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DISORDERS OF GLUTAMATE METABOLISM

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The significant role the amino acid glutamate assumes in a number of fundamental metabolic pathways is becoming better understood. As a central junction for interchange of amino nitrogen, glutamate facilitates both amino acid synthesis and degradation. In the liver, glutamate is the terminus for release of ammonia from amino acids, and the intrahepatic concentration of glutamate modulates the rate of ammonia detoxification into urea. In pancreatic β-cells, oxidation of glutamate mediates amino acid-stimulated insulin secretion. In the central nervous system, glutamate serves as an excitatory neurotransmittor. Glutamate is also the precursor of the inhibitory neurotransmittor GABA, as well as glutamine, a potential mediator of hyperammonemic neurotoxicity. The recent identification of a novel form of congenital hyperinsulinism associated with asymptomatic hyperammonemia assigns glutamate oxidation by glutamate dehydrogenase a more important role than previously recognized in β-cell insulin secretion and hepatic and CNS ammonia detoxification. Disruptions of glutamate metabolism have been implicated in other clinical disorders, such as pyridoxine-dependent seizures, confirming the importance of intact glutamate metabolism. This article will review glutamate metabolism and clinical disorders associated with disrupted glutamate metabolism.

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DISORDERS OF GLUTAMATE METABOLISM

The amino acid glutamate plays a central role in nitrogen metabolism and participates in multiple biochemical pathways. Glutamate metabolism is linked with aminotransferase reactions, ureagenesis, the tricarboxylic acid (TCA) cycle, GABA synthesis, and glutathione synthesis. The clinical importance of glutamate metabolism has been highlighted by the recent discovery of a dominantly-expressed defect in glutamate metabolism, the hyperinsulinism/ hypermmonemia syndrome (HI/HA) [Weinzimer et al., 1997; Stanley et al., 1998; 2000]. Defects in glutamate metabolism have been implicated in other disorders as well, but these associations are less well established. This paper briefly reviews the pathways of glutamate metabolism and discusses the HI/HA syndrome and other potential disorders of glutamate metabolism.

A. Pathways of Glutamate Metabolism

Figure 1 outlines the central position of glutamate in multiple metabolic pathways. Glutamate plays a key role in protein synthesis and degradation. Glutamate is the central transit site for the interchange of amino nitrogen amongst amino acids. Several different aminotransferses mediate the transfer of amino nitrogen to and from glutamate through fully reversible reactions. The action of these enzymes conserves amino nitrogen for the synthesis of other amino acids and maintains the equilibration amongst the pools of nonessential amino acids for protein synthesis. Protein degradation also relies on exchange of nitrogen through the aminotransferases to glutamate. While amino nitrogens are channeled through glutamate before being released as free ammonia, the carbon skeletons that remain after amino nitrogen removal can be oxidized to complete amino acid catabolism.

Glutamate completes amino acid degradation through its oxidative deamination by glutamate dehydrogenase (GDH) to α-ketoglutarate and free ammonia [Brusilow and Horwich, 1995]. [Glutamate + H_20 + $NAD(P)^+$ $\leftarrow \rightarrow$ α-KG + NH_4^+ + NAD(P)H]. By linking amino acid and carbohydrate metabolism glutamate supports energy production. This energy generating process has implications for insulin secretion and will be considered in more detail under gain of function disorders of GDH. As shown in Figure 1, the rate of glutamate oxidation via GDH also controls the terminal release of ammonia and, thus, the rate of protein oxidation. In the liver, the free ammonia that is generated from glutamate oxidation enters the urea cycle for ultimate disposal.

GDH can potentially salvage free ammonia through the reductive amination of α-KG to glutamate since the reaction is reversible in vitro. However, the significance of this reverse reaction has generated some confusion. In prokaryotes the GDH reaction is solely toward glutamate synthesis. Some reviews have suggested that the GDH reaction also favors glutamate formation in mammals [Hudson and Daniel, 1993]. Nissim has argued that, since flux through GDH is in the direction of reductive amination [Nissim, 1999], the enzyme plays an insignificant role in supplying ammonia to the urea cycle [Nissim, 1999; Nissim et al., 1999]. Others have argued that the GDH reaction favors oxidative deamination of glutamate. For example, Cooper has demonstrated that ammonium from the portal vein quickly equilibrates with glutamate, alanine, and aspartate, and that GDH activity subsequently provides ammonia for irreversible conversion to urea [Cooper et al., 1987; 1988]. As discussed

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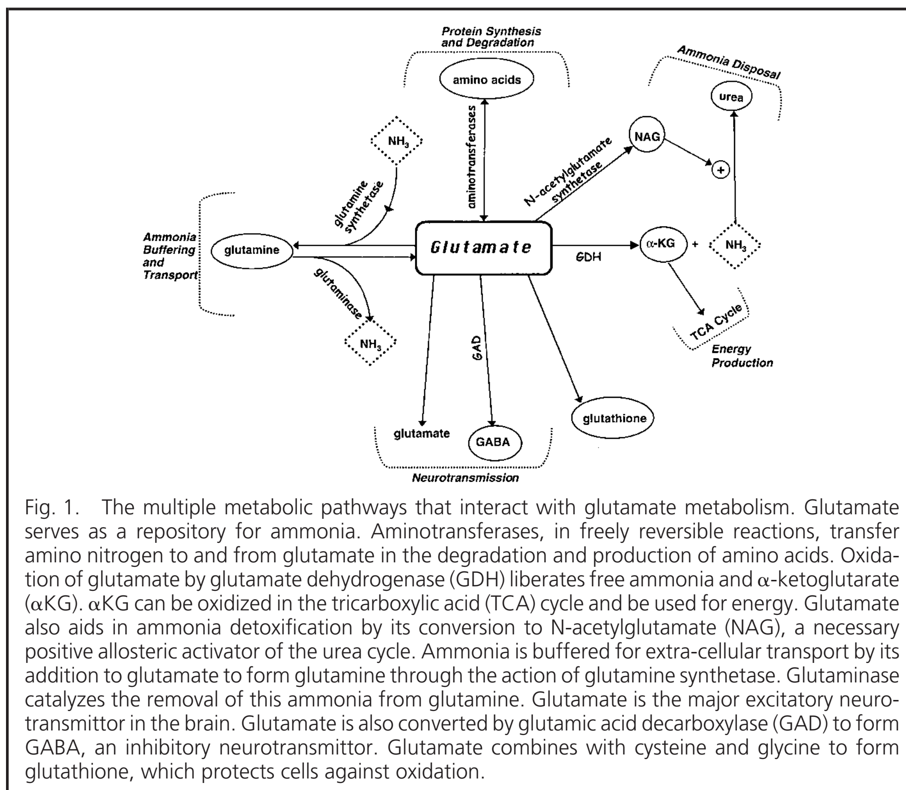

Fig. 1. The multiple metabolic pathways that interact with glutamate metabolism. Glutamate serves as a repository for ammonia. Aminotransferases, in freely reversible reactions, transfer amino nitrogen to and from glutamate in the degradation and production of amino acids. Oxidation of glutamate by glutamate dehydrogenase (GDH) liberates free ammonia and α-ketoglutarate (αKG). αKG can be oxidized in the tricarboxylic acid (TCA) cycle and be used for energy. Glutamate also aids in ammonia detoxification by its conversion to N-acetylglutamate (NAG), a necessary positive allosteric activator of the urea cycle. Ammonia is buffered for extra-cellular transport by its addition to glutamate to form glutamine through the action of glutamine synthetase. Glutaminase catalyzes the removal of this ammonia from glutamine. Glutamate is the major excitatory neurotransmittor in the brain. Glutamate is also converted by glutamic acid decarboxylase (GAD) to form GABA, an inhibitory neurotransmittor. Glutamate combines with cysteine and glycine to form glutathione, which protects cells against oxidation.

later, the features of the HI/HA syndrome fit best with the concept that GDH operates in the direction of oxidative deamination as shown in Figure 1.

Glutamate has a second role in ammonia disposal which further supports protein degradation (Fig. 1). In addition to being an intermediate for ammonia production, glutamate is the substrate for a critical urea cycle allosteric activator [Brusilow and Horwich, 1995]. In the liver, the intramitochondrial concentration of glutamate determines the rate of production of N-acetyl glutamate (NAG) by N-acetylglutamate synthetase (NAGS) and, thus, governs the rate of ureagenesis.

Glutamate also provides a substrate for ammonia buffering and transport of this potentially toxic metabolite. Glutamine synthetase adds ammonia to glutamate to form glutamine [Brosnan et al., 1996; Dejong et al., 1996; Nissim et al., 1996; Norenberg et al., 1997; Sonnenwald et al., 1997]. Glutamine is the major inter-organ carrier of ammonia and can transport ammonia that is generated, for example, in skeletal muscle or the intestinal lumen to the liver for detoxification. Glutamate can then be regenerated through phosphate-dependent glutaminase, which removes the amide nitrogen from glutamine and, thus, releases free ammonia for ureagenesis.

In the liver, the enzymes of glutamine metabolism are found in a heterogeneous but strategic arrangement that supports intercellular cycling of glutamine. Periportal hepatocytes are rich in glutaminase and urea cycle enzymes whereas a smaller subpopulation of perivenous cells contain glutamine synthetase. This organization permits ammonium detoxification via ureagenesis to occur predominantly in the periportal hepatocytes. Any ammonia that escapes can be scavenged by perivenous hepatocytes and complexed with glutamate through the activity of glutamine synthetase. The glutamine that results is released to the systemic circulation. Subsequently, it can be re-introduced to periportal hepatocytes and to ureagenesis.

In the setting of acidosis, ureagenesis by periportal hepatocytes is suppressed in favor of glutamine production by the perivenous hepatocytes. Glutamine is then transported to the kidney to aid in reversing acidosis through regeneration of free ammonia to be excreted with hydrogen ions [Haussinger, 1989; 1998; Haussinger et al., 1990].

In the central nervous system, a similar glutamate-glutamine shuttle is present and is essential for the nontoxic recycling of glutamate from astrocytes to neurons. Glutamate is the major excitatory neurotransmittor of the central nervous system. Astrocyte uptake of glutamate released from neural synapses occurs through sodium-dependent and independent pathways and is crucial for preventing toxic extracellular accumulations of glutamate [Anderson and Swanson, 2000; Kvamme et al., 2000]. Glutamate taken up by astrocytes is "neutralized" by conversion to glutamine. This glutamine can then be released for re-uptake by neurons and used to regenerate glutamate for neurotransmission. During states of hyperammonemia, formation of glutamate and glutamine may act as a sump for ammonia [Kanamori et al., 1996; Lapidot and Gopher, 1997], leading to accumulation of glutamine in the brain to millimolar concentrations and causing brain swelling through shifts in intracellular osmoles. This explanation for the toxic effects of hyperammonemia has been supported by the findings of cerebral edema and glutamine accumulation in rats made acutely hyperammonemic [Takahshi et al., 1991; Willard-Mack et al., 1996].

As Figure 1 demonstrates, glutamate is a precursor to GABA, an inhibitory neurotransmittor in the CNS. Glutamic acid decarboxylase (GAD) is responsible for the conversion of glutamate to GABA.

Glutamate is also a precursor to glutathione. Cysteine and and glycine are consecutively added to glutamate to produce glutathione. Glutathione protects cells against oxidative damage by reacting with hydrogen peroxide and organic peroxides [Meister and Larsen, 1995].

B. Disorders of Glutamate Metabolism

1. The hyperinsulinsim/hyperammonemia syndrome—gain of function mutations of GDH

GDH-the enzyme. GDH is a mitochondrial matrix enzyme found in substantial amounts only in the brain, liver, pancreas, and kidney. GDH is a homohexamer encoded by *GLUD1*, which is located on chromosome 10q and composed of 13 exons [Nakatani et al., 1988; Michaelidis et al., 1993].

In mammals, the reversible oxidative deamination of glutamate to α-ketoglutarate by GDH is highly regulated. GDH is allosterically inhibited by GTP [Cho et al., 1996] and is allosterically activated by ADP, ATP [Wrzeszczynski and Colman, 1994], and the amino acid, leucine [Gylfe, 1976; Fahien et al., 1980; Sener and Malaisse, 1980; Sener et al., 1981; Erecinska and Nelson, 1990].

A potential second gene for GDH has been reported. *GLUD2* is an intronless gene located on the X-chromosome.

It has over 95% amino acid sequence homology with *GLUD1* and, thus, is likely a retroposon [Shashidharan et al., 1994]. *GLUD2* is suggested to be expressed in retinal and testicular tissue and in lesser amounts in the brain [Shashidharan et al., 1994], but a role for this gene remains uncertain.

HI/HA—the syndrome. An unusual form of congenital hyperinsulinism (HI) associated with dominantly-expressed, gain of function mutations of GDH has recently been identified and is referred to as the hyperinsulinism/hyperammonemia syndrome (HI/HA) [Zammarchi et al., 1996; Weinzimer et al., 1997; Stanley et al., 1998; Yorifuji et al., 1999; Miki et al., 2000; Stanley et al., 2000]. Affected children suffer from recurrent hypoglycemia due to inappropriate secretion of insulin. They also have persistent hyperammonemia from which they appear to be asymptomatic.

Table 1 shows the clinical presentation of infants with HI/HA. These presentations are dramatically different from those of infants with the more commonly recognized form of HI, which is due to defects of the sulfonylurea receptor/potassium channel complex (K-channel) [Thornton et al., 1998]. The latter children present with large for gestational age birthweight and have intractable hypoglycemia within the first few days of life. In contrast, HI/HA children are of normal birth weight, and their hypoglycemia often does not become apparent until later in infancy [Stanley et al., 2000]. The hypoglycemic symptoms of HI/HA range from subtle to severe. Some affected individuals have only been diagnosed with HI/HA in adulthood, after an affected relative was identified. Fortunately, unlike K-channel HI, this form of HI is responsive to diazoxide. This medication inhibits insulin secretion at the sulfonylurea receptor/potassium channel complex of the β-cell (Fig. 2). HI/HA may be associated with only subtle defects of fasting; Figure 3 shows the response to fasting in a 15-year-old HI/HA girl. As shown in this same figure, HI/HA is characterized by post-prandial hypoglycemia induced by protein consumption [Hsu et al., 2001].

Table 1. Clinical Manifestations in HI/HA Children With GDH Exon 11 or 12 Mutations

n	25
Sex (F/M)	10/15
Birth weight (n = 22)	3,500 g (2,180–4,310)*
Age of onset (n = 25)	4 months (1 h-15 months)^
Plasma ammonium (n = 21)	135 μmol/L (58–360)
Diazoxide responsive	23/23
Developmental status (n = 22)	
Normal	11
Delayed	11
Transmittance	
Familial	7
Sporadic	18

Data are n or medians (range).
*Normal mean = 3,200 g; ^normal value = 9–35 μmol/l.

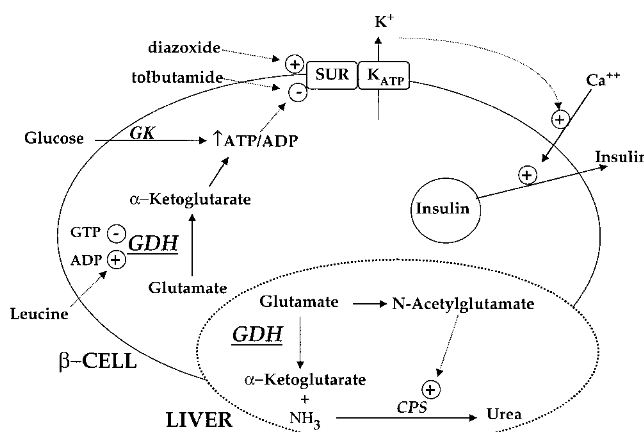

Fig. 2. The role of glutamate dehydrogenase (GDH) activity in hepatic ureagenesis and β-cell insulin secretion. In the hepatocyte, GDH activity generates ammonia through oxidation of glutamate. Glutamate is necessary for production of N-acetylglutamate, a necessary allosteric activator of carbamoylphosphate synthetase (CPS), the rate-controlling first step of the urea cycle. In the pancreatic β-cell, activation of GDH by leucine stimulates insulin secretion. Upon entering the β-cell, glucose is activated by glucokinase (GK) and undergoes oxidation to increase the ATP to ADP ratio which then causes closure of an inwardly-rectifying K_{ATP} channel, composed of a sulfonylurea receptor (SUR1)/ potassium channel (Kir6.2) complex. Depolarization of the cell membrane then occurs, allowing calcium (Ca^{++}) to enter the β-cell and stimulate insulin secretion. GDH stimulates insulin secretion through oxidation of glutamate to α-KG and generation of ATP, which subsequently activates the same cascade of insulin release as glucose. GDH is allosterically activated by leucine and ADP and allosterically inhibited by GTP.

Hyperammonemia is a distinguishing feature of HI/HA. Serum ammonium concentrations are persistently elevated to 2–10 times normal (Table 1). Affected children and adults appear to be entirely asymptomatic from their hyperammonemia. Unlike individuals with urea cycle defects or hyperammonemia secondary to liver disease, patients with HI/HA do not manifest lethargy or vomiting from their hyperammonemia. Although they have not been formally tested for subtle neurologic defects, affected patients can lead normal lives and appear only to have cognitive difficulties attributable to hypoglycemia.

In further contrast to the hyperammonemia associated with urea cycle enzyme disorders, the plasma amino acid profiles are normal in HI/HA. Most notably, plasma glutamine concentrations are not elevated despite the hyperammonemia. Plasma ammonium concentrations are stable in HI/HA patients and are not affected by protein feeding or blood glucose concentrations [Zammarchi et al., 1996; Weinzimer et al., 1997; Miki et al., 2000]. Treatments with protein restriction or benzoate, an alternate pathway treatment for hyperammonemia, are not effective in lowering serum ammonium [Weinzimer et al., 1997; Yorifuji et al., 1999; Huijmans et al., 2000]. In two patients, N-carbamylglutamate, a NAG analogue, reduced serum ammonium concentrations by 50%. These patients did not show any apparent clinical improvement from the lower serum ammonium concentrations [Zammarchi et al., 1996, Huijmans et al., 2000].

Because GDH connects carbohydrate metabolism and ammonia detoxification, a defect of this enzyme was con-

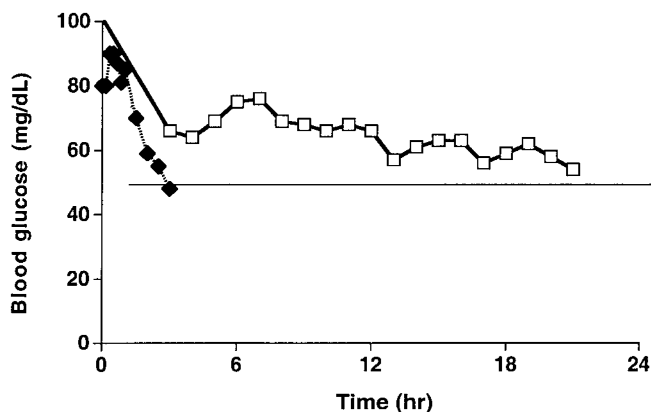

Fig. 3. Blood glucose responses to fasting and protein feeding in HI/HA. Shown are the concentrations of blood glucose during a fasting test (open squares) and following a protein meal (solid squares) in a 15-year-old girl with an Arg269His GDH mutation. Unlike this patient, normal individuals do not become hypoglycemic before 36 to 48 hours of fasting and do not develop hypoglycemia after a protein meal.

sidered an attractive candidate for the unusual combination of hyperinsulinism and hyperammonemia. The postulated mechanism of HI/HA involves a gain of function mutation of GDH that simultaneously causes excessive insulin secretion while impairing ureagenesis.

Insulin secretion in HI/HA. Oxidative deamination of glutamate by GDH supplies α-KG to the TCA cycle and generates ATP. As shown in the simplified diagram of insulin secretion by the β-cell (Figure 2), this increase in the ATP/ADP ratio triggers insulin release through the sulfonylurea receptor/potassium channel complex [Matschinsky and Sweet, 1996]. Therefore, overactivity of GDH in the direction of α-KG could drive unregulated insulin secretion.

Ammonia detoxification in HI/HA. Overactivity of GDH in the direction of glutamate oxidation can also account for the hyperammonemia associated with gain of function mutations of GDH. Figure 2 demonstrates the dual roles which glutamate performs in hepatic ammonia metabolism: ammonia generation and NAG synthesis. Overactivity of hepatic GDH would lead to excessive oxidative deamination of glutamate and excessive ammonia production. In the setting of an intact urea cycle, periportal hepatocytes would be expected to compensate for this excessive ammonia production by increased ureagenesis. However, if NAG synthesis is deficient as a result of depleted glutamate stores from overactive GDH, ammonia detoxification via urea will also be impeded. In addition, perivenous hepatocytes, which are crucial for ammonia detoxification via glutamine synthetase, may be less able to compensate if glutamate pools have been depleted by this excessive GDH activity. To date, the glutamate content of β-cells and hepatocytes from HI/HA patients has not been measured to confirm these hypotheses.

An alternative hypothesis for the hyperammonemia of HI/HA is that abnormal muscle catabolism generates excessive ammonia. The findings of normal portal vein, hepatic vein, and arterial ammonium but "subnormal" peripheral venous ammonium in one anesthetized patient with HI/HA [Kaumon et al., 1998] suggested to the authors a muscular origin for the hyperammonemia of HI/HA. However, this report must be interpreted cautiously. Serum ammonium concentrations, although consistently elevated, do fluctuate in HI/HA patients. The apparent "normalization" of peripheral serum ammonium in this patient may actually reflect the normal fluctuations of serum ammonium concentrations in the HI/HA syndrome.

Enzymatic studies of GDH from HI/HA patients support the postulated mechanism of GDH overactivity. Table 2 shows the results of enzymatic studies performed on GDH isolated from HI/HA patient lymphoblasts. Mutant GDH from patients has normal basal activity. Sensitivity of the GDH mutants to allosteric stimulation by ADP and leucine is also largely unaffected. However, as shown in Table 2 and in Figure 4, GDH from HI/HA patients displays decreased sensitivity to inhibition by GTP [Stanley et al., 1998; 2000] with IC_{50} values 2–10 times more than that of normal controls. This loss of inhibitory regulation of GDH by GTP results in a gain of GDH function in patients with the HI/HA syndrome.

Mutational analysis of *GLUD1* further supports the above of role of GDH in HI/HA. Multiple mutations have been reported in either exons 11 or 12 of *GLUD1* [Stanley, 1998; Fujioka, 2000; Miki, 2000; Ohura, 2000]. Additional mutations have been found in exons 6 and 7 [Miki, 2000; Stanley, 2000; Ohura, 2000; MacMullen, 2001] and exon 10 [Yorifuji, 1999]. Based upon the recently determined three-dimensional structure of bovine GDH, which has more than 95% homology with human GDH, these exons encode areas important for allosteric regulation [Peterson and Smith, 1999]. As shown in Figure 5, the amino acid residues mutated in HI/HA patients lie in close proximity to the GTP inhibitory allosteric site and likely interfere with GTP binding.

These regulatory mutations of GDH are anticipated to allow unrestrained allosteric activation of GDH by leucine. Indeed, HI/HA patients have exaggerated insulin secretion in response to leucine stimulation. In contrast, patients with the common K-channel form of HI are not leucine-sensitive [Kelly et al., 2001]. Hence, exaggerated leucine-stimulated insulin secretion results from these GDH gain of function mutations and likely accounts for the hypoglycemia that occurs with protein ingestion in HI/HA (Fig. 3).

This enhanced sensitivity of GDH to the allosteric effects of leucine likely accounts for former reports of "leucine-sensitive hypoglycemia of infancy and childhood." In 1956, Cochrane et al., reported a father and his two daughters as developing hypoglycemia following ingestion of protein or of the amino acid leucine. Four years later, Grumbach and Kaplan showed that this leucine-induced hypoglycemia was due to excessive serum insulin. Subsequently, leucine-sensitivity was assumed to be synonymous with congenital HI, and all children with HI were instructed to avoid protein. The identification of a number of the molecular defects responsible for HI, including the gain of function mutations of GDH in HI/HA, has helped clarify the leucine-sensitivity issue. Although leucine-sensitive insulin secretion is not entirely specific for HI/HA, some patients previously described as "leucine sensitive" may have had gain of function mutations of GDH. The finding of leucine sensitivity in HI/HA aids in its management: pure protein loads are avoided.

The correlation of GDH mutations with the HI/HA syndrome has a

Table 2. Activity and Allosteric Responses of Lymphoblast GDH With Exon 11 and 12 Mutations

	Mutation Sites								
(n)	Phe440Leu (1)	Gln441Arg (1)	Ser445Leu (2)	Gly446Arg (1)	Gly446Asp (1)	Ser448Pro (4)	Lys450Glu (2)	His454Tyr (1)	Control (7)
Basal activity ($nmol \cdot mg^{-1} \cdot min^{-1}$)	22	22	29*, 28*	21	23	10 ± 17**	19, 20	19	22 ± 2.3
Allosteric activators									
•ADP SC_{50} (μmol/l)	21	20	23*, 19	18	28*	34 ± 2.3**	24*, 25*	13	19 ± 3.4
Maximum activity ($nmol \cdot mg^{-1} \cdot min^{-1}$) (with ADP 200 μmol/l)	39*	49*	48, 45	46	49	36 ± 5**	55*, 51*	43	46 ± 4.5
•Leucine SC_{50} (mmol/l)	0.7	0.83	0.90, 0.7	0.75	0.80	1 ± 0.1**	1*, 0.98*	0.71	0.8 ± 0.12
Maximum activity ($nmol \cdot mg^{-1} \cdot min^{-1}$) (with leucine 6 mmol/l)	37	50*	47, 47	36	49*	29 ± 5.3**	35*, 49*	38	42 ± 6.5
Allosteric inhibitors									
•GTP IC_{50} (nmol/l)	190*	94*	280*, 340*	300*	480*	290 ± 34**	230*, 190*	280*	48 ± 17
Residual activity ($nmol \cdot mg^{-1} \cdot min^{-1}$) (% basal with GTP 3 μmol/l)	2.4	0.83	14*, 8*	20*	26*	9.2 ± 3.6	22*, 21*	19*	2.4 ± 2.3

Data are means ± SD.
*Beyond 95% confidence limit of normal control subjects.
**vs. control $P < 0.01$.

number of implications for our understanding of GDH activity. The primary direction of GDH activity in β-cells has been the subject of recent debate [Maechler and Wollheim, 1999; MacDonald and Fahien, 2000, Maechler et al., 2000] and thought to favor glutamate production [Maechler and Wollheim, 1999; Maechler et al., 2000]. However, the findings of hyperinsulinism and hyperammonemia in the setting of gain of function mutations of GDH suggest that the reaction operates in the direction of oxidative deamination of glutamate to α-KG.

The association of HI/HA with regulatory mutations of GDH suggests this enzyme plays a more important role in insulin secretion than previously recognized. Unlike other amino acids, leucine does not require the presence of glucose to stimulate insulin secretion [Newgard and Matschinsky, 1999]. This glucose independence may allow leucine to serve as a signal of protein consumption/catabolism to the β-cell. Through its allosteric activation, leucine may confer GDH the role of β-cell amino acid "sensor," just as glucokinase is the β-cell glucosensor [Matschinsky and Sweet, 1996].

In addition, through its allosteric inhibition, GTP may confer GDH the role of maintaining basal insulin secretion. In vitro studies in isolated rat islets reveal that glucose deprivation enhances glutamate oxidation and insulin secretion [Gao et al., 1999]. The corollary is that in vivo, fasting is expected to enhance glutamate oxidation to maintain insulin secretion. This hypothesis is based upon decreases in β-cell ATP and GTP that accompany declines in plasma glucose during fasting. Lowering of the phosphorylated energy state of the β-cell removes GTP inhibition of GDH and is anticipated to stimulate glutamate oxidation.

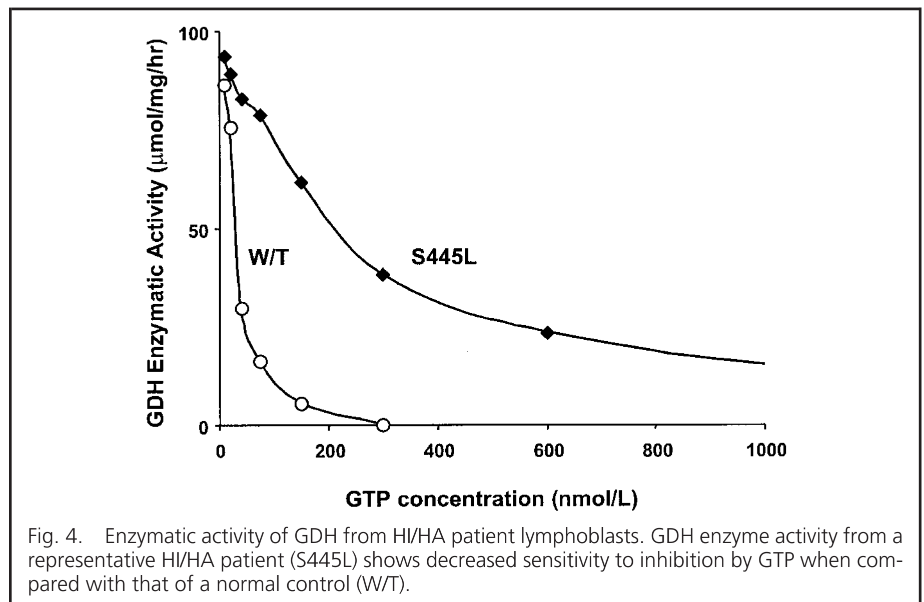

Fig. 4. Enzymatic activity of GDH from HI/HA patient lymphoblasts. GDH enzyme activity from a representative HI/HA patient (S445L) shows decreased sensitivity to inhibition by GTP when compared with that of a normal control (W/T).

Conversely, additional studies in isolated rat islets demonstrated that exposure to high glucose concentrations suppresses glutamate oxidation and insulin secretion [Gao et al., 1999]. This suppression by glucose occurs presumably through changes in the phosphorylated energy state of the β-cell that make GTP available for inhibition of GDH. These in vivo findings are supported by studies in HI/HA patients whose excessive insulin responses were suppressed during hyperglycemia [Kelly et al., 2001].

The finding of GDH gain of function mutations in the setting of asymptomatic hyperammonemia has implications for the etiology of hyperammonemic encephalopathy. As described earlier, the coupling of excess ammonia to glutamine and glutamate may result in 1) neurotoxicity from excessive glutamate production and 2) astrocyte swelling due to glutamine accumulation. As diagrammed in Figure 6, overactivity of GDH may deplete glutamate and, thus, circumvent glutamate intoxication in HI/HA patients. In addition, without abundant glutamate stores, excessive glutamine pro-

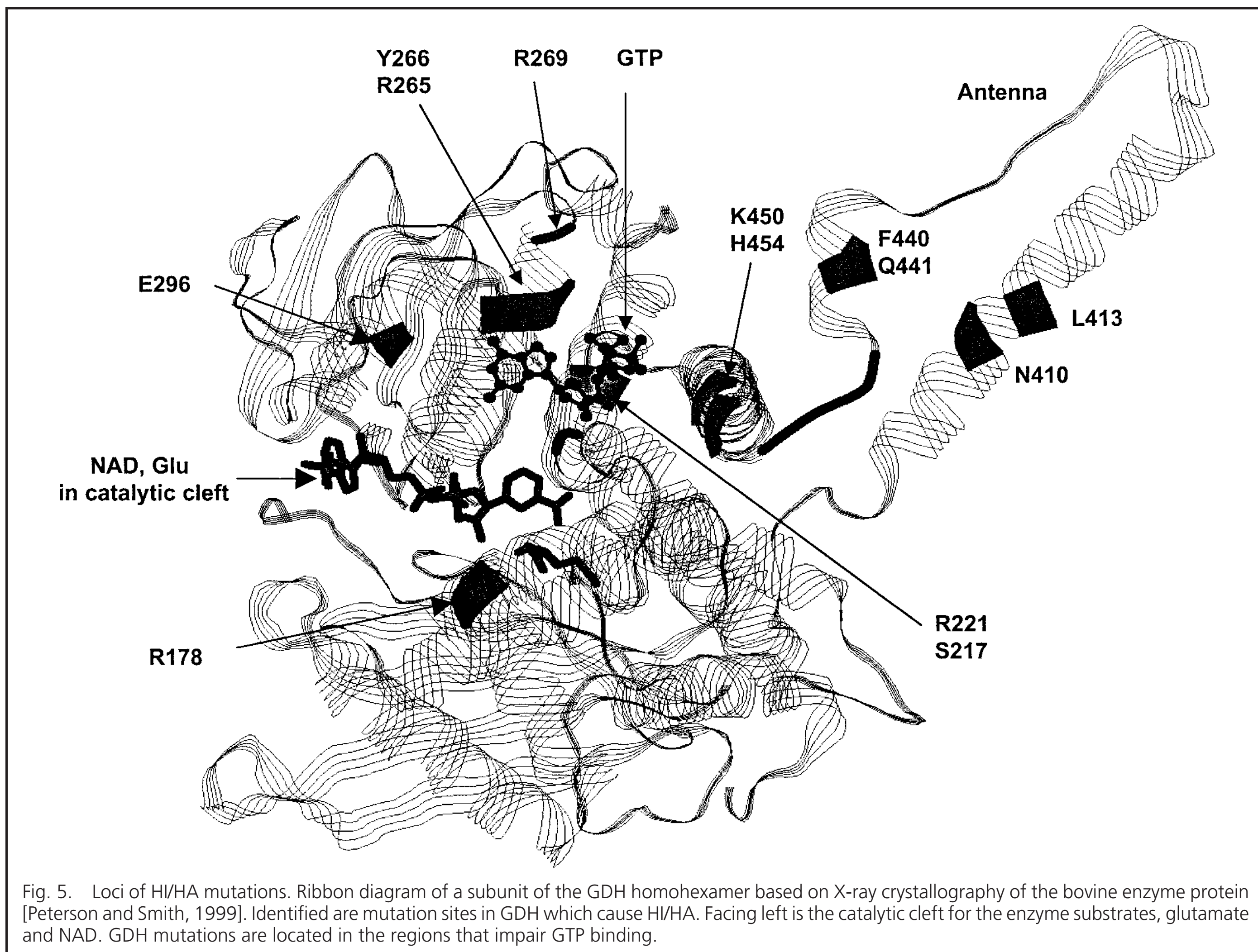

Fig. 5. Loci of HI/HA mutations. Ribbon diagram of a subunit of the GDH homohexamer based on X-ray crystallography of the bovine enzyme protein [Peterson and Smith, 1999]. Identified are mutation sites in GDH which cause HI/HA. Facing left is the catalytic cleft for the enzyme substrates, glutamate and NAD. GDH mutations are located in the regions that impair GTP binding.

duction and the brain swelling that accompanies it would be avoided.

2. Isolated hyperammonemia—N-acetylglutamate synthetase [NAGS] deficiency

NAGS—the enzyme. In the rate-controlling step of the urea cycle, carbamyl-phosphate synthetase-I (CPS-I), a mitochondrial matrix enzyme, catalyzes the synthesis of carbamyl-phosphate from ammonia and bicarbonate in the liver [Stewart and Walser, 1980; Brusilow and Horwich, 1995]. CPS-I requires activation by its allosteric activator, NAG. NAG is synthesized from glutamate and acetyl-CoA by the mitochondrial enzyme N-acetylglutamate synthetase (NAGS). Because the K_m of NAGS for glutamate (3 mM) is close to the intracellular concentration of this amino acid, changes in glutamate concentrations control the rate of ureagenesis.

The disorder. Several putative cases of NAGS deficiency have been reported [Bachmann et al., 1982; Bachmann et al., 1989; Epleleg et al., 1990; Schubiger et al., 1991; Vockley et al., 1992; Plecko et al., 1998]. All cases have included hyperammonemia but otherwise have had variable clinical presentations. The hyperammonemia varies from normal to mid-200 μmol/L range. The hyperammonemia is not exacerbated by protein intake. Symptoms of NAGS deficiency are not typical of the hyperammonemia that accompanies urea cycle enzyme defects. Plasma and urine amino acids are relatively normal. The mild abnormalities that are present are not diagnostic of typical urea cycle enzyme disorders. Treatment with N-carbamylglutamate has been suggested to lower plasma ammonium concentrations [Plecko et al., 1998].

Activity of NAGS from liver biopsies of these patients has ranged from none to approximately 60% of normal [Bachmann et al., 1989; Epleleg et al., 1990; Plecko et al., 1998]. Despite having only 10% of normal NAGS activity, the liver of the one patient in whom it was measured had normal NAG content [Vockley et al., 1992]. Tuchman and Holzknecht have noted that measurements of NAG content and NAGS activity in the liver may be inaccurate as a result of the indirect assays employed [1990]. As a result, the reports of decreased NAGS activity in these patients must be regarded as inconclusive.

A review of the reported NAGS deficiency cases suggests that some of these children may have had the HI/HA syndrome. The presentations of these patients with acute changes in mental status after intercurrent illnesses or after protein loads suggest that these episodes may have been due to hypoglycemia. In the initial presentation of one patient, hypoglycemia (35 mg/dL) was documented and responded to intravenous glucose treatment [Vockley et al., 1992].

The finding of hyperammonemia in the absence of the usual signs and symptoms that accompany urea cycle enzyme defects should prompt one to evaluate not only for NAGS deficiency but also regulatory mutations of GDH. Hy-

poglycemia serves as a clue to the latter and should not be discounted.

3. Olivopontocerebellar atrophy—loss of GDH activity proposed

Olivopontocerebellar atrophy (OPCA) is a form of Multiple System Atrophy, commonly accompanied by ataxia or Parkinsonism-like symptoms. Inferior olivary nuclei and cerebellar pathology are consistently present but pontine and spinal anterior motor horn pathology are variable. One proposed mechanism for this neurodegenerative process has been GDH deficiency [Plaitakis et al., 1980; Plaitakis and Yahr, 1986; Plaitakis et al., 1993], but findings have been inconsistent, and direct evidence is lacking.

A rat model of OPCA, generated by systemic administration of the nicotinamide antagonist 3-acetylpyridine, led to the suggestion that GDH may be involved in the pathology of OPCA [Plaitakis et al., 1993]. This agent is thought to mediate its neurotoxic effects through inhibition of NADP-dependent oxidoreductases; GDH is one such candidate enzyme. Subsequent evaluation of a patient with OPCA revealed decreased GDH activity in cultured skin fibroblasts and leukocytes (22% of activity in controls) [Plaitakis et al., 1980]. Additional patients with variable clinical features of OPCA were found to have decreased GDH activity (50% of control GDH activity) in peripheral blood leukocytes. Blood leukocytes from patients with atypical Parkinsonism and progressive supranuclear palsy also had decreased GDH activity (85% of control) [Plaitakis and Yahr, 1986]. A number of other studies have examined GDH activity in lymphocytes, platelets, fibroblasts, and muscle [Yamaguchi et al., 1982; Finocchiaro et al., 1986; Sorbi et al., 1986; 1989]. Partial deficiencies in GDH have been identified but not associated with a specific OPCA phenotype.

The role of GDH in OPCA has not been settled. The GDH deficiencies are only partial and lack specific disease correlates. Despite findings in peripheral tissues, evidence of deficient GDH activity in brain is lacking. In one patient with OPCA, the concentration of a GDH isoprotein in the brain was selectively reduced [Hussain et al., 1989]. Conceivably, the variant GDH could represent the GLUD2 protein. In contrast, another patient with OPCA had repeatedly low leukocyte GDH activity. However, postmortem examination of the brain of this patient revealed no differences in GDH activity, mRNA size, or mRNA amount when compared with that of controls [Chokroverty et al., 1990]. At least one study suggests GDH deficiency may be an epiphenomenon. In this report, decreased activities of other mitochondrial enzymes were found but not to the same degree as that of GDH [Sorbi et al., 1989]. Finally, despite the findings of GDH deficiency, mutations of GDH have yet to be reported in OPCA.

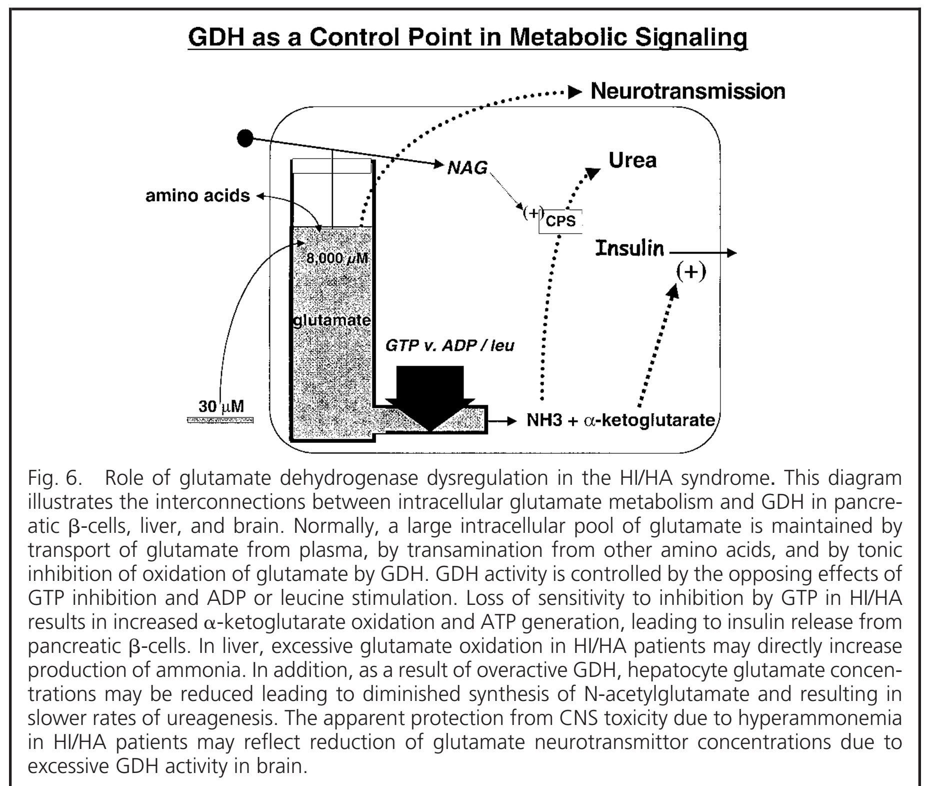

Fig. 6. Role of glutamate dehydrogenase dysregulation in the HI/HA syndrome. This diagram illustrates the interconnections between intracellular glutamate metabolism and GDH in pancreatic β-cells, liver, and brain. Normally, a large intracellular pool of glutamate is maintained by transport of glutamate from plasma, by transamination from other amino acids, and by tonic inhibition of oxidation of glutamate by GDH. GDH activity is controlled by the opposing effects of GTP inhibition and ADP or leucine stimulation. Loss of sensitivity to inhibition by GTP in HI/HA results in increased α-ketoglutarate oxidation and ATP generation, leading to insulin release from pancreatic β-cells. In liver, excessive glutamate oxidation in HI/HA patients may directly increase production of ammonia. In addition, as a result of overactive GDH, hepatocyte glutamate concentrations may be reduced leading to diminished synthesis of N-acetylglutamate and resulting in slower rates of ureagenesis. The apparent protection from CNS toxicity due to hyperammonemia in HI/HA patients may reflect reduction of glutamate neurotransmittor concentrations due to excessive GDH activity in brain.

Glutamate and its metabolism by GDH play key roles in the CNS. The association of CNS disturbances with defects of GDH activity is conceptually attractive. Whether a deficit of GDH activity is causal or the result of another primary defect in OPCA has yet to be determined.

4. Pyridoxine-dependent seizures—a disorder of glutamic acid decarboxylase?

GAD—the enzyme. Glutamic acid decarboxylase is responsible for the conversion of glutamate to GABA, a major inhibitory neurotransmittor [Scriver and Gibson, 1995]. Two GAD enzymes have been identified in the brain, GAD_{65} and GAD_{67}. GAD_{65} is encoded on chromosome 10, depends on the coenzyme pyridoxyl phosphate, and is located in the nerve terminal where it is thought to be responsible for the generation of GABA for neuro-inhibition. GAD_{67} is encoded on chromosome 2, is relatively independent of pyridoxyl phosphate, and is located in the cell body where its production of GABA is important for cellular metabolism [Scriver and Gibson, 1995].

Pyridoxine-dependent seizures—the disorder. Pyridoxine-dependent seizures are an autosomal recessively inherited form of seizures that usually present in the neonatal period [Gospe, 1998]. These seizures are unresponsive to conventional anticonvulsant agents but cease after pharmacological doses of intravenous pyridoxine (vitamin B6). Atypical forms have been recognized. These forms may present later in infancy, respond initially to conventional anticonvulsants, or be associated with prolonged seizure-free periods after withdrawal of pyridoxine. In utero seizure activity has even been described. The types of seizures associated with pyridoxine-dependency vary and may be partial, generalized, atonic, myoclonic, tonic-clonic, or even infantile spasms. Not uncommonly, a period of irritability or vomiting precedes the seizure [Gospe, 1998].

The etiology of pyridoxine-dependent seizures remains unresolved, but a disorder in the synthesis of GABA, an inhibitory neurotransmittor considered important in the control of seizures, is suspected [Scriver and Whelan, 1969]. Elevated concentrations of glutamate and low concentrations of GABA detected in the brain and cerebrospinal fluid of affected patients suggest that glutamate

conversion to GABA is impaired [Lott et al., 1978; Kurlemann et al., 1992; Baumeister et al., 1994]. Similar demonstrations of low GABA concentrations in the brain and cerebrospinal fluid (CSF) during states of pyridoxine-deficiency [Coursin, 1954] as well as during states of pyridoxyl phosphate inhibition [Gospe, 1998], e.g. isoniazid treatment, lend credence to this theory. However, low GABA has been detected in other, non-pyridoxine dependent seizure disorders [Rating et al., 1983; Loscher and Siemes, 1985]. In one patient, transient resolution of seizure activity occurred with intravenous GABA administration [Kurlemann et al., 1987]. In another patient, resolution of seizure activity with pyridoxine administration was associated with a rise in CSF GABA concentrations [Baumeister et al., 1994].

Because GAD activity accounts for GABA production and because the GAD_{65} isoenzyme requires pyridoxyl phosphate, a binding defect of GAD_{65} to its coenzyme has been proposed as responsible for pyridoxine-dependent seizures. Such a defect would impair GABA synthesis. The success of pharmacological dosing of pyridoxine in ameliorating this disorder would lie in its ability to overcome this impaired binding. In support of this hypothesis, studies of cultured skin fibroblasts demonstrated reduced pyridoxyl-phosphate dependent GABA synthesis but an unaltered pyridoxyl-independent pathway [Gospe, 1998]. However, genetic linkage studies to GAD_{65} and GAD_{67} have excluded both these enzymes as the responsible genes. A genome-wide search of five affected families has linked the disorder to chromosome 5q31 [Cormie-Daire et al., 2000]. However, the gene involved has yet to be identified.

C. Summary and Conclusions

Intact regulation of glutamate metabolism is crucial for amino acid synthesis and oxidation, for ureagenesis, and for normal function of the CNS. The identification of dysregulated glutamate metabolism as the etiology of HI/HA attests to the significance of glutamate metabolism. Although speculative, the findings of abnormal glutamate metabolism in association with other disorders suggests glutamate may contribute to their pathology as well. ■

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