Overexpression of constitutively activated glutamate dehydrogenase induces insulin secretion through enhanced glutamate oxidation

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Am J Physiol Endocrinol Metab 286: E280–E285, 2004. First published October 7, 2003; 10.1152/ajpendo.00380.2003—Glutamate dehydrogenase (GDH) catalyzes reversible oxidative deamination of l-glutamate to α-ketoglutarate. Enzyme activity is regulated by several allosteric effectors. Recognition of a new form of hyperinsulinemic hypoglycemia, hyperinsulinism/hyperammonemia (HI/HA) syndrome, which is caused by gain-of-function mutations in GDH, highlighted the importance of GDH in glucose homeostasis. GDH266C is a constitutively activated mutant enzyme we identified in a patient with HI/HA syndrome. By overexpressing GDH266C in MIN6 mouse insulinoma cells, we previously demonstrated unregulated elevation of GDH activity to render the cells responsive to glutamine in insulin secretion. Interestingly, at low glucose concentrations, basal insulin secretion was exaggerated in such cells. Herein, to clarify the role of GDH in the regulation of insulin secretion, we studied cellular glutamate metabolism using MIN6 cells overexpressing GDH266C (MIN6-GDH266C). Glutamine-stimulated insulin secretion was associated with increased glutamine oxidation and decreased intracellular glutamate content. Similarly, at 5 mmol/l glucose without glutamine, glutamine oxidation also increased, and glutamate content decreased with exaggerated insulin secretion. Glucose oxidation was not altered. Insulin secretion profiles from GDH266C-overexpressing isolated rat pancreatic islets were similar to those from MIN6-GDH266C, suggesting observation in MIN6 cells to be relevant in native β-cells. These results demonstrate that, upon activation, GDH oxidizes glutamate to α-ketoglutarate, thereby stimulating insulin secretion by providing the tricarboxylic acid (TCA) cycle with a substrate. No evidence was obtained supporting the hypothesis that activated GDH produced glutamate, a recently proposed second messenger of insulin secretion, by the reverse reaction, to stimulate insulin secretion.

hypoglycemia; hyperinsulinism/hyperammonemia syndrome; islet of Langerhans

THE MITOCHONDRIAL MATRIX ENZYME glutamate dehydrogenase (GDH; EC 1.4.1.3) catalyzes reversible oxidative deamination of l-glutamate to α-ketoglutarate with NAD(P) as a cofactor. The activity of this enzyme is regulated positively and negatively by several allosteric effectors, including amino acids (leucine, isoleucine, valine, methionine), ADP, and GTP. In pancreatic β-cells, GDH has been suggested to be involved in the regulation of insulin secretion, especially leucine-stimulated insulin secretion (18, 19). The importance of GDH in glucose homeostasis is also evident from recent findings that gain-of-function mutations in the GLUD1 gene, which encodes GDH, cause hyperinsulinism/hyperammonemia (HI/HA) syndrome (9, 20, 21, 22, 25, 29).

Previously, we identified a GLUD1 gene mutation, Y266C, in a patient with HI/HA syndrome (22). The activity of the mutant GDH (GDH266C) was constitutively elevated, and allosteric regulations by ADP and GTP were severely impaired. Using GDH266C as a tool, we showed unregulated elevation of GDH activity in MIN6 insulinoma cells to render the cells responsive to glutamine. Glutamine stimulated insulin secretion from these cells in the absence of leucine, an allosteric activator of GDH. We also demonstrated insulin secretion to be exaggerated in these cells at low glucose concentrations (22). Glutamine alone, to which the plasma membrane is permeable and which is readily converted to glutamate intracellularly, does not normally stimulate insulin release. However, it remarkably stimulates insulin secretion in the presence of leucine. It is generally accepted that, in pancreatic β-cells, activation of GDH by allosteric effectors, such as leucine, enhances glutamate oxidation and increases ATP production by providing the tricarboxylic acid (TCA) cycle with α-ketoglutarate and thereby stimulates insulin secretion (18, 19). Physiologically, GDH is also suggested to play an important role in basal insulin secretion (2, 5). Our previous observations (22) in MIN6 cells are in good agreement with this theory.

On the other hand, mitochondrially derived glutamate was suggested to be a second messenger in glucose-stimulated insulin secretion, acting directly on insulin-secretory granules (4, 11, 17). This theory assumes reverse flux through GDH in the direction of glutamate formation, and glutamate-induced insulin secretion was suggested to correlate with the level of GDH expression (4, 10). However, this hypothesis is controversial and has been contradicted by other studies (2, 8). Furthermore, it was recently demonstrated that cellular glutamate content did not correlate with the amplification of insulin secretion (1, 7). Most previous studies have investigated the role of GDH in insulin secretion by activating intracellular..
GDH with allosteric activators such as leucine or β-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid. Identification of constitutively activated mutant GDH (GDH266C) enabled us to study the effects of elevated intracellular GDH activity on insulin secretion more directly by introducing the mutant enzyme into cells. Our present study was designed to investigate directly the correlations among insulin secretion, GDH activity, and cellular glutamate metabolism. We also studied changes in insulin secretion profiles caused by unregulated elevation of GDH activity in native β-cells to confirm the physiological relevance of our findings in MIN6 cells. Our results further clarify the role of GDH in the regulation of insulin secretion and provide insights into the pathophysiology of the HI/HA syndrome.

MATERIALS AND METHODS

Analysis of glutamine and glucose oxidation. MIN6 cells overexpressing the mutant GDH via retrovirus-mediated gene transfer (MIN6-GDH266C) and control lacZ-overexpressing cells (MIN6-lacZ) were used for these experiments (22). Cells were seeded onto a 6-cm dish at a concentration of 4.0 × 10⁶ cells/dish and cultured in DMEM-MIN6 medium (Sigma, St. Louis, MO) containing 25 mM lactate supplemented with 15% heat-inactivated fetal calf serum, 72 μM β-mercaptoethanol, 50 μg/ml penicillin, and 50 μg/ml streptomycin. Sixty hours later, glutamine or glucose oxidation was assayed. After a 30-min preincubation in HEPES-balanced Krebs-Ringer bicarbonate buffer (HB-KRBB; in mmol/l: 10 HEPES, 120 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 20 NaHCO₃, and 2 CaCl₂; pH 7.4) containing 0.5% BSA and 5 mmol/l glucose, radioactive [1-¹⁴C]glutamine (0.05 μCi, 261 Ci/mmol; Amersham, Buckinghamshire, UK) or radioactive [6-¹⁴C]glucose (0.04 μCi (for 5 mmol/l glucose) or 0.20 μCi (for 25 mmol/l glucose), 38.0 Ci/mmol; Amersham) was added to 4 ml of fresh HB-KRBB containing 0.5% BSA and various concentrations of glutamine or glucose. Then, MIN6-GDH266C or MIN6-lacZ cells in the culture dishes were placed immediately in sealed glass containers (7 cm diameter × 10 cm height) filled with 100% oxygen and incubated for 30 min (glutamine oxidation) or 1 h (glucose oxidation) at 37°C. At completion of the incubations, 0.5 ml of 10% HClO₄ was added to the medium by means of a long 21-gauge needle through rubber stoppers on the top of the container, allowing CO₂ gas (containing radioactive [¹⁴C]CO₂) to evaporate and be trapped in 2 ml of 10% KOH solution in a small glass cup suspended above the medium in a sealed glass container. The glass containers were incubated for another 30 min, and the KOH solution was then transferred to scintillation vials containing 10 ml of scintillation fluid and various concentrations of glutamine or glucose. After an additional 30-min incubation at 37°C, the buffer was collected, and the supernatant was kept at −80°C for the glutaamine measurement. Cells in one of the wells were homogenized in PBS and used for protein determination. The amount of glutamate was determined, using an aliquot of the cell extract, by high-performance liquid chromatography with precolumn o-phthalaldehyde derivatization, separation on a reverse-phase Resolve C18 column (3.9 × 150 mm; Waters, Toronto, ON, Canada), and fluorescence detection (3, 27, 28).

Construction of recombinant adenoviruses and adenovirus-mediated gene transfer. pcDNA3-hGDHWT- and pcDNA3-hGDH266C (22) were digested with NotI and SnaBI. The fragments containing GDH cDNA were then ligated into NotI- and SnaBI-digested pShuttle vectors (14, 15). The resultant plasmids, pShuttle-hGDHWT and pShuttle-hGDH266C, were then digested with I-CeuI/Pl-Srcl and ligated into I-CeuI/Pl-Srcl-digested pAdHDM4 (14, 15) to produce pAd-hGDHWT and pAd-hGDH266C. They were then linearized with PscI and transfected into 293 human embryonic kidney cells with FuGENE6 (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. Recombinant adenoviruses expressing GDH WT and GDH266C (Ad-hGDHWT and Ad-hGDH266C) were thus obtained and amplified via infection of 293 cells. As a control, we also constructed an adenoviral vector to express enhanced green fluorescent protein (eGFP; Ad-eGFP). Titers of the recombinant adenovirus stocks were 6.0 × 10⁷ (Ad-hGDHWT), 5.5 × 10⁷ (Ad-hGDH266C), and 9.5 × 10⁷ plaque-forming units (pfu)/ml (Ad-eGFP).

Pancreatic islets were isolated by collagenase digestion as described previously (6, 24). Isolated islets were cultured on a 60-mm tissue culture dish with RPMI 1640 medium containing 11 mmol/l glucose supplemented with 1% fetal calf serum, 50 U/ml penicillin, and 50 μg/ml streptomycin (RPMMI-islet) and maintained at 37°C in humidified 5% CO₂-95% air. Twenty-four to thirty-six hours after isolation, groups of 50–100 islets were incubated with the recombinant adenoviruses at a multiplicity of infection (moi) of ~4 × 10⁵ pfu/islet. After a 1-h incubation with the adenovirus at 37°C, the medium was removed, and the islets were washed once with phosphate-buffered saline (PBS). The islets were then further incubated on a 60-mm tissue culture dish with RPMI-islet medium. Experiments were performed 24 h after infection.

GDH enzyme assay. COS-7 cells and isolated pancreatic islets were infected with recombinant adenoviruses at an moi of ~10 pfu/cell or 4 × 10⁵ pfu/islet, respectively. Forty-eight (COS-7) or 24 (islets) h after the infection, cells were washed, suspended in PBS, and sonicated to prepare crude cell extract. GDH activity was measured by the oxidation of NADH (ε₃₄₀ nm = 6.22 × 10³ mol⁻¹·l⁻¹·cm⁻¹), as described previously (26), with a Beckman Coulter (Fullerton, CA) Spectrophotometer model DU-640 at 25°C. The assay solution (1 ml) consisted of 10 mmol/l Tris-acetate (pH 8.0), 10 μmol/l EDTA, 100 μmol/l NADH, 50 mmol/l NH₄Cl, and 5 mmol/l L-α-ketoglutarate. ADP, GTP, or leucine was added to the solution at various concentrations. The reaction was started by adding appropriate amounts (30–50 μl) of cell extracts, and the decrease in absorbance at 340 nm was measured for 5 min. During this incubation period, the reaction was linear, and there was no indication of GTP hydrolysis, substrate depletion, or product saturation. The activity was determined in duplicate for each sample.

Analysis of insulin secretion. Groups of 10–30 islets overexpressing the mutant GDH (Islets-GDH266C) and eGFP (Islets-eGFP) via adenovirus-mediated gene transfer were used for each assay. Insulin secretion was examined by the static incubation method (23, 24). In brief, after a 30-min preincubation in HB-KRBB supplemented with 0.5% BSA and 5 mmol/l glucose, the preincubation buffer was replaced with fresh HB-KRBB containing 0.5% BSA and various concentrations of glutamine or glucose. After an additional 30-min incubation at 37°C, the buffer was collected, and immunoactive insulin was measured by radioimmunoassay using rat insulin (Linco Research, St. Charles, MO) as a standard. The amounts of secreted insulin were corrected by the amounts of cell protein in each well.
RESULTS

Glutamine and glucose metabolism in MIN6-GDH266C cells. We investigated the metabolic changes associated with elevated GDH activity. We used MIN6-GDH266C as a model. When the cells were incubated in the presence of glutamine (1 mmol/l) with a tracer of L-[U-14C]glutamine, glutamine oxidation was increased in MIN6-GDH266C compared with control MIN6-lacZ (P < 0.02, unpaired t-test; Fig. 1A). In agreement with the enhanced glutamine oxidation, intracellular glutamate content in MIN6-GDH266C was lower than that in MIN6-lacZ (P < 0.01 at 1.0 mmol/l; Fig. 1B).

At low glucose concentrations (without exogenous glutamine), insulin secretion was augmented in MIN6-GDH266C (22). We then studied metabolic changes under these conditions. Glucose oxidation did not differ between MIN6-GDH266C and MIN6-lacZ ([4.0 ± 0.7 vs. 4.0 ± 0.9 pmol/μg protein at 5 mmol/l glucose (P > 0.9; Fig. 2A) and 7.9 ± 2.0 vs. 6.8 ± 1.9 pmol/μg protein at 25 mmol/l glucose (P > 0.6; unpaired t-test)]. Cellular glutamine oxidation, however, was significantly enhanced in MIN6-GDH266C [46.7 ± 1.2 vs. 27.2 ± 1.6 pmol/μg protein at 5 mmol/l glucose (P < 0.001; Fig. 2B) and 31.7 ± 4.1 vs. 16.4 ± 2.0 pmol/μg protein at 25 mmol/l glucose (P < 0.03; unpaired t-test)]. The corresponding intracellular glutamate content was decreased in MIN6-GDH266C compared with that in MIN6-lacZ [10.3 ± 2.0 vs. 25.4 ± 2.3 pmol/μg protein at 5 mmol/l glucose (P < 0.002; Fig. 2C) and 14.2 ± 1.3 vs. 36.6 ± 2.1 pmol/μg protein at 25 mmol/l glucose (P < 0.001; unpaired t-test)]. These results indicate that elevated GDH activity enhances glutamine oxidation and probably increases ATP synthesis via the TCA cycle, thereby stimulating insulin secretion.

Characterizations of GDH266C expressed in COS-7 cells and in isolated rat pancreatic islets. To overexpress GDH266C in isolated rat pancreatic islets, we constructed a recombinant adenovirus, Ad-hGDH266C. As we previously demonstrated using the enzyme expressed in COS-7 cells (22), basal activity (activity in the absence of allosteric effectors) of GDH266C was elevated, and inhibition by GTP and activation by ADP were blunted compared with those of the wild-type enzyme. Here, we further characterized activation by leucine by using COS-7 cell extracts in which wild-type GDH or GDH266C was overexpressed by adenovirus-mediated gene transduction. Activation of GDH266C by leucine was only twofold (from 2,850 ± 120 to 5,520 ± 190 nmol NADH·mg protein−1·min−1 at 3 mmol/l leucine), whereas that of wild-type GDH was more than 35-fold (from 130 ± 20 to 4,670 ± 70 nmol NADH·mg protein−1·min−1 at 3 mmol/l leucine). Maximal activity of GDH266C in the presence of leucine was nearly the same as that of the wild-type enzyme in the crude cell extracts, although analysis of the purified enzyme was necessary for the strict quantitative comparison.

Next, we overexpressed GDH266C in isolated islets (Islets-GDH266C) using the adenovirus-mediated gene transfer system to investigate the role of GDH in native β-cells. Islets overexpressing eGFP (Islets-eGFP) were used as a control. Transfer of exogenous genes with adenovirus vector to islets was very efficient, and most of the islet cells expressed eGFP when they were infected with Ad-eGFP, as confirmed by observation under fluorescence microscopy (Ref. 24 and data not shown). The basal GDH activities in the crude extracts of Islets-eGFP and Islets-GDH266C were 20 ± 3 and 2,890 ± 670 nmol NADH·mg protein−1·min−1, respectively, when activity was measured without allosteric effectors in the reaction mixture (Table 1). As expected, ADP activated GDH activity 20-fold in the crude extract of Islets-eGFP (510 ± 50 nmol NADH·mg protein−1·min−1 at 200 μmol/l ADP), whereas activation in the extract of Islets-GDH266C was less than twofold (4.570 ± 650 nmol NADH·mg protein−1·min−1 at 200 μmol/l ADP). GTP did not inhibit GDH activity in Islets-GDH266C (2,840 ± 600 nmol NADH·mg protein−1·min−1 at 25 μmol/l GTP). Therefore, in Islets-GDH266C, GDH activity was constitutively elevated.

Profiles of insulin secretion from Islets-GDH266C. It is known that in normal pancreatic β-cells glutamine stimulates
insulin secretion only in the presence of leucine, an allosteric activator of GDH. As shown in Fig. 3A, glutamine alone did not stimulate insulin secretion from Islets-eGFP, as was observed with intact islets. On the other hand, it stimulated insulin secretion from Islets-GDH266C in a dose-dependent manner.

Glucose-stimulated insulin secretion was also studied. Insulin secretion from Islets-GDH266C was significantly exaggerated at low glucose concentrations compared with control Islets-eGFP [0.16 ± 0.03 (Islets-eGFP) vs. 0.34 ± 0.09 ng insulin/µg protein (Islets-GDH266C) at 2 mmol/l glucose (P < 0.05, n = 16); 0.29 ± 0.06 (Islets-eGFP) vs. 0.48 ± 0.07 ng insulin/µg protein (Islets-GDH266C) at 5 mmol/l glucose (P < 0.001, n = 15); 0.55 ± 0.08 (Islets-eGFP) vs. 0.78 ± 0.15 ng insulin/µg protein (Islets-GDH266C) at 8 mmol/l glucose (P < 0.05, n = 14); paired t-test] but not at higher glucose concentrations (Fig. 3B).

**DISCUSSION**

A mutant GDH, GHD266C, which was identified in a Japanese patient with HI/HA syndrome, is a constitutively activated enzyme: basal activity is elevated, and activation by ADP and inhibition by GTP are blunted compared with the wild-type enzyme (22). In addition, we have herein demonstrated activation by leucine also to be blunted. It has been suggested that ADP binds to and activates GDH by opening the catalytic cleft of the enzyme (16). Leucine is thought to bind at the active site (26). It is possible that in GDH266C the catalytic cleft is almost fully open in the basal state, such that binding of ADP or leucine only minimally activates this mutant enzyme. We used the GDH266C, rather than wild-type GDH, as a tool to examine the effects of elevated cellular GDH activity in the regulation of insulin secretion, because with this mutant, GDH activity is thought to be elevated regardless of phosphate potential (GTP and ATP-to-ADP and P, ratio) (2, 5) in the cells.

We previously demonstrated glutamine to stimulate insulin secretion from MIN6 cells overexpressing GDH266C (MIN6-GDH266C) in the absence of leucine. In addition, and very interestingly, insulin secretion from MIN6-GDH266C cells was exaggerated at low glucose concentrations (2–5 mmol/l) in the absence of glutamine in the incubation buffer (22). To investigate the mechanism by which elevated cellular GDH activity leads to the stimulation of insulin secretion, we studied changes in glutamate metabolism in cells in which GDH activity was constitutively elevated.

In association with the stimulation of insulin secretion by glutamine, cellular glutamate oxidation was elevated (Fig. 1A),

### Table 1. GDH activity in Islets-GDH266C and Islets-eGFP

<table>
<thead>
<tr>
<th>Condition</th>
<th>ADP, µmol/l</th>
<th>Glucose, mmol/l</th>
<th>GTP, µmol/l</th>
<th>Glutamate production (µmol)</th>
<th>GDH Activity, nmol NADH/mg protein min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Islets-eGFP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>20±3 2.890±670</td>
</tr>
<tr>
<td>Islets-GDH266C</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>510±50</td>
<td>4.570±650</td>
</tr>
<tr>
<td>Islets-GDH266C</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td>2.840±600</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3. GDH, glutamate dehydrogenase; Islets-GDH266C, islets overexpressing a constitutively activated mutant GDH; Islets-eGFP, islets overexpressing enhanced green fluorescent protein (control). *Below assay sensitivity.
GDH ENHANCES INSULIN SECRETION VIA GLUTAMATE OXIDATION

and intracellular glutamate content was lower (Fig. 1B) in MIN6–266C cells. This observation is consistent with the hypothesis that elevated GDH activity enhances the oxidative deamination of glutamate to α-ketoglutarate to supply TCA cycle substrates (18, 19). It is noteworthy that, at the basal glucose concentration (5 mmol/l) without glutamine in the medium, exaggerated insulin secretion was also associated with enhanced glutamine oxidation, and a decrease in the intracellular glutamate content reflected utilization of the substrate. At this glucose concentration, glutamine (or glutamate) from the intracellular pool would likely be utilized as a substrate of GDH to fuel the TCA cycle and thereby stimulate insulin secretion. At a higher glucose concentration (25 mmol/l), glutamine oxidation also increased, and the cellular glutamate content was lower in MIN6-GDH266C than in MIN6-lacZ cells, although insulin secretion did not differ. Under these conditions, the effect of increased glutamate oxidation on insulin secretion was probably undetectable because glucose stimulation was more potent.

Although the MIN6 cell line is one of the best models of native β-cells (13), it is derived from an insulinoma, and insulin-secretory profiles are known to change over several passages (12). Therefore, this cell line may not accurately reflect native β-cells in some respects. Therefore, we wished to confirm the effect of unregulated elevation of GDH activity on insulin secretion in a more physiological model: isolated rat pancreatic islets. Islets-GDH266C secreted insulin in response to glutamine in a dose-dependent manner (Fig. 3A). More importantly, in Islets-GDH266C, insulin secretion was also significantly enhanced at low glucose concentrations (2–8 mmol/l) compared with control Islets-eGFP (Fig. 3B), just as we observed in MIN6-GDH266C cells (22). Recently, Kelly et al. (5) reported an H454Y-GDH transgenic mouse. In this animal model, an HI/HA syndrome patient-derived mutant, H454Y-GDH, the activity of which was not inhibited by GTP, was specifically expressed in β-cells. Random blood glucose concentrations were lower than in control mice, and amino acid- and leucine-stimulated insulin secretions from perfused islets were markedly enhanced in these mice. Although glucose-stimulated insulin secretion was reported to be similar to that of control mouse islets, detailed data, including basal insulin secretion at low glucose concentrations, have not been presented. Because basal GDH activity was more than 100 times higher than that in control islets in our model (Table 1), the enhancement of basal insulin secretion might have been more prominent in our model than in the transgenic mouse model.

Physiologically, GDH is an important regulator of glutaminolysis (glutamate oxidation), which may contribute to the interprandial basal insulin secretion (2, 5). Glutaminolysis is regulated by allosteric regulation of GDH with amino acids such as leucine, isoleucine, and methionine. In addition, it is also precisely regulated by glucose metabolism through changes in concentrations of other important allosteric regulators of GDH, GTP, ATP, and ADP. According to this hypothesis, at glucose concentrations near or below its threshold to stimulate insulin secretion (5 mmol/l), GDH is activated by a decrease in the GTP/ADP ratio and drives basal insulin secretion, at least in part (2, 5). Insulin-secretory profiles of MIN6-GDH266C cells and Islets-GDH266C are in agreement with this hypothesis. Constitutively activated GDH rendered the cells responsive to glutamine in insulin secretion in the absence of leucine and enhanced insulin secretion at low glucose concentrations. Furthermore, they would be reflected in the fasting and protein meal-induced hyperinsulinemic hypoglycemia in patients with HI/HA syndrome, although in patients β-cells elevation of GDH activity at low glucose concentrations would be modest compared with that in MIN6-GDH266C cells and in Islets-GDH266C. On the other hand, in our previous and present studies, glucose-stimulated insulin secretion was not enhanced in either MIN6-GDH266C cells (22) or Islets-GDH266C (Fig. 3B). On the basis of the insulin secretion profiles and glutamate metabolism, our data do not support the hypothesis that glutamate, derived from the reverse GDH reaction (flux from α-ketoglutarate to glutamate), is a second messenger of glucose-stimulated insulin secretion (1, 2, 5, 8, 9), although we neither measured the flux directly nor tested
the messenger action of glutamate in glucose-stimulated insulin secretion, and therefore the messenger role of glutamate is not completely excluded.

This is the first study, to our knowledge, in which insulin secretion and glutamate metabolism were analyzed simultaneously under conditions of direct and constitutive cellular GDH activity elevation. Our results illustrate the importance of GDH in amino acid-stimulated insulin secretion and possible contribution to the regulation of basal insulin secretion. Furthermore, we have provided additional evidence that, at least under our experimental conditions, the metabolic flux through GDH is in the direction of α-ketoglutarate production in pancreatic β-cells. No evidence was obtained to suggest that glutamate produced by the reverse GDH reaction enhanced insulin secretion.

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