β-Cell Secretory Products Activate α-Cell ATP-Dependent Potassium Channels to Inhibit Glucagon Release

Isobel Franklin,1 Jesper Gromada,2 Asllan Gjinovci,1 Sten Theander,1 and Claes B. Wollheim1

Glucagon, secreted from islet α-cells, mobilizes liver glucose. During hyperglycemia, glucagon secretion is inhibited by paracrine factors from other islet cells, but in type 1 and type 2 diabetic patients, this suppression is lost. We investigated the effects of β-cell secretory products zinc and insulin on isolated rat α-cells, intact islets, and perfused pancreata. Islet glucagon secretion was markedly zinc sensitive (IC50 = 2.7 μmol/l) more than insulin release (IC50 = 10.7 μmol/l). Glucose, the mitochondrial substrate pyruvate, and the ATP-sensitve K+ channel (KATP channel) inhibitor tolbutamide stimulated isolated α-cell electrical activity and glucagon secretion. Zinc opened KATP channels and inhibited both electrical activity and pyruvate (but not arginine)-stimulated glucagon secretion in α-cells. Insulin transiently increased KATP channel activity, inhibited electrical activity and glucagon secretion in α-cells, and inhibited pancreatic glucagon output. Insulin receptor and KATP channel subunit transcripts were more abundant in α- than β-cells. Transcript for the glucagon-like peptide 1 (GLP-1) receptor was not detected in α-cells nor did GLP-1 stimulate α-cell glucagon release. β-Cell secretory products zinc and insulin therefore inhibit glucagon secretion most probably by direct activation of KATP channels, thereby masking an α-cell metabolism secretion coupling pathway similar to β-cells. Diabetes 54:1808–1815, 2005

From the 1Department of Cell Physiology and Metabolism, University Medical Centre, Geneva, Switzerland; and 2Lilly Research Laboratories, Hamburg, Germany.

Address correspondence and reprint requests to Dr. Claes B. Wollheim, Department of Cell Physiology and Metabolism, University Medical Centre, 1211 Geneva 4, Switzerland. E-mail: claes.wollheim@medicine.unige.ch.

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level of the perfused pancreas, intact and dispersed islets, and fluorescence-activated cell sorter (FACS)-isolated adherent α-cells. The suppressive action of β-cell secretory products on glucagon release substantiated here emphasizes the requirement for drug development aimed at restoring normal control of glucagon secretion in diabetic patients.

RESEARCH DESIGN AND METHODS

Islet isolation and culture and pancreatic perfusion. Islets were isolated from 200- to 300-g male Wistar rats by digestion with collagenase (Roche Diagnostics, Rotkreutz, Switzerland) or, for molecular analyses, liberase (Roche) to improve α-cell yield. Unless stated otherwise, handpicked islets or FACS-isolated α-cells were maintained overnight in 2.5 mmol/l glucose/RPMI-1640 (Invitrogen, Basel, Switzerland) supplemented with 10% FCS (Bun
dschweig, Basel, Switzerland), 100 units/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml gentamicin. Pancreatic perfusions were performed on 300- to 350-g male Wistar rats (6). Animal care and experimentation was approved by the Swiss Academy of Medical Sciences and performed with the permission of the Canton of Geneva Veterinary Office.

Isolation of islet cells. To obtain α- or β-cell fractions for molecular analyses, islets cultured overnight were dispersed (16) and then subjected to two successive FACS purifications essentially as described elsewhere (22). The first sort, on the basis of FAD (flavine adenine dinucleotide) content, yielded β- and α-cell fractions, and a subsequent sort, based on the NAD(P)H content of the α-cell fraction, gave more pure α- and non-α-cell populations. Quantitative RT-PCR analyses showed that glucagon transcript was 14-fold more abundant in α- than β-cell RNA extracts and conversely that insulin transcript was 5-fold more enriched in the β-cell RNA fraction (α × 4, P < 0.005). To gain sufficient α-cells for hormone secretion assays, fresh islets were immediately dispersed and subjected to a single FACS analysis on the basis of FAD content, as above. This α-cell fraction contained undetectable levels of insulin when hormone content was analyzed.

Hormone secretion assays. FACS-isolated α-cells were seeded on polyomiti
dhène-coated 24-well plates (~10,000 per well) and cultured overnight. Cells were washed with 0.5 ml of Krebs-Ringer bicarbonate HEPES buffer (KRBH; [6]) supplemented with 2.5 mmol/l or 0 mmol/l (Fig. 3) glucose then preincubated in 0.5 ml of the same buffer for 1 h at 37°C. After a second wash, cells were incubated at 37°C for 15 min (Fig. 5) or 30 min in the same buffer supplemented with additional reagents, as indicated, before the supernatant was aspirated and the hormone content analyzed. Dispersed islet assays were as above, except the incubation time was extended to 60 min. Islets cultured overnight in 11.5 mmol/l glucose were washed and incubated at 37°C for 60 min in KRBH supplemented with 2.5 mmol/l glucose. Subsequently, hormone secretion from 10 (Fig. 1C and D) or 15 size-matched islets per condition was measured after a 60-min incubation at 37°C in 0.3 ml (anti-insulin assays), 0.2 ml (Fig. 1), or 1 ml (all other assays) of KRBH with 0.4% BSA (fraction V; Sigma, St. Louis, MO), the indicated concentration of glucose, and other reagents (kemptide and chloramphenicol-purified human insulin containing <0.01% zinc, a gift from Dr. G. Seipke, Aventis Pharma, Frankfurt/Main, Germany; rat insulin antisemur from Linco Research, St. Charles, MO). For static α-cell, dispersed and intact islet assays, secreted hormone was calculated as a percentage of total cellular hormone content, the latter extracted with acid ethanol. Hormone concentrations were measured by radioimmunoassay: glucagon using anti-glucagon (Dako Diagnostics, Zug, Switzerland) insulin as previously described (23), and C-peptide using a kit (Linco Research).

Quantitative RT-PCR. Total RNA was extracted from α- and β-cell fractions using RNeasy Mini kit (Qiagen, Basel, Switzerland) and converted into cDNA using Superscript reverse transcriptase (Gauthier, 1999 no. 2311). Primers (available on request) were designed to amplify the insulin receptor, Kir6.2, and sulfonlurea receptor (SUR-1) (accession nos. NM_017071, AF057013, and AB052294, respectively) glucagon; insulin; and cyclophilin transcripts. Quantitative real-time PCR was performed using an ABI 7000 Sequence Detection System (Applied Europe) with SYBR Green. At least four independent experiments, comparing relative abundance in α- and β-cell fractions, were performed for each transcript of interest. Each dataset was normalized to cyclophilin transcript content.

Zinc secretion assay. All solutions were prepared fresh, avoiding contact with glass, to reduce contamination with exogenous zinc. Islets cultured overnight were incubated as for hormone secretion, with 15 islets per 0.3 ml KRBH (0.1% BSA) for 60 min at 37°C. A 20-μl sample of supernatant was then removed for analysis of secreted insulin and the remainder pooled with seven similar samples for overnight desiccation under vacuum. Islet insulin content was determined as above. Desiccated samples were resuspended in 0.5 ml deionized water and their zinc concentrations determined with a Perkin-Elmer Cetus Instrument 2380 atomic absorption spectrophotometer. Background zinc levels were measured in parallel assays without islets.

Electrophysiology. Electrical activity in α-cells cultured for 4–1 days was analyzed using the perforated patch configuration of the patch-clamp technique, as before (24). Pipette resistance was between 2 and 6 MΩhm. Zero-voltage currents were cancelled electronically before seal formation. Amphotericin B (25) was used for patch perforation. The superfusion rate was

FIG. 1. Quantitative comparison of zinc (A) and insulin (B) secretion from batch-incubated islets in response to 2.5 or 16 mmol/l glucose. Data are means ± SE, n = 3, *P < 0.005. Dose response of glucagon (C) or insulin (D) secretion from static-incubated islets to zinc in the presence of 0 (C) or 10 (D) mmol/l glucose. E and F: Membrane potential recordings from isolated α-cells using the perforated-patch whole-cell configuration. Response of electrical activity to zinc (0.3–30 mmol/l, E) and zinc (30 μmol/l) with Ca²⁺ EDTA (2.5 mmol/l, F). G: Dose response of spike frequency to zinc. Data are means ± SE, n = 6, *P < 0.05. **P < 0.01.

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1–1.5 ml/min and bath temperature 33°C. The extracellular medium consisted of 138 mmol/l NaCl, 5.6 mmol/l KCl, 1 mmol/l MgCl₂, 2.6 mmol/l CaCl₂, and 5 mmol/l HEPES (pH 7.40 with NaOH), 0 mmol/l glucose, and additional agents where indicated. The pipette solution for perforated patch recordings was 76 mmol/l K₂SO₄, 10 mmol/l NaCl, 10 mmol/l KCl, 1 mmol/l MgCl₂, and 5 mmol/l HEPES (pH 7.35 with KOH). K<sub>ATP</sub> channel current was monitored using the standard whole-cell configuration and was elicited by 10-mV voltage excursions (duration: 200 ms; pulse interval: 2 s) from a holding potential of -70 mV. The pipette solution for measurements of whole-cell K<sub>ATP</sub> channel activity consisted of 125 mmol/l KCl, 30 mmol/l KOH, 10 mmol/l EGTA, 5 mmol/l HEPES, 1 mmol/l MgCl₂, 0.3 mmol/l Mg-ATP, and 0.3 mmol/l K-ADP (pH 7.15).

Statistical analyses. The statistical significance of the difference between two groups was calculated using the two-tailed, homoscedastic, Student's t-test. Static incubations were performed in triplicate, and n values refer to separate experiments.

RESULTS

Zinc release, hormone secretion, and electrical activity in islet cells. Atomic absorption spectrophotometry was used to detect secreted zinc from rat islets subjected to static incubations (Fig. 1A). When incubated in high glucose, islet zinc secretion increased ~6.5-fold over basal levels (from 0.17 to 1.1 pmol islet⁻¹ h⁻¹ in 16 vs. 2.5 mmol/l glucose).

FIG. 3. A: Glucagon secretion from isolated α-cells incubated for 30 min in basal (0 mmol/l) glucose conditions. Where indicated, incubation medium included glucose (16 mmol/l) or pyruvate (5 mmol/l) or arginine (10 mmol/l) ± zinc (30 μmol/l). Secreted glucagon was calculated as percent of content and is expressed relative to basal (100%). Data are means ± SE, n = 3, *P < 0.05. Whole-cell patch-clamp recordings of K<sub>ATP</sub> channel current activity in isolated α-cells, exposed to zinc (30 μmol/l) (B) or zinc and Ca<sup>2+</sup> EDTA (2.5 mmol/l) and subsequently diazoxide (0.1 mmol/l) (C). Traces are representative of seven or more experiments. D: Relative increases in K⁺ current amplitude in response to zinc, where I = current in presence of zinc and Io = current under control conditions. EC<sub>50</sub> = 2.2 μmol/l with cooperativity factor of 1.1. Data are means ± SE, n = 7 for each point. Diazoxide (100 μmol/l) increased relative current amplitude (∆Io/∆o) to 3.2 ± 0.3, n = 6. E: Relative transcript abundance of K<sub>ATP</sub> channel subunits Kir6.2 and SUR1 in FACS-isolated α- and β-cells, quantified by real-time RT-PCR. Data are presented relative to β-cells (1), n = 3, *P < 0.05.
mmol/l glucose, respectively). This increase paralleled that of secreted insulin (−3.5-fold, Fig. 1F) and reinforced the concept that islet α-cells are exposed to increased concentrations of zinc during hyperglycemia. Extracellular zinc inhibited islet glucagon secretion (IC50 = 2.7 μmol/l) during static incubations when glucose was absent (Fig. 1C). Insulin secretion, provoked by high glucose (10 mmol/l), was also inhibited by zinc (Fig. 1D) in agreement with earlier studies (26), although with an apparently lower sensitivity than glucagon (IC50 = 10.7 μmol/l). Patch-clamp recordings from isolated α-cells often exhibited spontaneous electrical activity in the absence of glucose (Fig. 1E). Importantly, increasing concentrations of zinc (0.3–30 μmol/l, IC50 = 1.4 μmol/l, Fig. 1G) reversibly inhibited this activity. The specificity of the observed zinc inhibition was confirmed by the protective effect of Ca2⁺ EDTA (Fig. 1F). These findings imply that α-cells are markedly zinc sensitive and that zinc may ultimately inhibit glucagon secretion by preventing calcium influx.

**Characterization of glucagon secretion from isolated α-cells.** To permit detailed analyses of the mode of action of both candidate inhibitors and stimulators of glucagon secretion, a technique was developed to study hormone release from FACS-isolated adherent α-cells. In these experiments, glucagon secreted during a 30-min static incubation revealed that rather than being inhibitory, glucose (16 mmol/l) was mildly stimulatory (33 ± 10%, n = 9) in the absence of other islet cell types (Fig. 2A). Moreover, monomethylsuccinate stimulated glucagon secretion to a greater extent (50 ± 4%, n = 3). Monomethylsuccinate is a mitochondrial substrate previously found to be incapable of inducing islet glucagon secretion despite its ability to increase free ATP levels in α-cells of intact islets (6). Parallel static incubations of dispersed (unsorted) islets yielded glucagon secretion results resembling those of intact islets (6), where high glucose was associated with inhibition of glucagon release (26 ± 5%, Fig. 2A inset). In patch-clamp recordings of isolated α-cells, high glucose triggered membrane depolarisation after several minutes and a subsequent increase in electrical activity (Fig. 2B). In support of the concept that, like β-cells, increased ATP in α-cells leads to KATP channel closure, subsequent membrane depolarization, calcium influx, and glucagon secretion, tolbutamide, a KATP channel inhibitor, strongly provoked glucagon release (60 ± 14%, n = 3, Fig. 2A) and membrane depolarization (Fig. 2C) in isolated α-cells.

Pyruvate stimulated glucagon secretion from isolated α-cells (67 ± 14%, n = 11, Fig. 2A), as previously observed for whole islets and perfused rat pancreata (6). In accord with the hypothesis that metabolized pyruvate raises ATP levels, thereby closing α-cell KATP channels, patch-clamp recordings of isolated α-cells revealed that pyruvate induced a rapid and sustained depolarization of the plasma membrane, stimulating electrical activity (Fig. 2C). Pyruvate-induced α-cell glucagon secretion was also found to be Ca2⁺-dependent (Fig. 2D), implicating a role for voltage-dependent calcium channels. GLP-1, a potentiator of glucose-induced insulin secretion, had no effect on pyruvate-stimulated glucagon release (Fig. 2A) nor did it effect basal glucagon secretion (not shown). Moreover, transcript encoding the GLP-1 receptor was not detected in

**Zinc stimulation of α-cell KATP channel activity.** Zinc inhibited both glucose and pyruvate-stimulated glucagon secretion (by 87 ± 35% and 66 ± 12%, respectively) from isolated α-cells in basal (0 mmol/l) glucose conditions (Fig. 3A). The cationic amino acid arginine is known to depolarize islet cells independently of KATP channel activity (28). Arginine triggered glucagon secretion in isolated α-cells (Fig. 3A). Importantly, arginine-induced glucagon release was not inhibited by zinc, indicating that when KATP channel activity is bypassed, zinc is unable to block hormone secretion. Thus, the site of action is unlikely to be voltage-dependent calcium channels. Moreover, extracellular zinc (30 μmol/l) had no effect on intracellular calcium levels in voltage-clamped α-cells loaded with fura 2/AM, although a pronounced increase in calcium was observed on the addition of adrenaline (n = 11, not shown). Patch-clamp recordings of KATP channel activity in isolated α-cells (Fig. 3B and C) revealed that zinc reversibly
increased current amplitude above control conditions in a dose-dependent manner (EC$_{50}$ = 2.2 μmol/l, Fig. 3D). This effect was comparable to but less potent than the action of diazoxide (100 μmol/l, Fig. 3C). Transcripts encoding K$_{ATP}$ subunits Kir6.2 and SUR1 were found to be more abundant (2 ± 0.4-fold and 3.8 ± 1.5-fold, respectively, n = 3) in FACS-isolated α- than β-cells when analyzed by quantitative RT-PCR (Fig. 3E).

Intrislet insulin directly inhibits glucagon secretion. α-Cell expression of insulin receptor transcript was demonstrated by quantitative RT-PCR analysis of FACS-isolated islet cells (Fig. 4A). Insulin receptor transcript was relatively abundant in α-cells, 3.6 ± 0.8-fold more than β-cells, similar to liver (3.7 ± 0.7), suggesting that α-cells may be a bona fide target for insulin. Previous in situ studies of perfused rat pancreas associated insulin with inhibition of glucagon secretion (12). At the level of the isolated islet, we confirm that inclusion of insulin antisem prevents inhibition of glucagon secretion during exposure to high glucose (Fig. 4B). Importantly, the presence of antisem did not alter endogenous insulin secretion, measured as C-peptide release, in basal glucose conditions (not shown). Similarly, insulin antisem does not alter somatostatin release in batch-incubated islets (29). Of note, replacement of antisem with a zinc chelator elevated endogenous insulin secretion in basal conditions and simultaneously lowered glucagon release, indicating that in this experimental system endogenous zinc was already inhibitory (not shown). Commercially available insulin usually contains high concentrations of zinc. To specifically analyze insulin action on glucagon release, we obtained insulin with minimal zinc content. In islet incubations, exogenous insulin inhibited glucagon secretion at low glucose to the extent observed at high glucose (25 ± 6%, Fig. 4C). In this experimental setup, endogenous insulin secretion (measured as C-peptide release) was not altered by exogenous insulin in basal conditions or when a secretagogue was present (Fig. 4D). These findings support the concept that insulin acts as a direct paracrine inhibitor of glucagon release.

Insulin inhibition of α-cell hormone secretion and electrical activity is transient. Physiological levels of exogenous insulin (100 ng/ml) (30) partially inhibited pyruvate-stimulated glucagon secretion in perfused rat pancreata (~36%, Fig. 5A). Inhibition exhibited a rapid onset (within 1 min) but was only transient (desensitization apparent after 5 min) in contrast with our earlier observations for zinc, where sustained inhibition was observed (6). The inhibitory effects of zinc (6) and insulin were not additive (not shown). Patch-clamp recordings of isolated α-cells revealed that exogenous insulin inhibited spontaneous electrical activity (Fig. 5B). Again, desensitization was apparent, ~6 min after the onset of inhibition (Fig. 5B and C), although maximal inhibition was stronger (~86%) than that observed for glucagon secretion in the pancreas. With a short incubation time (15 min), insulin completely inhibited (100 ± 13%) pyruvate-stimulated glucagon secretion in isolated α-cells (Fig. 5D). Interestingly, this inhibition was not prevented by the inclusion of phosphatidylinositol 3-kinase inhibitor wortmanin (Fig. 5D) and was not observed with longer time-course experiments (not shown), providing further evidence for the
transient nature of this inhibitory effect. Wortmannin alone was without effect (not shown). Patch-clamp recordings of K<sub>ATP</sub> channel activity in isolated α-cells revealed that insulin transiently increased K<sup>+</sup> current amplitude with a maximal effect 6 min after application (Fig. 5E), when channel activity was increased by 41 ± 7%. The inclusion of wortmannin did not prevent the stimulatory effect of insulin (not shown). These results indicate that although insulin is a genuine inhibitor of glucagon release that most probably activates K<sub>ATP</sub> channels and thereby blocks electrical activity and calcium influx at the plasma membrane, its effect on α-cells is transient and possibly independent of phosphatidylinositol 3-kinase signaling.

**DISCUSSION**

We have addressed the effects of β-cell secretory products zinc and insulin on α-cell glucagon release and electrical activity. Our results show that rat islet α-cells are exposed to greatly increased concentrations of extracellular zinc in high-glucose conditions and that zinc can directly exert a sustained inhibitory effect on α-cell electrical activity and hormone secretion most probably by opening K<sub>ATP</sub> channels. Insulin directly inhibits α-cell electrical activity and glucagon secretion, although only transiently. The inhibitory effect of insulin also seems to be the result of activation of K<sub>ATP</sub> channels. Importantly, we have uncovered a stimulatory effect of glucose and tolbutamide on glucagon secretion from isolated α-cells. These findings catapult the role of islet paracrine signaling to the primary regulatory mechanism governing glucagon secretion in the rat islet micro-organ and redefine our understanding of stimulus secretion coupling in the α-cell. This study provides a clear explanation for the hyperglucagonemia associated with diabetes, whereby loss of β-cell function permits hyperactivity and eventually glucose responsiveness in neighboring α-cells (2). The endogenous pathways uncovered here, by which glucagon secretion is inhibited in vivo, could be considered as suitable starting points for the design of drugs aimed at reducing postprandial α-cell activity.

The rate of zinc secretion we recorded from isolated islets in low glucose, 0.17 pmol · islet<sup>−1</sup> · h<sup>−1</sup>, was comparable with that calculated by others (31), 0.11 pmol · islet<sup>−1</sup> · h<sup>−1</sup>. We detected and quantified increased zinc secretion in response to high glucose. The increase in zinc relative to insulin, 2:1, corresponds to the predicted ratio that should secreted zinc originate from β-cell granules (31). Hormone secretion from islet α-cells was more sensitive than β-cells to extracellular zinc. Spontaneous electrical activity in α-cells and glucose and pyruvate-stimulated glucagon secretion were all inhibited by physiologically relevant concentrations of the metal ion. These findings extend our earlier studies in the perfused pancreas, where zinc (30 μmol/l) exhibited a sustained inhibitory effect on pyruvate-stimulated glucagon secretion and zinc chelation unmasked monomethylsuccinate-evoked glucagon release (6). Zinc action probably results from direct activation of α-cell K<sub>ATP</sub> channels rather than inhibition of Ca<sup>2+</sup> channels. A similar finding was reported for rat β-cell line RINm5F, where zinc inhibition of electrical activity was traced to K<sub>ATP</sub> rather than Ca<sup>2+</sup> channels (32), and recent work (33) suggests that the site of zinc action may be located on the SUR subunit. The greater abundance of transcripts encoding the K<sub>ATP</sub> channel subunits in rat islet α-cells relative to β-cells has been previously noted (25). We have quantified this difference for both Kir6.2 and SUR1 (two- and fourfold, respectively), suggesting an important role for K<sub>ATP</sub> channels in α-cell electrical activity.

The high level of insulin receptor expression in α-cells, similar to liver, a major target tissue for insulin, suggests that α-cells are also important sites of insulin action. However, the transient inhibitory effect of insulin on electrical activity, glucagon secretion, and indeed K<sub>ATP</sub> channel activation in isolated α-cells and glucagon secretion in the perfused pancreas indicates that it is unlikely to be responsible for the sustained α-cell suppression observed during hyperglycemia in vivo (1). Our pancreatic perfusion results with “zinc-free” insulin are in accord with others who demonstrated an inhibitory effect of exogenous insulin on glucagon secretion in the perfused pancreata of streptozotocin-induced diabetic rats (34), rats perfused in the retrograde but not anterograde direction (35), and alloxan diabetic dogs (5). Interestingly, in our static islet experiments, insulin appeared to account for all of the high-glucose–associated inhibition during a 1-h incubation. However, this probably reflected an accumulation of secreted zinc in the static incubation such that zinc-sensitive hormone secretion was already strongly inhibited in basal conditions. Other secretory products capable of influencing glucagon release in this assay would have included somatostatin (9,36,37) and γ-aminobutyric acid, released from the synaptic-like microvesicles of β-cells (38,39). Such complexity of paracrine signaling dictates caution when interpreting results from static islet assays. The effect of γ-aminobutyric acid on glucagon release from isolated α-cells was not tested here, as the physiological conditions under which γ-aminobutyric acid is released remain to be defined.

The signaling pathway by which insulin receptor activation attenuates α-cell electrical activity appears to involve K<sub>ATP</sub> channel activation. Preliminary investigations indicate that this may not involve phosphatidylinositol 3-kinase. In rat hypothalamic neurons, insulin can induce K<sub>ATP</sub> channel activation (40), and insulin has also been shown to activate K<sub>ATP</sub> channels in mouse pancreatic β-cells (41). Modulation of ion channel activity would permit paracrine signals to have a rapid and precise effect on hormone secretion. Functional α-cell K<sub>ATP</sub> channels have been recently demonstrated as a requirement for normal regulation of glucagon secretion in mice (42).

The possibility that GLP-1 is able to directly modulate glucagon release now seems unlikely given our inability to detect transcript encoding the GLP-1 receptor in purified α-cells, in accord with earlier attempts by others (20). Moreover GLP-1 had no effect on pyruvate-induced glucagon secretion in isolated α-cells, in agreement with the earlier study where cAMP levels in α-cells were unaffected by GLP-1 (20). These findings correlate with studies in type 1 diabetic subjects where GLP-1 had no effect on plasma glucagon levels during a hyperinsulinemic-euglycemic clamp (21). Assertions that GLP-1 acts as a direct inhibitor of glucagon secretion are founded upon studies where paracrine factors may have mediated an apparent
suppression. For example, where GLP-1-associated inhibition of glucagon secretion was observed in the perfused rat pancreas, simultaneous activation of insulin release occurred both at low and high glucose (43) or at intermediate glucose in rat and dog pancreas (44).

We believe that stimulus secretion coupling in the α-cell mirrors that of the β-cell. In support of this, we demonstrate that glucose moderately stimulates glucagon secretion and depolarizes the plasma membrane of isolated α-cells. Secretion is also stimulated by monomethylsucinate and more strongly by the KATP channel inhibitor tolbutamide; the latter has been reported to stimulate glucagon release in the perfused rat pancreas (45). Our observations are supported by in vivo studies (1,2) of subjects with type 1 diabetes, where glucose has been found to stimulate glucagon secretion and in vitro studies (35) showing glucose-induced glucagon release in the rat pancreas perfused in the retrograde direction. α-Cells express both glucokinase and the glucose transporter GLUT1, a lower capacity isofrom than GLUT2, expressed in β-cells (46,47). Although steady-state glucose utilization is the same (47), glucose oxidation in α-cells is only 30% of that in β-cells (48). This indicates that glucose is a relatively poor substrate for mitochondrial ATP generation in α-cells, as measured in intact islets (6). However, ATP must be produced from glucose to provoke glucagon secretion during a 30-min static incubation. An earlier study (36) reported glucose inhibition of arginine-stimulated glucagon release from isolated α-cells. Many technical disparities between this study and ours, including both culture and assay conditions, may have been responsible for differences in both the rate of glucagon secretion (<1% of content/h, detected only in the presence of arginine [36]) and glucose action. An inhibitory effect of high glucose on spontaneous electrical activity in isolated rat α-cells has been reported (25). It could be that, as in β-cells, glucose-induced membrane depolarization is preceded by a transient hyperpolarization that is the result of initial ATP consumption by glucokinase in β-cells (49) and notably INS-1E cells in supraphysiological glucose conditions (24). In α-cells, this could occur over a longer time course (minutes rather than seconds) due to the relatively slow rate of glucose oxidation. The concept that, as for β-cells, an increase in intracellular ATP leads to KATP channel closure, membrane depolarization, calcium influx, and glucagon release is supported by our observation that tolbutamide triggers α-cell electrical activity and hormone secretion, consistent with other studies (25). Tolbutamide also increased circulating glucagon levels in patients with advanced type 1 diabetes (50). In conclusion, this study demonstrates that only by removing α-cells from the repressive environment of the islet micro-organ can we begin to identify direct effectors of glucagon secretion and characterize the stimulus-secretion coupling pathways that lead to glucagon release.

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