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Paradoxical Stimulation of Glucagon Secretion by High Glucose Concentrations

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Short running title: Glucose stimulation of glucagon secretion

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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 mM glucose, we discovered that mouse islets showed paradoxical stimulation of glucagon release at 25-30 mM and In-R1-G9 cells at 12-20 mM of the 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. Since U-shaped dose-response relationships for glucose-regulated glucagon secretion were observed in normal islets as well as 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.

Hyperglycemia in diabetes mellitus 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 counter-regulation (6). Secretion occurs in response to hypoglycemia and is inhibited already when the glucose concentration reaches 4-6 mM (7, 8). Since 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 mM 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 mM 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 GmbH, Mannheim, Germany) from C57 BL/6 mice. Local ethics committees approved the experimental procedures. The animals were killed by decapitation under anesthesia with CO₂. 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 Ca²⁺-deficient medium containing 0.5 mM EDTA and 0.05% trypsin (Invitrogen, Carlsbad, CA) followed by brief shaking. The cells were suspended in RPMI 1640 medium with 5.5 mM glucose (Gibco Ltd., Paisley, Scotland) supplemented with 10% fetal calf serum (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 Co., St. Louis, MO) circular 25 mm cover slips. The cover slips were then kept for 60 min in a culture incubator at 37°C with a humidified atmosphere of 5% CO₂ 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 kindly provided by Dr. Björn Olde, Lund University, Sweden with permission from Dr Jacques Philippe,
University of Geneva, Switzerland. The cells were cultured on plastic dishes or culture bottles in GlutaMAX™-containing RPMI 1640 medium with 11 mM glucose (Gibco) supplemented with 10% fetal calf serum, 100 IU/ml 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 40 min incubation at 37°C in a buffer containing 0.5 mg/ml bovine serum albumin (Sigma), 125 mM NaCl, 4.8 mM KCl, 1.2 mM MgCl₂, 1.28 mM CaCl₂, 3 mM glucose, 1 μM fura-2 acetoxymethyl ester (Molecular Probes Inc., Eugene, OR; 0.1% dimethylsulphoxide, Sigma) and 25 mM HEPES (Sigma) with pH adjusted to 7.4 with ~13 mM NaOH. The cover slips 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 40x 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 Ltd., Faversham, UK) with rapid grating and slit width adjustment and a 150W 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 Corp., 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 340/380 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- (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 pre-incubated for 30 min at 37°C in 1 ml of Krebs-Ringer buffer (pH 7.4) supplemented with 10 mM HEPES, 0.1% bovine serum albumin and 1 mM 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 mM K or 30 mM K\(^+\) plus 250 μM 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 mM glucose (1 ml per 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 GmbH, Erkrath, Germany).

RESULTS

Fig. 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 mM glucose and the maximal effect was reached at 7-8 mM (Fig. 1, upper left panel). With further rise of glucose to 16-20 mM there was a small but statistically significant reduction of this inhibition (P<0.005-0.05). However, above 20 mM inhibition was dramatically reversed into a small stimulation at 25-30 mM glucose. In parallel insulin measurements from the same islets threshold stimulation of insulin release (18%) was obtained at 7 mM glucose and maximal secretion at 25-30 mM of the sugar (Fig. 1, lower left panel). Stimulation of glucagon secretion at 25-30 mM glucose could not be attributed to an unspecific action of the high sugar concentrations, since 30 mM of the non-metabolizable glucose transport analogue 3-o-methyl glucose was inhibitory (Fig. 1, upper left panel). Separate control measurements under identical conditions revealed that somatostatin secretion was stimulated by glucose and significantly higher at 30 than at 20 mM of the sugar (data not shown), indicating that glucose stimulation of glucagon secretion was not due to decreased release of somatostatin. There was an U-shaped dose-response relationship for glucose-regulated glucagon secretion also in In-R1-G9 cells with maximal inhibition at 7 mM (Fig. 1, right panel). In this case the stimulatory component was left-shifted with significant
stimulation already at 12 mM glucose. The In-R1-G9 cells did not secrete detectable amounts of insulin or somatostatin.

Rise of $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$. In 57% (8 of 14; P<0.01) of individual mouse $\alpha$-cells selected for showing $[\text{Ca}^{2+}]_i$ oscillations at 1 mM glucose $[\text{Ca}^{2+}]_i$ was reduced to basal levels at 20 mM of the sugar (Fig. 2). After further elevation to 30 mM glucose 86% (12 of 14; P<0.001) of the $\alpha$-cells were inhibited.

To clarify the role of $[\text{Ca}^{2+}]_i$ 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 $\mu$M diazoxide, which clamps the membrane potential close to the equilibrium potential for $K^+$ by activating the ATP-sensitive $K^+$ ($K_{\text{ATP}}$) channels that are present in all types of islet cells (12, 14, 16, 19). Hyperpolarization in the absence of glucose had no effect on $[\text{Ca}^{2+}]_i$ of resting $\beta$-cells but lowered $[\text{Ca}^{2+}]_i$ of oscillating $\alpha$-cells (not shown). In the presence of diazoxide introduction of 7 mM glucose resulted in a small reduction of basal $[\text{Ca}^{2+}]_i$ in 71% of the $\alpha$-cells (14 of 18; P<0.001) and all of 12 $\beta$-cells (P<0.001; Fig. 3). Further elevation of glucose to 30 mM had no effect on the $\alpha$-cells but induced a more pronounced additional reduction of $[\text{Ca}^{2+}]_i$ in 23 of 25 $\beta$-cells (P<0.001). These data are consistent with stimulated sequestration and outward transport of $\text{Ca}^{2+}$ (14, 20, 21) being saturated at lower glucose concentrations in $\alpha$- (14) than in $\beta$-cells (21). Measurements of hormone secretion under similar conditions demonstrated that hyperpolarization with diazoxide reduced glucagon release at 0-1 mM glucose by 70-80% of the maximal glucose
inhibition (Fig. 4, upper panel). Nevertheless, the additional inhibition obtained by raising the sugar concentration to 7 mM 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 mM range. Basal insulin secretion at 0-7 mM glucose was unaffected by the hyperpolarization (Fig. 4, lower panel), whereas insulin secretion at 12-30 mM glucose was reduced by about 70% compared to control.

Glucose initiates insulin release by raising $[Ca^{2+}]_i$ in β-cells. The sugar has also 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 mM K$^+$ + diazoxide) to activate voltage-dependent Ca$^{2+}$ influx. We used this approach to clarify if glucose has an amplifying effect also on glucagon secretion. Fig. 5 shows that depolarization by combining diazoxide with 30 mM K$^+$ resulted in rapid increases of $[Ca^{2+}]_i$ in both α- and β-cells. Subsequent introduction of 3 mM 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 mM 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 mM (Fig. 6, lower panel). Glucose did not affect the depolarization-induced insulin secretion in the 0-7 mM range but higher concentrations
of the sugar had a pronounced amplifying effect. The corresponding glucagon data demonstrated that 30 mM K⁺ plus diazoxide markedly stimulated glucagon secretion in the 0-30 mM range (Fig. 6, upper panel). Glucose elevation to 7 mM 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, it was evident from the present study of mouse pancreatic islets and clonal hamster InR1-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 Kₐ₅p channels leading to depolarization and voltage-dependent Ca²⁺ influx, glucose stimulation of glucagon secretion from mouse islets occurred independent of Kₐ₅p channels and did not require elevation of [Ca²⁺]. A reason for the
different $[Ca^{2+}]_i$ responses is apparently that rat $\alpha$-cells have a high density of $K_{ATP}$ channels, exceeding that in rat $\beta$-cells (24), whereas mouse $\alpha$-cells show little $K_{ATP}$ channel expression (25, 26) corresponding to 2% of that in mouse $\beta$-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 $\alpha$-cells, but tolbutamide-induced elevation of $[Ca^{2+}]_i$ is observed only in a small minority of mouse $\alpha$-cells (14). Moreover, whereas glucose increases the electrical activity and $[Ca^{2+}]_i$ of rat $\alpha$-cells (24) the sugar hyperpolarizes mouse $\alpha$-cells (14, 27, 29) and, as now confirmed, lowers $[Ca^{2+}]_i$ (14).

The present study of mouse $\alpha$-cells and islets and clonal hamster glucagon-releasing cells provide arguments that glucose has direct effects on the $\alpha$-cells both 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, as compared to about 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 GABA (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 involves 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).

Since 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 has also 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 these actions by showing: 1. Stimulated insulin secretion after [Ca\(^{2+}\)]\(_i\) elevation due to depolarization with 30 mM 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. 3. Maintenance of some glucose-
stimulated insulin secretion under hyperpolarizing conditions when the sugar lowered [Ca\textsuperscript{2+}]\textsubscript{i} below the basal level.

The Ca\textsuperscript{2+}-dependent and independent pathways for stimulated secretion were present also in mouse \(\alpha\)-cells: 1. Elevation of [Ca\textsuperscript{2+}]\textsubscript{i} in isolated \(\alpha\)-cells correlated to enhanced glucagon release from pancreatic islets. 2. High glucose concentrations stimulated glucagon secretion under hyperpolarizing conditions although [Ca\textsuperscript{2+}]\textsubscript{i} of the \(\alpha\)-cells was lowered below basal levels. It was recently reported that glucose amplifies Ca\textsuperscript{2+}-initiated glucagon secretion from purified rat \(\alpha\)-cells (8). However, the presently observed stimulatory effect of glucose on glucagon secretion from mouse islets did not depend on the [Ca\textsuperscript{2+}]\textsubscript{i} level of the \(\alpha\)-cells, indicating that glucose initiates rather than amplifies secretion. It may seem surprising that the \(\alpha\)-cells can sense high concentrations of glucose since they express low \(K_m\) glucose transporter GLUT1 but lack high \(K_m\) GLUT2 (38). The situation is reminiscent of that in human \(\beta\)-cells, which, unlike the GLUT2-expressing rodent \(\beta\)-cells, predominantly express GLUT1 (39). Apparently glucose transport via GLUT1 is not rate limiting in these cases and the sensing is explained by presence of the high \(K_m\) glucokinase (39, 40). The signal transduction 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 an U-shaped dose-response-relationship for glucose regulation of secretion. A similar regulation of human \(\alpha\)-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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Legends to Figures

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 to absence of the sugar. ## $P<0.01$, ### $P<0.001$ for the effect of 3-o-methylglucose compared to absence of the sugar. Brackets indicate observations with identical significance levels.

FIG. 2. Effects of raising the glucose concentration from 1 to 20 and 30 mM on $[\text{Ca}^{2+}]_i$ of individual mouse $\alpha$-cells. Representative responses for 14 $\alpha$-cells from 4 preparations.

FIG. 3 Effects of raising the glucose concentration from 0 to 7 and 30 mM on $[\text{Ca}^{2+}]_i$ of hyperpolarized individual mouse $\alpha$- and $\beta$-cells. Diazoxide (250 $\mu$M), which hyperpolarizes the cells in 4.8 mM $K^+$, was present throughout. Representative responses for 18 $\alpha$-cells and 25 $\beta$-cells from 6 preparations.
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 μM diazoxide and 4.8 mM K⁺ (white squares). 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 to absence of the sugar. +++ P <0.001 for the effect of hyperpolarization with diazoxide versus control. The bracket indicates observations with identical significance levels.

FIG. 5. Effects of depolarization by 30 mM K⁺ and increase of the glucose concentration from 0 to 3, 7 and 30 mM on [Ca²⁺]ᵢ of individual mouse α- and β-cells. Diazoxide (250 μM) was added to activate Kₐₜₚ channels before raising the K⁺ concentration from 4.8 to 30 mM. Representative responses for 19 α-cells and 16 β-cells from 4 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 μM diazoxide and 30 mM K⁺ (black squares). 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 8 experiments. *P<0.05, **P<0.01, ***P<0.001 for the effect of glucose compared to absence of the sugar. +++P<0.001 for the effect of depolarization with 30 mM K⁺ plus diazoxide versus control and hyperpolarizing conditions. Brackets indicate observations with identical significance levels.
Glucagon secretion pg/(islet · h)

Insulin secretion ng/(islet · h)

Glucose or (---) 3-o-Methylglucose concentration (mM)

Mouse islets

IN-R1-G9 cells

FIG. 1
FIG. 2
FIG. 3
Glucose concentration (mM)

Glucagon secretion pg/(islet·h)

Insulin secretion ng/(islet·h)

Control □ 250 μM Diazoxide

FIG. 4
FIG. 5
Glucose concentration (mM)

Glucagon secretion pg/(islet · h)

Insulin secretion ng/(islet · h)

Control

- - 250 μM Diazoxide

■ 250 μM Diazoxide + 30 mM K+

+++ ***

FIG. 6