Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4088–4092, June 1990
Biochemistry

Regulation of β-cell glucose transporter gene expression

LING CHEN*†‡, TAUSIF ALAM*†‡, JOHN H. JOHNSON*†‡, STEVE HUGHES*§, CHRISTOPHER B. NEWGARD*§, AND ROGER H. UNGER*†‡

*Center for Diabetes Research, Gifford Laboratories, and Departments of †Internal Medicine and §Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235; and ‡Department of Veterans Affairs Medical Center, Dallas, TX 75216

Contributed by Roger H. Unger, March 5, 1990

ABSTRACT **It has been postulated that a glucose transporter of β cells (GLUT-2) may be important in glucose-stimulated insulin secretion. To determine whether this transporter is constitutively expressed or regulated, we subjected conscious unrestrained Wistar rats to perturbations in glucose homeostasis and quantitated β-cell GLUT-2 mRNA by *in situ* hybridization. After 3 hr of hypoglycemia (glucose at 29 ± 5 mg/dl), GLUT-2 and proinsulin mRNA signal densities were reduced by 25% of the level in control rats. After 4 days (blood glucose at 57 ± 7 mg/dl vs. 120 ± 10 mg/dl in saline-infused control rats), GLUT-2 and proinsulin mRNA densities were reduced by 85% and 65%, respectively ($P = 0.001$). After 12 days (glucose at 54 ± 8 mg/dl), GLUT-2 mRNA signal density was undetectable whereas proinsulin mRNA was reduced by 51%. After 12 days of hypoglycemia, the K_m for 3-*O*-methyl-D-glucose transport in isolated rat islets, normally 18–20 mM, was 2.5 mM. This provides functional evidence of a profound reduction of high K_m glucose transporter in β cells. In contrast, GLUT-2 was only slightly reduced by hypoglycemia in liver. To determine the effect of prolonged hyperglycemia, we also infused animals with 50% (wt/vol) glucose for 5 days (glucose at 200 ± 50 mg/dl). Hyperglycemic clamping increased GLUT-2 mRNA by 46% ($P = 0.001$) whereas proinsulin mRNA doubled ($P = 0.001$). We conclude that GLUT-2 expression in β cells, but not liver, is subject to regulation by certain perturbations in blood glucose homeostasis.**

In recent years biochemical and molecular studies have revealed the existence of a family of glucose transporters with distinct functional properties but with related primary sequences (1, 2). Glucose uptake into hepatocytes (3) and pancreatic β cells (4) occurs with a K_m of 17–20 mM and is likely mediated by the so-called glucose transporter 2 (GLUT-2) (5) or L type (6) glucose transporter that has identical primary sequence in the two tissues (4, 7). In most other tissues the K_m for glucose uptake is in the 1–2 mM range (8) and is mediated by the low K_m transporter that is termed glucose transporter 1 (GLUT-1) (5) or the E type (6). Normal β cells express GLUT-2 but not GLUT-1 (1, 4, 7) whereas in clonal insulinoma cell lines such as RINm5F, GLUT-1 mRNA predominates (1). The lack of responsiveness to glucose exhibited by such insulinoma lines has been attributed to a reduction in glucose transport that in normal β cells is believed not to be rate-limiting (9). Replacement of GLUT-2 with GLUT-1 in insulinoma cells may render glucose transport rate-limiting, thereby contributing to their glucose insensitivity. Expression of the high K_m GLUT-2 transporter may thus be required for the normal insulin response to glucose, which in turn is essential for normal blood glucose homeostasis. Indeed, loss of glucose-stimulated insulin secretion is the earliest known defect in both autoimmune and nonautoimmune forms of diabetes (10–12).

It is not known at present if the GLUT-2 gene is constitutively expressed or if it is regulated by perturbations of glucose homeostasis. We, therefore, report on the expression of the β-cell GLUT-2 gene after two such perturbations, chronic hypoglycemia induced by insulin infusion and chronic hyperglycemia induced by infusion of 50% (wt/vol) glucose.

MATERIALS AND METHODS

All infusion experiments were conducted in intact unrestrained Wistar rats. In the hypoglycemic experiments rats were infused with either normal saline or with insulin for 4 days or 12 days. Regular porcine insulin (Eli Lilly) was infused at a rate of 1–2 units/24 hr through silastic tubing (Dow Corning) previously implanted in a jugular vein under sodium pentothal anesthesia, as described (13). During these infusions insulin levels averaged >200 microunits/ml. The daily blood glucose levels were maintained at or below 50 mg/dl. In a 3-hr hypoglycemic experiment, 5 units of regular insulin or saline was injected intraperitoneally in anesthetized rats. In the hyperglycemic studies, 50% glucose was infused continuously for 5 days, at a rate varied so as to maintain the blood glucose levels at about 200 mg/dl (13). Control rats received an infusion of 5% glucose that did not raise blood glucose levels. In all groups pancreata were excised under anesthesia and processed for *in situ* hybridization using the method of Simmons *et al.* (14).

Radiolabeled antisense RNA probes were prepared by using the Sp6 promoter of pGEM-7 (Promega) to transcribe nucleotides 1841–1 of the rat liver glucose transporter cDNA (a gift of B. Thorens and H. Lodish, Whitehead Institute, Boston) and the T7 promoter of pGEM-3Z to transcribe a 381-base-pair insert from the rat insulin II cDNA (15). Probes for *in situ* hybridization were prepared using cytidine 5′-[α-[^{35}S]thio]triphosphate or uridine 5′-[α-[^{35}S]thio]triphosphate, whereas those for Northern blot analysis were labeled with cytidine 5′-[α-^{32}P]triphosphate. For Northern blot analysis with total RNA, control experiments were carried out with an 18S rRNA antisense oligonucleotide probe, labeled using [γ-^{32}P]ATP and polynucleotide kinase. Hybridization of the ^{35}S-labeled probes to frozen tissue sections was carried out by the method of Simmons *et al.* (14) with the probe (5×10^6 dpm/ml) in 50% (vol/vol) formamide at 55°C overnight. The slides were treated with RNase, washed under stringent conditions, dipped in NTB3 emulsion, and stored in the dark for 1 week. After developing, fixing, and counterstaining, density of insulin mRNA and glucose transporter mRNA was quantitated by reading the exposure time of individual islets in the dark-field image (×10), on the spot meter of a Nikon microscope photographic UFXIIA system at constant light setting and an ASA setting of 100. Reciprocals of these

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GLUT-1 and GLUT-2, glucose transporter 1 and 2, respectively.

numbers were used as representative of the signal grain density after correcting for the background. The density of 20 randomly picked islets from pancreatic tails of each group was measured after each islet had been centered in the metering area.

For Northern blot analysis, whole pancreas or liver samples were harvested and immediately frozen in liquid N_2. Pancreas samples were ground to a powder with a mortar and pestle under liquid N_2. Total RNA was extracted from liver as described (16) and from powdered pancreas by the guanidine thiocyanate method of MacDonald *et al.* (17), which is designed for tissues with high levels of RNase. Poly(A)$^+$ mRNA was prepared from whole pancreas. Total RNA (liver/pancreas) and poly(A)$^+$ RNA (pancreas) were resolved on formaldehyde gels, transferred to MSI nylon membrane filters, and probed with the ^{32}P-labeled antisense RNA probes in 50% formamide buffer (16) at a temperature of 65°C for 20 hr. The blot was washed four 20-min periods in 0.1× SSC/0.1% SDS at 65°C (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

To determine the characteristics of glucose transport, islets were isolated from seven insulin-infused rats (200–250 g) by using a modification of the Ficoll gradient method of Naber *et al.* (18). The method of islet isolation, islet cell dispersion, and assay of 3-*O*-methylglucose uptake are described in detail elsewhere (19). Initial rates of uptake were measured in duplicate for 3, 6, and 15 sec at 3-*O*-methylglucose concentrations of 1, 5, 15, and 30 mM.

RESULTS

Effects of Extended Hyperinsulinemic Hypoglycemia on GLUT-2 and Proinsulin mRNA of Rat β cells. mRNA encoding the liver/islet high K_m glucose transporter (GLUT-2) was quantitated in β cells by *in situ* hybridization, a technic used to quantitate proinsulin and proglucagon mRNA of islets (20, 21). In pancreata of control rats, the signal for GLUT-2 mRNA was readily apparent (Fig. 1) and hybridization of adjacent sections for proinsulin mRNA revealed that the GLUT-2 mRNA was localized to β cells (Fig. 1*C*).

In the pancreas of animals infused with insulin for 4 or 12 days (Fig. 1 and Table 1), the GLUT-2 mRNA was profoundly reduced to levels that were more than 85% below controls. Proinsulin mRNA was also sharply reduced by the insulin clamp (Table 1) in rats with insulin-induced hypoglycemia for 12 days, confirming previous work (20). In contrast 3 hr of hypoglycemia induced by intraperitoneal injection of insulin reduced GLUT-2 mRNA by only 23% ($P = 0.053$) and proinsulin mRNA by 19% ($P = 0.009$; Table 1). The results obtained by *in situ* hybridization after 4 days of insulin infusion were confirmed by Northern blot analysis on whole pancreas (Fig. 2 *Left*).

Effect of a 12-Day Hyperinsulinemic Hypoglycemic Infusion on Kinetics of Glucose Transport in Islets. To determine if the reduction of GLUT-2 mRNA associated with chronic insulin-induced hypoglycemia would reduce high K_m glucose transport in islet cells, we performed an Eadie–Hofstee transformation of the 3-*O*-methylglucose concentration-dependence data for 3-*O*-methylglucose uptake as shown in Fig. 3. We employed pooled islets isolated from seven rats infused with insulin for 12 days and compared these with data from 40 normal control experiments performed in other studies (4, 19). We found a dramatic reduction in high K_m 3-*O*-methylglucose uptake without a change in low K_m uptake. The overall apparent K_m for 3-*O*-methylglucose uptake was reduced from the normal 17–20 mM range (4) to 2.5 mM. This

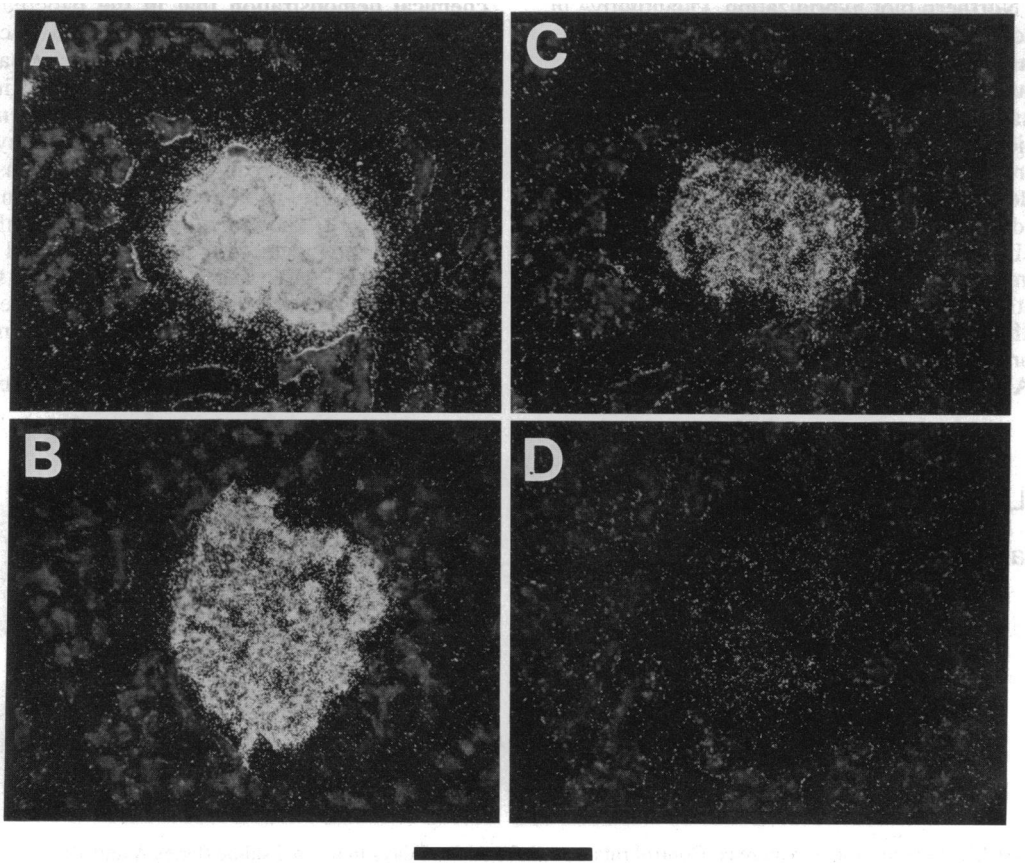

FIG. 1. Dark-field photomicrographs of adjacent sections of pancreas hybridized *in situ* with ^{35}S-labeled antisense RNA probes for insulin (*A* and *B*) or GLUT-2 (*C* and *D*). Pancreata were obtained from normoglycemic control rats (*A* and *C*) and from hypoglycemic rats by a 12-day insulin infusion (*B* and *D*). (Bar = 200 μm.)

Table 1. Effects of insulin-induced hypoglycemia of various durations and of glucose-induced hyperglycemia upon GLUT-2 mRNA of β cells

Infusion	*n*	Duration	Blood glucose, mg/dl	Proinsulin mRNA Density	Proinsulin mRNA % change	GLUT-2 mRNA Density	GLUT-2 mRNA % change
			Insulin-induced hypoglycemia				
Control	4		91 ± 6	0.93 ± 0.15		0.26 ± 0.09	
Insulin	4	3 hr	29 ± 5	0.75 ± 0.25	−19*	0.20 ± 0.10	−23†
Control	3		120 ± 10	1.01 ± 0.16		0.28 ± 0.15	
Insulin	2	4 days	54 ± 7	0.35 ± 0.28	−65	0.04 ± 0.03	−85
Control	3		122 ± 12	0.61 ± 0.03		0.13 ± 0.05	
Insulin	4	12 days	54 ± 8	0.30 ± 0.03	−51	0	−100‡
			Glucose-induced hyperglycemia				
Control	3		120 ± 10	0.46 ± 0.11		0.22 ± 0.07	
Glucose	3	5 days	200 ± 50	0.92 ± 0.18	+100	0.32 ± 0.08	+46

mRNA density values (mean ± SD) are from 20 islets from each group of islets. Blood glucose levels (mean ± SD) are the final determination before sacrifice of the animals. Density number is the reciprocal of the exposure time showing on the light meter. Statistical analysis was carried out by the Student *t* test for two groups ($n = 20$). *n* refers to number of islets in each group. mRNA density is also expressed as percent change from the mean of the control values in each group to permit comparison of changes between experimental groups; differences in specific activity of the probes and in the exposure times employed preclude intergroup comparisons of the mRNA density values. *P* values are 0.001 for control versus treated groups except as indicated by *.

*$P = 0.009$.

†$P = 0.053$.

‡*P* values not determined.

profound change suggests that very few functional GLUT-2 transporters remain on rat β cells after this treatment.

Effect of a 12-Day Hyperinsulinemic Hypoglycemic Infusion on Hepatic GLUT-2 mRNA. To determine whether the reduction in β-cell GLUT-2 mRNA signal is tissue-specific or if it also occurs in the liver, one of the tissues known to express this type of transporter (1), we analyzed total RNA isolated from liver samples taken from saline- or insulin-infused rats by Northern blot hybridization. Quantitative *in situ* hybridization was not employed in liver because it is difficult to quantitate mRNA microscopically when it is distributed throughout an organ rather than sharply localized as in islets. As shown in Fig. 2 (*Right*), hypoglycemic hyperinsulinemic clamping for 4 days or 12 days did not result in a consistent reduction in GLUT-2 mRNA levels in liver.

Effect of Prolonged Hyperglycemic Clamping on GLUT-2 Expression. To determine the effects of prolonged hyperglycemia upon GLUT-2 expression, we compared the GLUT-2 and proinsulin mRNA signal densities in islets of hyperglycemic Wistar rats with blood glucose levels clamped at 200 ± 50 mg/dl with those of a normoglycemic control group. As reported (21), proinsulin mRNA density doubled (Table 1). GLUT-2 mRNA signal density increased by 46% above the controls ($P = 0.001$). Typical islets from a control and a hyperglycemic rat are shown in Fig. 4.

DISCUSSION

This study of rat pancreas demonstrates by *in situ* hybridization that mRNA for the liver-β-cell-type glucose transporter (GLUT-2) is present only in islet cells that also contain proinsulin mRNA. This confirms earlier immunocytochemical demonstration that in the pancreas this glucose transporter is present only in insulin-positive cells of the islet (1, 22). The work further reveals a marginal reduction in GLUT-2 mRNA within the first 3 hr of insulin-induced hypoglycemia and a profound decline to extremely low levels in rat β cells after 4 days or more of modest hypoglycemia in the range of 50 mg/dl. After a 12-day insulin infusion, GLUT-2 mRNA was undetectable *in situ* under the conditions employed. The reduction of GLUT-2 mRNA in β cells exposed to protracted hyperinsulinemia and hypoglycemia was associated with a dramatic reduction in the 17 mM K_m uptake of 3-*O*-methylglucose by islets, evidence that functioning GLUT-2 transporters had been profoundly reduced or kinetically altered. It was not determined if the down-regulation of GLUT-2 mRNA was the consequence of hy-

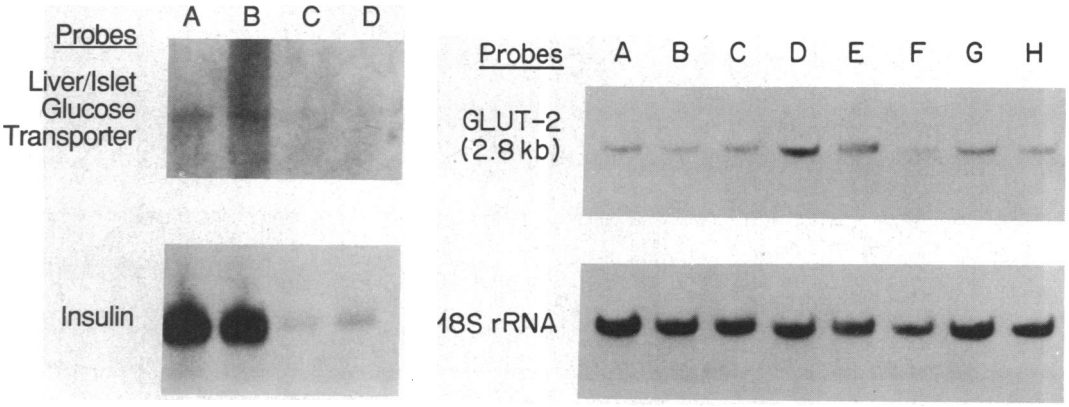

FIG. 2. (*Left*) GLUT-2 expression in pancreas. Control rats were infused for 4 days in normal saline (lanes A and B) or insulin (lanes C and D). Poly(A)$^+$ RNA was prepared from samples of whole pancreas taken from separate animals and 3 μg was loaded in each lane. (*Right*) GLUT-2 expression in liver. Rats were infused for 12 days with insulin (lanes A and B) or saline (lanes C and D) or for 4 days with insulin (lanes E and F) or saline (lanes G and H). Total RNA was prepared from individual livers and 20 μg was loaded in each lane. The liver blots were also probed with an 18S rRNA as a control.

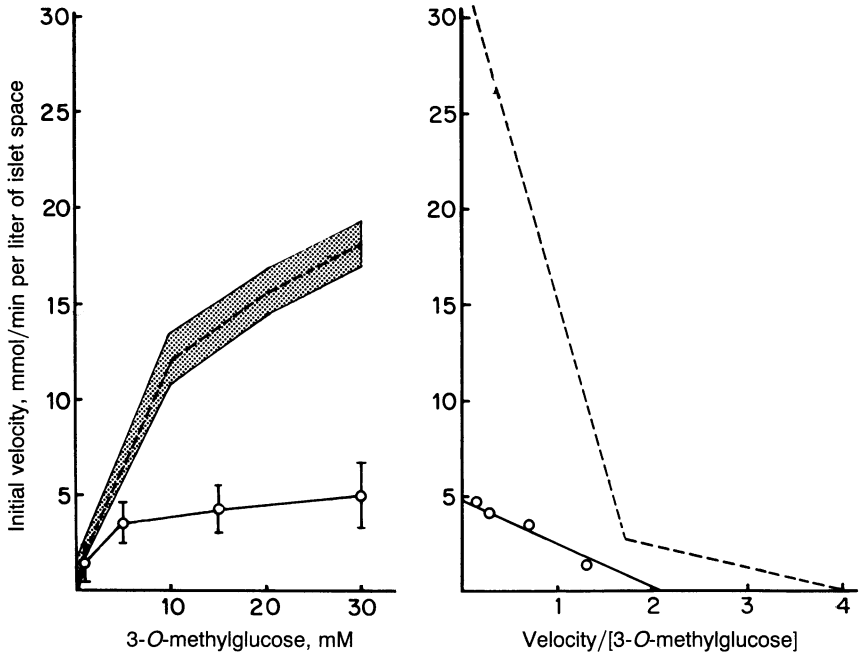

FIG. 3. (*Left*) Concentration dependence of 3-*O*-methylglucose uptake in islets isolated from insulin-infused rats ($n = 7$, solid line) and normal Wistar rats ($n = 12$, dashed line). (*Right*) Eadie–Hofstee plot of data on the *Left* for insulin-infused rats (solid line) and normal Wistar rats (dashed line), showing the decrease in high K_m glucose transport in islets from insulin-infused animals.

poglycemia and/or hyperinsulinemia or other unidentified factors that were perturbed by the manipulations of this study. GLUT-2 mRNA expression in liver was not significantly reduced by hypoglycemic insulin clamping.

Hyperglycemic clamping for 5 days significantly increased GLUT-2 mRNA in rat islets. This maneuver has been shown to increase proinsulin mRNA and to increase the number of β cells and the size of the β-cell mass (20).

Previous studies have demonstrated regulation of expression of other glucose transporter genes in other tissues by platelet-derived growth factor (23, 24), fibroblast growth factor (24), epidermal growth factor (24), insulin (25), and perturbations such as streptozotocin-induced diabetes and fasting (26). The present findings provide evidence that expression of the GLUT-2 transporter in β cells also can be regulated. In contrast to its effects on GLUT-1 expression in 3T3 cells (25), long-term insulin infusion *in vivo* causes a decrease in steady-state concentrations of GLUT-2 mRNA in islet β cells. In this study of insulin-induced hypoglycemia, the effects on the GLUT-2 transporter appear to be tissue-specific, as evidenced by the relative lack of effect on expression of GLUT-2 in liver.

It appears that, when GLUT-2 is not expressed by β cells, β cells become less responsive to glucose. In the perfused pancreata of hypoglycemic insulin-treated rats, such as were studied here, glucose-stimulated insulin secretion averages only 25% of the normal response (A. Komiya and R.H.U., unpublished observations). Clonal insulinoma cell lines (RINm5F) have reduced expression of the high K_m GLUT-2 molecule and aberrant expression of the low K_m GLUT-1 or E-type transporter. These lines are glucose-unresponsive, as are β cells of the Zucker diabetic fatty rat that also is deficient

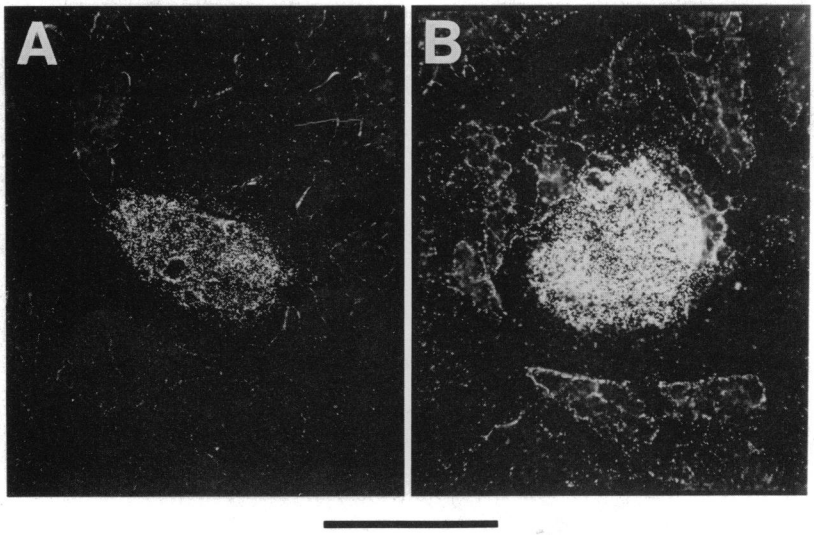

FIG. 4. Dark-field photomicrographs of pancreatic sections hybridized *in situ* with ^{35}S-labeled antisense glucose transporter RNA probe obtained from normoglycemic control rats infused with 5% glucose for 5 days (*A*) and hyperglycemic rats infused with 50% glucose for 5 days (*B*). (Bar = 200 μm.)

in immunodetectable GLUT-2 molecules (J.H.J., A. Ogawa, and R.H.U., unpublished observations). Since the loss of glucose-stimulated insulin release is the earliest known functional deficit in diabetes (11–13), down-regulation of GLUT-2 expression may be an important early event in the pathophysiology of the disease. However, it remains to be demonstrated if regulation of GLUT-2 expression plays an important physiologic role in β-cell function, since the regulating events (hyperinsulinemic hypoglycemia clamping or hyperglycemic clamping) employed in this study represent extreme perturbations without counterparts in normal physiology.

We thank Drs. Bruce Beutler, Dennis Stone, and Kenneth Luskey for critical reading of the manuscript and Ms. Teresa Autrey for expert secretarial assistance. These studies were supported by National Institutes of Health Grant DK02700-30, Veterans Administration Institutional Research Support Grant 549-8000, the Diabetes Research and Education Foundation (to R.H.U.), a Research and Development Award from the American Diabetes Association, and a grant from the American Heart Association, Texas Affiliate (to C.B.N.). L.C. is the recipient of a postdoctoral fellowship from the Juvenile Diabetes Foundation.

1. Thorens, B., Sarkar, H. K., Kaback, H. R. & Lodish, H. F. (1988) *Cell* **55**, 281–290.
2. Gould, G. W. & Bell, G. I. (1990) *Trends Biochem. Sci.* **15**, 18–23.
3. Axelrod, J. D. & Pilch, P. F. (1983) *Biochemistry* **22**, 2222–2227.
4. Johnson, J. H., Newgard, C. B., Milburn, J. L., Lodish, H. F. & Thorens, B. (1990) *J. Biol. Chem.*, in press.
5. Fukumoto, H., Seino, S., Imura, H., Seino, Y., Eddy, R. L., Fukushima, Y., Byers, M. G., Shows, T. B. & Bell, G. I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5434–5438.
6. Pilch, P. F. (1990) *Endocrinology* **126**, 3–5.
7. Permutt, M. A., Koranyi, L., Keller, K., Lacy, P. E., Scharp, D. W. & Mueckler, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8688–8692.
8. Wheeler, T. J. & Hinkle, P. C. (1981) *J. Biol. Chem.* **256**, 8907–8919.
9. Meglasson, M. D., Manning, C. D., Najafi, H. & Matschinsky, F. M. (1986) *Diabetes* **35**, 1340–1344.
10. Pfeiffer, M. A., Halter, J. B. & Porte, D., Jr. (1981) *Am. J. Med.* **70**, 579–588.
11. Srikanta, S., Ganda, O. P., Eisenbarth, G. S. & Soeldner, J. S. (1983) *N. Engl. J. Med.* **308**, 322–325.
12. Tominaga, M., Komiya, I., Johnson, J. H., Inman, L., Alam, T., Moltz, J., Crider, B., Stefan, Y., Baetens, D., McCorkle, K., Orci, L. & Unger, R. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9749–9753.
13. Komiya, I., Baetens, D., Kuwajima, M., Orci, L. & Unger, R. H. (1990) *Metabolism* **39**, in press.
14. Simmons, D. M., Arriza, J. L. & Swanson, L. W. (1989) *J. Histotechnol.* **12**, 169–181.
15. Chan, S. J., Noyes, B. E., Agarwal, K. L. & Steiner, D. F. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5036–5040.
16. Newgard, C. B., Nakano, K., Hwang, P. K. & Fletterick, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8132–8136.
17. MacDonald, R. J., Swift, G. H., Przybyla, A. E. & Chirgwin, J. M. (1987) *Methods Enzymol.* **152**, 219–227.
18. Naber, S. P., McDonald, J. M., Jarret, L., McDaniel, M. L., Ludvigsen, C. W. & Lacy, P. E. (1980) *Diabetologia* **19**, 439–444.
19. Johnson, J. H., Crider, B. P., McCorkle, K., Alford, M. & Unger, R. H. (1990) *N. Engl. J. Med.* **322**, 653–659.
20. Chen, L., Komiya, I., Inman, L., McCorkle, K., Alam, T. & Unger, R. H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1367–1371.
21. Chen, L., Komiya, I., Inman, L., O'Neil, J., Appel, M., Alam, T. & Unger, R. H. (1989) *J. Clin. Invest.* **84**, 711–714.
22. Orci, L., Thorens, B., Ravazzola, M. & Lodish, H. F. (1989) *Science* **245**, 295–297.
23. Rollins, B. J., Morrison, E. D., Usher, P. & Flier, J. S. (1988) *J. Biol. Chem.* **263**, 16523–16526.
24. Hiraki, Y., Rosen, O. M. & Birnbaum, M. J. (1988) *J. Biol. Chem.* **263**, 13655–13662.
25. Garcia de Herreros, A. & Birnbaum, M. J. (1989) *J. Biol. Chem.* **264**, 9885–9890.
26. Berger, J., Biswas, C., Vicarie, P. P., Strout, H. V., Saperstein, R. & Pilch, P. F. (1989) *Nature (London)* **340**, 70–72.