Amylin modulates β -cell glucose sensing via effects on stimulus–secretion coupling

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ABSTRACT The release of insulin from the pancreatic β cell is dependent upon a complex interplay between stimulators and inhibitors. Recently, amylin, a peptide secreted by pancreatic β cells, has been implicated in the development of type II (noninsulin dependent) diabetes through its modulation of the peripheral effects of insulin. However, the effect of amylin on insulin secretion from the β cell has remained controversial. It is reported here that in single β cells exhibiting normal glucose sensing, amylin causes membrane hyperpolarization, increases in net outward current, and reductions in insulin secretion. In contrast, in cells with abnormal glucose sensing (e.g., from *db/db* diabetic mice), amylin has no effect on electrical activity or secretion. Thus, amylin's effects on excitation–secretion coupling in the β cell of the pancreas appear to be linked to the cell's capacity for normal glucose sensing.

Although the mysteries underlying the effects of glucose and other secretagogues on pancreatic β -cell electrical and secretory activity are rapidly unfolding, the mechanisms by which hormones and peptides modulate these activities remain unclear. Certain neuropeptides, released by nerves supplying the pancreas, decrease insulin secretion. This inhibition involves coupling between receptors and ion channels (1). In contrast to such neurotransmitters, it is not known whether and how peptides secreted from the β cells themselves affect insulin secretion. Amylin, a 37-aa peptide, is colocalized with insulin in β -cell secretory granules of human islets (2) and is cosecreted with insulin. It is also the major component of pancreatic islet amyloid commonly found in the pancreases of patients with non-insulin-dependent diabetes mellitus (3). In this disease, loss of normal glucose sensing is one of the earliest defects. Although peripheral actions of amylin have been demonstrated, the influence of amylin on insulin secretion is still debated (4–7). Though the overall role of amylin in regulating insulin secretion and in the development of diabetes may remain unclear for some time, it is critical to determine the effects of amylin on stimulus–secretion coupling at the single-cell level. Thus, we have examined the effects of amylin on excitation and insulin secretion in single isolated pancreatic β cells that exhibit normal or abnormal glucose sensing.

METHODS

To determine the effects of both glucose and amylin on membrane electrical parameters of single β cells, we used the nystatin perforated patch technique (8) to record membrane potentials and ion currents. Detection of insulin secretion from single β cells was accomplished using a sequential-cell immunoblot method (9) whereby cells are stimulated to secrete insulin into peptide-retentive immunoblot material

and secreted hormone is quantified by standard densitometric methods. Details of these procedures follow.

A control strain of diabetic mouse (C57BL/KsJ-m+/+db, “*db* control”) and a diabetic strain (C57BL/KsJ-db+/+db, “*db/db*”) (the Jackson Laboratory) were used for single-cell and islet assays. Control *db* mice were 6–12 weeks of age and had an average weight of 22 g and a serum glucose of 118 mg/dl. Diabetic *db/db* mice of the same age weighed $\approx$ 50 g and had an average serum glucose level of 374 mg/dl. For rat islet experiments, Sprague–Dawley rats (200–250 g, Charles River Breeding Laboratories) were used. Pancreatic islets were selected by hand from dispersed pancreas obtained after digestion with collagenase (0.5 mg/ml). Islets were treated for 1 min with 0.05% trypsin and triturated to disperse them into single cells. Cells were plated on poly(L-lysine)-coated coverslips and cultured for 2–6 days (37°C, 5% CO₂/95% air) in RPMI 1640 medium/10% (vol/vol) fetal bovine serum/2 mM L-glutamine/2.5% Nu-Serum (Collaborative Research). Rat insulinoma (RIN-38) cells (kindly provided by W. L. Chick and S. A. Clark, University of Massachusetts) were maintained in medium 199/5% fetal bovine serum/1% penicillin/streptomycin and used from passages 20 to 22.

Membrane voltage and whole cell currents were measured using an Axopatch 1C amplifier (Axon Instruments, Burlingame, CA). Access resistance was monitored and series resistance compensation was performed as described (10). Currents were acquired by a 12-bit analog/digital converter (Indec Systems, Santa Cruz, CA) and analyzed with custom programs written in C. Data were also recorded on a pulse-code modulated videotape recorder and analyzed with AXOTAPE (Axon Instruments) software. The pipette solution contained 55 mM KCl, 95 mM potassium aspartate, 5 mM MgCl₂, 1 mM EGTA, and 20 mM Hepes, adjusted to pH 7.3. In addition, the electrode contained nystatin (50 μ g/ml), which was freshly prepared and sonicated every 2 hr. The bath solution was continuously perfused and contained 147 mM NaCl, 2 mM CaCl₂, 5 mM KCl, 1 mM MgCl₂, 5 mM Hepes, and glucose (0–20 mM), adjusted to pH 7.4. Electrophysiology experiments were performed at room temperature.

Rat amylin (Bachem) was stored lyophilized and used fresh in each experiment. Except in immunoblot experiments, amylin and bovine serum albumin (0.5 mg/ml) were dissolved into the external recording solution immediately prior to application to single cells via a U-tube (11). Amylin can precipitate from solution and adheres avidly to both plastics and glass, thus actual concentrations of the delivered peptide are likely lower than those reported. Tolbutamide, glibenclamide, and diazoxide were obtained from Sigma and were also applied to single cells via the U-tube.

Immunoblot experiments were performed using a modified sequential cell immunoblot assay (9). Coverslips with at-

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tached cells were preincubated for 1 h at 37°C in a modified Krebs–Ringer buffer containing 2 mM glucose. After preincubation, coverslips of cells were inverted and gently placed onto poly(vinylidene difluoride) transfer membranes (Millipore) with 30 μ l of a challenge solution separating each coverslip and membrane. Challenge solutions contained either glucose (2–10 mM) or glucose plus test drug. The coverslip–membrane “sandwiches” were incubated for 1 h at 37°C, trapping secreted insulin onto the transfer membranes. Coverslips were then gently removed from the membranes and allowed to incubate for 1 h in control conditions. In some experiments, coverslips were rechallenged on a second membrane. Cell density was periodically checked under phase optics during the blot sequence with subsequent fixing and Giemsa staining to match specific cells with insulin blots and confirm that sequential blots were in register. Serially diluted rat insulin (Novobiolabs, Bagsvaerd, Denmark) standards were run in parallel for each experiment. After hormone transfer, membranes were exposed (4 h) to Tris-buffered saline with 10% bovine serum albumin to block unoccupied binding sites. Then, sequentially, membranes were incubated with guinea pig anti-porcine insulin serum (1:40,000 dilution, Novo Biolabs, 12 h), biotinylated affinity-purified anti-guinea pig immunoglobulin (1:500 dilution, Jackson ImmunoResearch), and streptavidin-alkaline phosphatase conjugate (1:3000 dilution), and then with the color reagent for 3–10 min. Control experiments replacing antiserum with normal guinea pig serum or preabsorbing antiserum with excess insulin eliminated insulin reactivity. Light microscopic images of immunoreactive insulin were captured with a charge-coupled device camera (Dage-MTI) and digitally analyzed (Image 1, Universal Imaging). Gray levels from photonegative images of the insulin standards were background-subtracted to construct a calibration curve yielding insulin in femtograms per pixel. In some cases (e.g., see Fig. 6), relative gray level values are reported, as all values fell in the linear region of the calibration curve. Insulin secretion was also measured from whole pancreatic islets cultured for 24 h. After a preincubation in 2 mM glucose, islets (20 islets per well) were incubated for 90 min at 37°C in either glucose or glucose plus amylin. RIN-38 cell insulin assays were performed on cells plated for 72 h and then rinsed twice in modified Ringer’s solution (without serum). After preincubation in 0 mM glucose, RIN-38 cells were challenged with glucose or glucose plus amylin at 37°C in a humidified incubator. Insulin was measured by a scintillation proximity assay using protein A-bound fluoromicrospheres and ^{125} I-labeled insulin (Amersham).

RESULTS

Glucose Effects on Electrical Activity in Individual β Cells.

We first examined the glucose sensitivity of cells from *db* control and *db/db* mice for comparison with responses previously reported for β cells from these and other sources. To establish a reproducible baseline of activity, β cells were equilibrated in 0–2 mM glucose for 15–45 min prior to recordings. After equilibration, resting potentials in *db* control cells were -70 ± 6 mV (mean $\pm$ SD; $n = 86$). Elevating glucose in the bathing solution to 5–15 mM induced a gradual depolarization, which was followed by phasic bursts of action potentials (Fig. 1A and B). After this brief phasic activity, >50% of *db* control cells developed a maintained depolarization with continuous spike activity during exposure to the elevated glucose (Fig. 1B). Since the majority of cells responded robustly to 8 mM glucose with phasic bursting, this concentration was used for all subsequent comparisons. After return to low glucose, the cells recovered their control resting potentials (Fig. 1A).

FIG. 1. Electrical responses of single β cells to glucose or amylin. Membrane potentials were recorded via nystatin-permeabilized patches. Cells were equilibrated for 40 min in 2 mM glucose prior to the indicated solution changes. Open bars, transitions from 2 mM to 8 mM glucose. (A) A single *db* control β cell responds to and recovers from a U-tube application of 8 mM glucose. A recording gap of 8 min is represented by the break in the trace. (B) Glucose at 8 mM induced bursting and continuous spiking in another *db* control cell. Amylin (5 nM, solid bar) restored phasic bursting in this cell. (C) Glucose at 8 mM was without effect in a *db/db* cell, which was spontaneously active in 2 mM glucose. (D) Glucose induced depolarization and action potentials (but no phasic bursts) in another *db/db* cell, whereas amylin (500 nM, solid bar) slightly increased firing frequency.

In contrast to *db* control β cells, cells from *db/db* mice had resting potentials averaging -55 ± 11 mV ($n = 16$) in 2 mM glucose. Approximately half of these cells generated spontaneous action potentials in low glucose, even after 90 min of equilibration (Fig. 1C). Glucose induced either no effect (Fig. 1C) or a depolarization and simple increase in firing frequency (Fig. 1D), rather than the bursting pattern typical of *db* controls. Similar observations have been reported from microelectrode impalements in whole intact *db/db* islets (12), suggesting that neither single-cell dissociation nor perforated patch recordings altered glucose responsiveness.

Effects of Glucose on Currents in β Cells. Currents underlying the changes in membrane potential in β cells were evaluated by transiently switching from current clamp to voltage clamp recording mode. In *db* control cells exposed to low glucose (0–2 mM), a large K current that reversed direction near the K^+ equilibrium potential (E_K) was revealed by ramp command voltages (Fig. 2A and B). This current–voltage relationship was essentially linear between -100 and -20 mV. Elevating external glucose (Fig. 2A) or adding tolbutamide (Fig. 2B) reduced this current in *db* control cells, consistent with observations on ATP-sensitive K (K_{ATP}) currents in other pancreatic β cells (e.g., refs. 13 and 14).

In contrast to the *db* control cells, the linear component of K current (between -100 and -20 mV) in *db/db* cells was smaller in low glucose, consistent with the observed abnormal membrane potentials. Moreover, glucose induced only modest decreases in this current in *db/db* cells ($n = 15$) (Fig. 2C). The smaller current in low glucose does not reflect an absence of K_{ATP} channels, since diazoxide (500 μ M), which induced membrane repolarization in *db/db* cells (Fig. 2D *Inset*), increased the K current dramatically (Fig. 2D).

Effects of Amylin on β Cell Membrane Potential. After initiation of glucose-induced action potential activity in *db* control cells, the subsequent addition of amylin (0.1–500 nM) hyperpolarized 78% (25 of 32 cells) of cells within 15–90 s. Amylin either restored the original bursting pattern (Fig. 1B) or completely eliminated glucose-induced action potentials (Fig. 3A). After removal of amylin, only 60% of cells resumed their preamylin activity; therefore, single-cell dose–response curves were impossible to obtain. We observed, however,

FIG. 2. Current responses of single β cells to voltage-ramp commands (-100 to $+20$ mV). Slope conductances (g_s) were calculated between -100 and -40 mV. (A) Ramp currents in a *db* control cell obtained after the switch from current clamp to voltage clamp mode while monitoring membrane potential. Records obtained during the application of either 2 mM glucose ($g_s = 1.7$ nS) or 8 mM glucose ($g_s = 0.51$ nS) for at least 10 min. (B) Effects of tolbutamide ($30 \mu\text{M}$; $g_s = 0.33$ nS) on a *db* control β cell recorded in 0 mM glucose ($g_s = 3.4$ nS). Same cell as in A. (Inset) Effect of tolbutamide (open bar) on the voltage recording. (C) Ramp currents obtained from a *db/db* cell after prolonged exposure to 0 mM ($g_s = 0.65$ nS) or 8 mM ($g_s = 0.57$ nS) glucose. (D) Currents and voltage recording (Inset) in a second *db/db* β cell exposed to 8 mM glucose ($g_s = 0.11$ nS) or glucose plus diazoxide ($300 \mu\text{M}$, open bar, $g_s = 1.57$ nS). Conditions were identical for A–D, except as otherwise noted. (Insets) The horizontal time marker represents 1 min, and vertical increments are 40 mV. All cells were $12 \mu\text{m}$ in diameter.

that decreases in firing frequency and hyperpolarization induced by subnanomolar concentrations of amylin were qualitatively identical to those obtained at the highest concentrations tested, suggesting that amylin is effective at extremely low concentrations.

In contrast to the results in *db* control cells, amylin never hyperpolarized *db/db* cells. Only 2 of 16 *db/db* cells responded to amylin in any manner, and only then at the highest concentrations. In 1 of these cells, action potential frequency was slightly decreased, whereas in the other it was slightly increased (Fig. 1D).

Amylin Increases Outward Currents in *db* Control but Not *db/db* β Cells. In Fig. 3A, the current clamp recording was interrupted at time points a–d to apply 200-ms voltage ramps. The resulting current–voltage curves are shown in Fig. 3B and C. In 2 mM glucose, β cells exhibited a net outward current (trace a) at voltages more positive than the resting potential. Increases in external glucose, sufficient to induce bursts of action potentials, decreased outward current between the resting potential and 0 mV, and produced a net inward current, which peaked at approximately -10 mV (trace b). This change in current pattern is due to a block of K_{ATP} channels (15, 16) and increases in Ca or nonselective cation currents (17, 18). When the cell was subsequently exposed to amylin (despite the continued presence of elevated glucose), net outward current above the resting potential was increased (trace c), as if the cell no longer sensed the presence of glucose. Both the increase in outward current and the accompanying hyperpolarization were reversed by the sulfonylurea K_{ATP} channel blocker glibenclamide (trace d). However, in each of four cells tested, the net inward current between -20 and 0 mV was not restored by sulfonylurea treatment. Only partial reversal of the amylin effects

FIG. 3. Changes in net membrane current associated with changes in electrical activity in response to glucose, amylin, or glibenclamide. (A) Membrane potential recorded from a *db* control β cell during elevation of glucose from 2 to 8 mM (open bar) and the subsequent addition of 10 nM amylin (solid bar) and then of $1 \mu\text{M}$ glibenclamide (hatched bar). Membrane potential was recorded continuously from a single cell. However, only three segments of the recording are shown. Traces a–d indicate brief periods during which the cell was voltage clamped and ramp commands were given to yield the current–voltage curves shown in B and C. (B) Ramp currents in 2 mM (trace a) or 8 mM (trace b) glucose exhibiting slope conductances (g_s) of 0.82 and 0.34 nS, respectively. (C) Currents in 8 mM glucose (trace b), 10 mM amylin plus 8 mM glucose (trace c; $g_s = 0.92$ nS), and glucose, amylin, and $1 \mu\text{M}$ glibenclamide (trace d; $g_s = 0.29$ nS).

by sulfonylureas in this critical voltage region implies that amylin may have effects on currents other than K_{ATP} and may explain the failure of action potential amplitudes to completely recover in glibenclamide (Fig. 3A). Accordingly, in some cells amylin decreased firing without increasing glibenclamide-sensitive currents (data not shown). In these cells, net outward current between -30 and 0 mV was still increased by the peptide. In contrast to the findings in *db* control cells, amylin failed to produce any detectable changes in net current in *db/db* cells ($n = 14$; data not shown). The absence of changes in current is consistent with the failure of amylin to modulate membrane potential in the diabetic cells.

Secretion from Individual β Cells Is Modulated by Sulfonylureas, Glucose, and Amylin. We sought to determine whether changes in insulin secretion from individual control and diabetic β cells were effected under the various conditions that altered electrical responses. We first measured insulin secretion from individual *db* control cells exposed to 1 mM glucose, with or without tolbutamide (10 – $50 \mu\text{M}$). Consistent with its ability to depolarize membrane potential, stimulate action potentials, and block K currents, tolbutamide triggered a dramatic increase in insulin secretion (Fig. 4). This increase in secretion was composed primarily of increases in the number of secreting cells, and to a lesser extent, increases in secretion from individual cells.

In Fig. 5, three-dimensional gray scale images of sequential immunoblots illustrate the effects of glucose or glucose plus amylin on insulin secretion in *db* control or *db/db* cells. For comparative purposes, secretion was quantified by integration of the gray levels over the entire field of cells in each image. Fig. 5A–F shows insulin blots from *db* control cells, and Fig. 5G–I is from *db/db* cells. Fig. 5A, D, and G shows basal insulin secretion when cells are exposed for 1 h to 2 mM glucose. Consistent with the spontaneous action potential behavior of *db/db* β cells in low glucose (e.g., Fig. 1C), basal

insulin secretion from individual *db/db* cells was somewhat greater (higher peaks) than from control cells. Upon exposure to 8 mM glucose, insulin secretion from *db* control β cells was markedly enhanced (compare Fig. 5 A and D with B and E), again consistent with their robust glucose-induced electrical activity. In contrast, despite having slightly more basal secretion, isolated *db/db* cells responded only weakly to elevated glucose (compare Fig. 5 G and H; 4-fold increase in total secretion as compared to 22-fold for controls) consistent with previous observations on intact *db/db* islets (19).

Sequential immunoblots were performed on *db* control β cells exposed first to 8 mM glucose and then to either 8 mM glucose or glucose plus amylin (10–200 nM). The second glucose challenge (after 1 h in 2 mM glucose) yielded a modest 27% increase in insulin secretion (Fig. 5 B and C). This increase is consistent with the priming effect seen in pancreatic islets, perfused pancreases, and whole animals during consecutive exposures to glucose (20, 21). In contrast, in six of eight experiments in which the second glucose challenge included amylin, the number of cells secreting insulin was greatly reduced (Figs. 5 E and F and 6A). Consistent with these observations, insulin secretion from intact *db* control islets was also inhibited, dose-dependently, by amylin (Fig. 6A), whereas β cells and intact islets from *db/db* mice showed nonsignificant or inconsistent changes due to amylin ($n = 4$; Figs. 5 H and I and 6A).

Comparing the effects of amylin on *db* control cells (which respond robustly to glucose) with those from *db/db* cells (which respond poorly) suggests that the effect of this peptide is restricted to those cells with normal glucose-sensing. We further tested this hypothesis by assessing amylin actions on the electrical and secretory responses of other cells with normal (rat β cells or islets) or abnormal (RIN-38) glucose sensing. Sprague-Dawley rat β cells and islets have been shown by many groups to have vigorous electrical and secretory responses to variations in glucose between 2 and 15 mM. In rat islets, amylin significantly decreased insulin secretion (Fig. 6B). In contrast, RIN-38 cells have abnormal insulin response patterns with no glucose dependency above 0.5 mM glucose (22). Consistent with this abnormal secretory pattern, in zero glucose, their average resting potential was -50 ± 10 mV ($n = 18$) and they frequently exhibited spontaneous action potentials. These cells did not show a

FIG. 4. Pseudocolor representations of insulin secretion (by sequential-cell immunoblot assay) from single β cells of *db* control mice. Insulin immunoblots were from separate coverslips incubated for 1 h in either 1 mM glucose (Left) or 1 mM glucose plus 50 μ M tolbutamide (Right). Calibrated levels of insulin range from 1.8 fg per pixel (light blue) to 95 fg per pixel (white).

FIG. 5. Three-dimensional representations of immunoreactive insulin secreted from single β cells. In some cases the image field represents a sequential determination of secretion from the same cells, but in all cases the density of cells is equivalent. The maximum gray level (lightest tone) corresponds to 45 fg of insulin per pixel. The total integrated gray level was determined for each image field (all cells) as a relative measure of insulin secretion and is given in units of gray level ($\times 10^6$). Cell types, solutions, and secretion (gray level units) are as follows. (A) *db* control, 2 mM glucose, 0.116. (B) *db* control, 8 mM glucose, 2.705. (C) Sequential assay of B, 8 mM glucose, 3.437. (D) *db* control, 2 mM glucose, 0.113. (E) *db* control, 8 mM glucose, 1.778. (F) Sequential assay of E, 8 mM glucose plus 100 nM amylin, 0.47. (G) *db/db*, 2 mM glucose, 0.498. (H) *db/db*, 8 mM glucose, 1.69. (I) Sequential assay of H, 8 mM glucose plus 100 nM amylin, 1.46. (Bar = 100 μ m.)

consistent electrical response to glucose nor a bursting electrical pattern. As hypothesized, in these cells, amylin, at concentrations up to 500 nM, rarely reduced the firing rate (1 of 10 cells) and was without effect on insulin secretion (Fig. 6C).

FIG. 6. Effects of amylin on insulin secretion. (A) Insulin secretion vs. amylin concentration for *db* control or *db/db* islets as determined by scintillation proximity assay (bars; $n = 4$ each, mean $\pm$ SD). For comparison, the total integrated gray levels from the immunoblots (Fig. 5 E, F, H, and I) are shown (circles). For both measurements, effects of amylin are not dose-related in *db/db* cells as compared to *db* control cells ($P \leq 0.05$ for amylin concentrations >10 nM for *db* control cells). (B) Effects of amylin on insulin secretion from rat islets exposed to 8 mM glucose. (C) Effects of amylin on insulin secretion from RIN-38 cells exposed to 0.5 mM glucose. Insulin secretion in B and C was determined by scintillation proximity assay (mean $\pm$ SEM, $n = 3$).

DISCUSSION

The pancreatic β cell of the control *db* mouse exhibits an electrical response to extracellular glucose that is qualitatively similar to that of β cells from many other sources. In contrast, cells isolated from the diabetic *db/db* mouse strain exhibit abnormal electrical activity and glucose responsiveness (ref. 12 and present study). The insulin secretory responses of these two β -cell types correlate well with their respective electrophysiological behavior. It is intriguing that cells with normal glucose responses (*db* control mice and Sprague-Dawley rats) also exhibit an amylin response, whereas cells with abnormal glucose responses (*db/db* mice and RIN-38 cells) are insensitive to amylin.

Amylin appears to act in part through an increase in glibenclamide-sensitive K current. It is unlikely that amylin directly modulates K_{ATP} channel activity but, rather, alters the β cell's ability to sense or metabolize glucose and, in turn, regulate K_{ATP} channel activity. This is suggested by the long delay (15–90 s) for full expression of the channel effects (perhaps reflecting an essential metabolic step) and the absence of effects in whole-cell dialysis experiments as compared to perforated patch recordings (data not shown). Furthermore, since both normal and abnormal cells apparently express K_{ATP} channels, the poor sensitivity of *db/db* and RIN-38 cells to glucose and amylin does not reflect an absence of this requisite channel target. Recently, glucose transporter Glut2 expression has been shown to be decreased in β cells from *db/db* mice (23). It is possible that loss of this transporter and loss of amylin responsiveness are related.

Amylin, by decreasing but not eliminating the β cell's capacity for electrical activity, may serve a protective function. As β cells are exposed to variations in circulating glucose levels and respond with variations in insulin secretion, co-secreted amylin may limit prolonged depolarization and constantly elevated intracellular Ca^{2+} , thus modulating the phasic electrical, Ca^{2+} , and insulin bursts. In the normal pancreas, amylin may act in concert with other peptides or hormones to establish the set point for glucose sensing. In the diabetic pancreas, as the β cell loses its glucose sensing capability, it may coincidentally lose its ability to respond to amylin. In advanced type II diabetes, local amylin excesses (24) could further contribute to the inability of the β cell to accurately sense and respond to normal variations in glucose levels. Although it is impossible to precisely measure the local concentration of amylin as it is secreted from the β cell, local concentrations up to 10^{-8} M have been estimated (25, 26). Effects seen in this study were robust at this concentration, as well as at lower and higher concentrations (0.1–500 nM).

Galanin and somatostatin also suppress insulin release. Furthermore, these peptides also act at least in part via a repolarization-induced reduction in intracellular Ca^{2+} . These effects are believed to be due to opening of K_{ATP} channels or to direct interactions with guanine nucleotide binding proteins coupled to ion channels or to exocytotic machinery (13, 27). Similarly, calcitonin-gene-related peptide (CGRP), which shares 46% sequence homology with amylin and modulates K and Ca currents in muscle and nerve (28–31), reduces insulin secretion from both single rat β cells and islets (32, 33). Although it is possible that local β -cell effects of amylin are mediated through low-affinity CGRP receptors (34), both amylin secretion from the β cell and its link to type II diabetes make it unique among peptide and hormone modulators of the β cell (35, 36). Unlike other peptides that are either released from nerve terminals in the endocrine pancreas or from other pancreatic cell types, amylin is cosecreted with insulin from the β cell in response to glucose. Amylin release is, therefore, modulated by the stimulus that it may ultimately play a role in regulating. Nonetheless, it will be of interest to investigate this entire family of peptides, including calcitonin and CGRP, as to

their regulation of specific β -cell ion channels, comparing their affinities and potencies, and identifying the points at which they may interact with the glucose-sensing mechanism of the pancreatic β cell.

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