Vol. 214, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 5, 1995 Pages 239-246

EXPRESSION OF CD38 GENE, BUT NOT OF MITOCHONDRIAL GLYCEROL-3-PHOSPHATE DEHYDROGENASE GENE, IS IMPAIRED IN PANCREATIC ISLETS OF GK RATS

Taka-aki Matsuoka, Yoshitaka Kajimoto*, Hirotaka Watada, Yutaka Umayahara, Minoru Kubota, Ryuzo Kawamori#, Yoshimitsu Yamasaki and Takenobu Kamada

First Department of Medicine, Osaka University School of Medicine, Suita 565, Japan

#Department of Medicine, Metabolism and Endocrinology, Juntendo University School of Medicine, Tokyo 113, Japan

Received July 11, 1995

Goto-Kakizaki (GK) rat, a rodent model of spontaneously occurring non-insulin dependent diabetes mellitus (NIDDM), exhibits impaired glucose-stimulated insulin secretion. To explore the background of the β-cell dysfunction in NIDDM, we investigated whether and how the expression pattern of factors that would potentially be involved in the glucose-stimulated insulin secretion machinery is changed in GK rats. Using quantitative reverse transcription-PCR (RT-PCR) method, we found that the gene expression of CD38, a type 2 membrane protein which has ADP-ribosyl cyclase activity, is reduced by approximately 50% in islets of GK rats. Despite previous studies showing reduction in the FAD-linked mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) activity in GK rats, the mGPDH mRNA amounts were equal to those in the control Wistar rats, suggesting a difference that arose post-transcriptionally. These observations support the idea that multiple defects of the glucose-responsive insulin secreting machinery are involved in the development of diabetes in GK rats.

In NIDDM patients, glucose-stimulated insulin secretion is impaired from the early stage of the disease. To date, various factors have been shown to be involved in the glucose-stimulated insulin-secreting machinery in pancreatic β-cells. Among them are GLUT2, a high-Km facilitative glucose transporter; glucokinase, the key enzyme located at the entrance of the glucose metabolism (1); mGPDH, the key enzyme of the glycerol phosphate shuttle (2); and CD38, a type 2 membrane protein with ADP-ribosyl cyclase activity (3,4). However, the pathological significance of the individual factors in causing β-cell defects observed in NIDDM is not known.

The Goto-Kakizaki (GK) rat, a model of spontaneously occurring non-obese NIDDM, is characterized by impaired glucose-stimulated insulin secretion (5,6). The etiology of GK rats is thought to be genetic, because they resulted from repetitive selective breeding of

*To whom correspondence should be addressed. Fax: +81-6-879-3639.

0006-291X/95 $12.00

Wistar rats with abnormal glucose tolerance and became stably diabetic after several generations (7). To date, this animal model has provided useful information regarding various pathological aspects of NIDDM and its complications, such as impaired function of the ATP-sensitive K^+ channel in pancreatic β cells in NIDDM (8) and reduced sodium-potassium ATPase activity that may cause diabetic neuropathy (9).

As a step toward elucidating pancreatic β-cell defects in NIDDM, we examined whether and how expression of the potential regulators of glucose-stimulated insulin secretion is modified in islets of GK rats. The results of our present studies revealed that multiple defects in the signaling machinery for glucose-stimulated insulin secretion may underlie the impaired β cell function in GK rats.

MATERIALS AND METHODS

Pancreatic islet isolation - Four male 8-week-old Goto-Kakizaki rats were used together with four male age-matched Wistar rats as control. After 24-hour fasting, islets were isolated by collagenase digestion and Ficoll gradient as described elsewhere (10).

Reverse transcription - Total RNA was extracted from pancreatic islets by the guanidinium thiocyanate-phenol/chloroform procedure. Extracted RNA (100 ng) was reverse-transcribed at 42 C for 1 hr in a 30-μl reaction mixture containing 1.0 mM TrisCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 5 units of ribonuclease inhibitor, 1 mM each of dNTP, 50 μM of each RT primer (for β-actin, GLUT2, glucokinase, mGPDH, CD38, and insulin genes; shown in Fig. 1), and 10 units of AMV reverse transcriptase. The reaction was terminated by heating at 95 C for 5 min, and then 1% of the sample was used for the competitive PCR.

Competitive PCR - Competitor DNA fragments, which were designed to be amplified by the same set of primers as were the original first strand cDNAs and were shorter than the cDNAs by 72-200 bp, were produced by the procedure described previously (Fig. 1; ref.11). All the PCR primers were designed to bypass at least one intron so that amplification of genomic DNA could be avoided (Fig. 1). The first strand cDNA synthesized as described above was amplified together with a pre-estimated number of copies of the

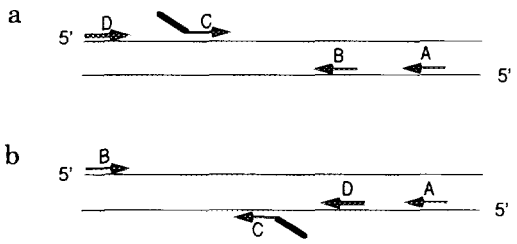

Fig. 1. Competitive RT-PCR strategy.
Panel **a**, oligonucleotide primers designed for the β-actin, GLUT-2, glucokinase, mGPDH, and CD38 genes. Panel **b**, insulin gene. The nucleotide numbers located at the 5'-end of each primer were as follows: β-actin (24), primer A, nucleotide 629, B, 382, C, 231, D,132; GLUT-2 (25), A, 1657, B, 1353, C, 1063, D, 951; glucokinase (26), A, 635, B, 442, C, 233, D, 161; mGPDH (27), A, 1733, B, 1592, C, 1207, D, 1061; CD38 (28), A, 542, B, 502, C, 310, D, 170; insulin (29), A, 386, B, 352, C, 213, D, 13. First strand cDNAs were synthesized using all the A primers together. The competitors were synthesized using each set of B and C primers, and, as a template, the first strand cDNA (11). The 5'-half (20 nucleotides) of the C primers were designed to correspond to the sequences of the D primers, so that both the competitors and original cDNAs could be amplified with the same primers (11).

competitor (12,13). Two products, one derived from the original cDNA and the other from the competitor, were separated on an agarose gel, and the point of equivalence, i.e. where the ratio of the band intensity derived from cDNA to that of the competitor is closest to 1, was determined.

RESULTS

Animal data - The background data of GK and Wistar rats used in the experiments are shown in Table 1. There is no significant difference in body weight, fasting plasma glucose, immunoreactive insulin (IRI), glucagon, or free fatty acid (FFA) level between the two strains. The intraperitoneal glucose tolerance test indicated that glucose-stimulated insulin secretion was blunted in GK rat (Fig. 2), in agreement with previous reports (5,6).

Establishment of a quantitative RT-PCR method for pancreatic islets - We utilized the quantitative RT-PCR strategy (12,13) to investigate gene expression of factors that could potentially be involved in the glucose-stimulated insulin secretion machinery. The genes examined were GLUT2, glucokinase, mGPDH, CD38, insulin, and β-actin genes. To examine the sensitivity of the method, β-actin mRNA that was contained in 1 ng or 0.5 ng of pancreatic islet total RNA of a GK rat was quantified by this method. The results indicated that the 1 ng total RNA contained twice as many copies of β-actin mRNAs as those contained in 0.5 ng total RNA ($1x\ 10^4$ copies vs. $0.5x\ 10^4$ copies, data not shown), providing support for the high sensitivity of the method that can distinguish a twofold difference.

Quantification of mRNA amounts in pancreatic islets of GK rats - As described above, the amount of β-actin mRNA was $1x\ 10^4$ copies per ng total RNA in pancreatic islet of GK rats, and the same result was obtained for the control Wistar rats.

Insulin mRNA amounts were not different between GK and Wistar rats (4.6×10^7 copies per ng islet total RNA (data not shown); Fig. 5). Although glucose-responsive insulin secretion is blunted in 8-week-old GK rats, insulin biosynthesis seemed to be intact at least at the level of gene transcription. Similarly, there was no difference observed for glucokinase mRNA amounts (2.2×10^2 copies per ng islet total RNA (data not shown); Fig. 5).

Despite previous reports showing reduced enzymatic activity of mGPDH in pancreatic islets of GK rats (14), we detected no difference in mGPDH mRNA amounts between GK and Wistar rats. The copy numbers of the mGPDH mRNA in both animals

Table 1. Background of GK and Wistar rats

	Body weight (g)	FPG (mg/dl)	IRI (μU/ml)	Glucagon (pg/ml)	FFA (mEq/l)
GK rat (n=4)	260.0 ±7.1	142.2 ±21.3	6.4 ±1.0	79.5 ±9.5	0.90 ±0.22
Wistar rat (n=4)	247.5 ±4.3	125.3 ±17.7	7.4 ±1.4	64.0 ±7.6	0.61 ±0.08

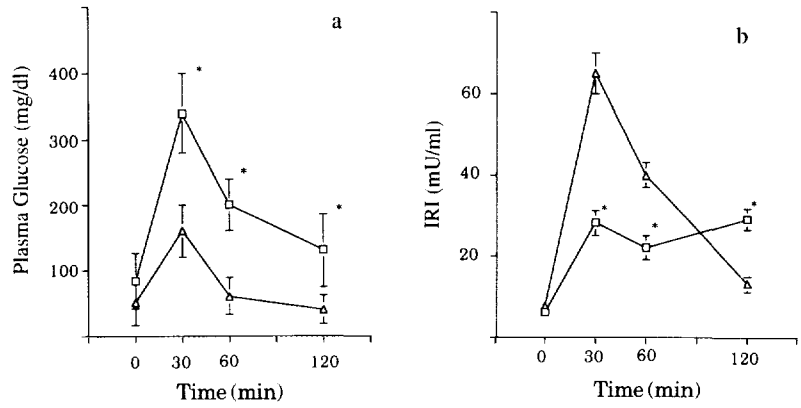

Fig. 2. Intraperitoneal glucose tolerance tests in GK and control rats.
Plasma glucose (panel **a**) and immunoreactive insulin (IRI; panel **b**) concentrations before and after intraperitoneal administration of glucose (2 g / kg body weight) in GK rats (□, n=4) and Wistar rats (Δ , n=4). The rats were fasted for 24 hrs before the tests. Values are expressed as mean ± SEM. Significant differences ($p < 0.05$) from the value for control rats are indicated by asterisks.

were identical at 4.4 x 10 copies per ng islets total RNA (Fig. 3 & Fig. 5), revealing a discrepancy between the mRNA amounts and the enzymatic activities of mGPDH.

In contrast, reduction in the mRNA amounts in GK rats was observed in two of the six genes that were investigated. One is the GLUT2 mRNA, for which 50% reduction was observed in the pancreatic islets of GK rats (8×10^3 vs. 1.6×10^4 copies per ng islet total RNA in GK and Wistar rats, respectively; data not shown; Fig. 5).

The other mRNA that was decreased in pancreatic islets of GK rats was CD38 mRNA; the gene expression was reduced by approximately 50% in comparison with the control rats. The copy number of the mRNA in GK rats was 4 x 10 copies per ng islet total RNA, whereas it was 8 x 10 copies in Wistar rats (Fig. 4 & Fig. 5).

DISCUSSION

NIDDM is characterized by impaired glucose-stimulated insulin secretion and insulin resistance. The glucose sensing of β cells is mediated via a complex signal transduction pathway (1), starting with glucose uptake into β cells by GLUT2, a high-Km facilitative glucose transporter. It has been reported that GLUT2 was decreased, in terms of activity, in some NIDDM model animals such as db/db (15) and ZDF (16), and, in terms of mRNA expression, in GK rats (17). The last observation, which was obtained by in situ hybridization, was supported by the data from our present RT-PCR experiments. Since the magnitude of the underexpression of GLUT2 alone may be insufficient to explain the reduced glucose-stimulated insulin secretion in GK rats (17), the pathological significance of the reduction remains obscure.

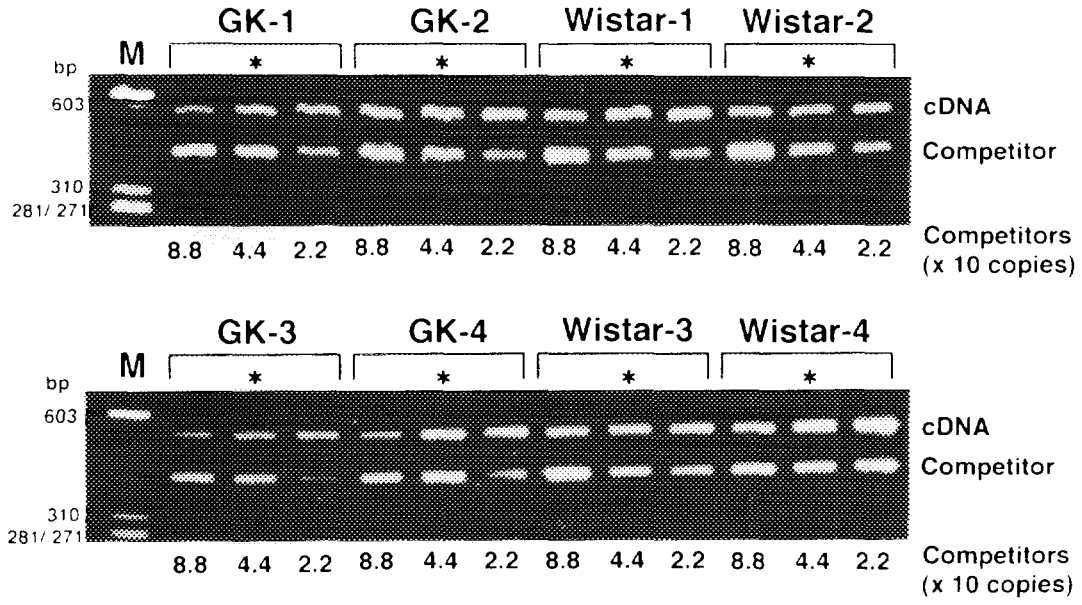

Fig. 3. Competitive RT-PCR for mGPDH mRNA.
Expression of mGPDH mRNA in the pancreatic islets was compared between GK (n=4; GK-1~4) and control rats (n=4; Wistar-1~4). Copy numbers of the competitors added to each sample are indicated below each lane. The upper bands (532 bp) correspond to amplified original cDNAs and the lower bands (406 bp) to amplified competitors. Asterisks indicate points of equivalence.

Glucose phosphorylation by glucokinase appears to be a rate-limiting step for glucose metabolism of β cells (1,18), and glucokinase therefore has been suggested as the glucose sensor. Physiological significance of this enzyme has been supported by the fact that some MODY or NIDDM cases are caused by the defects in the glucokinase gene (19). The results of the present study, revealing no difference in mRNA amounts of glucokinase between GK and normal rats, suggest that GK rats have no major rearrangements or loss of the glucokinase gene, and are consistent with the previous observation that the enzyme activity of glucokinase was not impaired in GK rats (8).

It has been suggested that approximately 24 to 40% of glucose-stimulated ATP production in the pancreatic β cell depends on the glycerol phosphate shuttle, a mitochondria-linked pathway that facilitates conversion of NADH to NAD^+ in cytoplasm and that of FAD to $FADH_2$ in mitochondria (20). Accordingly, mGPDH, the key enzyme of this shuttle, may account for the preferential alteration of the β-cell insulin secretory response to glucose. Reduced activity of mGPDH was recently observed in islets at least in two models of inherited diabetes, GK rat (14) and db/db mouse (21). The results of the present study, detecting no difference between GK and the control in terms of the mGPDH mRNA amount (Fig. 3 & Fig. 5), suggest that the reduction of the enzyme activity may be a post-transcriptional event. Further studies are necessary to determine the exact cause of the reduced enzyme activity and to clarify the physiological significance of the reduction.

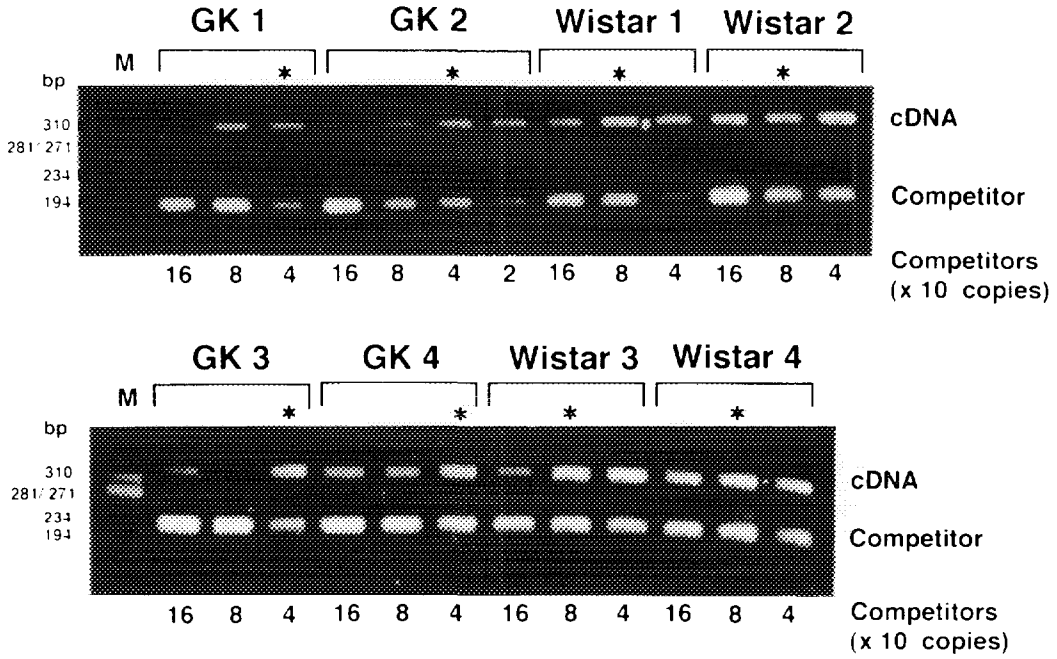

Fig. 4. Competitive RT-PCR of CD38 mRNA.
Expression of CD38 mRNA in the pancreatic islets was compared between GK (n=4; GK-1~4) and control rats (n=4; Wistar-1~4). Copy numbers of the competitors added to each sample are indicated below each lane. The upper bands (333 bp) correspond to amplified original cDNAs and the lower bands (213 bp) to amplified competitors. Asterisks indicate points of equivalence.

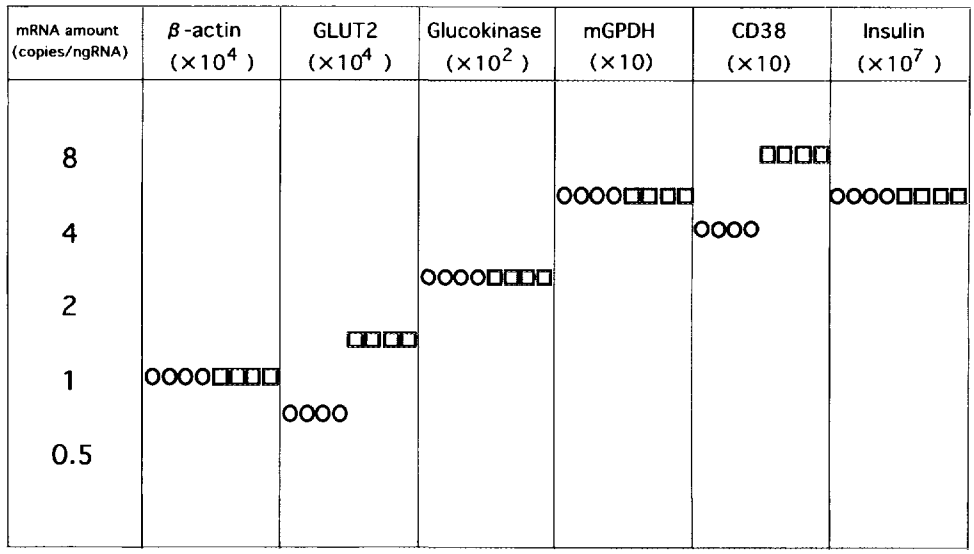

Fig. 5. Amount of mRNA in pancreatic islets of GK and Wistar rats.
Copy numbers of β-actin, GLUT2, glucokinase, mGPDH, CD38, and insulin mRNAs expressed in the pancreatic islets of GK rats and Wistar rats are illustrated. Each open circle and square represents a datum of a GK rat and a Wistar rat, respectively.

Cyclic ADP-ribose (cADPR) is a recently described second messenger that induces calcium release from the endoplasmic reticulum (3,22). CD38, a type 2 membrane protein first recognized as a surface antigen of T lymphocytes, has both ADP-ribosyl cyclase activity and cADPR-hydrolyzing activity (4). With the latter enzyme activity being suppressed by ATP, cADPR accumulation was observed in response to ATP. Accordingly, CD38 has been suggested to be a putative second messenger for glucose-stimulated insulin secretion (4). The 50% reduction in the CD38 gene expression observed in this study therefore suggests that the bluntness of the glucose-responsive cADPR accumulation that would be induced by the reduced CD38 expression may partially explain the impaired insulin secretion in GK rats. It should be noted that there is a previous report of no difference in the CD38 mRNA between GK and Wistar rats (23). Although the exact cause of this discrepancy is not known, it is conceivable that differences in the experimental design, such as the use of a competitor in our PCR experiments but not in theirs (23), may have caused a difference in the sensitivity of the experiments.

In conclusion, our present observations support the idea that multiple defects of the glucose-responsive insulin-secreting machinery are involved in the development of diabetes in GK rats.

ACKNOWLEDGMENTS

We thank Dr. Hitoshi Ikeda, Takeda Chemical Industry, for kindly providing the GK rats, Drs. Masayoshi Nishihara, Yoshihiko Ohta, Takayuki Fukuzaki and Mitsukazu Goto, Biomedical Research Center, Osaka University School of Medicine, for valuable advice on the isolation of pancreatic islets, Drs. Yoichi Hamada and Eiji Yamato, Department of Geriatrics, Osaka University School of Medicine, for useful discussions, and Ms. Noriko Fujita for the excellent technical assistance.

REFERENCES

1. Henquin, J. C. (1994) In Joslin's Diabetes Mellitus, 13th. edition (C. R. Kahn, and G. C. Weir, Eds.) pp. 57-64. Lea & Febiger Press, Malvern, PA.
2. Sener, A., and Malaisse, W. J. (1992) J. Biol. Chem. 267, 13251-13256.
3. Takasawa, S., Nata, K., Yonekura, H., and Okamoto, H. (1993) Science 259, 370-373.
4. Takasawa, S., Tohgo, A., Noguchi, N., Koguma, T., Nata, K., Sugimoto, T., Yonekura, H., and Okamoto, H. (1993) J. Biol. Chem. 268, 26052-26054.
5. Kimura, K., Toyota, T., and Kakizaki, M. (1982) Tohoku J. Exp. Med. 137, 453-459.
6. Portha, B., Serradas, P., Bailbe, D., Suzuki, K., Goto, Y., and Giroix, M. H. (1991) Diabetes 40, 486-491.
7. Goto, Y., Kakizaki, M., and Masaki, N.(1975) Proc. Jpn. Acad. 51, 80-85.
8. Tsuura, Y., Ishida, H., Okamoto, Y., Kato, S., Sakamoto, K., Horie, M., Ikeda, H., Okada, Y., and Seino, Y. (1993) Diabetes 42, 1446-1453.
9. Greene, D. A., Lattimer, S. A., and Sima, A. A. (1987) N. Eng. J. Med. 316, 599-606.
10. Gotoh, M., Maki, T., Kiyoizumi, T., Satomi, S., and Monaco, A. P. (1985) Transplantation 40, 437-441.
11. Celi F. S. , Zenilman, M. E., and Shuldiner, A. R. (1993) Nucl. Acids. Res. 21, 1047.
12. Nobuhito, A., Robinson, F. D., and Nishimoto, S. K. (1993) J. Bone. Miner. Res. 8, 313-322.
13. Iwashima, Y., Kondoh-Abiko, A., Seino, S., Takeda, J., Eto, M., Polonsky, K. S., and Makino, I. (1994) Endoclinology 135, 1010-1017.
14. Ostenson, C. G., Abdel-Halim, S. M., Rasschaert, J., Malaisse-Lagae, F., Meuris, S., Sener, A., and Efendic, S. (1993) Diabetologia 36, 722-726.

15. Thorens, B., Wu, Y. J., Leahy, J. L., and Weir, G. C. (1992) J. Clin. Invest. 90, 77-85.
16. Orci, L., Ravazzola, M., Baetens, D., Inman, L., Amherdt, M., Peterson, R. G., Newgard, C. B., Johnson, J. H., and Unger, R. H. (1990) Proc. Natl. Acad. Sci. USA. 87, 9953-9957.
17. Ohneda, M., Johnson, J. H., Inman, L. R., Chen. L., Suzuki, K., Goto, Y., Alam T., Ravazzola, M., Orci, L., and Unger, R. H. (1993) Diabetes 42, 1065-1072.
18. Ishihara, H., Asano, T., Tsukuda, K., Katagiri, H., Inukai, K., Anai, M., Kikuchi, M., Yazaki, Y., Miyazaki, J., and Oka, Y. (1994) J. Biol. Chem. 28, 3081-3087.
19. Sakura, H., Eto, K., Kadowaki, H., Shimokawa, K., Ueno, H., Koda, N., Fukushima, Y., Akanuma, Y., Yazaki, Y., and Kadowaki T. (1992) J. Clin. Endocrinol. Metab. 75, 1571-1573.
20. Sener, A., and Malaisse, W. J. (1987) Biochem. J. 246, 89-95.
21. Malaisse, W. J. (1993) Acta Diabetol. 30, 1-5.
22. Galione, A., Lee, H. C., and Busa, W. J. (1991) Science 253, 1143-1146.
23. Li, Q., Yamada, Y., Yasuda, K., Ihara, Y., Okamoto, Y., Kaisaki, P. J., Watanabe, R., Ikeda, H., Tsuda, K., and Seino, Y. (1994) Biochem. Biophys. Res. Commun. 202, 629-636.
24. Tokunaga, K., Taniguchi, H., Yoda, K., Shimizu, M., and Sakiyama, S. (1986) Nucleic Acids Res. 14, 2829.
25. Thorens, B., Sarkar, H. K., Kabck, H. R., and Lodish, H. F. (1988) Cell 55, 281-290.
26. Andreone, T. L., Printz, R. L., Pilkis, S. J., Magnuson, M. A., and Granner, D. K. (1989) 264, 363-369.
27. Brown, L. J., MacDonald, M. J., Lehn, D. A., and Moran, S. M. (1994) J. Biol. Chem. 269, 14363-14366.
28. Koguma, T., Takasawa, S., Tohgo, A., Karasawa, T., Furuya, T., Yonekura, H., and Okamoto, H. (1994) Biochim. Biophys. Acta. 1223, 160-162.
29. Ullich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W. J., and Goodman, H. M. (1977) Science 196, 1313-1319.