Glucose metabolism and glutamate analog acutely alkalinize pH of insulin secretory vesicles of pancreatic β-cells

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glutamate metabolism; alkalinization of vesicular pH; regulation of insulin secretion

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cose was capable of acutely alkalining secretory vesicle pH and that this effect was dependent on glucose metabolism but not on cytosolic [Ca\(^{2+}\)] elevation. Glutamate dimethyl ester (GME), a cell-permeable analog of glutamate (10, 46, 55), potentiated glucose-stimulated insulin secretion without changing cellular ATP content or cytosolic [Ca\(^{2+}\)]. Application of GME reproduced the alkalining effect of glucose on vesicular pH at the basal glucose concentration. These results suggested that glucose metabolism increased secretory vesicle pH, at least in part, through generation of glutamate, which may be related to an alternative pathway of insulinotropic effect of glucose to the K\(_{\text{ATP}}\) channel-dependent pathway.

MATERIALS AND METHODS

**Materials.** Insulin radioimmunoassay kit and [U-\(^{14}\)C]glucose were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden); lysosomal green DND-189, fura 2-acetoxyethyl ester, and 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-acetoxymethyl ester were from Molecular Probes (Eugene, OR); collagenase, quinacrine, 3-methylglucoside were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden); collagenase, quinacrine, 3-methylglucoside, monoiodoacetic acid, oligomycin, diazoxide, glibenclamide, and ATP bioluminescent assay kit were from Sigma (St. Louis, MO); and GME was from Fluka (Buchs, Switzerland).

**Plasmid construction.** Plasmid phogrin-Ds red was generated by subcloning an AgeI-NotI fragment bearing Ds Red cDNA from plasmid prepro-atrial natriuretic factor (kindly provided by Dr. David K. Apps, University of Edinburgh, Scotland, UK) into phogrin-EGFP, previously digested to remove the EGFP-encoding region.

**Preparation of islets.** Islets were isolated by collagenase digestion and manual picking from the pancreata of C57BL/6J mice at 3–4 mo old (54). These islets were used for experiments immediately after isolation.

**Insulin secretion from islets.** Insulin secretion from pancreatic islets was measured in static incubation or perfusion incubation with Krebs-Ringer bicarbonate (KR) buffer composed of (in mM) 129 NaCl, 4.8 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 2.5 CaCl\(_2\), 5 NaHCO\(_3\), 10 HEPES (pH 7.4), and 0.2% bovine serum albumin (15). In static incubation, batches of 10 islets were preincubated at 37°C for 30 min in KR buffer containing 2.8 mM glucose. The preincubation solutions were replaced with KR buffer containing test agents, and batches of islets were incubated at 37°C for 60 min. Insulin released into supernatants was measured by radioimmunoassay.

In perfusion incubation, 30 islets were suspended in 500 μl of Bio-Gel G-10 beads in each perfusion chamber and perfused with KR buffer at 37°C at a flow rate of 0.6 ml/min. Islets were perfused for 30 min in the presence of 2.8 mM glucose before stimulation. Effluent fractions were periodically collected, and insulin in these samples was measured by radioimmunoassay.

**Fluorescence study.** For monitoring intraluminal pH of insulin-secreting vesicles, islets were loaded with 2 μM DND-189 for 30 min at 37°C in Sol All buffer composed of (in mM) 150 NaCl, 5 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), and 10 HEPES (pH 7.4) (3, 20). After completion of the dye loading, a single islet on a glass coverslip in a perfusion chamber was placed on the stage of an inverted fluorescence microscope IX70 (Olympus, Osaka, Japan) equipped with a charge-coupled device camera (Photometrics). The islet was viewed under a water immersion objective LUMPlanFL lens (Olympus) with an excitation wavelength of 373 nm. Images of 256 × 256 pixels (170 × 170 μm) were obtained every 20 s with an exposure time of 100 ms. Image analysis was carried out using the IPLab program (Signal Analysis). A standard curve for DND-189 fluorescence was obtained by dissolving DND-189 in the intracellular solution (in mM: 136 KCl, 4 NaCl, 5 MgCl\(_2\), 5 glucose, and 20 HEPES) with varying pH values from 5.0 to 6.0. For monitoring cytosolic pH, islets were loaded with 1 μM BCECF for 30 min at 37°C and excited with alternate wavelengths of 440 and 490 nm (23, 44, 47, 48). The emission intensity from islets was excited at 490 nm compared with that at 440 nm of BCECF’s isobestic point was measured and plotted on a standard curve made with the intracellular solution with varying pH values from 6.0 to 8.0. For monitoring cytosolic [Ca\(^{2+}\)]\(_{\text{cyt}}\), islets were loaded with 15 μM fura 2-acetoxyethyl ester at 37°C for 60 min in Sol All buffer and excited with alternate wavelengths of 355 and 380 nm (4, 17). Cytosolic [Ca\(^{2+}\)]\(_{\text{cyt}}\) was expressed as ratios of the emission intensities excited at 355/380 nm. For double staining of β-cells with DND-189 and phogrin-Ds red, a confocal microscopic examination was performed with the FLUOVIEW system and an oil-immersion objective UPlanApo lens (Olympus, Japan). Dispersed single β-cells on a coverslip were transfected with 0.4 μg of plasmid encoding cDNA for phogrin-Ds red by Effectene transfection reagent (Qiagen, Hilden, Germany) (37, 38). After 48 h, islets were loaded with 1 μM DND-189 for 30 min at 37°C. The fluorescence of DND-189 was excited at 488 nm, and that of phogrin-Ds red was excited at 568 nm. The image analysis was performed with FLUOVIEW software (version 2.0.32, Olympus).

**Glucose oxidation.** Oxidation of [U-\(^{14}\)C]glucose in islets was measured by generation of \(^{14}\)CO\(_2\) (54). Batches of 10 islets were incubated at 37°C for 90 min in KR buffer containing the isotope. The \(^{14}\)CO\(_2\) generated in the buffer was made volatile by an addition of HCl, captured in NaOH, and measured with liquid scintillation counting.

**ATP content.** To determine ATP content in islets, batches of 10 islets were incubated at 37°C for 60 min in KR buffer. The incubation was stopped by addition of ice-cold HClO\(_4\), and islets were homogenized by sonication. The homogenates were neutralized by addition of NaOH. ATP content in the supernatants was measured with an ATP bioluminescence assay kit, using known amounts of ATP as internal controls (54).

**Statistical analysis.** Statistical analysis was performed using Student’s t-test for unpaired comparisons and analysis of variance. Values were presented as means ± SE.

RESULTS

Glucose acutely alkalizes secretory vesicle pH. We first studied whether glucose was able to acutely modify the intraluminal pH of secretory vesicles in mouse pancreatic β-cells. For this purpose, β-cells were loaded with a fluorescent probe, lysosensor green DND-189. This probe accumulates in intracellular acidic organelles and emits an increasing intensity of fluorescence in response to acidifying change of the organelle pH when its fluorescence is excited at 373 nm (3, 20). Upon microscopic examination of β-cells loaded with DND-189, the fluorescence was observed in the cytosolic space with a fine granular pattern, which was consistent with the probe’s distribution in secretory vesicles (Fig. 1A, left). To more precisely assess the distribution of DND-189, pancreatic β-cells were transfected with the plasmid phogrin-Ds red. It was constructed to express a fluorescent protein, Ds Red, connected to a...
phogrin that was targeted to secretory vesicle membranes (37, 38). We found that localization of DND-189 fluorescence well overlapped that of phogrin-Ds red (Fig. 1A, right), supporting that DND-189 fluorescence originated from secretory vesicles. Quinacrine has been known to stain lysosomes as well as secretory vesicles (13, 39). When /H9252-cells were loaded with both quinacrine and phogrin-Ds red, the two fluorescences stained almost completely identical spots (data not shown), indicating that a contribution of lysosomes to the DND-189 fluorescence was negligible compared with that of secretory vesicles. Previous reports also showed that each /-cell was tightly packed with ~10,000 secretory vesicles and that the other acidic organelles were much fewer than secretory vesicles in number (21, 43).

Next, islets loaded with DND-189 were examined under perfusion conditions. When the glucose concentration of the perfusate was raised from 2.8 to 22.2 mM, DND-189 fluorescence was decreased by 0.66 ± 0.10% at 148.9 ± 16.0 s (Fig. 1C). Because the pH of secretory vesicles has been reported to be ~5.5 (21), we estimated that the fluorescence decrease corresponded to an increase in pH by 0.016 ± 0.002 unit, using a standard curve in Fig. 1B, in which a decrease in DND-189 fluorescence by 0.41% indicated an increase in pH by 0.010 unit at ~pH 5.5. For comparison, the cytosolic pH of islet cells was monitored with a pH-sensitive fluorescent probe, BCECF (23, 44, 47, 48). In response to 22.2 mM glucose stimulation, cytosolic pH immediately and transiently rose by 0.02 ± 0.01 unit (Fig. 1D). After the pH returned to baseline level, it was further decreased below the level by ~0.04 ± 0.01 unit but was finally restored to the baseline level. Thus the profile of cytosolic pH change in response to glucose was distinct from that of secretory vesicles in their phasic pattern and time course.

Glucose-induced vesicular alkalinization is dependent on glucose metabolism. To study the mechanism of glucose-induced alkalinization of secretory vesicles, we

Fig. 1. Changes of intravesicular and cytosolic pH in islets. A: confocal microscopic examination of /-cells. Dispersed /-cells were cultured on a glass coverslip overnight, transfected with plasmid phogrin-Ds red, and cultured for 48 h. Then, cells were loaded with 1 M fluorescent pH indicator, lysosensor green DND-189, for 30 min at 37°C, and fluorescence was observed with excitation at 488 nm (left). For detection of phogrin-Ds red fluorescence in the same field, excitation wavelength 568 nm was used (right). Scale bar, 0.5 μm. B: standard curve of DND-189 fluorescence was plotted against varying values of pH in the intracellular solution. At pH 5.5, the pH increase by 0.01 unit corresponded to the fluorescence decrease by 0.41%. C: change of intravesicular pH of islets in response to elevating the perfusate glucose concentration from 2.8 to 22.2 mM at the indicated point. Trace shown is representative of 14 experiments. D: cytosolic pH of islets was monitored with fluorescence of 2', 7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). Glucose concentration in the perfusate was changed from 2.8 to 22.2 mM at the indicated point. Trace shown is representative of 6 experiments.
examined effects of glucose metabolism and cytosolic 
\([\text{Ca}^{2+}]\) on the phenomenon. The nonmetabolizable glu-
cose analog 3-\(\text{O}\)-methylglucose, at 19.4 mM, was un-
able to induce a decrease in DND-189 fluorescence
(Fig. 2A) (30). Inhibitor of glycolysis moniodoacetic acid at 1 mM (data not shown) or inhibitor of mitochon-
drial ATP synthase oligomycin at 10 \(\mu\)g/ml (Fig. 2B) 
completely blocked the effect of glucose on secretory
vesicle pH (16, 24, 57). These results indicated that
the vesicular alkalinization was dependent on glucose-metab-
olizing steps finally leading to ATP generation in
mitochondria. We next studied a relationship between
elevating cytosolic \([\text{Ca}^{2+}]\) and vesicular alkalinization.
Diazoxide is known to suppress glucose-induced \(\text{Ca}^{2+}\)
influx into the cytosol by opening the K\text{ATP} channels on
the plasma membranes (19, 45). Indeed, the drug at
250 \(\mu\)M completely inhibited glucose-stimulated insu-
lin secretion (data not shown), indicating that \(\text{Ca}^{2+}\)
influx into the cytosol failed to occur. The alkalinizing
change of vesicles in response to 22.2 mM glucose was
preserved in the presence of 250 \(\mu\)M diazoxide (Fig.
2C). When islets were stimulated with 50 mM KCl in
the presence of 2.8 mM glucose, no changes in DND-
189 fluorescence were observed (Fig. 2D). Gliben-
clamide at 5 \(\mu\)M, which closed the K\text{ATP} channels and
thereby activated \(\text{Ca}^{2+}\) influx into the cytosol, was also
unable to induce the fluorescence change at 2.8 mM
glucose (data not shown). These results indicated that
cytosolic \([\text{Ca}^{2+}]\) elevation was neither necessary nor
sufficient for the glucose-induced vesicular alkaliniza-
tion and that the alkalinizing effect of glucose was
mediated through its metabolic processes but indepen-
dently of the closure of K\text{ATP} channels and subsequent
elevation of cytosolic \([\text{Ca}^{2+}]\).

GME enhances glucose-stimulated insulin secretion
without modulating the K\text{ATP} channel-dependent path-
way. In the next series of experiments, we examined
the possibility that the effect of glucose on secretory
vesicle alkalinization might be relevant to intracellular
generation of glutamate. It has been reported that
cellular concentrations of glutamate were increased
after stimulation of \(\beta\)-cells with glucose (29), probably
via breakdown of glucose molecules through glycolysis
and the TCA cycle, generation of \(\alpha\)-ketoglutarate in the
cycle, and its conversion to glutamate by the enzyme
 glutamate dehydrogenase. Insulin secretion in re-

![Fig. 2. Changes of intravesicular pH in islets. A: at indicated time point, 19.4 mM 3-O-methylglucose was applied
to perifusate in presence of 2.8 mM glucose. Trace shown is representative of 9 experiments. B: glucose
concentration was elevated from 2.8 to 22.2 mM in presence of inhibitor of mitochondrial ATP synthase oligomycin
at 10 \(\mu\)g/ml throughout the perifusion. Trace shown is representative of 12 experiments. C: effect of elevating
concentration of glucose from 2.8 to 22.2 mM was monitored in presence of ATP-sensitive K\textsuperscript{+} (K\text{ATP}) channel opener
diazoxide at 250 \(\mu\)M. Trace shown is representative of 12 experiments. D: depolarizing stimulation with 50 mM
KCl was applied in presence of 2.8 mM glucose. Trace shown is representative of 6 experiments.]

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sponse to 22.2 mM glucose was potentiated by GME (Fig. 3A), a cell-permeable analog of glutamate (10, 46, 55). The effect of GME was dose dependent until 1 mM, but the higher dose of 5 mM was employed thereafter in the present study. This was because the dose makes it easier to compare our results with the previous ones (29, 46). GME at 5 mM increased the secretion by 123 ± 29%. This potentiating effect of GME at 5 mM was not observed at 2.8 or 5.6 mM glucose but was evident at higher concentrations of glucose, indicating that GME was not an initiator of insulin secretion but a potentiator of glucose stimulation (Fig. 3B). In a perifusion study, 5 mM GME potentiated both the first-phase and the second-phase secretions in response to 22.2 mM glucose (Fig. 3C).

Next, we examined whether this effect of GME was mediated through activated oxidation of glucose, which should lead to an increase in ATP content of islets. [U-14C]glucose oxidation was not affected by 5 mM GME at 2.8 mM glucose (Fig. 4A). However, GME decreased [U-14C]glucose oxidation by 28 ± 8% at 22.2 mM glucose (Fig. 4A). These results suggested that application of GME might decrease glucose-stimulated generation of ATP in islets. Thus we next measured ATP content of islets (Fig. 4B) (54). Stimulation of islets with 22.2 mM glucose significantly increased ATP content from 10.2 ± 0.4 to 13.9 ± 1.2 pmol/islet. In the presence of 5 mM GME, ATP content was 13.7 ± 0.3 pmol/islet at 22.2 mM glucose, and this value was comparable to that in the absence of GME. GME at 0.5–2.0 mM also failed to significantly change ATP content of islets stimulated with 22.2 mM glucose (Fig. 4B). Thus, although application of GME decreased glucose oxidation, we assumed that overall ATP generation from oxidation of both glucose and GME-derived glutamate remained largely unchanged. ATP is known to play a crucial role in inhibiting the KATP channels, thereby depolarizing the plasma membranes and elevating cytosolic [Ca2+] (1, 5). Consistent with the unchanged ATP content of islets, elevation of cytosolic [Ca2+] in response to 22.2 mM glucose (from 0.37 ± 0.04 to 0.55 ± 0.06; Fig. 4C, top) was not changed by a simultaneous application of 5 mM GME (from 0.36 ± 0.03 to 0.53 ± 0.05; Fig. 4C, bottom). These results suggested that GME’s potentiating effect on glucose-stimulated insulin secretion was exerted not through augmenting ATP generation or elevating cytosolic [Ca2+] but mainly through nonoxidative utilization of glutamate in the β-cells.

GME mimics the alkalinizing effect of a high concentration of glucose. We next examined whether GME modified the pH of secretory vesicles in perifusion experiments. At 2.8 mM glucose, application of 5 mM GME decreased DND-189 fluorescence by 0.83 ± 0.19%
(an increase in pH by 0.020 ± 0.005 unit) at 37.9 ± 2.6 s (Fig. 5), which was comparable in magnitude to that of 22.2 mM glucose stimulation (Fig. 1C). However, the time required for attaining the level was significantly less ($P < 0.01$) than that of 22.2 mM glucose stimulation (Fig. 1C).

**DISCUSSION**

Here, we report that, by use of a pH-sensitive fluorescent probe specifically targeted to the vesicular lumen, glucose stimulation acutely alkalinized the intravesicular pH of insulin-containing secretory granules by ~0.016 unit within ~3 min in pancreatic β-cells. This was the first observation demonstrating that proton transfer across the vesicle membranes was regulated dynamically in response to the fuel insulin secretagogue. Until now, only chronic-phase acidification of the intravesicular pH has been known to progress

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**Fig. 4.** Effects of GME on glucose metabolism and K<sub>ATP</sub> channel-dependent pathway of glucose-stimulated insulin secretion. A: [U-<sup>14</sup>C]glucose oxidation of islets at 2.8 or 22.2 mM glucose in absence or presence of 5 mM GME ($n = 6$). *$P < 0.05$ compared with controls without GME. B: cellular ATP content of islets was measured after 60-min incubation with indicated concentrations of glucose and GME ($n = 6$). **$P < 0.01$ compared with controls with 2.8 mM glucose. C: cytosolic Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]) changes in response to 22.2 mM glucose in absence (top) or presence (bottom) of 5 mM GME were monitored with fura 2 fluorescence. Cytosolic [Ca<sup>2+</sup>] was expressed as ratios of emission intensities excited at 355/380 nm. Traces shown are representative of 6 experiments.

**Fig. 5.** Changes of intravesicular pH of islets in response to GME. At indicated point, 5 mM GME was applied to perifusate containing 2.8 mM glucose. Trace shown is representative of 10 experiments.
during maturation steps of the vesicle (21, 22, 34, 35, 42). They included a processing of proinsulin molecules by prohormone convertases such as PC2, PC3, and carboxypeptidase H, the pH optimum of which was close to 5.5 (21). Moreover, an aggregation of insulin monomers to hexamers and finally into crystallization was known to be pH sensitive (14, 51). The acute-phase vesicular alkalinization by glucose was dependent on its metabolism, because the use of a nonmetabolizable glucose analog failed to manifest such effect, and the addition of inhibitor of glycolysis or mitochondrial ATP synthesis completely blocked the glucose-induced pH changes. The effect of glucose was preserved in the presence of diazoxide and was not reproduced by pure Ca\(^{2+}\) stimulations without glucose, suggesting that the elevation of cytosolic [Ca\(^{2+}\)] was neither necessary nor sufficient for the phenomenon. It is notable that diazoxide has been known not to interfere with glucose oxidation (8), although the drug opens the K\(_{ATP}\) channels and thus inhibits glucose-stimulated insulin secretion.

The alkalinizing change of vesicular pH in response to a glucose challenge was monotonous and lasted more than 10 min, and thus it was totally distinct from the pH change of the cytosolic space in response to glucose (Fig. 1D), which has been reported to have a biphasic pattern (44, 48). Upon stimulation with glucose, cytosolic pH quickly moved to an initial transient alkalinization. This was explained by mitochondrial consumption of H\(^+\) ions in the course of glucose metabolism. Next, the pH shifted to acidification, which was thought to be coupled with Ca\(^{2+}\) influx from the extracellular space and the action of HCO\(_3\)/Cl\(^-\) exchanger. These results further supported that the fluorescence of DND-189 originated from secretory vesicles but was not contaminated by the fluorescence of the cytosolic space, as was also demonstrated by the copresence of the DND-189 fluorescence with that of vesicular membrane-bound phogrin-Ds red (Fig. 1A) (37, 38).

We assumed glutamate to be a candidate for molecular links between glucose metabolism and acute vesicular alkalinization. Intracellular concentration of glutamate was reported to be increased in β-cells exposed to glucose (29), probably via generation of α-ketoglutarate through a series of steps in glycolysis and the TCA cycle, and finally via its reductive amination reaction into glutamate catalyzed by glutamate dehydrogenase. In fact, glutamate, which was derived from GME by intracellular removal of dimethyl maleoyl (10, 46, 55), mimicked the alkalinizing effect of glucose on the vesicular pH (Fig. 5). However, the times required to attain these fluorescence changes were quite different. The longer time required for the effect of glucose to occur probably corresponded to a time for metabolizing glucose until it becomes glutamate through the intermediate substrate α-ketoglutarate. It was known that it took ~2 min for the mitochondrial electrochemical membrane potential to be fully formed after glucose stimulation (16), which well reflected the activity of the TCA cycle and thus generation of α-ketoglutarate in the cycle.

We observed that GME at 5 mM was effective in potentiating insulin secretion in response to 22.2 mM glucose in mouse islets. Previous reports indicated that the potentiating effect of GME was exhibited at low-to-moderate concentrations of glucose but disappeared at higher concentrations (16.7 to 25 mM) in rat islets and rat β-cell-derived INS-1 cells (29, 46). One possible interpretation of these discrepancies is the difference in efficiencies of intrinsic glutamate generation from glucose. Thus rat β-cells might be active in generating glutamate from glucose and an additional application of external glutamate, as a form of GME, failed to show the potentiating effect. In contrast, the effect was manifested in our study with mouse β-cells because the intrinsic generation of glutamate from glucose might not reach the threshold required for the potentiation. It is well known that the amplitude of the second-phase insulin secretion to glucose is much higher in rat β-cells than in mouse β-cells. A recent report showed that the expression of glutamate dehydrogenase was indeed lower in mouse islets than in rat islets and supported our data because GME induced the secretory response even at 16.7 mM glucose in the mouse islets (27).

At present, a causal relationship between the vesicular alkalinization and glucose-stimulated insulin secretion or glutamate-induced potentiation of the insulin secretion is unclear. In fact, addition of GME did not further alkalinize the pH of secretory vesicles attained by glucose treatment (data not shown), indicating that the potentiating effect of GME on glucose-stimulated insulin secretion was, at least in part, not directly linked to the vesicular alkalinization. However, the following observations prompted us to consider that the alkalinization would not be merely an event that coincided with those increases in insulin secretion but would be, at least in part, causally related to them. Maechler et al. (29) reported that a direct application of glutamate to the vesicles evoked increases in insulin secretion and cytosolic [ATP] and that these increases were blocked by an inhibitor of the vesicular glutamate transporters. They argued that the vesicles themselves were the ATP source of the increase in cytosolic [ATP], probably through a reverse reaction of the vacuolar-type H\(^+\)-ATPase. In fact, similar effects were observed with an inhibitor of vacuolar-type H\(^+\)-ATPase (29). If this were the case, an outward flux of protons across vesicular membranes would reduce the proton gradient, and the luminal pH of the vesicles would tend to be alkalinized, which is what we observed in the present study. Generation of ATP in this manner from the vesicles was assumed to elevate the cytosolic [ATP] immediately near the exocytotic machinery composed of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex and thus to be favorable to the priming and final fusion processes of the vesicles (11, 52). Another merit of the vesicular alkalinization during exocytosis might be prompting the crystals of Zn\(^{2+}\)-insulin hexamers stored inside the vesicles to
dissolve for faster diffusion into the bloodstream. Interestingly, Aspinwall et al. (6) demonstrated with amperometric detection of insulin molecules that inhibiting the vacuolar-type H^+-ATPase and thereby increasing the vesicular pH caused insulin to be more rapidly extruded from the vesicles. Thus alkalinizing the vesicular pH could be a form of priming process of the vesicles for exocytosis, in which the insulin crystals were “primed” for the forthcoming dissolution, although the extent of the alkalinization by glutamate was small.

Lately, it has been found that glutamate is taken up into synaptic vesicles by brain-type Na^+-dependent inorganic phosphate transporter (BNPI) (7, 53) to prepare for its later release in excitatory neuronal cells. Another Na^+-dependent phosphate transporter, differentiation-associated Na^+-dependent inorganic phosphate transporter (DNPI), which has a close resemblance in amino acid sequence to BNPI, was also cloned in neural tissues (2). We screened for these two transporters in a cDNA library made from insulin-producing INS-1 cells, resulting in isolation of both the types of transporters in the β-cells (Yamashita T, Eto K, and Kadowaki T, unpublished observations). It was thus assumed that β-cells also exhibited glutamate uptake activities via these transporters. It is suggested that H^+ may play a role as a counter ion during the glutamate uptake by those transporters (36), where the outward efflux of H^+ should contribute to alkalization of the intravesicular pH, although precise expression and function of BNPI and DNPI in β-cell secretory vesicles remain to be addressed.

In summary, β-cell secretory vesicle alkalinization occurred acutely after a glucose challenge depending on glucose metabolism but independently of cytosolic [Ca^{2+}] elevation or the change of cytosolic pH. Our results suggest the possibility that one of the mediators of this glucose effect was glucose-derived glutamate, because the cell-permeable glutamate analog GME reproduced such alkalinization at a basal concentration of glucose. GME potentiated glucose-stimulated insulin secretion without affecting the K_{ATP} channel-dependent pathway of the secretion. Thus it is likely that glutamate exhibited such a potentiating effect on the secretion by acting directly on secretory vesicles, which may be coupled to changes of proton transport across the vesicle membrane and their acute alkalinization. It was reported that insulin-secretory vesicles were organelles actively importing and releasing Ca^{2+} via certain types of Ca^{2+}-ATPases and ryanodine receptors, respectively, in response to glucose (32). Further elucidation of acute-phase regulation of the ionic milieu in the secretory vesicles will be needed for a comprehensive understanding of the exocytotic mechanism of glucose-stimulated insulin secretion in a normal state and in pathological states such as insulin-deficient type 2 diabetes and PHHI caused by mutations of glutamate dehydrogenase.

DISCLOSURES

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