COMMENTARY

Glutamate dehydrogenase, insulin secretion, and type 2 diabetes: a new means to protect the pancreatic β-cell?

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Abstract

In this issue of *Journal of Endocrinology*, Dr Han and colleagues report a protective effect of the glutamate dehydrogenase activator 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) under diabetes-like conditions that impair β-cell function in both a pancreatic β-cell line and *db/db* mice. Based on these observations, the authors suggest that BCH could serve as a novel treatment modality in type 2 diabetes. The present commentary discusses the importance of the findings. Some additional questions are raised, which may be addressed in future investigations, as there is some concern regarding the BCH treatment of β-cell failure.

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Glutamate dehydrogenase and insulin secretion

Glutamate dehydrogenase (GDH) is a pivotal enzyme in the control of substrate flux from glutamine to α-ketoglutarate over glutamate (Karaca *et al*. 2011). This pathway is essentially an anaplerotic/cataplerotic pathway, where the tricarboxylic acid (TCA) cycle either is replenished or serves as a source of carbons and intermediates for amino acid metabolism (Fig. 1). The directionality of the reaction is controlled by the fuel status of the cell. For instance, a high energy level, signaled by an abundance of GTP, inhibits the enzyme, while ADP activates it. Leucine is an allosteric activator of GDH (Sener *et al*. 1981). Substrate availability is a critical factor: an abundance of amino acids will drive the reaction toward the TCA cycle; a surplus of TCA cycle intermediates will favor the exit of carbons from the cycle via α-ketoglutarate and GDH (Smith & Stanley 2008).

Of interest here, GDH has been implicated in stimulus–secretion coupling in pancreatic β-cells by Claes Wollheim, Pierre Maechler, Charles Stanley, and others (Maechler & Wollheim 1999, Li *et al*. 2003). Insulin secretion is thought to be provoked by the combination of a triggering pathway and an amplifying pathway (Henquin 2009). Thus, glucose is transported into the β-cell proportionally to its extracellular concentration by GLUT2 (rodents) and GLUT1 and -2 (humans). Its subsequent metabolism in glycolysis and the TCA cycle leads to ATP production in the mitochondria via oxidative phosphorylation. A rise in the ATP/ADP ratio closes an ATP-dependent potassium channel (K_{ATP}), which depolarizes the plasma membrane. Consequently, exocytosis is triggered.

However, it has long been realized that this triggering pathway of glucose-stimulated insulin secretion (GSIS) is not sufficient to sustain secretion. There must exist other mechanisms that account for the amplification of GSIS; these have been summarized as the amplifying or K_{ATP}-independent pathway (Gembal *et al*. 1992). Several metabolic pathways linked to that of glucose have been suggested to serve this role (Sato & Henquin 1998). One of these, which has received quite substantial support, is the glucose-dependent intracellular formation of glutamate (Karaca *et al*. 2011). How formation of glutamate actually brings about exocytosis is unclear but is thought to involve a direct effect on the secretory granules (Maechler & Wollheim 1999).

The critical role of GDH in insulin secretion has been further emphasized by the condition persistent hyperinsulinemia and hyperammonemia in infancy (PHHI; Stanley 2006). This is a rare congenital hereditary disease caused by activating mutations in GDH. These children exhibit hypersecretion of insulin and elevated circulating levels of ammonium. In terms of pathogenicity, this suggests that GDH in this condition drives the reaction toward α-ketoglutarate, since NH_4^+ is formed. Functional genomics approaches in mice have shown that overexpression of GDH exaggerates insulin secretion (Carobbio *et al*. 2004), whereas disruption of GDH perturbs insulin secretion (Carobbio *et al*. 2009).

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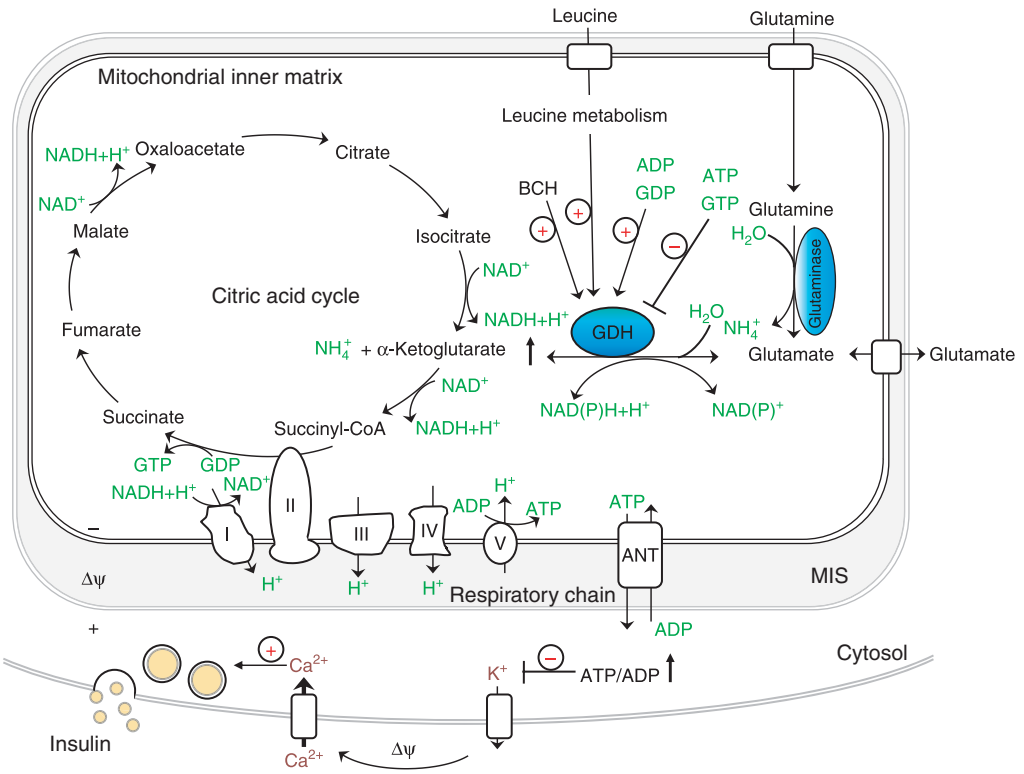

Figure 1 GDH activation stimulates the conversion of glutamate to α-ketoglutarate, resulting in enhanced substrate oxidation in the citric acid cycle. When GDH is activated, it catalyzes deamination of glutamate to α-ketoglutarate and ammonia by oxidation, accepting either NAD^+ or $NADP^+$ as the redox coenzyme. An increase in mitochondrial α-ketoglutarate stimulates its conversion in the citric acid cycle, resulting in increased ATP production during respiration. An increase in the cytosolic ATP/ADP ratio eventually results in the closure of K_{ATP} channels, initiating plasma membrane depolarization and influx of Ca^{2+} ions. The intracellular rise in Ca^{2+} ions triggers insulin release. GDH activity is regulated by multiple allosteric inhibitors and activators controlling glutamate oxidation depending on the intracellular energy level and the supply of amino acids. ANT, adenine nucleotide translocator; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; GDH, glutamate dehydrogenase; MIS, mitochondrial intermembrane space; $\Delta\Psi_m$, membrane potential.

Could activation of GDH protect β-cells in type 2 diabetes?

In this issue of *Journal of Endocrinology*, Dr Han and colleagues report on an exciting novel function of GDH in chronic pathological situations relevant for type 2 diabetes. They have used 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH), which is a well-known activator of GDH (Sener *et al.* 1981), to explore whether manipulation of glutamine–glutamate–α-ketoglutarate metabolism affects β-cell function *in vitro* and *in vivo*. Their hypothesis was that promotion of anaplerosis in β-cells may stabilize β-cell function and perhaps make them more resistant in a stressful metabolic environment. Their assumption was based on a previous study where they showed that BCH increased the levels of TCA cycle intermediates, restored ATP levels, enhanced the oxidation rate, and protected against high glucose/palmitic acid (HG/PA)-induced apoptosis in INS-1 cells (Choi *et al.* 2011). Presently, they first confirmed that BCH is an efficient potentiator of GSIS. Upon culture of INS-1 cells at a chronic high glucose concentration for 24 h, GSIS as well as insulin content was reduced. Interestingly, the long-term addition of BCH ameliorated this detrimental effect of glucose. Moreover, BCH also blocked the reduction in insulin mRNA in INS-1 cells exposed to a combination of high glucose and palmitate, i.e. glucolipotoxicity. It is widely accepted that there is increased cell death in β-cells under glucolipotoxic conditions, foremost apoptosis. The authors also examined whether BCH would affect this adverse event in β-cells. Indeed, BCH halved the apoptotic processes in INS-1 cells, as measured by DNA fragmentation; this was accompanied by reduced phosphorylation of the stress-activated c-Jun N-terminal kinase, a process previously shown to be involved in β-cell apoptosis (Maedler *et al.* 2008).

After this overture, the authors moved to the *in vivo* situation. They employed *db/db* mice, which lacked

functional leptin signaling. Consequently, the mice overate, became obese and developed insulin resistance. After 6 weeks of treatment with BCH, glucose tolerance in the mice improved. This was accounted for by increased insulin secretion; body weight was unaffected. The untreated *db/db* mice displayed altered islet morphology, with α-cells dispersed throughout the islets and reduced β-cell number. Upon treatment with BCH, the proportion of islet β-cells increased, and that of α-cells decreased. Moreover, the rate of apoptosis appeared to have diminished because the frequency of cleaved caspase-3 was lower in islets from BCH-treated *db/db* mice. Based on their results, the authors suggested that activation of GDH could become a new treatment modality in type 2 diabetes.

Future perspectives

Although it is clearly too early to surmise that activation of GDH is potentially a new therapeutic avenue in type 2 diabetes, the studies by Han and colleagues highlight the potential effects of changing/improving the metabolic milieu of β-cells, and thereby preventing cell loss and deteriorating function. An increased load of glucose and fatty acids on β-cells is toxic. Some of this may be caused by an incapacity of the electron transport chain, resulting in the formation of reactive oxygen species. The results by Han and colleagues suggest that some of this overload may be balanced by the metabolism of other fuels in the TCA cycle. Although the previous studies by the group indicated that mitochondrial metabolism to some extent was normalized by culture in BCH (Choi *et al*. 2011), a more detailed analysis is warranted. The following questions remain to be answered: is GDH activity and/or expression affected under pathological conditions? Are glutamine and glutamate consumed upon BCH stimulation, while α-ketoglutarate is formed, or the reversed? In addition, pancreatic islets convert glucose into fatty acids by *de novo* synthesis via citrate production and export from the mitochondria. This will lead to the inhibition of fatty acid oxidation. Fatty acids play a dual role in β-cell stimulus–secretion coupling under acute and chronic conditions (Prentki & Corkey 1996). Blocking these processes long term may have unforeseen effects. It would be a topic for further investigation whether BCH treatment and the consequent increase in TCA cycle metabolism positively affects fatty acid oxidation and synthesis. Moreover, while it was demonstrated that β-cell loss in *db/db* mice was ameliorated, we do not know whether improved glucose tolerance also can be attributed to improved β-cell function. It would be interesting to see whether GSIS and insulin content are preserved in isolated islets in this animal model. Furthermore, analysis of the ATP/ADP ratio rather than ATP content measurements is a more relevant parameter to assess with respect to β-cell stimulus–secretion coupling. It is well known that the ATP/ADP ratio is reduced in diabetic islets and upon chronic high glucose culture (Patane *et al*. 2002, Anello *et al*. 2005). Furthermore, ADP is an allosteric activator of GDH. Finally, the relationship between protein metabolism and β-cell function is a neglected area in diabetes research. It has recently been reported that BCH, like glucose stimulates [^{3}H]palmitate incorporation into protein (Abdel-Ghany *et al*. 2010), presumably due to protein palmitoylation. Protein acylation is a post-translational modification of proteins and plays an important role in cell signaling. Whether this process is disturbed under high glucose/palmitate conditions and whether it is preserved by BCH is not yet known but could be addressed in future investigations. Increased circulating levels of amino acids are most frequently observed in catabolic situations. Here, the requirements for insulin are low. What are the implications of the findings by Han and colleagues for this circumstance? Will GDH be activated in β-cells under starvation (ADP rises?) and in which direction will the reactions flow? Given that ADP is an activator of GDH, one would assume that GDH may become activated during starvation. This is clearly the case in renal cells, where catabolism of glutamine is an important process in adaptation to starvation.

Therapeutic implications of GDH activation

The authors suggest that GDH activation by BCH could be used as a treatment in diabetes. As mentioned above, activating mutations in GDH (PHHI) result in hyperinsulinism (Stanley 2006). Thus, caution is called for when applying GDH activators. Already 6-week-old *db/db* mice develop hyperinsulinemia (Kobayashi *et al*. 2000) and whether this was altered in *db/db* mice during BCH treatment was not further addressed. Moreover, the effect of BCH on wild-type mice was not reported. As shown by the authors in INS-1 cells (Choi *et al*. 2011), BCH is a strong insulinotropic agent (Sener *et al*. 1981). While BCH may preserve β-cell function, it may also act as a permanently stimulating agent, resulting in elevated blood insulin levels and potentially hypoglycemia. As the authors have applied BCH as a protective agent in INS-1 cells before the detrimental effects of chronic high glucose were established, it could readily be determined whether insulin progressively accumulates in the medium within 24 h. This would also be interesting and relevant for other reasons. Can BCH more permanently stimulate and preserve insulin secretion in the presence of high glucose or high glucose/palmitate without affecting insulin content in a profound way? That would show that reduced insulin content has contributed to impaired glucose and/or fatty acid metabolism rather than β-cell exhaustion.

Summary

The effects of BCH in combination with high glucose and high glucose/palmitate highlight that the mechanisms by which glucose and fatty acid metabolism impair β-cell

function are still largely unknown. It is not unlikely that it may not solely be attributable to β-cell overstimulation. Impaired cellular signaling and induction of apoptotic processes may also be involved.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Abdel-Ghany M, Sharp GW & Straub SG 2010 Glucose stimulation of protein acylation in the pancreatic β-cell. *Life Sciences* **87** 667–671. (doi:10.1016/j.lfs.2010.09.021)

Anello M, Lupi R, Spampinato D, Piro S, Masini M, Boggi U, Del Prato S, Rabuazzo AM, Purrello F & Marchetti P 2005 Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia* **48** 282–289. (doi:10.1007/s00125-004-1627-9)

Carobbio S, Ishihara H, Fernandez-Pascual S, Bartley C, Martin-Del-Rio R & Maechler P 2004 Insulin secretion profiles are modified by over-expression of glutamate dehydrogenase in pancreatic islets. *Diabetologia* **47** 266–276. (doi:10.1007/s00125-003-1306-2)

Carobbio S, Frigerio F, Rubi B, Vetterli L, Bloksgaard M, Gjinovci A, Pournourmohammadi S, Herrera PL, Reith W, Mandrup S *et al.* 2009 Deletion of glutamate dehydrogenase in β-cells abolishes part of the insulin secretory response not required for glucose homeostasis. *Journal of Biological Chemistry* **284** 921–929. (doi:10.1074/jbc.M806295200)

Choi SE, Lee YJ, Hwang GS, Chung JH, Lee SJ, Lee JH, Han SJ, Kim HJ, Lee KW, Kim Y *et al.* 2011 Supplement of TCA cycle intermediates protects against high glucose/palmitate-induced INS-1 beta cell death. *Archives of Biochemistry and Biophysics* **505** 231–241. (doi:10.1016/j.abb.2010.10.011)

Gembal M, Gilon P & Henquin JC 1992 Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. *Journal of Clinical Investigation* **89** 1288–1295. (doi:10.1172/JCI115714)

Henquin JC 2009 Regulation of insulin secretion: a matter of phase control and amplitude modulation. *Diabetologia* **52** 739–751. (doi:10.1007/s00125-009-1314-y)

Karaca M, Frigerio F & Maechler P 2011 From pancreatic islets to central nervous system, the importance of glutamate dehydrogenase for the control of energy homeostasis. *Neurochemistry International* **59** 510–517. (doi:10.1016/j.neuint.2011.03.024)

Kobayashi K, Forte TM, Taniguchi S, Ishida BY, Oka K & Chan L 2000 The db/db mouse, a model for diabetic dyslipidemia: molecular characterization and effects of western diet feeding. *Metabolism* **49** 22–31. (doi:10.1016/S0026-0495(00)90588-2)

Li C, Najafi H, Daikhin Y, Nissim IB, Collins HW, Yudkoff M, Matschinsky FM & Stanley CA 2003 Regulation of leucine-stimulated insulin secretion and glutamine metabolism in isolated rat islets. *Journal of Biological Chemistry* **278** 2853–2858. (doi:10.1074/jbc.M210577200)

Maechler P & Wollheim CB 1999 Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis [see comments]. *Nature* **402** 685–689. (doi:10.1038/45280)

Maedler K, Schulthess FT, Bielman C, Berney T, Bonny C, Prentki M, Donath MY & Roduit R 2008 Glucose and leptin induce apoptosis in human β-cells and impair glucose-stimulated insulin secretion through activation of c-Jun N-terminal kinases. *FASEB Journal* **22** 1905–1913. (doi:10.1096/fj.07-101824)

Patane G, Anello M, Piro S, Vigneri R, Purrello F & Rabuazzo AM 2002 Role of ATP production and uncoupling protein-2 in the insulin secretory defect induced by chronic exposure to high glucose or free fatty acids and effects of peroxisome proliferator-activated receptor-γ inhibition. *Diabetes* **51** 2749–2756. (doi:10.2337/diabetes.51.9.2749)

Prentki M & Corkey BE 1996 Are the β-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* **45** 273–283. (doi:10.2337/diabetes.45.3.273)

Sato Y & Henquin JC 1998 The K^+-ATP channel-independent pathway of regulation of insulin secretion by glucose: in search of the underlying mechanism. *Diabetes* **47** 1713–1721. (doi:10.2337/diabetes.47.11.1713)

Sener A, Malaisse-Lagae F & Malaisse WJ 1981 Stimulation of pancreatic islet metabolism and insulin release by a nonmetabolizable amino acid. *PNAS* **78** 5460–5464. (doi:10.1073/pnas.78.9.5460)

Smith TJ & Stanley CA 2008 Untangling the glutamate dehydrogenase allosteric nightmare. *Trends in Biochemical Sciences* **33** 557–564. (doi:10.1016/j.tibs.2008.07.007)

Stanley CA 2006 Hypoglycemia in the neonate. *Pediatric Endocrinology Reviews* **4** (Suppl 1) 76–81.

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