*Original Article*

# Paradoxical Stimulation of Glucagon Secretion by High Glucose Concentrations

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**Hypersecretion of glucagon contributes to the dysregulation of glucose homeostasis in diabetes. To clarify the underlying mechanism, glucose-regulated glucagon secretion was studied in mouse pancreatic islets and clonal hamster In-R1-G9 glucagon-releasing cells. Apart from the well-known inhibition of secretion with maximal effect around 7 mmol/l glucose, we discovered that mouse islets showed paradoxical stimulation of glucagon release at 25–30 mmol/l and In-R1-G9 cells at 12–20 mmol/l sugar. Whereas glucagon secretion in the absence of glucose was inhibited by hyperpolarization with diazoxide, this agent tended to further enhance secretion stimulated by high concentrations of the sugar. Because U-shaped dose-response relationships for glucose-regulated glucagon secretion were observed in normal islets and in clonal glucagon-releasing cells, both the inhibitory and stimulatory components probably reflect direct effects on the α-cells. Studies of isolated mouse α-cells indicated that glucose inhibited glucagon secretion by lowering the cytoplasmic $Ca^{2+}$ concentration. However, stimulation of glucagon release by high glucose concentrations did not require elevation of $Ca^{2+}$, indicating involvement of novel mechanisms in glucose regulation of glucagon secretion. A U-shaped dose-response relationship for glucose-regulated glucagon secretion may explain why diabetic patients with pronounced hyperglycemia display paradoxical hyperglucagonemia. *Diabetes* 55:2318–2323, 2006**

Hyperglycemia in diabetes is primarily due to inappropriate or no secretion of insulin. However, there is ample evidence that hypersecretion of glucagon plays an important role in initiating and maintaining hyperglycemic conditions in diabetic animals and humans (1,2). Considerable attention is therefore given to glucagon signaling as a target in the treatment of diabetic hyperglycemia (2,3). The elevated glucagon concentrations in diabetes can be reduced but not normalized by insulin treatment (4,5), and the mechanisms underlying the hyperglucagonemia have not been fully elucidated.

Glucagon has an important role in glucose counterregulation (6). Secretion occurs in response to hypoglycemia and is inhibited already when the glucose concentration reaches 4–6 mmol/l (7,8). Because such concentrations are at or below the threshold for stimulation of insulin secretion, it is apparent that release of insulin or other β-cell factors does not suffice to explain inhibition of glucagon secretion. We now discovered that the dose-response relationships for glucose-regulated glucagon secretion from mouse pancreatic islets and clonal hamster glucagon-releasing In-R1-G9 cells are U-shaped with inhibition at 7–8 mmol/l glucose followed by stimulation at high concentrations of the sugar. Studying the mechanism underlying these effects in mouse α-cells, we found that inhibition of secretion was associated with a lowering of the cytoplasmic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), whereas the stimulation at high glucose concentrations occurred independently of $[Ca^{2+}]_i$ elevation. In the mouse islets, the stimulatory component was most pronounced above 20 mmol/l glucose when insulin secretion is maximally stimulated. A similar regulation of human α-cells may explain why diabetic patients with hyperglycemia display paradoxical hyperglucagonemia and why a further glucose challenge has been found to stimulate glucagon release (9–11).

## RESEARCH DESIGN AND METHODS

**Preparation and culture of pancreatic islets and cells and clonal glucagon-releasing cells.** Islets of Langerhans were isolated with collagenase (Boehringer Mannheim, Mannheim, Germany) from C57 BL/6 mice. Local ethics committees approved the experimental procedures. The animals were killed by decapitation under anesthesia with $CO_2$. The peritoneal cavity was opened, and a collagenase solution was injected into the bile-pancreatic duct to expand the pancreas (glucagon secretion experiments). The pancreas was excised and cut into small pieces, which were digested with collagenase to obtain free islets of Langerhans. The lower duodenal part of the pancreas was rejected to avoid islets with cells producing pancreatic polypeptide (12). The freshly isolated islets were used for studies of glucagon and insulin secretion or preparation of free cells. Free cells were obtained by incubating the islets for 4 min at 37°C in $Ca^{2+}$-deficient medium containing 0.5 mmol/l EDTA and 0.05% trypsin (Invitrogen, Carlsbad, CA) followed by brief shaking. The cells were suspended in RPMI 1640 medium with 5.5 mmol/l glucose (Gibco, Paisley, Scotland) supplemented with 10% FCS (Gibco), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 30 μg/ml gentamicin. Small samples of this suspension (15 μl) were applied to the centers of poly-L-lysine-coated (Sigma Chemical, St. Louis, MO) circular 25-mm coverslips. The coverslips were then kept for 60 min in a culture incubator at 37°C with a humidified atmosphere of 5% $CO_2$ to allow cells to settle and begin attachment. More medium was then cautiously added, and the cells were cultured for 1–3 days.

Glucagon-secreting clonal hamster In-R1-G9 cells (13) were provided by Dr. Björn Olde (Lund University) with permission from Dr. Jacques Philippe (University of Geneva, Geneva, Switzerland). The cells were cultured on plastic dishes or culture bottles in GlutaMAX-containing RPMI 1640 medium with 11 mmol/l glucose (Gibco) supplemented with 10% FCS, 100 IU/ml

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$[Ca^{2+}]_i$, cytoplasmic $Ca^{2+}$ concentration; $K_{ATP}$ channel, ATP-sensitive $K^+$ channel.

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penicillin, and 100 μg/ml streptomycin. For secretion studies, the In-R1-G9 cells were seeded at a density of 100,000 cells/well in 48-well plates and cultured as described above for 3 days.

**Measurements of $[Ca^{2+}]_i$.** The cells were loaded with the $Ca^{2+}$ indicator fura-2 during a 40-min incubation at 37°C in a buffer containing 0.5 mg/ml BSA (Sigma), 125 mmol/l NaCl, 4.8 mmol/l KCl, 1.2 mmol/l $MgCl_2$, 1.28 mmol/l $CaCl_2$, 3 mmol/l glucose, 1 μmol/l fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR) (0.1% dimethylsulfoxide; Sigma), and 25 mmol/l HEPES (Sigma) with pH adjusted to 7.4 with ~13 mmol/l NaOH. The coverslips with the attached cells were used as exchangeable bottoms of an open chamber. The chamber volume was 0.16 ml, and the cells were superfused at a rate of 1 ml/min. The superfusion chamber was placed on the stage of an inverted Nikon Diaphot microscope equipped with an epifluorescence illuminator and a 40× oil immersion fluorescence objective. The chamber holder and the objective were maintained at 37°C by custom-built thermostats. The epifluorescence illuminator was connected through a 5-mm-diameter liquid light guide to an Optoscan monochromator (Cairn Research, Faversham, U.K.) with rapid grating and slit width adjustment and a 150 W xenon arc lamp. The monochromator provided excitation light at 340 nm (2.8-nm half-bandwidth) and 380 nm (2.5-nm half-bandwidth), and emission was measured at >515 nm by an intensified CCD camera. The Metafluor software (Universal Imaging, Downingtown, PA) controlled the monochromator acquiring fluorescence images of 30 accumulated frames at 340 and 380 nm every 4 s. $[Ca^{2+}]_i$ images were calculated from 340-to-380 nm ratio images as previously described (14).

**Identification of α-cells.** The α-cells were initially selected by their small size and $[Ca^{2+}]_i$ response to epinephrine (Sigma) (14,15), which is not shared by β-cells (14) and δ-cells (16). Only α-cells confirmed by immunostaining were included in the analyses (14).

**Hormone secretion.** Batches of 8–12 freshly isolated islets were preincubated for 30 min at 37°C in 1 ml Krebs-Ringer buffer (pH 7.4) supplemented with 10 mmol/l HEPES, 0.1% BSA, and 1 mmol/l glucose. Each incubation vial was gassed with 95% $O_2$ and 5% $CO_2$ to obtain constant pH and oxygenation. The islets were then incubated for 1 h at 37°C in the Krebs-Ringer buffer containing different glucose or 3-*o*-methyl glucose concentrations and 4.8 mmol/l K or 30 mmol/l $K^+$ plus 250 μmol/l diazoxide. At the end of the incubation, aliquots of the medium were removed and frozen pending the radioimmunoassay of glucagon and insulin (17).

The 3-day cultured In-R1-G9 cells were carefully washed and preincubated for 30 min at 37°C in the Krebs-Ringer buffer containing 1 mmol/l glucose (1 ml/well). The medium was then replaced by one containing different glucose concentrations and incubated for 60 min at 37°C. Aliquots of the medium were removed and frozen pending assay of glucagon, insulin, and somatostatin (17,18).

**Statistical analysis.** Dose-response relationships for the effect of glucose on glucagon and insulin secretion were analyzed with ANOVA and paired Student's *t* test. The reaction patterns in individual α-cells were analyzed with two-tailed Fisher's exact test. All calculations were made by SigmaStat software (Systat Software, Erkrath, Germany).

## RESULTS

Figure 1 shows how glucagon and insulin secretion from mouse islets and In-R1-G9 cells depend on the glucose concentration. In the mouse islets, glucagon secretion was more than half-maximally inhibited by 4 mmol/l glucose, and the maximal effect was reached at 7–8 mmol/l (Fig. 1, *top left panel*). With further rise of glucose to 16–20 mmol/l, there was a small but statistically significant reduction of this inhibition ($P < 0.005$–$0.05$). However, above 20 mmol/l, inhibition was dramatically reversed into a small stimulation at 25–30 mmol/l glucose. In parallel insulin measurements from the same islets, threshold stimulation of insulin release (18%) was obtained at 7 mmol/l glucose and maximal secretion at 25–30 mmol/l sugar (Fig. 1, *bottom left panel*). Stimulation of glucagon secretion at 25–30 mmol/l glucose could not be attributed to an unspecific action of the high sugar concentrations because 30 mmol/l nonmetabolizable glucose transport analog 3-*o*-methyl glucose was inhibitory (Fig. 1, *top left panel*). Separate control measurements under identical conditions revealed that somatostatin secretion was stimulated by glucose and significantly higher at 30 than at 20 mmol/l sugar (data not shown), indicating that glucose

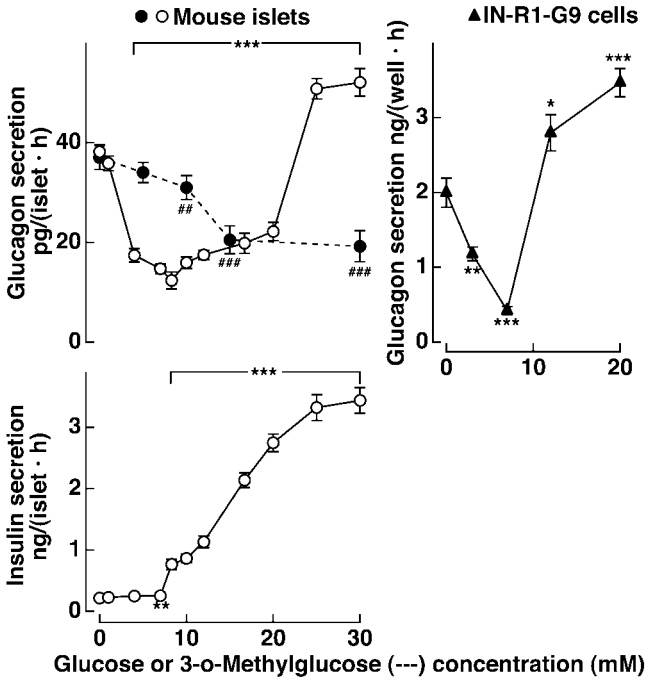


**FIG. 1. Glucose dependence of glucagon and insulin secretion from mouse pancreatic islets and hamster In-R1-G9 cells. Glucagon and insulin secretion from mouse islet and glucagon secretion from In-R1-G9 cells were measured after 60-min incubations at indicated glucose concentrations. Glucagon secretion was also measured from mouse islets exposed to 3-*o*-methylglucose (dashed line). Data are presented as means ± SE of 6–12 experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for the effect of glucose compared with absence of the sugar. ##$P < 0.01$, ###$P < 0.001$ for the effect of 3-*o*-methylglucose compared with absence of the sugar. Brackets indicate observations with identical significance levels.**

stimulation of glucagon secretion was not due to decreased release of somatostatin. There was a U-shaped dose-response relationship for glucose-regulated glucagon secretion also in In-R1-G9 cells with maximal inhibition at 7 mmol/l (Fig. 1, *right panel*). In this case, the stimulatory component was left-shifted with significant stimulation already at 12 mmol/l glucose. The In-R1-G9 cells did not secrete detectable amounts of insulin or somatostatin.

Rise of $[Ca^{2+}]_i$ is probably the most important trigger of pancreatic islet hormone secretion. Accordingly, the inhibition of glucagon secretion obtained with elevation of glucose was associated with lowering of $[Ca^{2+}]_i$. In 57% (8 of 14; $P < 0.01$) of individual mouse α-cells selected for showing $[Ca^{2+}]_i$ oscillations at 1 mmol/l glucose, $[Ca^{2+}]_i$ was reduced to basal levels at 20 mmol/l sugar (Fig. 2). After further elevation to 30 mmol/l glucose, 86% (12 of 14; $P < 0.001$) of the α-cells were inhibited.

To clarify the role of $[Ca^{2+}]_i$ in glucose-regulated glucagon secretion, mouse islets were studied also under hyperpolarizing conditions, preventing voltage-dependent influx of the ion. This was achieved by exposure to 250 μmol/l diazoxide, which clamps the membrane potential close to the equilibrium potential for $K^+$ by activating the ATP-sensitive $K^+$ channels ($K_{ATP}$ channels) that are present in all types of islet cells (12,14,16,19). Hyperpolarization in the absence of glucose had no effect on $[Ca^{2+}]_i$ of resting β-cells but lowered $[Ca^{2+}]_i$ of oscillating α-cells (not shown). In the presence of diazoxide, introduction of 7 mmol/l glucose resulted in a small reduction of basal $[Ca^{2+}]_i$ in 78% of the α-cells (14 of 18; $P < 0.001$) and all of 12 β-cells ($P < 0.001$; Fig. 3). Further elevation of glucose

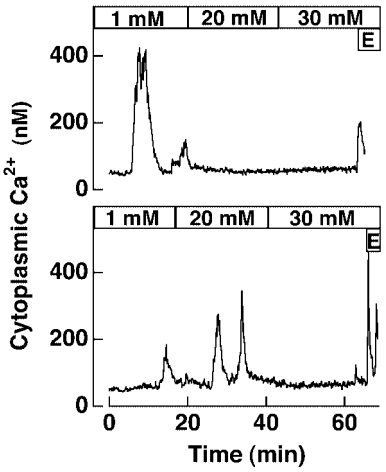


**FIG. 2. Effects of raising the glucose concentration from 1 to 20 and 30 mmol/l on $[Ca^{2+}]_i$ of individual mouse α-cells. Epinephrine (E; 5 μmol/l) was present as indicated. Representative responses for 14 α-cells from four preparations.**

to 30 mmol/l had no effect on the α-cells but induced a more pronounced additional reduction of $[Ca^{2+}]_i$ in 23 of 25 β-cells ($P < 0.001$). These data are consistent with stimulated sequestration and outward transport of $Ca^{2+}$ (14,20,21) being saturated at lower glucose concentrations in α-cells (14) than in β-cells (21). Measurements of hormone secretion under similar conditions demonstrated that hyperpolarization with diazoxide reduced glucagon release at 0–1 mmol/l glucose by 70–80% of the maximal glucose inhibition (Fig. 4, *top panel*). Nevertheless, the additional inhibition obtained by raising the sugar concentration to 7 mmol/l remained statistically significant. Glucagon secretion at higher glucose concentrations was not diminished during hyperpolarizing conditions and even slightly exceeded control secretion in the 12–30 mmol/l range. Basal insulin secretion at 0–7 mmol/l glucose was unaffected by the hyperpolarization (Fig. 4, *bottom panel*), whereas insulin secretion at 12–30 mmol/l glucose was reduced by ~70% compared with control.

Glucose initiates insulin release by raising $[Ca^{2+}]_i$ in β-cells. The sugar also has an amplifying effect on secretion, which is apparent when $[Ca^{2+}]_i$ is held constant at an elevated level (22). This situation can be achieved by clamping the membrane potential at a depolarized level (30 mmol/l $K^+$ plus diazoxide) to activate voltage-dependent $Ca^{2+}$ influx. We used this approach to clarify whether

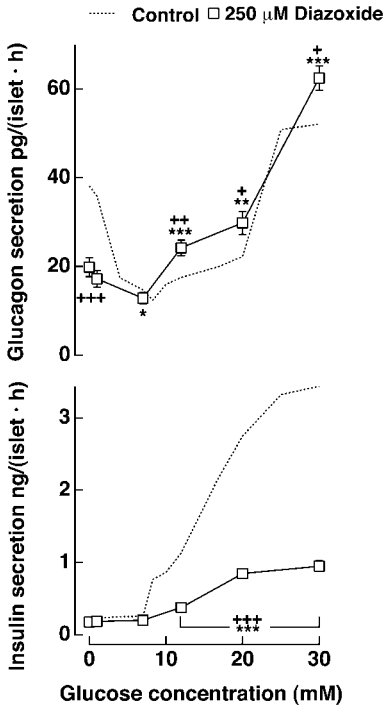


**FIG. 4. Glucose dependence of glucagon and insulin secretion from mouse pancreatic islets clamped at a hyperpolarized membrane potential. Glucagon and insulin secretion were measured after 60-min incubations at indicated glucose concentrations in the presence of 250 μmol/l diazoxide and 4.8 mmol/l $K^+$ (□). For comparison, control data were duplicated from Fig. 1 (dotted lines). Data are presented as means ± SE of 12 experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for the effect of glucose compared with absence of the sugar. +$P < 0.05$, ++$P < 0.01$, +++$P < 0.001$ for the effect of hyperpolarization with diazoxide versus control. The bracket indicates observations with identical significance levels.**

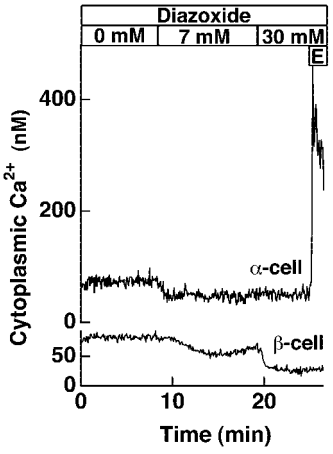


**FIG. 3. Effects of raising the glucose concentration from 0 to 7 and 30 mmol/l on $[Ca^{2+}]_i$ of hyperpolarized individual mouse α- and β-cells. Diazoxide (250 μmol/l), which hyperpolarizes the cells in 4.8 mmol/l $K^+$, was present throughout. Epinephrine (E; 5 μmol/l) was present as indicated. Representative responses for 18 α-cells and 25 β-cells from six preparations.**

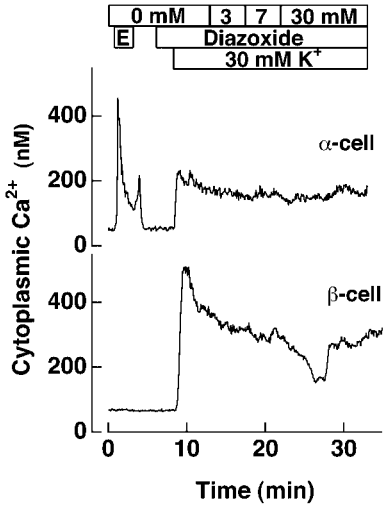


**FIG. 5. Effects of depolarization by 30 mmol/l $K^+$ and increase of the glucose concentration from 0 to 3, 7, and 30 mmol/l on $[Ca^{2+}]_i$ of individual mouse α- and β-cells. Diazoxide (250 μmol/l) was added to activate $K_{ATP}$ channels before raising the $K^+$ concentration from 4.8 to 30 mmol/l. Epinephrine (E; 5 μmol/l) was present as indicated. Representative responses for 19 α-cells and 16 β-cells from four preparations.**

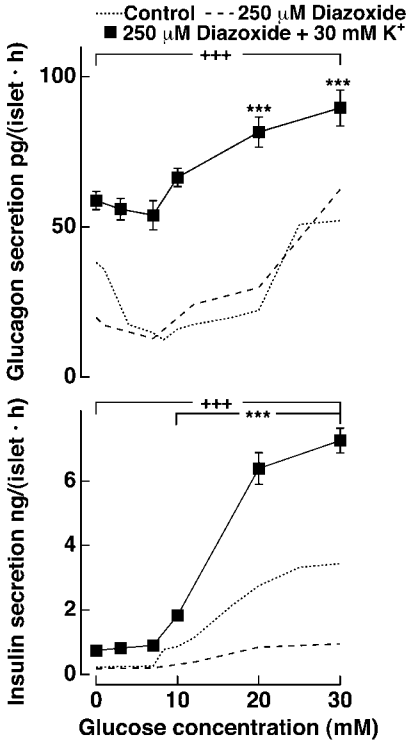


**FIG. 6. Glucose dependence of glucagon and insulin secretion from mouse pancreatic islets clamped at a depolarized membrane potential. Glucagon and insulin secretion were measured after 60-min incubations at indicated glucose concentrations in the presence of 250 μmol/l diazoxide and 30 mmol/l $K^+$ (■). For comparison, control data were duplicated from Fig. 1 (dotted lines) and data obtained under hyperpolarizing conditions from Fig. 4 (dashed lines). Data are presented as means ± SE of eight experiments. ***$P < 0.001$ for the effect of glucose compared with absence of the sugar. +++$P < 0.001$ for the effect of depolarization with 30 mmol/l $K^+$ plus diazoxide versus control and hyperpolarizing conditions. Brackets indicate observations with identical significance levels.**

glucose also has an amplifying effect on glucagon secretion. Figure 5 shows that depolarization by combining diazoxide with 30 mmol/l $K^+$ resulted in rapid increases of $[Ca^{2+}]_i$ in both α- and β-cells. Subsequent introduction of 3 mmol/l glucose caused a small reduction of $[Ca^{2+}]_i$ in 3 of 19 α-cells, but there was no change of $[Ca^{2+}]_i$ when increasing the glucose concentration from 0 to 3, 7, and 30 mmol/l in the remaining 16 α-cells. However, 14 of 16 β-cells responded with temporary reduction of $[Ca^{2+}]_i$ after introducing at the highest glucose concentration ($P < 0.001$), supporting previous observations that stimulated $Ca^{2+}$ sequestration and outward transport can transiently affect $[Ca^{2+}]_i$ in this cell type also when voltage-dependent influx is stimulated (23). Under these depolarizing conditions, basal insulin secretion increased 3.5-fold at 0–7 mmol/l (Fig. 6, *bottom panel*). Glucose did not affect the depolarization-induced insulin secretion in the 0–7 mmol/l range, but higher concentrations of the sugar had a pronounced amplifying effect. The corresponding glucagon data demonstrated that 30 mmol/l $K^+$ plus diazoxide markedly stimulated glucagon secretion in the 0–30 mmol/l range (Fig. 6, *top panel*). Glucose elevation to 7 mmol/l failed to induce significant inhibition of secretion. Higher concentrations of the sugar still stimulated glucagon release, but this effect was not more pronounced than under control or hyperpolarizing conditions.

## DISCUSSION

It is generally accepted that glucose is an inhibitor of glucagon secretion (1,2). However, its was evident from the present study of mouse pancreatic islets and clonal hamster In-R1-G9 glucagon-releasing cells that inhibition of glucagon secretion by intermediate glucose concentrations is followed by reversal of this effect and even stimulation at higher concentrations of the sugar. The U-shaped dose-response patterns are different from those obtained with perfused rat pancreas (7) and batch-incubated rat islets (8), which only show glucose inhibition of glucagon secretion. A recent observation that glucose stimulates glucagon secretion from purified rat α-cells was taken to indicate that this secretion is regulated in a β-cell–like manner and that the inhibitory effect of glucose requires release of paracrine factors from non–α-cells within the islets (8). The present observation of glucose-stimulated glucagon secretion from mouse islet is principally different from that in purified rat α-cells. Whereas the latter is attributed to a β-cell–like pathway with closure of $K_{ATP}$ channels leading to depolarization and voltage-dependent $Ca^{2+}$ influx, glucose stimulation of glucagon secretion from mouse islets occurred independent of $K_{ATP}$ channels and did not require elevation of $[Ca^{2+}]_i$. A reason for the different $[Ca^{2+}]_i$ responses is apparently that rat α-cells have a high density of $K_{ATP}$ channels, exceeding that in rat β-cells (24), whereas mouse α-cells show little $K_{ATP}$ channel expression (25,26), corresponding to 2% of that in mouse β-cells (27). Depolarization by tolbutamide-induced closure of the $K_{ATP}$ channels thus increases the electrical activity (24), $[Ca^{2+}]_i$ (8), exocytosis (28), and secretion (8) in rat α-cells, but tolbutamide-induced elevation of $[Ca^{2+}]_i$ is observed only in a small minority of mouse α-cells (14). Moreover, whereas glucose increases the electrical activity and $[Ca^{2+}]_i$ of rat α-cells (24), the sugar hyperpolarizes mouse α-cells (14,27,29) and, as now confirmed, lowers $[Ca^{2+}]_i$ (14).

The present study of mouse α-cells and islets and clonal hamster glucagon-releasing cells provides arguments that glucose has direct effects on the α-cells in terms of inhibition and stimulation of glucagon release. Although the In-R1-G9 cells are derived from an insulin-secreting cell line also producing glucagon (13), the presently used cells did not release detectable amounts of insulin. Considering dilutions and detection limits of the assays, this means that maximal theoretical insulin secretion on a molar basis is lower than or similar to glucagon secretion compared with an ~40-fold excess of insulin secretion in the mouse islets. Also, somatostatin secretion was undetectable, indicating that maximal theoretical secretion of this hormone corresponded to 0.5–4% of glucagon secretion, which is much lower than in the mouse islets (data not shown). It seems unlikely that phenotypic diversity among the In-R1-G9 cells explains inhibitory or stimulatory effects of glucose by paracrine routes, but an inhibitory effect of insulin is not entirely excluded. The observation that most inhibition of glucagon secretion from mouse islets is obtained at glucose concentrations, which fail to stimulate insulin release argues against the involvement of the proposed β-cell factors insulin (30), $Zn^{2+}$ (30,31), and γ-aminobutyric acid (32,33). Moreover, glucose stimulation of glucagon secretion was not diminished, and some glucose inhibition of glucagon secretion remained under conditions expected to hyperpolarize the islet cells and reduce release of paracrine factors.

Three alternative mechanisms have been proposed to mediate a direct glucose inhibition of the α-cell. In conflict with the hyperpolarizing effect of glucose (14,27,29), one of these proposals assumes that glucose paradoxically inhibits glucagon secretion by depolarizing mouse α-cells (26,34). The other two alternatives involve glucose-induced hyperpolarization by activation of the electrogenic Na,K-ATPase (35) or inhibition of a depolarizing store-operated cation influx by glucose-stimulated $Ca^{2+}$ sequestration in the endoplasmic reticulum (14).

Because the present and previous data (14) show that glucose lowers $[Ca^{2+}]_i$ in mouse α-cells, the question arises how high concentrations of the sugar stimulate glucagon secretion. In the insulin-releasing β-cells, glucose seems to stimulate insulin secretion by at least three mechanisms. Initiation of secretion is generally attributed to depolarization with increase of $[Ca^{2+}]_i$ after closure of $K_{ATP}$ channels (19). Glucose also has an amplifying effect, which does not require further elevation of $[Ca^{2+}]_i$ (22). In addition, there is evidence that increased metabolism can stimulate insulin release independent of $[Ca^{2+}]_i$ elevation (36,37). We now confirm all of these actions by showing *1*) stimulated insulin secretion after $[Ca^{2+}]_i$ elevation due to depolarization with 30 mmol/l $K^+$ in the presence of diazoxide; *2*) glucose amplification of this secretory response, although the sugar temporarily lowered $[Ca^{2+}]_i$ in isolated β-cells; and *3*) maintenance of some glucose-stimulated insulin secretion under hyperpolarizing conditions when the sugar lowered $[Ca^{2+}]_i$ below the basal level.

The $Ca^{2+}$-dependent and -independent pathways for stimulated secretion were present also in mouse α-cells. *1*) Elevation of $[Ca^{2+}]_i$ in isolated α-cells correlated to enhanced glucagon release from pancreatic islets. *2*) High glucose concentrations stimulated glucagon secretion under hyperpolarizing conditions, although $[Ca^{2+}]_i$ of the α-cells was lowered below basal levels. It was recently reported that glucose amplifies $Ca^{2+}$-initiated glucagon secretion from purified rat α-cells (8). However, the presently observed stimulatory effect of glucose on glucagon secretion from mouse islets did not depend on the $[Ca^{2+}]_i$ level of the α-cells, indicating that glucose initiates rather than amplifies secretion. It may seem surprising that the α-cells can sense high concentrations of glucose because they express low $K_m$ glucose transporter GLUT1 but lack high $K_m$ GLUT2 (38). The situation is reminiscent of that in human β-cells, which, unlike the GLUT2-expressing rodent β-cells, predominantly express GLUT1 (39). Apparently glucose transport via GLUT1 is not rate limiting in these cases, and the sensing is explained by the presence of the high $K_m$ glucokinase (39,40). The signal transduction of the intriguing glucose stimulation of glucagon secretion remains to be elucidated.

Hypersecretion of glucagon is an important feature of diabetes contributing to glucose dysregulation (1,2). The mechanism underlying this phenomenon is unclear. The present study of mouse islets and clonal hamster glucagon-releasing cells indicated that paradoxical hypersecretion of glucagon in hyperglycemia may depend on a U-shaped dose-response relationship for glucose regulation of secretion. A similar regulation of human α-cells may explain why diabetic patients with hyperglycemia display paradoxical hyperglucagonemia and why a further glucose challenge has been found to stimulate glucagon release (9–11).

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