Differential Effects of Overexpressed Glucokinase and Hexokinase I in Isolated Islets

EVIDENCE FOR FUNCTIONAL SEGREGATION OF THE HIGH AND LOW K_m ENZYMES*

(Received for publication, October 17, 1995, and in revised form, October 30, 1995)

Thomas C. Beckert†, Richard J. Noël‡, John H. J ohnson§, Ronald M. Lynch¶, Hiroshi Hirose‡§, Yoshiharu Tokuyama, Graeme I. Bell, and Christopher B. Newgard**

From the 1Gifford Laboratories for Diabetes Research and Departments of Biochemistry and Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75235, 2Veterans Administration Medical Center, Dallas, Texas 75216, 3Departments of Physiology and Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona 85724, and 4Howard Hughes Medical Institute and Departments of Biochemistry and Molecular Biology and Medicine, University of Chicago, Chicago, Illinois 60637

Glucose-stimulated insulin secretion is believed to require metabolism of the sugar via a high K_m pathway in which glucokinase (hexokinase IV) is rate-limiting. In this study, we have used recombinant adenoviruses to overexpress the liver and islet isoforms of glucokinase as well as low K_m hexokinase I in isolated rat islets of Langerhans. Glucose phosphorylating activity increased by up to 20-fold in extracts from islets treated with adenoviruses containing the cDNAs encoding either tissue isoform of glucokinase, but such cells exhibited no increase in 2- or 5-[3H]glucose usage, lactate production, glycogen content, or glucose oxidation. Furthermore, glucokinase overexpression enhanced insulin secretion in response to stimulatory glucose or glucose plus arginine by only 36–53% relative to control islets. In contrast to the minimal effects of overexpressed glucokinases, overexpression of hexokinase I caused a 2.5-4-fold enhancement in all metabolic parameters except glycogen content when measured at a basal glucose concentration (3 mM). Based on measurement of glucose phosphorylation in intact cells, overexpressed glucokinase is clearly active in a non-islet cell line (CV-1) but not within islet cells. That this result cannot be ascribed to the levels of glucokinase regulatory protein in islets is shown by direct measurement of its activity and mRNA. These data provide evidence for functional partitioning of glucokinase and hexokinase and suggest that overexpressed glucokinase must interact with factors found in limiting concentration in the islet cell in order to become activated and engage in productive metabolic signaling.

Glucose-stimulated insulin secretion (occurring at concentrations in excess of 5 mM) is believed to require metabolism of the sugar via a high K_m pathway in which glucokinase is rate-limiting (1, 2). Consistent with an important role for glucokinase in the high K_m regulatory pathway, it has recently been shown that mutations in this gene are associated with β-cell dysfunction in a subtype of non-insulin-dependent diabetes mellitus known as maturity-onset diabetes of the young (3). Furthermore, reduction of glucokinase activity by 70% in β-cells of transgenic mice expressing a glucokinase-specific ribozyme results in decreased glucose-stimulated insulin secretion (GSIS)1 (4). Low K_m hexokinases are also expressed in β-cells and appear to determine the amount of insulin secreted at basal glucose concentrations (1, 5–7) suggesting a functional segregation from the high K_m glucokinase-mediated glucose signaling pathway. Implicit in such a model is that protection against an inappropriately vigorous response to a glucose challenge might be achieved by regulating access of the sugar to the high K_m pathway by requiring that glucokinase couple to factors found in limiting concentration in the islet cell. Since the impact of glucokinase overexpression has not been studied, it is not known whether increased abundance of this enzyme will specifically enhance high K_m glucose metabolism and insulin secretion. In order to address this question, we have used the recombinant adenovirus system to overexpress two isoforms of glucokinase and hexokinase I in isolated islets of Langerhans.

MATERIALS AND METHODS

Preparation of Recombinant Adenovirus—Recombinant adenovirus containing the cDNA encoding human islet glucokinase (AdCMV-GKI) was prepared by inserting a full-length (2.4 kilobase pairs) BamHI fragment into the pACCMV.pL.pA plasmid (8), followed by co-transfection with the adenovirus plasmid pM17 (9), using previously described techniques (5, 8, 10). A virus containing the rat liver glucokinase cDNA (AdCMV-GKL) was prepared by coligation of a 480-base pair BamHI/NsiI fragment from the 5′-end of rat liver glucokinase (11) and a 1.05-kilobase pairs NsiI/BamHI fragment from the 3′-region of glucokinase common to the rat liver and islet into pACCMV.pL.pA, followed by recombination as described for the islet construct. Recombinant adenovirus containing the rat hexokinase I cDNA (AdCMV-HKI) was prepared as described (5).

Islet Isolation and Perfusion—Pancreatic islets were isolated from male Wistar rats (140–180 g) (12), transduced with recombinant adenoviruses, and cultured for 3–4 days prior to performing the assays described below (5). Groups of 500–1000 islets were perfused (5) in Hanks’ buffer or DMEM medium (Atlanta Biologicals) with additions as described in the figure legends. Effluent was collected in 0.7-ml aliquots and assayed for insulin by radi-immunoassay.

Assays of Glucokinase Expression—Glucokinase protein was measured by immunoblot analysis with antibody U343 (specific for islet glucokinase) or antibody V980 (reactive to a sequence common to islet and liver glucokinases), as described (11), and hexokinase I was detected with an antibody provided by Dr. J ohn Wilson, Michigan State

* These studies were supported by United States Public Health Service Grants DK 42582, DK 46492, and DK 44840, the American Diabetes Association, and the Howard Hughes Medical Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Gifford Laboratories for Diabetes Research, Y8.222, 5323 Harry Hines Blvd., Dallas, TX 75235. Tel.: 214-648-2939; Fax: 214-648-9131.

† The abbreviations used are GSIS, glucose-stimulated insulin secretion; DMEM, Dulbecco’s modified Eagle’s medium; PCR, polymerase chain reaction; ZDF, Zucker diabetic fatty.
University (13). For immunocytochemical measurements (14), a new glucokinase antibody (gGK-1) was raised in rabbits (Tana Biosystems, Houston, TX) against a purified rat islet glucokinase/glutathione S-transferase fusion protein prepared in the pGEX-2T expression vector (Pharmacia Biotech Inc.). The specificity of gGK-1 was indicated by its capacity to immunoprecipitate a single band co-migrating with glucokinase from metabolically labeled CV-1 (African green monkey kidney) cells transduced with AdCMV-GKI. 2 Total glucose phosphorylating and glucokinase activities were measured with a radioisotopic assay in crude cellular extracts or in cytosolic or mitochondrial fractions as described previously (5, 15). Glucose phosphorylation in intact cells was measured by incubating islets or CV-1 cells with Hanks’ solution containing 3 or 20 mM [U-14C]glucose (DuPont NEN) for 90 min at 37°C. The labeled medium was then replaced with 50 μl of 0.5% Triton X-100 and 100 μl of 3% methanol in 95% ethanol. All labeled and phosphorylated intermediates were captured by spotting an aliquot of such extracts onto DEAE ion exchange membranes (Schleicher & Schuell) and 3H2O was measured as described previously (5, 16), using approximately 350 islets/assay. Lactate production was measured (17) in groups of 400–500 islets incubated at 37°C in perfusion medium containing 3 or 20 mM glucose. Glycogen content was determined as described (17) after culturing islets for 3–4 days in 11 mM glucose. Glucose oxidation was determined by measuring 14CO2 production from [U-14C]glucose (14) with aliquots of approximately 25 islets incubated with 3 or 20 mM glucose for 3 h at 37°C.

Reverse Transcriptase-Polymerase Chain Reaction (PCR) Assay for Glucokinase Regulatory Protein Transcripts—Tissues were obtained from 6- and 12-week-old male Wistar rats, male Zucker diabetic fatty rats (fa/fa), and lean controls (fa−/− or −/−) (18). cDNA was prepared from a total of 2 μg of total liver or islet RNA and 50 pmol of random hexamer primers (Pharmacia), and glucokinase regulatory protein cDNA was amplified from 1 μl of this cDNA solution using previously described methods (19) and primers GR-A (5′-CCAATCCAGGCTCT-TCTGGAG-3′) and GR-B (5′-TCTAACAACCTCAAGACTGAA-3′) (20). The 276-base pair PCR product was identified by electrophoresis in a 1% agarose gel and quantified by densitometry after ethidium bromide staining (Molecular Dynamics, Sunnyvale, CA).

RESULTS AND DISCUSSION

A large body of evidence has accumulated in support of the notion that glucokinase represents the rate-limiting step in glucose metabolism in pancreatic islet β-cells. Based solely on kinetic considerations, one may have predicted that overexpression of glucokinase in islets would have a potent enhancing effect on glucose metabolism and, as a consequence, on GSIS. Indeed glucokinase activity, glucose usage, and GSIS increase coordinately in proportion to the glucose concentration of islet culture media (21, 22), and hyperglycemic infusion is reported to increase glucokinase activity and render islets more sensitive to glucose (23), leading to the suggestion that the increase in enzyme activity is the likely cause of the enhanced glucose response. Interpretation of these experiments is complicated by the fact that expression of a large number of islet proteins is increased by culture at high glucose (24). The present study was designed to evaluate the effect of glucokinase overexpression in a more specific manner by introducing the gene encoding this enzyme into isolated islets via recombinant adenovirus.

Overexpression of Glucose-phosphorylating Enzymes—We have previously shown that treatment of islets with recombinant adenovirus containing the bacterial β-galactosidase reporter gene (AdCMV-βGAL) results in gene transfer to islet cells (including β-cells) with an efficiency of approximately 70% (5). Overexpression of glucokinase with similar efficiency in islet cells is demonstrated by the data of Fig. 1. Sections from islets treated with AdCMV-GKI and exposed to anti-glucokinase antibody gGK-1 (Fig. 1B) exhibited a clear increase in immunofluorescence intensity in the majority of cells, including β-cells (identified by staining of serial sections with an insulin antibody, Fig. 1, C and D), relative to control sections from islets treated with AdCMV-βGAL (Fig. 1A).

Western blot analysis showed that treatment of islets with the AdCMV-GKI virus resulted in large increases in glucokinase protein relative to control islets, as detected with antibody U343 (11) (Fig. 2A), which is specific for the unique N-terminal segment of the islet isoform of glucokinase, or antibody V580 (Fig. 2B), raised against a region of the protein common to both liver and islet glucokinases (11). As shown in Fig. 2B, treatment of islets with the AdCMV-GKL virus resulted in levels of immunodetectable protein slightly higher than those achieved with the AdCMV-GKI virus. Finally, treatment of islets with the AdCMV-HKI virus resulted in consistent overexpression of hexokinase I protein (Fig. 2C), in agreement with our previous work (5).

Glucokinase represents less than half of the total glucose phosphorylating activity in control islets (4.4 ± 0.7 and 5.6 ± 1.0 units/g) measured at 20 mM glucose in the absence of Glc-6-P and 1.2 ± 0.4 and 2.3 ± 0.5 units/g in the presence of 10 mM Glc-6-P for untreated and AdCMV-βGAL-treated islets, respectively, consistent with previous reports (1, 25). Treatment of islets with AdCMV-GKI or AdCMV-GKL resulted in 14- and 19.4-fold increases in total glucose phosphorylation and 37.6- and 53.8-fold increases in glucokinase activity (measured in the presence of 10 mM Glc-6-P, respectively) (data represent the average of 6–8 groups of islets per condition). Overexpression of hexokinase I resulted in an 8-fold increase in total glucose phosphorylation relative to the two control groups, with nearly all of the observed increase sensitive to Glc-6-P inhibition.
The significance of mM glucose was increased by 40–51% in islets treated with As shown in Table I, insulin secretion in response to 20 nase—release during the 3 mM Glc basal period. Data represent the mean during stimulation with Glc or Glc arginine (Arg) is shown. Shown in parentheses is the insulin release shown previously contained only a single band (11). B, immunodetection with antibody V980, which recognizes a C-terminal region common to the liver and islet glucokinase isoforms (11). C, islets treated with AdCMV-HKI and immunodetection performed with an antibody specific for rat hexokinase I (13). Samples from three separate islet aliquots treated with AdCMV-HKI are shown.

TABLE I

| Insulin release/1000 islets | 3 mM Glc | 20 mM Glc | 30 mM Arg + 20 mM Glc | microunits/ml |
|-----------------------------|---------|-----------|----------------------|--------------|
| No treatment                | 40.4 ± 6.67 | 75.5 ± 10.4 | 111.2 ± 15.2 | (1.00) |
| AdCMV-μGAL                  | 33.3 ± 5.2  | 71.3 ± 15.1 | 103.7 ± 21.3 | (1.00) |
| AdCMV-GKI                   | 43.2 ± 7.8  | 107.9 ± 7.9* | 159.1 ± 14.7* | (1.00) |
| AdCMV-GKL                   | 46.2 ± 7.6  | 105.9 ± 15.1 | 151.2 ± 15.8* | (1.00) |
| AdCMV-HKI*                  | 60.1 ± 2.8  | 101.0 ± 5.6  | 141.7 ± 7.6  | (1.00) |

*Insulin release was increased relative to either control group of islets with a significance of p < 0.05.

Insulin secretion from islets overexpressing glucokinase—As shown in Table I, insulin secretion in response to 20 mM glucose was increased by 40–51% in islets treated with AdCMV-GKI or AdCMV-GKL and by 36–53% when such cells were challenged with 20 mM glucose plus 30 mM arginine. The differences noted between control islets and islets treated with AdCMV-GKL or AdCMV-GKI were of marginal statistical significance (see Table I for statistical analysis). As previously reported (5) basal insulin release was nearly doubled by treatment of islets with AdCMV-HKI.

Isolated islet preparations are removed from their normal pancreatic environment and may be deprived of certain nutritional, nervous, and hormonal signals that might be required to activate overexpressed glucokinase (26). To partially address this concern, we repeated the foregoing experiments with a rich tissue culture medium (DMEM) containing 5.5 mM glucose in the basal period and 20 mM glucose in the stimulation period. We found that basal insulin release from all islet groups was increased approximately 5-fold during perfusion with 5.5 mM glucose in DMEM (average of 190 microunits/ml/1000 islets) compared with insulin release in Hanks’ buffer containing 3 mM glucose (37 microunits/ml/1000 islets). Untreated islets or islets treated with AdCMV-μGAL, AdCMV-GKI, or AdCMV-GKL all responded similarly to 20 mM glucose by increasing insulin release by approximately 2-fold above this new base line (data not shown). Thus, while our data indicate a minimal secretory impact of overexpressed glucokinases in isolated islets, it remains possible that future studies of transgenic ani-
Glucose Metabolism—As shown in Fig. 3, treatment of islets with AdCMV-GKI or AdCMV-GKL had no effect on 5-[3H]glucose usage, 2-[3H]glucose usage, or lactate production relative to untreated or AdCMV-bGal-treated islets, regardless of whether measurements were made at 3 or 20 mM glucose. The lack of effect of overexpressed glucokinase is not explained by failure to overexpress the enzyme efficiently in β-cells, based on the results shown in Fig. 1 and on studies in which treatment of the well differentiated INS-1 cell line (27) with AdCMV-GKI also has no effect on 5-[3H]glucose usage studied at 20 mM glucose. The H label is released to water at the hexose-phosphate isomerase and triose-phosphate isomerase reactions, respectively, for 2- and 5-[3H]glycologos. 2-[3H]Glucose was used to test the possibility that glucokinase overexpression was specifically enhancing glucose ↔ Glc-6-P recycling without increasing flux through phosphofructokinase. The similar rates of usage attained with 2- and 5-[3H]glycologos clearly indicate that this is not the case. In contrast to the lack of effect of overexpressed glucokinase, treatment of islets with AdCMV-HKI resulted in a 2.2-fold increase in 5-[3H]glucose usage, a 2.6-fold increase in 2-[3H]glucose usage, and a 4.2-fold increase in lactate production at 3 mM glucose (Fig. 3). We also measured [U-14C]glucose oxidation and found that while overexpression of glucokinase did not cause an increase in this parameter, islets treated with AdCMV-HKI exhibited a 3.3-fold enhancement in this pathway at 3 mM glucose (4.0 ± 0.6 and 3.6 ± 2.9 fmol/h/islet for untreated and AdCMV-bGal-treated controls, respectively, versus 12.5 for AdCMV-HKI-treated islets). For three of the four measurements of glucose metabolism (2-[3H]glucose usage, lactate production, and glucose oxidation), the increase in rate observed in AdCMV-HKI-treated cells at 3 mM glucose was reflected in a similarly increased flux at 20 mM glucose. No change in glycogen content was noted in control islets (67 ± 2 and 63 ± 6 ng of glycogen/100 islets for untreated and AdCMV-bGal-treated groups, respectively) versus islets overexpressing glucokinase-phosphorylating enzymes (76 ± 1, 67 ± 3, and 68 ± 2 ng of glycogen/100 islets for AdCMV-GKI, AdCMV-GKL, and AdCMV-HKI-treated groups, respectively).

Intracellular Glucose Phosphorylating Activity—The lack of metabolic impact of overexpressed glucokinases is surprising in light of the high levels of enzyme activity measured in islet extracts. To determine whether the overexpressed enzyme was active within intact cells, we incubated AdCMV-GKI or AdCMV-bGal-treated islets with [U-14C]glucose and measured accumulation of phosphorylated glucose and its by-products. As a control, we also performed parallel experiments in the monkey kidney cell line CV-1. As shown in Fig. 4, intact islets overexpressing glucokinase failed to increase levels of [U-14C]glucose-derived glycolytic intermediates above those obtained in control islets, despite a 28-fold increase in glucose phosphorylating capacity in homogenates from these same cells. In contrast, similar overexpression of glucokinase in CV-1 cells resulted in a 4.7 ± 0.8-fold increase in phosphorylated products at 20 mM glucose relative to AdCMV-bGal-treated control cells. Metabolic impact (increased glycogen deposition, 5-[3H]glucose usage, and lactate production) is also clearly evident when glucokinase is overexpressed in hepatoma cells (28) or primary hepatocytes. These data suggest either that overexpressed glucokinase is inhibited by an islet-specific factor or that activation and metabolic coupling of the overexpressed enzyme does not occur in the islet environment.

Lack of Regulation of Overexpressed Glucokinase by the Glucokinase Regulatory Protein—A glucokinase regulatory protein that binds the enzyme and inhibits it in a hexose phosphate-sensitive manner has been described in liver (29). Glucokinase activity in islet extracts can be increased slightly by the addition of fructose 1-phosphate, an antagonist of the glucokinase regulatory protein (30), but no other information about the level of expression of the regulatory protein in islets has been presented. We found that 10 mM Fru-1-P modestly increased glucokinase phosphorylating activity measured at 20 mM glucose in islet extracts with overexpressed liver (from 205.3 ± 7.7 to 269.7 ± 5.3 units/g of protein) or islet (from 301.7 ± 6.3 to 329.8 ± 8.1 units/g) glucokinases, while Fru-6-P, an activator of the regulatory protein (29), had no effect at either 100 μM or 10 mM. We also found that islets from 6-week-old lean (fa/− or −/−) or obese (fa/fa) ZDF rats contained only 19 or 33% as much regulatory protein mRNA, respectively, as found in liver of Wistar rats or 12-week-old obese ZDF rats (Fig. 5). While the level of regulatory protein mRNA was maintained in islets of lean controls with age, 12-week-old ZDF rats (Fig. 5). While the level of regulatory protein mRNA was maintained in islets of lean controls with age, 12-week-old ZDF rats (Fig. 5). While the level of regulatory protein mRNA was maintained in islets of lean controls with age, 12-week-old ZDF rats (Fig. 5). While the level of regulatory protein mRNA was maintained in islets of lean controls with age, 12-week-old ZDF rats (Fig. 5). While the level of regulatory protein mRNA was maintained in islets of lean controls with age, 12-week-old ZDF rats (Fig. 5). While the level of regulatory protein mRNA was maintained in islets of lean controls with age, 12-week-old ZDF rats. Since the regulatory protein is thought to work stoichiometri-
cally (29), these data argue strongly that the known glucokinase regulatory protein is unlikely to be present at levels sufficient to inhibit the large excess of overexpressed glucokinase in islets. It remains possible that a glucokinase regulatory factor that is either an isozyme of the known protein or a member of a completely different gene family is operative in islets.

Partitioning of Overexpressed Glucose-phosphorylating Enzymes—One explanation for our findings is that a metabolic impact of overexpressed glucokinase requires assembly of the enzyme into a complex analogous with those described for sequential enzymes of the citric acid cycle (31). Indeed, Malaisse and Bodur (32) have pointed out that the rate of conversion of [2-3H]glucose to 3H2O is less than the predicted value deduced from known activities of the relevant enzymes and have suggested from this that early glycolytic substrates are "channeled" from one enzyme to another within a complex. The idea that metabolic impact may be linked to the physical partitioning of glucose-phosphorylating enzymes is consistent with our finding that overexpressed hexokinase I partitions in islets such that 41% of the total activity is associated with a mitochondrionally enriched fraction (5), while mitochondria-enriched fractions of both control and glucokinase-overexpressing islets are completely lacking in Glc-6-P-insensitive glucose phosphorylating activity (data not shown). Association of hexokinase with mitochondria causes activation of the enzyme by reducing its sensitivity to Glc-6-P as an allosteric inhibitor (33).

This compartmentation may also be important for functional segregation of the low Km, pathway of glucose metabolism that is responsible for "maintenance" metabolic activity and basal insulin secretion of the β-cell. A recent study has shown that glucokinase may localize to a discrete "perinuclear" compartment within β-cells (34). In light of this study, the lack of metabolic impact of overexpressed glucokinase in islets can be explained by a model in which discrete localization of glucokinase and its participation in high Km, signaling and glucose metabolism require binding of the enzyme to a limiting number of sites, which are fully occupied by the endogenous enzyme.

In summary, our studies show that overexpression of glucokinase in isolated islets has minimal effects on glucose metabolism and insulin release. These findings appear to be specific to the high Km, glucokinases, since changes in metabolic parameters and insulin secretion are easily detectable in islets overexpressing low Km, hexokinase I. Our results indicate that the known glucokinase regulatory protein is unlikely to be present in islets at levels sufficient to explain our findings. We therefore favor an alternative model, in which overexpressed glucokinase must interact with factors found in limiting concentrations in the islet cell in order to become activated and engage in productive metabolic signaling. This regulatory mechanism may be a unique feature of islet cells, since overexpressed glucokinase is clearly active within CV-1 or liver cells, and may explain why a decrease in β-cell glucokinase activity has demonstrable effects on glucose metabolism and insulin release, while overexpression of the enzyme has little impact.

Acknowledgments—We are grateful to Dr. Roger Unger, Dr. J. Denis McGarry, and Dr. Kenneth Polonsky for critical reading of this manuscript. We are also indebted to Donna Lehman and Dr. Chris Macllister for assistance with islet isolation, Kay McCorkle for purification of the glutathione S-transferase-glucokinase fusion protein.

REFERENCES

1. Meglasson, M. D. & Matschinsky, F. M. (1986) Diabetes Metab. Rev. 2, 163–214
2. Newgard, C. B. & McGarry, J. D. (1991) J. Biol. Chem. 267, 25219–25314
3. Frosdick, P., Zouali, H., Viollet, N., Veho, G., Vaillanterre, M., Sun, F., Lesage, S., Stoffel, M., Takeda, J., Passa, P., Permutt, M. A., Beckmann, J. S., Bell, G. I. & Cohen, D. (1993) N. Engl. J. Med. 329, 697–702
4. Ernfors, P., Leiser, M. W., Wu, Y.-J., Fusco-Defrane, D., Enram, D. A., Surana, M., Jett, T. L., Magnuson, M. A., Weir, G. & Fleischer, N. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 2051–2055
5. Becker, T. C., Beltrand-Rio, H., Noel, R. J., Johnson, J. H. & Newgard, C. B. (1994) J. Biol. Chem. 269, 21234–21238
6. Voss-McCowan, M. E., Xu, B. & Epstein, P. N. (1994) J. Biol. Chem. 269, 15814–15818
7. Ishihara, M., Asano, T., Tsukuda, K., Katagiri, H., Inukai, K., Anai, M., Kikuchi, M., Yazaki, Y., Miyazaki, J. & Oka, Y. (1994) J. Biol. Chem. 269, 3081–3087
8. Gomez-Fox, A. M., Coats, W. S., Baque, S., Alam, T., Gerard, R. D. & Newgard, C. B. (1992) J. Biol. Chem. 267, 25129–25314
9. Mcvitty, W. J., Bautista, D. S. & Graham, F. L. (1988) Virology 163, 614–617
10. Bodur, H., Noel, R. J., Coats, W. S., Gomez-Fox, A. M., Alam, T., Gerard, R. D. & Newgard, C. B. (1994) Methods Cell Biol. 43, 161–189
11. Quadea, C., Hughes, S. D., Coats, W. S., Sestak, A. L., Lynedjian, P. B. & Newgard, C. B. (1992) J. Biol. Chem. 267, 280, 471–477
12. J. Johnson, J. H., Crider, B. P., McCorrnie, K. Alford, M. & Unger, R. H. (1990) N. Engl. J. Med. 322, 653–659
13. Schwab, D. A. & Wilson, J. E. (1988) J. Biol. Chem. 263, 3220–3224
14. Milburn, J. L., Hirose, H., Lee, Y. H., Nagasawa, Y., Ogawa, A., Ohneda, M., Beltrand-Rio, H., Newgard, C. B., j., Johnson, J. H. & Unger, R. H. (1995) J. Biol. Chem. 270, 1295–1299
15. Kuwajima, M., Newgard, C. B., Foster, D. W. & McGarry, J. D. (1986) J. Biol. Chem. 261, 8849–8853
16. Hughes, S. D., Quadea, C., Johnson, J. H., Ferber, S. & Newgard, C. B. (1993) J. Biol. Chem. 268, 15205–15212
17. Newgard, C. B., Hirose, H., Foster, D. W. & McGarry, J. D. (1983) J. Biol. Chem. 258, 8046–8052
18. Johnson, J. H., Ogawa, A., Chen, L., Orii, L., Newgard, C. B., Alam, T. & Schwartz, R. H. (1990) Science 250, 43–47
19. Ishikawa, Y., Wugh, P., Deadi, M., Takeda, J., Seino, S., Bel, G. I. & Polonsky, K. S. (1993) Diabetes 42, 948–955
20. Dahaer, M., Van Schaftingen, E. (1993) FEBS Lett. 321, 111–115, Correction (1994) FEBS Lett. 339, 312
21. Liang, Y., Nafii, H., Smith, R. M., Zimmerman, E. C., Magnuson, M. A., Tal, M. & Matschinsky, F. M. (1992) Diabetes 41, 792–806
22. Chen, C., Hosokawa, H., Bumbalo, L. M. & Leachy, J. L. (1994) J. Clin. Invest. 94, 1616–1620
23. Chen, C., Bumbalo, L. & Leachy, J. L. (1994) Diabetes 43, 684–689
24. Colless, D., Nafii, H., Bhatt, C., Ronan, C. & Alford, J. (1993) J. Biol. Chem. 268, 1671–1676
25. Ghosh, A., Ronner, P., Cheong, E., Khalid, P. & Matschinsky, F. M. (1991) J. Biol. Chem. 266, 22887–22892
26. Asafari, M., Janjic, D., Meda, P., Li, G., Halban, P. A. & Wollheim, C. B. (1992) Endocrinology 130, 1671–1676
27. Valera, A. & Bosch, F. (1994) Eur. J. Biochem. 221, 533–539
28. Van Schaftingen, E., Deheuex, E. & Veiga da Cunha, M. (1990) FASEB J. 4, 414–419
29. Malaisse, W. J., Malaisse-Lagae, F., Davies, D. R., Vandercammen, A. & Van Schaftingen, E. (1990) Eur. J. Biochem. 190, 539–545
30. Sere, P. A. (1987) Annu. Rev. Biochem. 56, 89–124
31. Malaisse, W. J. & Bodur, H. (1993) Int. J. Biochem. 23, 1471–1481
32. Wilson, J. E. (1984) Regulation of Carbohydrate Metabolism (Beitner, R., ed) pp. 45–85, CRC Press, Inc., Boca Raton, FL
33. Nona, Y., Bonner-Weir, S., Latimer, J. B. & Weir, G. C. (1995) Diabetes 44, Suppl. 1, 21A
Differential Effects of Overexpressed Glucokinase and Hexokinase I in Isolated Islets: EVIDENCE FOR FUNCTIONAL SEGREGATION OF THE HIGH AND LOW K\textsubscript{m} ENZYMES

Thomas C. Becker, Richard J. Noel, John H. Johnson, Ronald M. Lynch, Hiroshi Hirose, Yoshiharu Tokuyama, Graeme I. Bell and Christopher B. Newgard

\textit{J. Biol. Chem.} 1996, 271:390-394.
doi: 10.1074/jbc.271.1.390

Access the most updated version of this article at \url{http://www.jbc.org/content/271/1/390}

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 17 of which can be accessed free at \url{http://www.jbc.org/content/271/1/390.full.html#ref-list-1}