Unregulated Elevation of Glutamate Dehydrogenase Activity Induces Glutamine-Stimulated Insulin Secretion

Identification and Characterization of a GLUD1 Gene Mutation and Insulin Secretion Studies With MIN6 Cells Overexpressing the Mutant Glutamate Dehydrogenase

Yukio Tanizawa,[1] Kazuaki Nakai,[1] Terumasa Sasaki,[1] Takatoshi Anno,[1] Yasuharu Ohta,[1] Hiroshi Inoue,[1] Kiyosato Matsuo,[2] Mayumi Koga,[1] Susumu Furukawa,[3] Yoshitomo Oka[1]

Glutamate dehydrogenase (GDH) is important in normal glucose homeostasis. Mutations of GDH result in hyperinsulinism/hyperammonemia syndrome. Using PCR/single-strand conformation polymorphism analysis of the gene encoding GDH in 12 Japanese patients with persistent hyperinsulinemic hypoglycemia of infancy (PHHI), we found a mutation (Y266C) in one PHHI patient. This mutation was not found in any of the control or type 2 diabetic subjects. The activity of the mutant GDH (GDH266C), expressed in COS-7 cells, was constitutively elevated, and allosteric regulations by ADP and GTP were severely impaired. The effect of the unregulated increase in GDH activity on insulin secretion was examined by overexpressing GDH266C in an insulinoma cell line, MIN6. Although glutamine alone did not stimulate insulin secretion from control MIN6-lacZ, it remarkably stimulated insulin secretion from MIN6-GDH266C. This finding suggests that constitutively activated GDH enhances oxidation of glutamate, which is intracellularly converted from glutamine to α-ketoglutarate, a tricarboxylic acid cycle substrate, which thereby stimulates insulin secretion. Interestingly, insulin secretion is also exaggerated significantly at low glucose concentrations (2 and 5 mmol/l) but not at higher glucose concentrations (8–25 mmol/l). Our results directly illustrate the importance of GDH in the regulation of insulin secretion from pancreatic β-cells.
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From the [1]Department of Bio-Signal Analysis, Yamaguchi University Graduate School of Medicine, Ube, Japan; the [2]Matsuo Clinic of Pediatrics, Yamaguchi, Japan; and the [3]Department of Reproductive, Pediatric and Infection Science, Yamaguchi University School of Medicine, Ube, Japan.

Address correspondence and reprint requests to Yukio Tanizawa, MD, PHD, Department of Bio-Signal Analysis, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami Kogushi, Ube, Yamaguchi 755-8505, Japan. E-mail: tanizawa@yamaguchi-u.ac.jp.

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DMEM, Dulbecco's modified Eagle's medium; GDH, glutamate dehydrogenase; HI/HA, hyperinsulinism/hyperammonemia; IC_{50}, half-maximal inhibitory concentration; KRBB, Krebs-Ringer bicarbonate buffer; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; SC_{50}, half-maximal stimulatory concentration; SSCP, single-strand conformation polymorphism; TCA, tricarboxylic acid.

Mitochondrial glutamate dehydrogenase (GDH; EC 1.4.1.3) catalyzes reversible oxidative deamination of L-glutamate to α-ketoglutarate. The activity of the enzyme is regulated positively and negatively by several allosteric effectors, such as leucine, ADP, and GTP. GDH is ubiquitously expressed, and its oxidative deamination reaction feeds the tricarboxylic acid (TCA) cycle by converting L-glutamate to α-ketoglutarate, whereas the reductive amination reaction supplies nitrogen for several biosynthetic pathways. In pancreatic β-cells, it is involved in the regulation of insulin secretion, especially amino acid–stimulated insulin secretion (1). For instance, leucine and its nonmetabolizable analog β-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) stimulate insulin secretion by allosterically activating GDH, thereby enhancing glutaminolysis (1,2). Glutamine alone, which is permeable to the plasma membrane and converted to glutamate intracellularly, does not stimulate insulin secretion. However, in the presence of leucine, glutamine does induce insulin secretion. Leucine-activated GDH is considered to enhance glutamate oxidation and increase ATP production by providing the TCA cycle with substrate (α-ketoglutarate) and therefore to stimulate insulin secretion.

The importance of GDH in normal glucose homeostasis in humans is also evident from the recent findings that mutations in the GLUD1 gene, which encodes GDH, cause hyperinsulinism/hyperammonemia (HI/HA) syndrome (3–5). HI/HA syndrome is characterized by hyperinsulinemic hypoglycemia accompanied by mild, asymptomatic hyperammonemia. Patients are usually diagnosed as having hypoglycemia, which frequently occurs after high-protein meals, several months after birth. Mutations were initially identified in exons 11 and 12 of the GLUD1 gene (3,4) and subsequently in exons 6 and 7 (5). All of the mutations identified in these patients impair allosteric inhibition by GTP and are therefore "gain of function" mutations. Hyperinsulinemia and hyperammomenia may be attributable to increased oxidative deamination of glutamate due to hyperactive GDH in pancreatic β-cells and hepatocytes (3). More recently, it was suggested that mitochondrially

derived glutamate potentiates insulin secretion, acting directly on insulin secretory granules (6). This theory assumes reverse flux through GDH in the direction of glutamate formation. However, the existence of this mechanism is controversial (7,8).

Herein, we examined the GLUD1 gene mutation in Japanese patients with persistent hyperinsulinemic hypoglycemia of infancy (PHHI). One mutation (Y266C) was identified in a patient with clinical features of HI/HA syndrome. This mutation gave the enzyme distinctive kinetic properties; basal enzymatic activity appeared to be higher and was not further activated or inhibited by ADP and GTP, respectively. By overexpressing this constitutively activated form of GDH in MIN6 cells, we directly examined the effect of altered GDH activity on the regulation of insulin secretion.

RESEARCH DESIGN AND METHODS

Study subjects. The 12 patients with PHHI were part of a cohort we previously studied (9). Patients with mutations in the SUR1 gene were excluded, and none of the 12 had mutations of the Kir6.2 gene. No information was available regarding plasma ammonium levels for these patients at the initiation of this study. Patients with type 2 diabetes and nondiabetic control subjects were recruited from Yamaguchi University Hospital and affiliated hospitals. At the time of recruitment, informed consent was obtained from each individual according to a protocol approved by the Human Studies Committee of Yamaguchi University School of Medicine, and the investigation was performed in accordance with the guidelines expressed in the Declaration of Helsinki.

PCR/single-strand conformation polymorphism analysis. All 13 coding exons of the human GLUD1 gene were examined by PCR/single-strand conformation polymorphism (SSCP) analysis. Each of the exons was PCR amplified from peripheral blood leukocyte DNA. Primers for exons 11 and 12 were synthesized according to Stanley et al. (3). Other primers were designed based on the public database sequences (10) (accession nos. X66300–X66309, X66311–X66319, and AL136982) (Table 1). SSCP analysis was performed at 15°C using a CleanGel DNA analysis kit and a Multiphor II electrophoresis system (Amersham Pharmacia Biotech, Tokyo) as previously described (9). Nucleotide changes were identified by direct sequencing of PCR products using a model 373A automated sequencer and BigDye terminators cycle sequencing kit (Applied Biosystems Japan, Tokyo).

Cloning of human GDH cDNA, expression in eukaryotic cells, and GDH enzyme assay. The full coding region of human GDH cDNA was obtained from 293 cell RNA by RT-PCR using primers 5′-CGC TTG TGG CCA TGT ACC-3′ (87–104, Genbank accession no. M20867) and 5′-TGT AAA CTT GTG ATA GGT CTG CAG-3′ (1,843–1,820). The GDH cDNA was cloned in pcDNA3 (Invitrogen, Carlsbad, CA) to make pcDNA3-hGDH, and the sequence was confirmed. A mutant GDH266C cDNA was made using a GeneEditor in vitro site-directed mutagenesis system (Promega, Tokyo) with mutagenesis primer 5′-CTC TAT GAG ATg TTT ACA TCG TTT TGG-3′ and a bottom-select oligonucleotide that was supplied in the kit.

The plasmids pcDNA3-hGDH and pcDNA3-hGDH266C were introduced into COS-7 cells by transfection with TransIT LT1 (PanVera, Madison, WI) according to the supplier's protocol. A reporter plasmid, pcDNA3-lacZ, was cotransfected. Forty-eight hours after the transfection, cells were collected, suspended in PBS, and disrupted by sonification. After a brief centrifugation, the supernatant was used for GDH enzyme assay. β-Galactosidase activity was also measured (11). In MIN6 cells, wild-type and mutant GDH were overexpressed using retrovirus-mediated gene transfer as described below. Samples for the GDH enzyme assay were prepared as for COS-7 cells.

GDH activity was measured as previously described (12) using 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 NH_4Cl, and 5 mmol/l α-ketoglutarate. ADP or GTP was added to the solution at various concentrations. The reaction was started by adding cell extracts, and the decrease in absorbance at 340 nm was measured for 5 min. The activity was determined in duplicate or in triplicate for each sample. The half-maximal stimulatory concentration (SC_{50}) and the half-maximal inhibitory concentration (IC_{50}) were determined graphically.

Production of recombinant retroviruses and establishment of MIN6 cells stably expressing GDH266C. The mutant GDH266C cDNA was inserted into the retrovirus vector pMX-puro (kindly provided by Dr. T. Kitamura, Institute of Medical Science, University of Tokyo) to make pMX-puro-GDH266C. Another retrovirus vector, pMX-puro-lacZ, which encodes the bacterial β-galactosidase gene, was also constructed and used as a control. Recombinant retroviruses were produced by transfecting these recombinant retrovirus vectors into a transient retrovirus packaging cell line, Plat-E cells (13), using FuGENE 6 (Roche, Indianapolis, IN) according to the supplier's protocol. Culture media containing infectious retrovirus particles were collected 48 h after transfection.

MIN6 cells (14) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St Louis, MO) containing 25 mmol/l glucose and supplemented with 15% of heat-inactivated FCS, 72 μmol/l β-mercaptoethanol, 50 unit/ml penicillin G, and 50 μg/ml streptomycin (DMEM-MING). MIN6 cells were infected with the recombinant retroviruses by incubating the cells with media containing the retroviruses for 24 h in the presence of 10 μg/ml polybrene (Sigma). Seventy-two hours after the initiation of infection, puromycin (2 μg/ml) was added to the culture medium, and MIN6 cells integrating the retroviral genome (MIN6-GDH266C and MIN6-lacZ) were selected.

Analysis of insulin secretion. MIN6 cells overexpressing the mutant GDH via retrovirus-mediated gene transfer (MIN6-GDH266C) and control lacZ-overexpressing cells (MIN6-lacZ) were seeded onto 24-well plates at a concentration of 3.5×10^5 cells/well and cultured in DMEM-MIN6 medium. Sixty hours later, insulin secretion was assayed by the static incubation method (15). In brief, after a 30-min preincubation in HEPES-balanced Krebs-Ringer bicarbonate buffer (KRBB) (10 mmol/l HEPES, 120 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 20 mmol/l $NaHCO_3$, and 2 mmol/l $CaCl_2$, pH 7.4) supplemented with 0.5% BSA and 5 mmol/l glucose, the preincubation buffer was replaced with fresh HEPES-balanced KRBB containing 0.5% BSA and various concentrations of glutamine or glucose. After an additional 2-h incubation at 37°C, the buffer was collected, and immunoreactive 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.

TABLE 1
Primers for the PCR-SSCP analysis of the human GLUD1 gene

Exon		Primer sequence
1		
	forward-1	5′-CGC ACC TCC CCT CCG CTT GT-3′
	reverse-1	5′-GCT TCT GCT CCT CGC TCT CC-3′
	forward-2	5′-GCG GCG CCA GCA TCG TGG AG-3′
	reverse-2	5′-GCG GGA CGG GGC CCG GCC TA-3′
2		
	forward	5′-CCA CCC CAA CAC ATT TTT TTA AAA AC-3′
	reverse	5′-TCC CCA GAG TTC TCA TTA GGC-3′
3		
	forward	5′-GGA ATA GCA ACC ATC AGC CT-3′
	reverse	5′-CAA CAA TAA AAA ACA AAT ATC-3′
4		
	forward	5′-TCT CAA GCG ATT TTT ATA TCA AT-3′
	reverse	5′-TTA TAC CAA AAA CTA TGT GGC T-3′
5		
	forward	5′-CAA GGA TCT TTG TGT TCG AGA AGA A-3′
	reverse	5′-AGA CAG CAA ATG CAG AAG CCT C-3′
6		
	forward	5′-TTG GAC TTG ACA TTT CAT TCT-3′
	reverse	5′-TGT CTA TCA GTT ATT AAG GAA ACT T-3′
7		
	forward	5′-ACT CTT GCT GTC TAT ACC AGG-3′
	reverse	5′-CTG CCA TTG ATT GAA AAT C-3′
8		
	forward	5′-TTA AAA CTC TGG TGC AGC TA-3′
	reverse	5′-CCC TAA CGT CAT TCA CAT TG-3′
9		
	forward	5′-TCA GTG GAG GGG ATT TGA CG-3′
	reverse	5′-TAT GAT TCT AAG TAG ATA TAA AGC C-3′
10		
	forward	5′-GCT TTT ACG AAA ACC ATC AT-3′
	reverse	5′-AGT GAG TTT GGC GAA CAA GA-3′
13		
	forward	5′-GGA TCA TCT TCA CTG CAT TT-3′
	reverse	5′-ATG TGA AGA GGA TAG TGA GG-3′

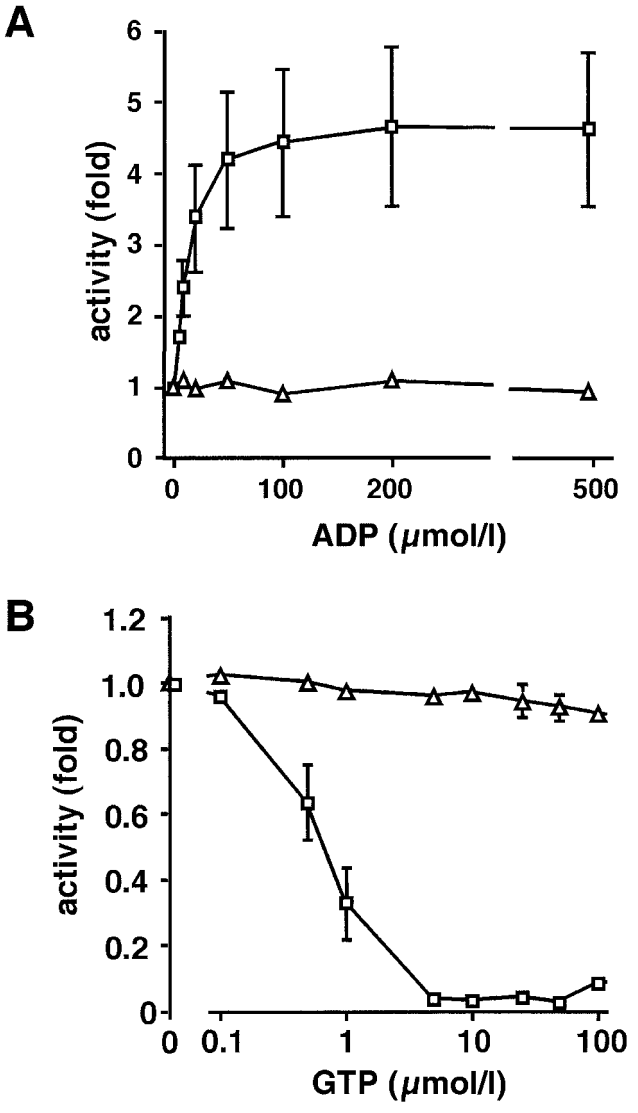

FIG. 1. Kinetic characteristics of GDH266C expressed in COS-7 cells. GDH activities in the crude extract of COS-7 cells were measured in the presence of various concentrations of ADP (*A*) or GTP (*B*). Relative activities at each concentration of allosteric effectors were plotted as ratios to the activity measured in the absence of the effectors. Experiments were repeated three times, and means ± SE are presented. □, COS-7 transfected with pcDNA3-hGDH (wild type); △, COS-7 cells transfected with pcDNA3-hGDH266C (mutant).

RESULTS

PCR/SSCP analysis of the human GLUD1 gene. Human mitochondrial GDH is encoded by 13 exons of the GLUD1 gene, which is located at 10q23.3. Several related pseudogenes (GLUDP1–5) have been recognized (10,16). Among them, the GLUDP2 and -3 genes contain three exons (exons 2–4) that are highly homologous to the GLUD1 gene (10). Due to the extensive similarity of intron sequences surrounding these exons among the GLUD1 gene and its pseudogenes, we carefully chose the primer sequences so that these GLUD1 gene exons could be specifically PCR amplified from genomic DNA (data not shown).

We identified three nucleotide substitutions in 12 Japanese patients with PHHI. Two of those were silent mutations: CTA (Leu) to CTG (Leu) at codon 261 and GTG (Val) to GTC (Val) at codon 496. Altogether, 5 of 12 PHHI patients (42%) and 50 of 94 control subjects (53%) were heterozygotes of CTA/CTG at codon 261. Although they were easily recognized on SSCP analysis and were confirmed by sequencing, the CTA/CTA homozygote and the CTG/CTG homozygote were barely distinguishable by SSCP patterns. Therefore, we did not determine the frequency of this single nucleotide polymorphism. One PHHI patient was a heterozygote at codon 496 (GTG/GTC). The frequency of this variation was not determined in control subjects. The third nucleotide variation, TAT to TGT at codon 266, was found in one patient with PHHI. This nucleotide substitution changes Tyr266 to Cys, and the patient was a heterozygote. This mutation was not found in 79 patients with type 2 diabetes or in 94 control subjects.

Clinical characteristics of the patient with the Y266C mutation and studies of her family. The female patient with the Y266C mutation was 21 years of age at the time of this study. She was born at 37 weeks and 5 days of gestation as the second of four daughters of nonconsanguineous parents. Pregnancy and delivery were normal. Her birth weight was 2,700 g. Her first seizure occurred at 7 months of age and was not responsive to anticonvulsants. Hypoglycemia (1.7 mmol/l) was disclosed at age 13 months, when a seizure occurred just after milk feeding. Oral administration of leucine (150 mg/kg) induced hypoglycemia, and diazoxide treatment was started under a diagnosis of leucine-sensitive hypoglycemia. After the initiation of diazoxide treatment, no hypoglycemic episodes were documented up to the time of this study, i.e., at 21 years of age. She has no family history of hypoglycemia, and her parents and three sisters were healthy. Her plasma ammonium levels, measured during this study, were mildly elevated (128 μmol/l, normal 12–84). Her parents and two of her sisters did not have the Y266C mutation, and their plasma glucose and ammonium levels were normal (data not shown).

Characterizations of GDH266C expressed in COS-7 cells. The wild-type and mutant GDH were expressed in COS-7 cells by transfection of pcDNA3-hGDH and pcDNA3-hGDH266C, respectively. A reporter plasmid pcDNA3-lacZ was cotransfected. GDH activities in the crude extracts of COS-7 cells transfected with pcDNA3-hGDH and pcDNA3-hGDH266C were 0.82 ± 0.22 and 3.27 ± 0.72 μmol · NADH · mg^{-1} · protein · min^{-1}, respectively, when activity was measured without ADP or GTP in the reaction mixture (means ± SE, $n = 3$). Endogenous GDH activity in untreated COS-7 cells (0.028 μmol/l · NADH · mg^{-1} · protein · min^{-1}) was <1/20 the total GDH activity in COS-7 cells transfected with pcDNA3-hGDH and thus negligible compared with the exogenous GDH expressed by the transfected plasmids. Therefore, the basal activity (activity in the absence of ADP or GTP) of GDH266C is elevated as compared with the wild-type enzyme. To exclude the possibility that this difference in the enzyme activity reflected the difference in the transfection efficiency, we measured β-galactosidase activities in the same cell extracts, and the GDH activity was normalized by the β-galactosidase activities. After the normalization, the basal activity of GDH266C was still 3.5 ± 1.2 times higher (means ± SE, $n = 3$) than that of wild-type GDH.

GDH is known to be regulated by various allosteric

TABLE 2
GDH activity in MIN6-GDH266C and MIN6-lacZ

ADP (μmol/l)	GTP (μmol/l)	GDH activity (nmol/l NADH consumption · mg^{-1} · protein · min^{-1} (n = 3, mean ± SE) MIN6-266C	MIN6-lacZ
0	0	110.7 ± 8.1	5.6 ± 0.5
200	0	240.3 ± 1.4	83.2 ± 9.7
0	15	97.6 ± 5.5	ND

Data are means ± SE. ND, not determined.

regulators. We examined whether the activity of GDH266C can be regulated by ADP, an allosteric activator, or GTP, an allosteric inhibitor. As shown in Fig. 1, ADP activated wild-type GDH (3.32 ± 0.12 μmol/l · NADH · mg^{-1} · protein · min^{-1} at 200 μmol/l ADP), with a SC_{50} of 11 μmol/l. On the other hand, the activity of GDH266C was not increased by ADP (3.45 ± 0.50 μmol/l · NADH · mg^{-1} · protein · min^{-1} at 200 μmol/l ADP). Similarly, although GTP inhibited the wild-type GDH activity, with a IC_{50} of 0.65 μmol/l, the activity of GDH266C was not inhibited by GTP at a concentration as high as 100 μmol/l. The activities of wild-type GDH and GDH266C at 100 μmol/l GTP were 2.7 ± 0.9 and 96.6 ± 1.6% of those without GTP, respectively.

Insulin secretion from MIN6 cells overexpressing GDH266C. We then overexpressed GDH266C in an insulinoma cell line, MIN6. Using a retrovirus-mediated gene transfer system, the mutant GDH gene was transferred to MIN6 cells along with the puromycin resistance gene. The cells stably integrating the retroviral genome were chosen by puromycin selection. MIN6 cells overexpressing bacterial β-galactosidase (MIN6-lacZ) were also established and used as a control.

Determined in crude cell extracts (Table 2), basal (in the absence of ADP) and inhibited (in the presence of GTP) GDH activities in MIN6-GDH266C were 20 times higher than the basal GDH activity in MIN6-lacZ. The values were even higher than activated MIN6-lacZ GDH activity (measured in the presence of 200 μmol/l ADP). GDH activity in MIN6-GDH266C was doubled in the presence of 200 μmol/l ADP, probably because intrinsic MIN6 GDH was activated. These results suggest that in MIN6-GDH266C, GDH activity is constitutively elevated and minimally regulated by allosteric regulators such as ADP and GTP.

Insulin secretion was analyzed in these cells. Glutamine, which is permeable to the plasma membrane and converted to glutamate inside β-cells, did not stimulate insulin secretion from MIN6-lacZ cells, as was reported for intact islets (7). On the other hand, it remarkably stimulated insulin secretion from MIN6-GDH266C in a dose-dependent manner (Fig. 2*A*). Glucose-stimulated insulin secretion was also studied. In MIN6-GDH266C, insulin secretion was significantly exaggerated at low glucose concentrations (2 and 5 mmol/l; $P = 0.009$ at 2 mmol/l and $P = 0.020$ at 5 mmol/l, unpaired Student's t test), as compared with control MIN6-lacZ, but not at higher glucose concentrations (8–25 mmol/l) (Fig. 2*B*).

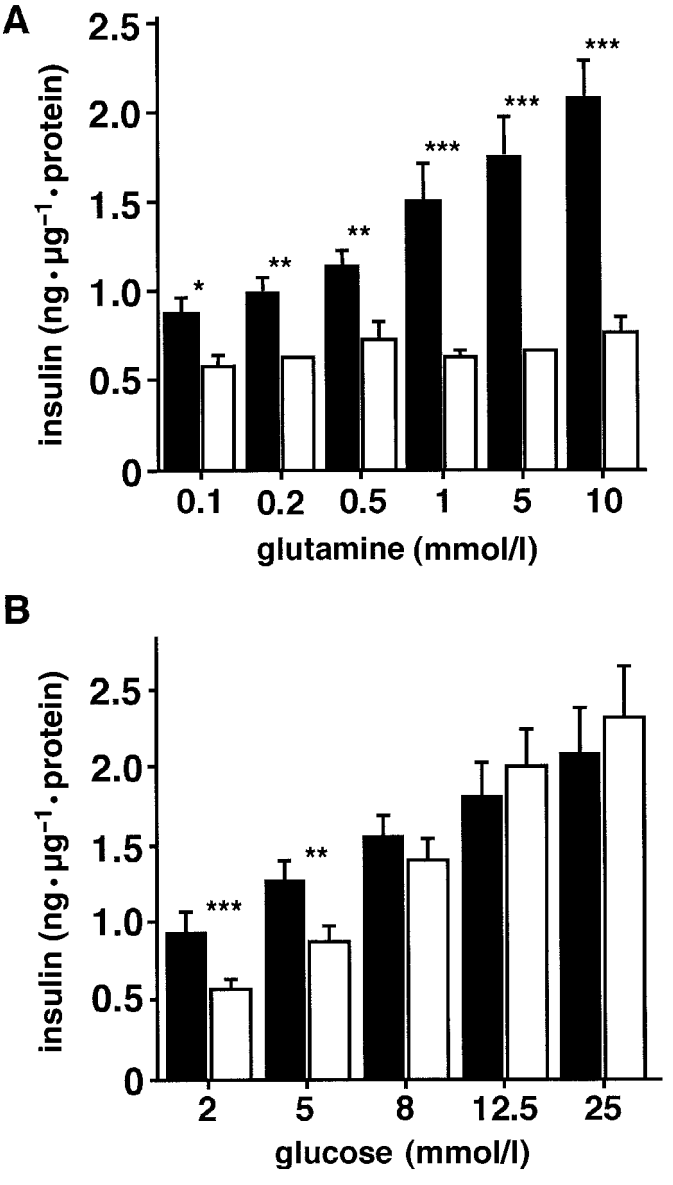

FIG. 2. Glutamine- and glucose-stimulated insulin secretion. MIN6-GDH266C (■) and MIN6-lacZ cells (□) were incubated in the presence of various concentrations of glutamine (*A*) or glucose (*B*) for 2 h, and insulin released into the incubation buffer was measured. Each assay was performed in duplicate or triplicate wells, and values are means ± SE of three (0.1 and 0.2 mmol/l glutamine), four (0.5 and 10 mmol/l glutamine), five (1 and 5 mmol/l glutamine) (*A*), and seven (2 mmol/l glucose) to nine experiments (other glucose concentrations) (*B*). *P < 0.05, **P < 0.03, and ***P < 0.01 in comparison between MIN6-GDH266C and MIN6-lacZ (unpaired Student's t test).

DISCUSSION

We found one patient with the GLUD1 gene mutation, Y266C, in a cohort of 12 patients with PHHI. The patient was heterozygous for the mutation and had clinical features of HI/HA syndrome (3–5). The Y266C mutation appeared to be a de novo mutation in this patient. The same mutation was recently described in a sporadic HI/HA syndrome patient (5), although the kinetic properties of the mutant enzyme have not been studied. It has been reported that Y262 of bovine GDH, corresponding to Y266 of the human enzyme, is in the GTP binding pocket and interacts with the γ-phosphate moiety of GTP (5,17–19). Chemical modification of this residue resulted in reduced

sensitivity to GTP inhibition and ADP activation (20). The reduced sensitivity to ADP activation is probably attributable to a secondary effect of modification of the GTP binding site because the affinity of ADP for the chemically modified GDH was not altered (20). We expressed GDH266C in COS-7 cells and examined the effect of this mutation on kinetic properties. Strikingly, the allosteric regulation of GDH266C by GTP and ADP was more severely impaired than that of the enzyme in which the corresponding amino acid was chemically modified. GDH266C was essentially insensitive to GTP inhibition or ADP activation (Fig. 1). Basal GDH266C activity, measured in the absence of GTP or ADP, appeared to be higher than that of the wild-type GDH when it was estimated in crude cell extracts (see RESULTS). Although quantitative evaluation of kinetic properties requires studies using purified enzyme rather than analysis in the crude cell extract, GDH266C appeares to be a constitutively activated enzyme.

The nearly total insensitivity of GDH266C to GTP and ADP contrasts with the kinetic characteristics of other mutants that were recently identified in patients with HI/HA syndrome. In previous studies, the IC_{50} elevation for GTP was 2- to 10-fold, with the IC_{50} being <2 μmol/l (3–5), and activation by ADP was mostly intact. The difference is probably attributable to the nature of the mutations. In addition, it may also be explained by the fact that the previous studies were performed using patients' lymphoblasts in which both mutant and wild-type GDH existed at a 1:1 ratio, and therefore the effects of mutations on kinetic properties were less prominent.

Despite the differences in the kinetic properties of the enzyme, the clinical characteristics of our patient and the previously reported patient with the same mutation (5) were very similar to those of patients with other mutations. It was recently reported that there is a genotype-phenotype correlation in regard to plasma ammonium levels but not the severity of hypoglycemia (5). Hypoglycemia in our patient was medically manageable with diazoxide, and the plasma ammonium level was only mildly elevated (128 μmol/l, normal 12–84).

Using GDH266C, which has unique kinetic properties, as a tool, we examined the role of GDH in the regulation of insulin secretion and the pathophysiology of β-cells from HI/HA syndrome patients. We established MIN6 cells stably overexpressing the mutant GDH and analyzed glutamine- and glucose-stimulated insulin secretion. GDH, upon stimulation by allosteric activators such as leucine, presumably enhances glutamate oxidation and thereby stimulates insulin secretion (1,2). Herein, we have directly shown for the first time that increased GDH activity confers on β-cells the ability to secrete insulin in response to glutamine. GDH266C-overexpressing MIN6 cells secreted insulin in response to glutamine in a dose-dependent manner, whereas control β-galactosidase–overexpressing MIN6 cells were unresponsive to glutamine stimulation. This observation clearly demonstrated the importance of GDH catalyzing oxidative deamination of glutamate for insulin secretion rather than glutamate formation from α-ketoglutarate by reverse reaction (6). Enhanced insulin secretion in response to glutamine from MIN6-GDH266C is directly relevant to the phenotype of HI/HA syndrome patients—they tend to develop hypoglycemia after a high-protein meal (21).

Physiologically, glutaminolysis may play more important roles in insulin secretion at low glucose concentrations and contribute to interprandial, basal insulin secretion (8). In pancreatic islets, glucose increases GTP levels while decreasing ADP levels in a concentration-dependent manner (22–24). At low glucose concentrations, flux through the TCA cycle via glucose metabolism is low, whereas glutaminolysis is activated because relatively low intracellular GTP concentrations and high ADP concentrations activate GDH, leading to sustained basal insulin secretion (8). In MIN6-GDH266C, in which GDH activity is supraphysiologically elevated, insulin secretion was exaggerated significantly at low glucose concentrations (2 and 5 mmol/l) compared with control MIN6-lacZ. This observation is consistent with a previous study (8) and may also explain the HI/HA syndrome patients' fasting hypoglycemia. At higher glucose concentrations, flux through the TCA cycle is probably high enough to mask the effect of enhanced glutaminolysis on insulin secretion in MIN6-GDH266C cells.

Recently, glutamate was suggested to be a mediator of glucose-stimulated insulin secretion, acting directly on insulin secretory granules (6). According to this hypothesis, GDH catalyzes the formation of glutamate from α-ketoglutarate rather than oxidative deamination of glutamate. However, our results from MIN6-GDH266C and previous evidence, including clinical data from HI/HA syndrome, do not support this hypothesis (3–5,7,8). Investigations to elucidate changes in intracellular glutamate concentrations and glutamine oxidation in MIN6-GDH266C will further clarify the role of GDH in the regulation of insulin secretion.

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