Glutamate dehydrogenase (GDH) plays an important role in insulin secretion as evidenced in children by gain of function mutations of this enzyme that cause a hyperinsulinism-hyperammonemia syndrome (GDH-HI) and sensitize β-cells to leucine stimulation. GDH transgenic mice were generated to express the human GDH-HI H454Y mutation and human wild-type GDH in islets driven by the rat insulin promoter. H454Y transgene expression was confirmed by increased GDH enzyme activity in islets and decreased sensitivity to GTP inhibition. The H454Y GDH transgenic mice had hypoglycemia with normal growth rates. H454Y GDH transgenic islets were more sensitive to leucine- and glutamine-stimulated insulin secretion but had decreased response to glucose stimulation. The fluxes via GDH and glutaminase were measured by tracing 15N flux from [2-15N]glutamine. The H454Y transgene in islets had higher insulin secretion in response to glutamine alone and had 2-fold greater GDH flux. High glucose inhibited both glutaminase and GDH flux, and leucine could not override this inhibition. 15NH4Cl tracing studies showed 15N was not incorporated into glutamate in either H454Y transgenic or normal islets. In conclusion, we generated a GDH-HI disease mouse model that has a hypoglycemia phenotype and confirmed that the mutation of H454Y is disease causing. Stimulation of insulin release by the H454Y GDH mutation or by leucine activation is associated with increased oxidative deamination of glutamate via GDH. This study suggests that GDH functions predominantly in the direction of glutamate oxidation rather than glutamate synthesis in mouse islets and that this flux is tightly controlled by glucose.

Glucose, fatty acids, and amino acids are fuels that stimulate pancreatic β-cell insulin secretion. Congenital hyperinsulinism (HI), 2 a group of disorders arising from mutations of genes encoding β-cell function, illustrates this basic phenomenon. For instance, gain of function mutations of glucokinase cause HI by lowering the threshold for glucose-stimulated insulin secretion (GSIS) and highlight the role of glucokinase as the glucosensor of the β-cell (1, 2). Recently a form of HI due to loss of function mutations in the enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase has been identified (3–6). Although the biochemical mechanisms of short-chain 3-hydroxyacyl-CoA dehydrogenase-HI are unknown, this disorder provides evidence of a role for fatty acid metabolism in insulin secretion. The ATP-dependent potassium channel (KATP), encoded by the sulfonylurea receptor 1 (SUR1) and Kir 6.2, transduces the energy state of the β-cell. Loss of function mutations in the ATP-dependent potassium channel cause the most common form of HI (KATP-HI) and confirm that the channel plays a key role in triggering insulin release (7–9). In 1998, we identified mutations of glutamate dehydrogenase (GDH) in children with a dominant form of hyperinsulinism (GDH-HI) and implicated this enzyme as a mediator of leucine-stimulated insulin secretion (LSIS) (10–12). GDH-HI mutations impair enzyme sensitivity to allosteric inhibition by GTP and ATP, resulting in a gain of function (10, 13). Amino acid-stimulated insulin secretion is conditional: most amino acids stimulate insulin release only in the presence of glucose (14). Leucine is an exception since glucose inhibits LSIS by elevating the β-cell phosphate potential (15, 16). Children with GDH-HI have excessive LSIS and protein-induced hypoglycemia (17, 18). Extension of these clinical observations to in vitro studies in isolated rat islets indicates that GDH serves as a pace maker for amino acid-stimulated insulin secretion and mediates glucose regulation of amino acid-stimulated insulin release (15, 16).

One of the GTP binding defects in GDH mutations is H454Y, which causes significant perturbations in enzyme kinetics and manifests clinically as a severe form of GDH-HI (10, 12, 13, 19). Specific expression of the GDH H454Y mutation in β-cell was employed to improve our understanding of the role of GDH in both amino acid- and glucose-stimulated insulin secretions.

**EXPERIMENTAL PROCEDURES**

Generation of Transgenic Mice—Two constructs were designed and were identical with the exception of a 1-nucleotide base change that either preserved the wild-type human GDH sequence or caused the H454Y mutation in GDH. The construct used the rat insulin promoter (obtained from Dr. Mark A. Magnuson) to drive β-cell-specific transcription of the human GDH cDNA sequence. The human growth hormone (huGH) genomic DNA sequence (received from Dr. Mark A. Magnuson) was used to stabilize the construct. The constructs were microinjected into fertilized eggs of B6SJLF1 mice, and the eggs were then transferred into the oviducts of pseudopregnant females (Trans-
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Generation of Transgenic Mice Expressing H454Y huGDH—Two lines of H454Y huGDH, TG-1 and TG-2, and one line of wild-type huGDH transgenic mice were generated. All three lines of transgenic animals yielded normal litter sizes. As shown in Fig. 1, body weights for...
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Both male and female mutant and wild-type huGDH transgenic mice were similar to those of their littermates. Random blood glucose concentrations were consistently lower in TG-1 mice after 12 weeks of age compared with controls (Fig. 1). TG-2 mice had low blood glucose concentrations by 4 weeks of age and were more severely hypoglycemic than TG1 mice. Hypoglycemia presumably impaired survival of both TG1 and TG2 lines compared with wild type. At 1 month, survival was 35% in TG1 and 33% in TG2 compared with 49% in wild-type and normal littermates (p < 0.01). At 4 months, survival rates were similar in TG1 and wild-type lines but further declined to 26% in TG2 (versus TG1, p < 0.05). Because of persistent hypoglycemia, both H454Y huGDH transgenic lines were difficult to breed. This was particularly true for TG2 mice, and only a limited number of studies could be carried out in this line before it could no longer be maintained.

Mutant huGDH Enzyme Activity in Islets—To demonstrate that the huGDH transgenes were expressed in islets, GDH enzyme activity and sensitivity to inhibition by GTP were measured in homogenates of isolated islets from the three transgenic lines. Assays were done in the presence of 200 μM ADP to achieve maximal basal GDH activity and avoid possible influences of allosteric effectors carried over in the preparation of tissues. Results of measurements of enzyme activities in homogenates of whole pancreas were not different between controls and the transgenic lines (data not shown). As shown in Table 1, total GDH activity in isolated islets was slightly increased in wild-type huGDH transgenic mice but was increased to two times that of normal controls in TG1 and to three times normal in TG2 mice indicating high levels of transgene expression in these two lines. Fig. 2 compares the GTP inhibition curves for GDH enzyme activity in islets from the transgenic lines and control mice. For comparison, the inhibition curve for purified H454Y huGDH is also shown. Inhibition of GDH enzyme activity by GTP in islets from wild-type huGDH transgenic mice was similar to that of normal control mice. In contrast, islet GDH from TG1 showed a right shift in the GTP inhibition curve with ~10% of total activity uninhibitable at 100 μM GTP. In the more severely affected TG2 transgenic mice, the GTP inhibition curve was shifted even further to the right very close to that of purified H454Y huGDH and about 45% of the total activity was uninhibited by 100 μM GTP. These results demonstrated that the mutant H454Y huGDH transgene was highly expressed in islets from the TG1 line and that expression was even greater in the TG2 line.

Glucose- and Leucine-stimulated Insulin Secretion—Fig. 3 shows the responses of isolated islets from the mutant H454Y huGDH transgenic mice to glucose and leucine stimulation. As shown in A, base-line insulin secretion in TG1 mouse islets was initially elevated compared with normal control islets (0.9 ± 0.04 ng/100 islets/min versus 0.4 ± 0.003 ng/100 islets/min, p < 0.001) and declined close to that of normal after 50 min of glucose- and amino acid-free perfusion. The threshold for GSIS was higher in TG1 islets than control islets (10 versus 6 μM), and total GSIS was significantly lower than controls in the time interval of 50–100 min (116 ± 3 versus 87 ± 6 ng/100 islets, p < 0.05). The response to glucose ramp stimulation was similar in wild-type huGDH transgenic and normal mouse islets (data not shown).
The response of TG1 islets to leucine ramp stimulation was further enhanced by glutamine (2 mM), as evidenced by increased insulin secretion (4.4 ± 0.1 ng/100 islets/min vs. 0.9 ± 0.04 ng/100 islets/min, p < 0.01). TG1 islets were more sensitive than control islets to leucine ramp stimulation (threshold: 1 vs. 4 mM). After subtracting base-line insulin release, total insulin secretion in TG1 islets during the period from 50 min to 100 min was not significantly different from normal islets (314 ± 124 ng/100 islets vs. 169 ± 42 ng/100 islets, p > 0.05). The insulin responses to depolarization with KCl were not different in islets from the TG1 line compared with islets from control littermates. Wild-type transgenic mouse islets showed responses to leucine ramp stimulation similar to that of normal mouse islets (data not shown).

Glutamine, AAM-stimulated Insulin Secretion, and Intracellular Calcium Responses—Since 2 mM glutamine appeared to cause an increase in base-line insulin secretion in islets from the H454Y huGDH transgenic mice but not from control littermates (Fig. 3), we examined the responses to a glutamine ramp alone. As shown in Fig. 4A, both TG1 and TG2 islets responded to glutamine ramp stimulation. In contrast, there was no response to glutamine alone by islets from normal mice and from wild-type huGDH transgenic mice (data not shown). As shown in Fig. 4A, TG2 islets were more sensitive to glutamine than TG1 islets, a finding consistent with the enzymatic data showing that GDH from TG2 islets was less sensitive to GTP inhibition than GDH from TG1 islets. Inhibition of glutaminase by 6-diazo-5-oxo-L-norleucine (DON), the enzyme responsible for deamination of glutamine to glutamate, completely abolished TG1 islet responsiveness to glutamine stimulation.

As shown in Fig. 4B, TG1 islets had enhanced insulin release in response to exposure of a complete mixture of amino acids, whereas islets from control littermates was insensitive to an amino acid ramp. The maximum insulin release by TG1 islets caused by the AAM stimulation was similar to that achieved by glutamine alone, suggesting that glutamine was responsible for most of the effect of the mixture of amino acids.

To confirm the above observations, the response of islet intracellular calcium concentrations ([Ca^{2+}]_i) to glutamine was measured dynamically by fluorescent microscopy. As shown in Fig. 5A, 10 mM glutamine alone caused an increase of [Ca^{2+}]_i, in TG2 islets but not in normal control islets. DON at 40 µM totally blocked this effect of glutamine. Similar results were obtained with islets from TG1 mice (B). These observations confirm that the stimulatory effect of glutamine on islets expressing the H454Y huGDH transgene depends on its conversion to glutamate, which can then be used as substrate for oxidation via GDH.

Changes of the ATP/ADP Ratio and Insulin Release—To directly assess the effect of the H454Y mutation on high energy phosphate generation in islets, we measured the ATP/ADP ratios and insulin release in batches of TG1 and control islets incubated for periods of 1 h (n = 6).
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FIGURE 5. Effects of the H454Y huGDH transgene on [Ca\(^{2+}\)] in isolated islets. Isolated islets from normal control mice, TG1 mice, and TG2 mice were cultured on coverslips with 10 mM glucose for 3 days. [Ca\(^{2+}\)] was continually measured by fura-2 fluorescence in response to glutamine with or without DON present. The sequence and concentrations of additions are shown in the figure. Representative experiments are displayed. All studies were repeated three times with comparable results.

TABLE 2
Insulin secretion, ammonia production and \(^{15}\)N enrichment, and flux through GDH and PDG in normal, TG1, and TG2 mouse islets

| Gene   | Ammonia production (total ammonia) | Ammonia \(^{15}\)N enrichments | GDH flux | PDG Flux |
|--------|-----------------------------------|--------------------------------|----------|---------|
|        | Nor | TG1 | TG2 | nor | TG1 | TG2 | nor | TG1 | TG2 | nor | TG1 | TG2 | nor | TG1 | TG2 |
| Control| 0.3 ± 0 | 1.4 ± 0.4 | 1.4 | 57 ± 8 | 112 ± 10 | 133 | 2.1 ± 0.2 | 4.9 ± 0.5 | 9.6 | 13 ± 1.2 | 25 ± 2.1 | 82 | 37 ± 4 | 46 ± 5 | 103 |
| 10 mM leucine | 3.7 ± 0.6 | 3.0 ± 0.2 | 2.6 | 45 ± 4 | 93 ± 12 | 118 | 3.9 ± 0.5 | 5.8 ± 0.7 | 7.4 | 36 ± 0.8 | 44 ± 4.9 | 87 | 49 ± 2.9 | 56 ± 7 | 98 |
| 25 mM glucose | 2.9 ± 0.8 | 2.3 ± 0.1 | 3.5 | 33 ± 4 | 55 ± 9 | 72 | 1.7 ± 0.5 | 1.1 ± 0.4 | 2.2 | 14 ± 7.7 | 4 ± 1.2 | 23 | 27 ± 8 | 20 ± 2 | 33 |
| 10 mM leucine/25 mM glucose | 3.2 ± 0.2 | 2.5 ± 0.1 | 1.8 | 26 ± 3 | 29 ± 5 | 392 | 1.3 ± 0.6 | 2.3 ± 0.3 | 3.1 | 9 ± 4.6 | 14 ± 1.7 | 24 | 20 ± 2 | 22 ± 2 | 59 |

### Notes:

* Versus control, p < 0.01.
* Versus normal, p < 0.05.
* Versus control, p < 0.05.
* Versus 10 mM leucine, p < 0.01.
* Versus 10 mM leucine, p < 0.05.

In the absence of added substrates, TG1 islet had an increased basal ATP/ADP ratio (4.4 ± 0.4 versus 2.8 ± 0.2, p < 0.05) and also showed a slightly increased basal insulin secretion (77 ± 26 versus 59 ± 13 ng/100 islets/h, not significant). In the presence of 10 mM glucose, compared with control islets, TG1 islets also had a higher ATP/ADP ratio (5.3 ± 0.3 versus 3.3 ± 0.4, p < 0.05) and increased insulin release (350 ± 34 versus 109 ± 33 ng/100 islets/h, p < 0.05). Exposure to the 4 mM AAM also stimulated insulin release in TG1 mice more effectively than in control islets (180 ± 22 versus 89 ± 33 ng/100 islets/h, p < 0.05) and resulted in a trend toward a higher ATP/ADP ratio (3.7 ± 0.5 versus 2.8 ± 0.6, not significant). Insulin content was similar in TG1 and control islets. These results are consistent with the concept that the H454Y gain of function mutation of GDH stimulates insulin release by increased oxidative flux through the enzyme generating elevated ratios of ATP to ADP both in the basal state and when exposed to the substrate precursor of the enzyme, leucine.

**[2-\(^{15}\)N]Glutamine Tracer Studies**—The above studies suggested that the H454Y huGDH gain of function mutation enhances insulin release by stimulating glutaminolysis (glutamine → glutamate → α-ketoglutarate) thus increasing flux into the tricarboxylic acid cycle. To directly examine the effects of the mutation on flux through GDH, stable isotope studies were carried out using [2-\(^{15}\)N]glutamine to trace its conversion to [2-\(^{15}\)N]glutamate via glutaminase and subsequently to \(^{15}\)Nammonia via deamination of glutamate in the GDH reaction.

As shown in Table 2, in the presence of 10 mM [2-\(^{15}\)N]glutamine, basal insulin secretion, ammonia production, \(^{15}\)Nammonia APE, and flux through GDH and glutaminase were all greater in islets from TG1 mice compared with normal mouse islets. In control islets, 10 mM leucine induced a 12-fold increase in insulin release, accompanied by increased rates of ammonia production and a 3-fold stimulation of flux through GDH. Leucine increased rates of insulin secretion and ammonia production in TG1 islets to approximately the same levels as in

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**Data:** mean ± S.E., n = 3.
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**TABLE 3**

$^{15}$N enrichment and $^{15}$N concentration of glutamate and aspartate in islets from normal, TG1, and TG2 mice

| Glutamate | Aspartate |
|-----------|-----------|
| $^{15}$N (APE) | $^{[15]}$N Glutamate | $^{15}$N (APE) | $^{[15]}$N Aspartate |
| Normal | TG1 | TG2 | Normal | TG1 | TG2 | Normal | TG1 | TG2 |
| nmol/1,000 islets | nmol/1,000 islets |

| Control | 15N leucine | 25 mM glucose | 10 mM leucine/25 mM glucose |
|---------|------------|---------------|-----------------------------|
| Alanine | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.1 | 2 | 1.4 ± 0.3 | 2 | 0.3 ± 0.5 | 2 | 0.4 ± 0.4 | 1 | 0.2 ± 0.1 |
| Aspartate | 25 ± 0.7 | 20 ± 2.2 | 23 | 17 ± 1.8 | 15 ± 1.9 | 15 | 14 ± 2.5 | 14 ± 0.2 | 14 | 12 ± 1.5 | 11 ± 0.2 | 41 | 0.2 ± 14 | 12 |
| GABA | 3 ± 0.1 | 2 ± 0.3 | 1 | 2 ± 0.0 | 2 ± 0.2 | 1 | 2 ± 0.4 | 1 ± 0.3 | 1 | 2 ± 0.4 | 0.2 ± 0.2 | 4 | 0.2 ± 14 | 12 |
| Glutamate | 17 ± 0.8 | 15 ± 1.9 | 18 | 14 ± 1.0 | 13 | 13 ± 0.7 | 14 ± 1.0 | 13 | 13 ± 1.6 | 12 ± 0.6 | 26 | 0.2 ± 14 | 12 |
| Glutamine | 28 ± 0.3 | 29 ± 2.2 | 35 | 17 ± 1.8 | 20 ± 2.9 | 15 | 30 | 4.1 | 37 ± 3.5 | 22 | 17 ± 3.8 | 22 ± 1.5 | 30 | 0.2 ± 14 | 12 |
| Glycine | 3 ± 0.4 | 4 ± 0.5 | 4 | 4 ± 0.3 | 4 ± 0.6 | 3 | 3 | 0.4 | 3 ± 0.3 | 3 | 3 ± 0.6 | 4 ± 0.4 | 3 | 0.2 ± 14 | 12 |
| Glucose | 0.3 ± 0.5 | 0.3 ± 0.0 | 0.5 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0 | 0.3 ± 0.1 | 0.4 ± 0.0 | 0.5 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.5 | 0.2 ± 14 | 12 |
| Leucine | 0.1 ± 0.1 | 0.2 ± 0.0 | 0 | (11 ± 4.5) | (16 ± 2.7) | (16.2) | 0 | 0.1 ± 0.0 | 0.5 | 0.1 | 0 | (11 ± 5.0) | (17 ± 2.0) | (15.1) | 0.2 ± 14 | 12 |
| Serine | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.4 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0 | 0.2 | 0.0 | 0.3 | 0.0 | 0 | 0.1 ± 0.1 | 0.2 ± 0.0 | 0.3 | 0.2 ± 14 | 12 |
| Sum | 78 ± 2.6 | 72 ± 6.9 | 84.9 | 57 ± 10 | 55 ± 9 | 49 | 64 | 3.5 | 72 ± 5.5 | 55.5 | 49 ± 5.8 | 52 ± 2.6 | 105.8 | 0.2 ± 14 | 12 |

- Versus control, $p < 0.01$.
- Versus 25 mM glucose, $p < 0.01$.
- Versus normal, $p < 0.05$.
- Versus 10 mM leucine, $p < 0.01$.

controls, suggesting that these values were close to maximal. Note that flux through GDH remained higher in TG1 islets, consistent with the high levels of GDH expression described above (Table I and Fig. 1). The apparent reduction in PDG flux associated with leucine stimulation of TG1 islets most likely reflects a dilution effect of the leucine $\alpha$-amino nitrogen.) Although the experiment was only performed once due to the difficulty of breeding this line, islets from TG2 transgenic mice showed an exaggeration of the trends seen with islets from TG1 mice. Note that leucine did not increase flux through GDH in the TG2 islets, consistent with the greater expression of mutant GDH in this line such that GDH flux reached its maximum with 10 mM glutamine alone.

*Effects of Glucose on GDH Flux*—Consistent with the perifusion studies (Fig. 2), additional studies of GSIS revealed that the presence of the H454Y huGDH transgene did not result in greater insulin release compared with control islets (Table 2). In both normal and transgenic islets glucose inhibited ammonia production, $^{15}$N enrichment of ammonia, PDG flux, and GDH flux. Leucine was unable to override this inhibitory effect of glucose.

As shown in Table 3, approximately half of the intracellular glutamate and aspartate pools were $^{15}$N-labeled under basal conditions with 10 mM [2, $^{15}$N]glutamine alone. The $^{15}$N enrichments of glutamate and aspartate were decreased in the presence of leucine, which, as noted above, reflects the contribution of unlabeled nitrogen from leucine through transamination. Glucose did not alter the $^{15}$N enrichment of the two amino acids in either the transgenic or normal islets.

Intracellular amino acid concentrations in islets from control and TG1 and TG2 transgenic mice are shown in Table 4. Apart from glutamine present in the incubation, glutamate and aspartate were the predominant intracellular amino acids. Aspartate levels were decreased by leucine, glucose, and the combination of leucine and glucose treatment in normal islets, and a similar effect was seen in islets from the TG1 mice. Intracellular glutamate concentrations remained largely unchanged under all conditions, reflecting the constant supply of glutamine. Alanine was increased by glucose in both normal mouse islets and H454Y huGDH transgenic islets, reflecting the transamination reaction from pyruvate to alanine. During stimulation with 10 mM leucine, as expected, intracellular leucine was very high and glutamine level was reduced.

*Studies with $^{15}$NH$_4$Cl to Measure Reverse Flux through GDH*—As noted above, the insulin response of TG1 islets to glucose stimulation was not greater than that of controls. Since it has been suggested that GSIS may involve reversal of the GDH reaction to generate glutamate from ammonia and $\alpha$-ketoglutarate, the transgenic islets provided an opportunity to evaluate the effect of increased GDH activity on flux in the reductive amination direction. Islets were incubated with 300 $\mu$M $^{15}$NH$_4$Cl to trace $^{15}$N flux into the amino nitrogen of glutamate and other amino acids. As shown in Table 5, in the presence of 300 $\mu$M $^{15}$NH$_4$Cl, glucose stimulated similar amounts of insulin release from control and TG1 islets. Addition of the leucine analog, BCH, to activate GDH did not stimulate insulin release in the presence of ammonia alone in control islets. Ammonia concentration in the media was lower in the presence of glucose and did not decrease with addition of BCH.

Table 6 shows the amino acid levels in islets from control and TG1 mice after incubation with 300 $\mu$M $^{15}$NH$_4$Cl. Media concentrations of amino acids were very low (1–10 nmol/liter) and remained essentially unchanged with the various incubations (data not shown). As seen previously with islets incubated with glutamine, glutamate and aspartate were the predominant intracellular amino acids. TG1 islets had lower levels of glutamate and aspartate than normal islets, consistent with increased oxidation through GDH. Glucose decreased islet aspartate and increased alanine levels. Glucose increased islet glutamate in normal islets, consistent with either inhibition of oxidation through GDH.
or increased reductive amination. However, glucose did not increase glutamate to a greater extent in TG1 compared with normal islets, suggesting that the increase reflected inhibition of oxidative deamination rather than stimulation of amination. Addition of the GDH activator, BCH, lowered glutamate during control incubations of normal islets but did not potentiate the increase in glutamate caused by glucose in these islets. These results suggested that, even in the presence of a supraphysiological concentration of ammonia, the effect of increased GDH activity by either the H454Y transgene or by BCH stimulation was to increase glutamate oxidation rather than its synthesis. When the incorporation of [15N]ammonia into intracellular and extracellular amino acids was examined, the only detectable increase noted was in alanine (maximum value, 1.6 APE). The 15N isotopic enrichments of glutamate, aspartate, and alanine were all below the limit of detection by gas chromatography-mass spectrometry. For purposes of estimating the possible rate of incorporation of ammonia into glutamate by reductive amination through GDH, we presumed that glutamate, aspartate, and alanine were in equilibrium with a 15N enrichment of 1.6 APE. Based on this assumption, the maximum GDH flux from ammonia to glutamate in islets incubated with 25 mM glucose and 300 μM ammonia was estimated to be only 0.2 nmol/1,000 islets/h. This value was less than 0.5% of the flux through GDH in the deamination direction (Table 2), indicating that, in mouse islets, the GDH reaction operates predominantly in the direction of oxidative deamination.

**DISCUSSION**

The results of these studies in mouse islets expressing the H454Y GTP-insensitive mutation of human GDH confirm clinical observations in affected children, which suggest that increased GDH activity is responsible for excessive insulin secretion in GDH-HI (11, 12, 22).

**TABLE 5**

Inulin secretion and media ammonia concentration in normal and TG1 islets incubated with 300 μM [15NH4]Cl

| n = 3 | Insulin secretion | Media ammonia |
| --- | --- | --- |
| | Normal | TG1 | Normal | TG1 |
| | μg/1,000 islets/2 h | nmol/ml |
| Control | 0.19 ± 0.03 | 0.19 ± 0.07 | 267 ± 8 | 285 ± 16 |
| 25 mM glucose | 2.47 ± 0.36a | 2.38 ± 0.55a | 229 ± 11b | 240 ± 6 |
| 10 mM BCH | 0.20 ± 0.02b | NA | 272 ± 8b | NA |
| 25 mM glucose/10 mM BCH | 3.92 ± 0.32a | NA | 240 ± 5 | NA |

* Versus control, p < 0.01.
* Versus 25 mM glucose, p < 0.01.
* Versus control, p < 0.05.
* Versus 25 mM glucose, p < 0.05.

**TABLE 6**

Intracellular concentrations of amino acids in islets incubated with [15N]ammonia (nmol/1,000 islets) in the absence of exogenous glucose

| n = 3 | Control | 25 mM glucose | 10 mM BCH (normal) | 25 mM glucose/10 mM BCH (normal) |
| --- | --- | --- | --- | --- |
| | Normal | TG1 | Normal | TG1 |
| Alanine | 0.5 ± 0.1 | 0.4 ± 0.1 | 1.4 ± 0.1a | 1.4 ± 0.1a | 0.5 ± 0.1 | 1.2 ± 0.2ac,bd |
| Aspartate | 5.2 ± 0.4 | 3.0 ± 0.3b | 3.2 ± 0.2a | 2.4 ± 0.3 | 1.2 ± 0.3bc | 2.5 ± 0.4b |
| Glutamine | 4.6 ± 0.3 | 2.5 ± 0.3b | 0.9 ± 0.1 | 0.7 ± 0.1 | 0.3 ± 0.0 | 1.1 ± 0.3 |
| Glutamate | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.6 ± 0.1b | 0.4 ± 0.0 | 0.2 ± 0.0 | 0.4 ± 0.1 |
| Glycine | 3.5 ± 0.4 | 4.6 ± 0.4 | 3.9 ± 0.1 | 3.2 ± 0.2 | 0.6 ± 0.9b | 4.2 ± 0.5 |
| Isoleucine | 0.5 ± 0.1 | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.4 ± 0.0 | NA | NA |
| Leucine | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.1 | ND | NA | NA |
| Serine | 0.2 ± 0.0 | 0.3 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.1 | 0.4 ± 0.1 |
| Sum | 16.2 ± 1.2 | 17.7 ± 0.9a | 18.1 ± 0.5 | 14.9 ± 0.8 | 11.4 ± 1.5ab | 15.6 ± 1.9 |

* Versus control-normal, p < 0.05.
* Versus TG1-control, p < 0.05.
* Versus 25 mM glucose-normal, p < 0.05.
* Versus BCH-normal, p < 0.05.
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children. The levels of transgene expression in the TG2 line, however, appeared to greatly exceed that of the endogenous enzyme, leading to substantial amounts of uninhibitable GDH activity. Compared with affected heterozygous children, the degree of impairment in GDH regulation is probably similar in the TG1 line, but the TG2 line more closely resembles the situation of homozygosity for the GDH gain of function mutations.

The present results are consistent with the suggestion by Sener and Malaise (27) that leucine and its non-metabolizable analog, BCH, act to stimulate insulin secretion by allosterically stimulating GDH activity to increase energy production through oxidation of glutamate. Leucine may also contribute to insulin secretion by direct oxidation via α-ketoadipate (16). The results in transgenic mice are consistent with our previous observations in rat islets suggesting that flux through GDH runs in the direction of oxidative deamination and that this flux is stimulated by leucine and suppressed by glucose (16). In the present study, the expression of a mutant GDH with impaired sensitivity to inhibition by GTP led to increased rates of oxidation of glutamine through glutamate and α-ketoglutarate. Under the conditions used in the present study and in the previous studies in normal rat islets, flux in the direction of glutamate formation by reductive amination was estimated to be no greater than 1% of flux toward oxidative deamination.

Maechler and Wollheim (28) have suggested that GDH is involved in GSIS by generating glutamate from α-ketoglutarate to enhance release of insulin from storage vesicles. Under the conditions used, we did not observe any evidence for such a reversal of flux through GDH. In both normal and transgenic mouse islets in the present experiments, glucose stimulation of insulin release was associated with a suppression of flux through GDH, consistent with the well known allosteric inhibitory effect of GTP and ATP on activity of the enzyme (13, 22, 29). We have previously presented evidence suggesting that glutamine, generated from glutamate by ATP in the glutamine synthetase reaction, contributes to GSIS by amplifying insulin release distal to elevations of cytosolic calcium (21). Interestingly, in the present study with both islet perfusion and batch incubations, transgenic islets with increased GDH activity appeared to be less, rather than more, responsive to glucose stimulation. This might reflect an effect of the increased oxidative flux through GDH to reduce levels of glutamate within the islets and, thus, limit the capacity for generating glutamine during exposure to glucose. It should be noted that our data do agree with the observation by Maechler and Wollheim (28), in which glucose increases glutamate level; however, our results suggest that this occurs through transamination rather than the GDH reaction.

The results of the present study, our previous study in isolated rat islets (16), and clinical information on children with hyperinsulinism due to H454Y and similar mutations of GDH (11–13) indicate that four enzymes (GDH, PDG, aminotransferases, and glutamine synthetase) are central to the regulation of insulin secretion by amino acids and to integrating the effects of amino acids with those of glucose and other fuels. As shown in Fig. 6, the activities of four of these enzymes are responsive to changes in cellular phosphate potential, in addition to the positive allosteric effects of leucine on GDH. Following a high protein feeding, leucine serves as an indicator of increased amino acid supply and activates oxidation of amino acids through transamination to glutamate and then into the tricarboxylic acid cycle via GDH to increase the ATP/ADP ratio and trigger insulin release. This pathway can be activated in the absence of glucose when the phosphate potential is low (15, 16), since a low ATP/ADP ratio increases glutaaminolysis through PDG and also sensitizes GDH to stimulation by leucine. During GSIS, the increased ATP/ADP ratio leads to inhibition of both PDG and GDH but activates the glutamine synthetase reaction to generate glutamine (21).

In conclusion, we successfully generated a mouse model expressing a GDH gain of function mutation that has a hypoglycemia phenotype and have confirmed that the H454Y mutation is disease causing. Our study of isolated islets from these mice shows that stimulation of insulin release either by the H454Y GDH mutation or by leucine activation is associated with increased oxidative deamination of glutamate via GDH. The results indicate that GDH functions predominantly in the direction of glutamate oxidation rather than glutamate synthesis in mouse islets and that this flux is tightly controlled by glucose.

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