were measured relative to baseline (y -axis = 0), and compared by Bonferroni posttest analyses (*G, H*). Two-way ANOVA showed significant difference between genotypes ($P < 0.001$) for (*A–E*). Bonferroni posttest analyses: * $P < 0.05$; *** $P < 0.001$.

to be delivered in a proportionate response to the circulating glucose. This permits glucose to be cleared efficiently and with minimal risk of hypoglycemia. To determine whether the PKA-mediated enhancement of the

acute insulin response was proportionate to the magnitude of the glucose challenge, mice were administered 0.5 g/kg or 3 g/kg glucose (Fig. 3*C–E*) and compared with the data obtained from mice administered 1 g/kg (Fig. 3*A, B*). At the

lowest dose of glucose (0.5 g/kg), insulin levels were elevated relative to littermates only at the 2-min time point, peaking at $1,147 \pm 120$ pg/mL. Increasing the magnitude of the glucose challenge increased peak insulin secretion at 2 min to $2,318 \pm 504$ pg/mL in response to 1 g/kg glucose and $3,192 \pm 749$ pg/mL in response to 3 g/kg glucose. Increasing the glucose challenge also prolonged the insulin secretory response. β -caPKA mice administered a 0.5 g/kg glucose challenge had insulin levels return to baseline by 5 min. In those receiving 1 g/kg glucose, insulin levels were still elevated at the 5-min time point and returned to baseline levels by 10 min. β -caPKA mice receiving 3 g/kg glucose showed a sustained response with insulin levels remaining elevated throughout the 20-min period. This model shows that PKA activity increases the acute insulin secretory response proportionately to the magnitude of the glucose challenge.

PKA activity affects β -cell function downstream of calcium entry. To determine whether the enhanced insulin secretory response to glucose was attributable to altered β -cell mass, β -cell area relative to pancreas area, islet number relative to pancreas area, and average islet size were measured. None of these parameters differed between β -caPKA and controls (Fig. 4A–C). Total pancreatic insulin content was also similar (Fig. 4D). These data indicate that the enhanced insulin secretion observed in β -caPKA mice is attributable to enhanced β -cell function and not attributable to increased β -cell mass. The effect of PKA on acute-phase insulin release (Fig. 3A) was consistent with PKA activity increasing the size of the readily releasable pool of insulin secretory granules. To test this hypothesis, mice were administered an arginine bolus (Fig. 5A) to depolarize the β -cells and release the readily releasable pool of insulin granules but not the reserve pool, which comprises the sustained phase. The arginine tolerance test showed an increase at 2 min in β -caPKA mice compared with controls ($3,364 \pm 666$ pg/mL vs. $1,270 \pm 256$ pg/mL, respectively). Thus, enhanced acute-phase insulin release in β -caPKA mice is, at least partly, attributable to a larger pool of granules in a readily releasable state. To determine whether cytosolic calcium influx was altered in response to increased β -cell PKA activity, whole islets isolated from β -caPKA and wild-type littermates were loaded with FURA-2. Imaging of these islet cells showed the expected increase in calcium in response to glucose over the 0- to 10-min acute phase, but calcium levels did not differ between β -caPKA and control mice (Fig. 5B). Likewise, 30 mmol/L KCl elicited a similar calcium influx in both β -caPKA and control islets (Fig. 5C). To determine whether β -cell electrical activity in response to glucose differs between β -caPKA and wild-type control littermates, β -cells from intact islets were analyzed by perforated patch electrophysiology. Membrane depolarization and action potential frequency was similar in β -caPKA and control islets (Fig. 5D and Supplementary Table 1). These data indicate that the effects of PKA activity on the acute phase of insulin release lie downstream of calcium signaling and plasma membrane depolarization.

Potentiation of acute insulin release by incretin hormones is PKA-dependent. Incretins provide potent new therapies to regain glucose control by potentiating both acute-phase and sustained-phase insulin secretion. These effects are, at least partly, achieved by increasing β -cell cAMP levels that will activate PKA. To compare the responses of insulin secretion to PKA activation and incretin hormones, two experiments were conducted. The

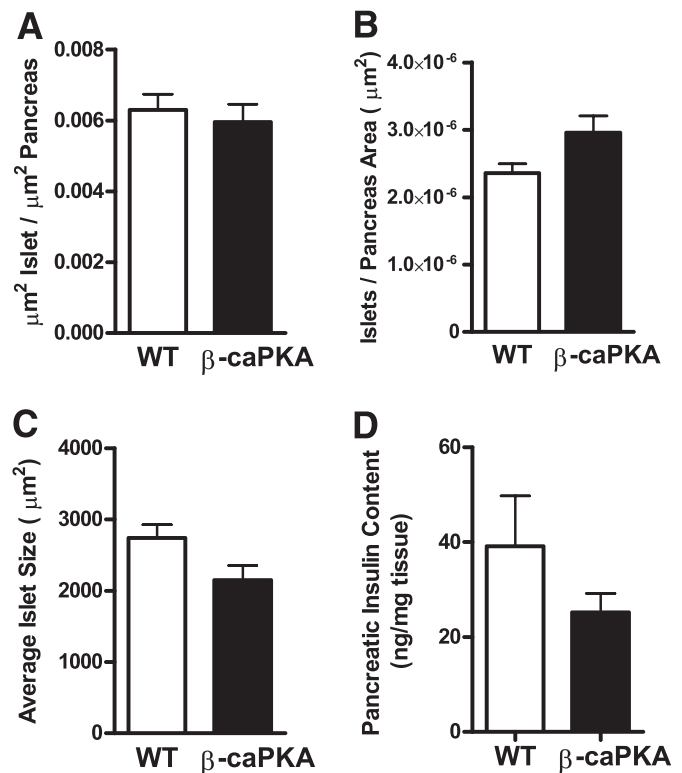


FIG. 4. Measurement of parameters of β -cell mass. Islet area relative to pancreas area (A), islet number relative to pancreas area (B), and average islet size (C) were measured on pancreatic sections prepared from paraformaldehyde-fixed/paraffin-embedded pancreata stained for insulin and glucagon. Total pancreatic insulin content (D) was measured in acid extracted pancreata by ELISA. Comparison between wild-type (WT) and β -caPKA littermates showed no significant differences ($P > 0.05$, t test).

first delivered oral glucose to induce the release of endogenous incretins (Fig. 6). The second used the administration of the GLP-1 receptor agonist, exendin-4 (Fig. 7). In control mice, oral glucose potentiated insulin release and improved glucose tolerance compared with intraperitoneal glucose delivery (Fig. 6A, B; $P < 0.05$). In contrast, oral delivery of glucose to β -caPKA mice did not augment the insulin release beyond that already potentiated by PKA and glucose tolerance was similar (Fig. 6C, D; $P > 0.05$). These data suggest that PKA is largely responsible for the incretin potentiation of insulin secretion at 2 min. However, a more potent activation of the cAMP signaling pathway in the β -cells may reveal PKA-independent effects. To achieve this, exendin-4 was administered at 5 $\mu\text{g}/\text{kg}$ body weight by intraperitoneal injection 15 min before a 2 g/kg i.p. glucose challenge to β -caPKA and control wild-type mice (Fig. 7A–D). This activation of the GLP-1 receptor increased acute-phase insulin release in control mice to levels similar to those in saline-treated β -caPKA mice. However, insulin secretion in β -caPKA mice was not potentiated beyond the level seen in saline-treated β -caPKA mice. Interestingly, exendin-4 treatment, but not PKA activation, increased insulin levels at later time points (10 and 15 min in control mice, 15 min in β -caPKA mice). Enhancement of sustained-phase insulin release with exendin-4 also led to glucose levels at 120 min declining below baseline values in both wild-type and β -caPKA mice (Fig. 7G). Overall, these data show that PKA activity is largely responsible for the acute insulin secretory

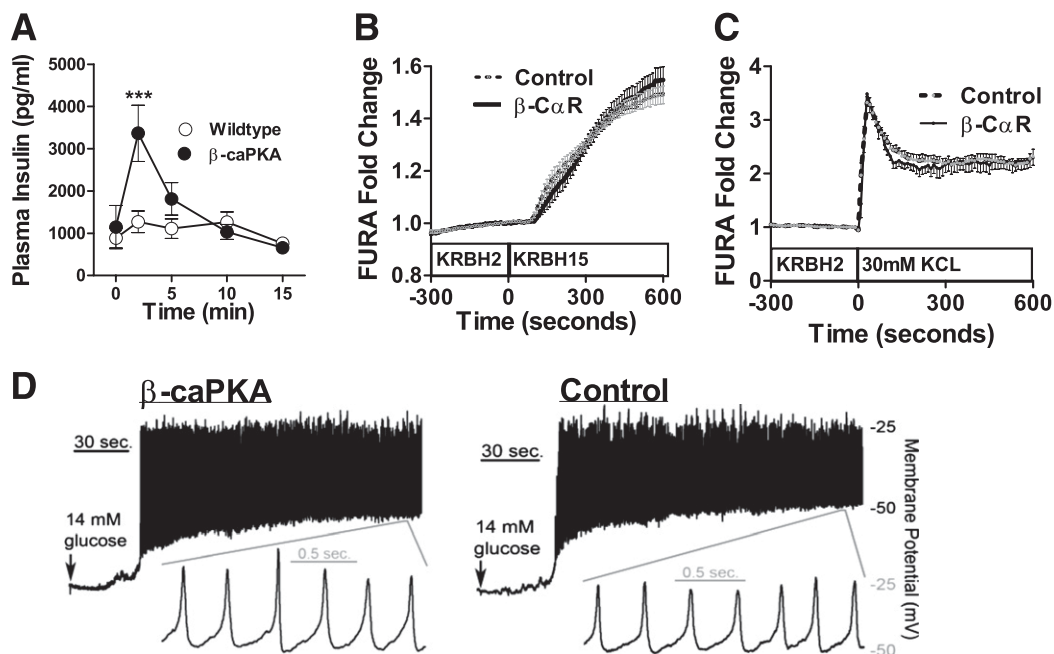


FIG. 5. Effects of β -cell PKA on the readily releasable granule pool and calcium signaling. *A*, Arginine tolerance tests were performed on overnight-fasted β -caPKA (●) and wild-type littermate control (○) mice by delivering 1 g/kg body weight i.p. L-arginine bolus in saline. *B* and *C*, Changes in cytoplasmic calcium was measured in response to a change from 2 mmol/L to 15 mmol/L glucose (*B*) and 30 mmol/L KCl in FURA-2-loaded islets isolated from β -caPKA (solid line) and control littermates (dotted line) mice. *D*, Membrane potential was measured by perforated patch electrophysiology in response to change in glucose from 2 mmol/L to 14 mmol/L glucose in islets isolated from β -caPKA (*left*) and control littermate (*right*) mice. Two-way ANOVA: *A*, $P < 0.001$; *B* and *C*, Not significant. Bonferroni posttest analysis: $***P < 0.001$.

response to incretins, but the GLP-1 receptor activation can prolong insulin secretion that may lead to an overshoot of glucose to levels below baseline values.

DISCUSSION

Progression to type 2 diabetes is characterized by a loss in β -cell function, a critical feature of which is reduced acute-phase insulin release (32,33). The acute phase of insulin release is a major determinant of the efficiency of glucose clearance because this initial burst of insulin accelerates insulin movement from the circulation to the interstitial space in skeletal muscle, where 80% of glucose disposal occurs (5). cAMP potentiates both acute-phase and sustained-phase insulin secretion (12,34–36) and provides a therapeutic target to restore glucose control. However, it is unclear whether cAMP regulates acute-phase insulin via PKA or EPAC, or both. Previous studies have relied on immortalized cell lines or dispersed primary cells, models that can exhibit altered phenotypes compared with β -cells in situ. Moreover, pharmacological agents for studying cAMP signaling pathways have significant off-target effects. The PKA inhibitors H89 and KT5720 inhibit a number of other kinases with similar or greater efficacy than PKA (37). The EPAC-specific cAMP analog, 8-pCPT-2'-O-Me-cAMP, also activates PKA, albeit with a 10-fold higher K_d than EPAC in cell free assays. In addition, 8-pCPT-2'-O-Me-cAMP inhibits phosphodiesterases 1, 2, and 6, and so this can increase cAMP and cGMP levels (38,39). Recently, a mouse model of activated β -cell PKA was reported that exhibited increased insulin secretion, supporting our findings, but the role of PKA activity specifically in the acute phase of insulin release in vivo was not studied (40). Moreover, PKA activity was driven by the pdx-Cre mouse strain, which directs Cre-recombinase expression to the

hypothalamus, as well as the β -cells (19), where it can have unintended effects on metabolism.

This study examined the role of β -cell PKA activity in regulating phasic insulin release using a tissue-specific and temporally regulated model. Two mouse strains were used: PKA-C α R mice, which carry a Cre-inducible activated PKA allele (18); and MIP-CreERT mice, which provide temporal regulation and β -cell specific Cre activity (19). An important aspect of this model was that the mutated PKA-C α allele (PKA-C α R) was targeted to the endogenous locus through a “knock-in” strategy. This ensured that integration did not affect expression of other genes and resulted in PKA-C α R expression at endogenous PKA-C α levels. The recently developed MIP-CreERT mice avoided central effects of PKA activation while providing a tamoxifen-inducible Cre system. This allowed tissue and temporal specificity of Cre expression, thereby avoiding developmental effects of increased PKA activity.

Induction of increased β -cell PKA activity potently increased both acute-phase and sustained-phase insulin release. However, a bolus delivery of glucose restricted the enhancement of insulin secretion solely to the acute phase. Enhanced acute-phase insulin delivered more efficient glucose clearance, with circulating glucose being reduced to 58% in β -caPKA mice relative to wild-type littermates (area under the curve, 1 g/kg glucose intraperitoneal glucose tolerance test; Fig. 3H), confirming the potency of acute-phase insulin in improving glucose tolerance. Acute-phase insulin release is responsive to the rate of change in circulating glucose. Thus, it is induced by increasing circulating glucose and acts to attenuate this increase in glucose. Here, in β -caPKA mice, the acute burst of insulin attenuates (2–5 min) before glucose peaks (5–10 min), consistent with the acute phase of insulin release being responsive to the rate of change of glucose, not to the absolute glucose

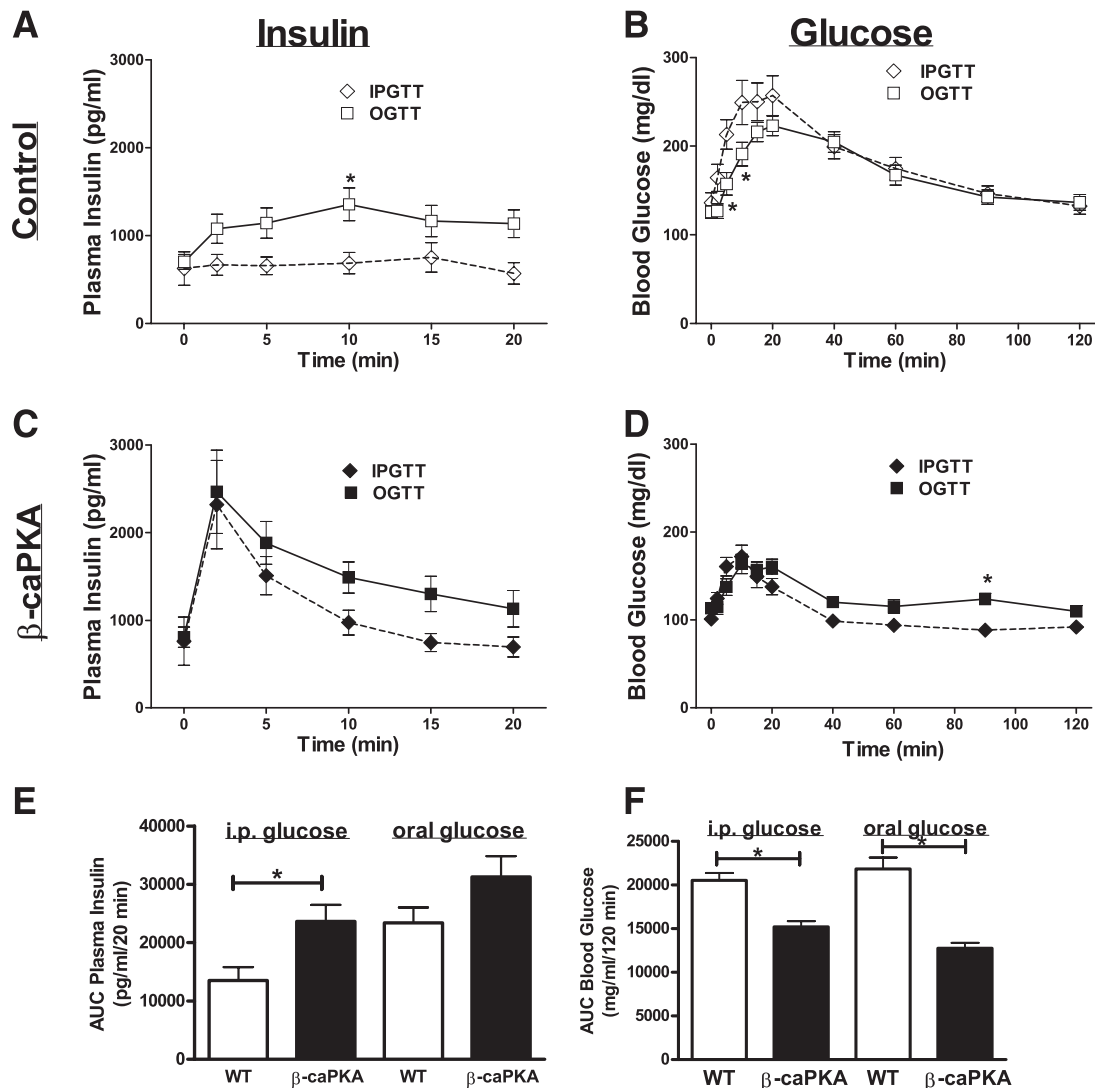


FIG. 6. Oral glucose tolerance tests (OGTTs). Overnight-fasted control littermates (*A, B*) and β -caPKA (*C, D*) mice were administered 1 g/kg body weight glucose by oral gavage at 0 min. Blood for blood glucose (*B, D*) and plasma insulin (*A, C*) was sampled from the tail vein. Intraperitoneal (i.p.) glucose tolerance test (IPGTT) data from Fig. 2 is provided as dotted lines for comparison. Plasma insulin was measured over the 0- to 20-min interval and blood glucose was measured over the 0- to 120-min interval. Area under the curve (AUC) was calculated for each curve, relative to a y-axis value of 0. IPGTT, diamonds; OGTT, squares; open symbols and bars, controls; closed symbols and bars, β -caPKA. Two-way ANOVA comparison of IPGTT and OGTT: $P < 0.05$ for (*A*); $P > 0.05$ for (*B–D*). WT, wild-type. Bonferroni posttest analysis: $*P < 0.05$.

concentration. In response to a modest glucose challenge (0.5 and 1.0 g/kg), this acute burst of PKA-induced insulin secretion is sufficient to normalize glucose. However, when the glucose challenge is sustained (the hyperglycemic clamp) or the glucose bolus is too large for acute-phase insulin to control (3 g/kg), then the sustained phase becomes essential to normalize circulating glucose.

The most promising therapies to restore biphasic insulin release are the incretin hormone-based therapies (34). The inability of oral glucose or exogenous exendin-4 to augment acute-phase insulin in β -caPKA mice (Figs. 6 and 7) indicates that incretins act via PKA to potentiate acute-phase insulin secretion. It seems unlikely that incretins act via other signaling molecules to potentiate the acute phase of insulin release in the presence of activated PKA. This is consistent with the EPACs not being involved in the acute response to incretin potentiation of insulin secretion, a conclusion that is supported by the EPAC2 knockout mice exhibiting no impairment of glucose control (41).

However, GLP-1 receptor activation prolonged insulin secretion beyond that seen with PKA activity alone, indicating that the potentiation of this phase of insulin release by incretins may be PKA-independent. Interestingly, although this sustained release of insulin brings down glucose levels more rapidly than PKA activity and reduces overall glucose exposure (Fig. 7*F*), glucose levels continue to decrease to below baseline values (Fig. 7*G*). These data reinforce that insulin secretion must be proportional to the glucose challenge, sufficient to control hyperglycemia but not so aggressive to risk hypoglycemia. Thus, PKA acts to enhance the efficacy of insulin release, promoting secretion at the acute phase, when insulin is most effective, while keeping insulin release tightly responsive to changes in circulating glucose.

The enhanced release of insulin in β -caPKA mice upon depolarization of β -cells in vivo by arginine shows that PKA acts downstream of calcium signaling to enhance acute-phase insulin secretion. This conclusion is supported

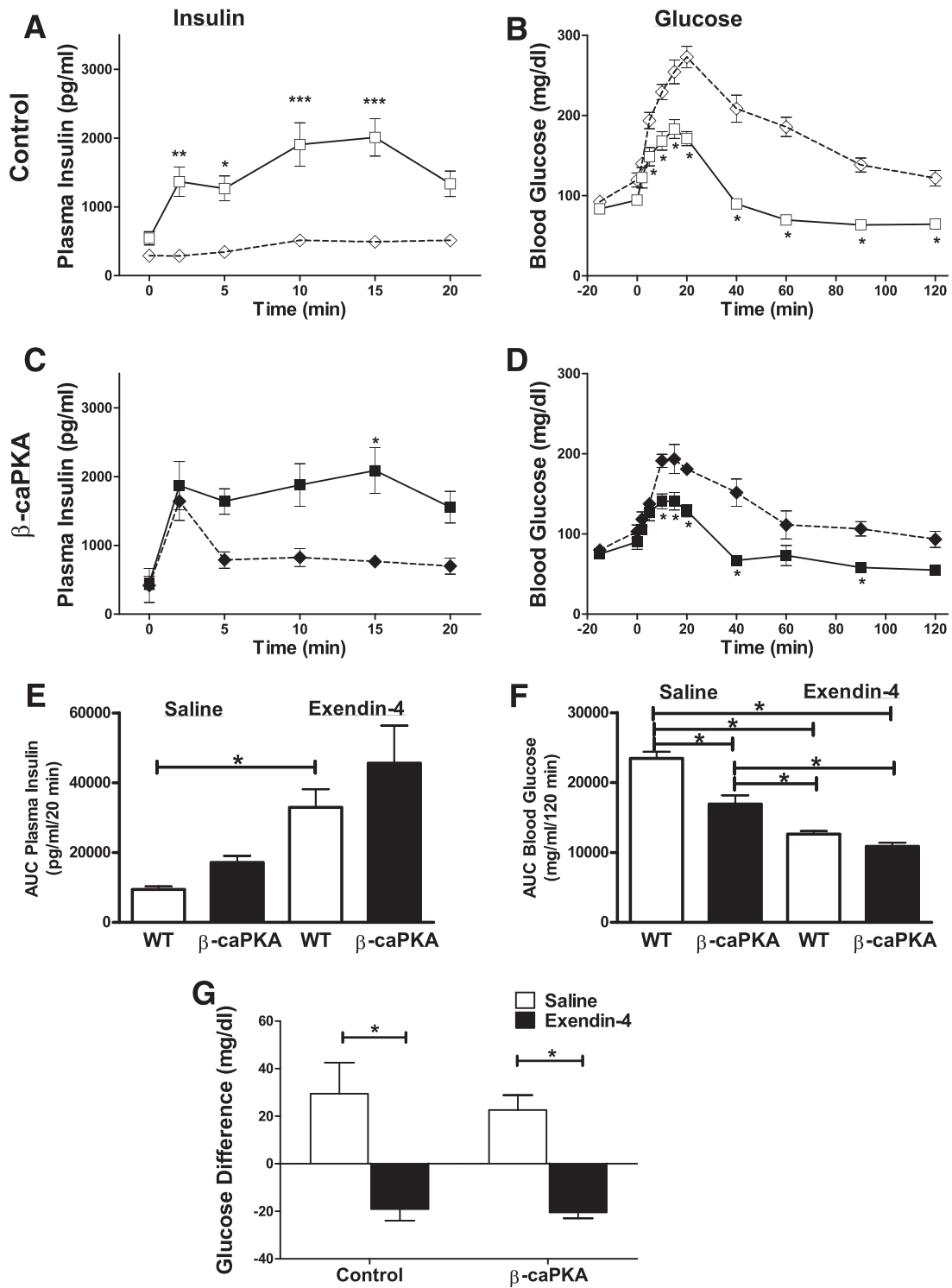


FIG. 7. Intra-peritoneal glucose tolerance tests (IGTTs) after exendin-4 administration. Overnight-fasted β -caPKA and wild-type (WT) littermate control mice were administered exendin-4 15 min before receiving an 2 g/kg i.p. glucose bolus. Plasma insulin was measured over the subsequent 20 min (0–20 min) and blood glucose was measured over the –15- to 120-min interval. Area under the curve (AUC) was calculated for each curve relative to a y-axis value of 0. Diamonds, saline treated; squares, exendin-4 treated; open symbols and bars, controls; closed symbols and bars, β -caPKA. Two-way ANOVA indicated significant difference between IGTT and oral glucose tolerance test ($P < 0.05$) in (A–D). Bonferroni posttest analysis: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

by calcium influx and membrane excitation being similar in β -caPKA and control mice (Fig. 5). The ability to rapidly activate the full potentiation of insulin secretion using exendin-4 15 minutes prior to a glucose bolus (Fig. 7) indicates that the effects of PKA activation do not lie in

chronic effects on the β -cells, for example, altered gene expression. Thus, it is most likely that PKA activity in the islet β -cells potentiates acute-phase glucose-stimulated insulin secretion by directly phosphorylating protein components of the insulin secretory machinery of the

β -cell. A number of these proteins have been identified as PKA targets, including SNAP25 (42,43), tomosyn-1 (44,45), tomosyn-2 (46), snapin (40), syntaxin-4 (47), and cysteine string protein (48–50). Consistent with this hypothesis, an increase in snapin phosphorylation was observed upon the expression of constitutive PKA activity (Supplementary Fig. 4). Molecular targets to therapeutically improve acute-phase release are unknown. This study reveals that targets of PKA activity regulate acute-phase insulin secretion in vivo and offer potential candidates for therapies to restore β -cell function. Although its ubiquitous expression makes PKA an unlikely therapeutic target, the data presented here show that G-protein-coupled receptors that activate PKA can enhance acute-phase insulin release. Future studies to understand the action of PKA upon the insulin secretory machinery are likely to identify proteins that can be targeted to restore acute-phase insulin release in the prediabetic or the diabetic state. Further studies also are needed to determine whether the downregulation of G-protein-coupled receptor signaling systems (including PKA) is associated with the loss of the acute-phase insulin response in the etiology of type 2 diabetes.

Overall, this study demonstrates that PKA activity controls acute-phase insulin release and is largely responsible for the effects of incretins on the acute phase of insulin release. In addition, PKA activity enhances sustained-phase insulin secretion. However, the data highlight that the augmentation of insulin secretion by PKA is tightly coupled to glucose levels and demonstrate that PKA activity enhances the efficiency of insulin release to control glucose in a safe manner that provides low risk for hypoglycemia.

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No potential conflicts of interest relevant to this article were reported.

K.A.K. and B.W. designed this study. K.A.K., L.M.D., D.A.J., and B.W. conducted the experimental research and analyzed the data. K.A.K., L.M.D., D.A.J., M.W.R., L.H.P., and B.W. contributed to the preparation of the manuscript. N.T., M.W.R., and L.H.P. provided the MIP-CreERT mice, an essential reagent for this study. B.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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