Expression of Constitutively Activated $G_{i2}$ in Vivo Ameliorates Streptozotocin-induced Diabetes*

(Received for publication, June 11, 1998)

Xi-Long Zheng, Jun hua Guo, Hsien-yu Wang, and Craig C. Malbon‡

From the Departments of Molecular Pharmacology and of Physiology and Biophysics, Diabetes and Metabolic Diseases Research Program, University Medical Center, SUNY/Stony Brook, Stony Brook, New York 11794-8651

Streptozotocin treatment in vivo generates a model of insulin-dependent diabetes mellitus via destruction of the pancreatic $\beta$-cells responsible for insulin secretion. Tissue-specific expression of the Q205L constitutively activated mutant form of the G-protein $G_{i2}$ in vivo ameliorates streptozotocin-induced insulin-dependent diabetes mellitus in transgenic mice. Conditional expression of Q205L $G_{i2}$ in vivo in liver, skeletal muscle, and adipose tissue markedly rectifies glucose tolerance, fasting glucose levels, and glycogen synthase activation in the mice with insulin-dependent diabetes mellitus, providing a novel therapeutic target for diabetes.

EXPERIMENTAL PROCEDURES

Transgenic Mice with Inducible, Targeted Expression of Constitutively Activated Q205L Mutant of $G_{i2}$—All animals were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee at SUNY/Stony Brook. Mice were maintained on a normal light/day cycle. The transgenic mice were constructed using the rat Q205L $G_{i2}$ under the control of PEPCK promoter, as described elsewhere (4). The PEPCK promoter is silent in utero and is strongly activated in birth yielding 2%–3% of cellular mRNA in tissues targeted for expression, such as liver, skeletal muscle, and adipose tissue. Tail DNA samples were extracted and the presence of Q205L $G_{i2}$ transgene detected by reverse transcription-PCR using primers described elsewhere (3, 4). Targeted tissues, such as epididymal white fat, were taken to monitor the tissue-specific expression of Q205L $G_{i2}$ with $G_{i2}$ antibody according to standard procedures (3, 4). Fasting glucose concentrations were analyzed by using a One Touch II glucometer (Life Scan Technologies, Milpitas, CA) during the period from 8:00 a.m. to 10:00 a.m. Serum insulin concentrations were assayed by use of an radioimmunoassay kit according to the protocol provided by the commercial supplier (NEN Life Science Products).

Glucose Tolerance Test—Glucose was loaded as a bolus (2.5 mg/g body weight, intraperitoneal injection) to transgenic and FVB mice with and without treatment of STZ. The glucose determination was made using a One Touch II glucometer at the time intervals indicated. All determinations were performed between the hours of 8:00 a.m. and 10:00 a.m. Insulin sensitivity was determined by measuring blood glucose concentrations after intraperitoneal insulin injection of increasing doses of insulin (0.05–0.4 IU/kg body weight).

Glycogen Synthase Activation—Skeletal muscle was excised from the thighs of each animal. Blood was collected at the end of week 12 for serum insulin determinations, verifying a sharp reduction in serum insulin levels in response to the streptozotocin. The muscle tissue was homogenized immediately after isolation in an ice-cold homogenization buffer (3, 4). Glycogen synthase activities were determined by measuring the incorporation of $[^{14}C]UDP$-glucose into purified glycogen in the absence or presence of 10 mM glucose-6-phosphate, as described previously (3, 4).

RESULTS AND DISCUSSION

Mice harboring the Q205L $G_{i2}$-expressing transgene under the PEPCK promoter were identified by PCR to amplify a 400-base pair fragment from the tail DNA preparations (Fig. 1A). Overexpression of $G_{i2}$ by transgenic (Q205L) as compared with nontransgenic littermates (FVB) was demonstrated in target (fat) but not nontarget (spleen) tissues by immunoblotting. STZ, an agent cytotoxic to pancreatic $\beta$-cells, was administered intraperitoneally at a dose of 40 mg/kg body weight daily for 5 days (8). Fasting blood glucose levels (mg/dl, mg%) increases dramatically during the 4 weeks following administration of STZ (Fig. 1B). By week 12, blood glucose levels of STZ-treated mice were ~400 mg%, as compared with <100 mg% in untreated, FVB controls. For mice harboring the Q205L $G_{i2}$ transgene, the results were quite remarkable. Fasting glucose levels were lower in the Q205L mice than their control FVB littermates. Upon treatment with STZ, both groups of mice display major reductions in serum insulin in either fed or fasting state (Fig. 1B). The presence of the Q205L $G_{i2}$ transgene markedly rectifies STZ-induced hyperglycemia, reducing the fasting glucose levels from ~400 mg% to ~150 mg%, in the absence of normal insulin levels.

Transgenic and control mice, both normal and STZ-treated, were challenged with a bolus of glucose intraperitoneally, and the ability of the mice to rectify the glucose loading was subsequently measured (Fig. 2A). Compared with FVB controls, untreated Q205L transgenic mice display an enhanced glucose tolerance, rectifying the hyperglycemia more rapidly. Not un-

* This work was supported by the American Cancer Society (to C. C. M.) and the National Institutes of Health (to J. H. G.). 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: Dept. of Pharmacology, HSC, SUNY/Stony Brook, Stony Brook, NY 11794-8651. Tel.: 516-444-7696; E-mail: craig@pharm.som.sunysb.edu.

1 The abbreviations used are: IDDM, insulin-dependent diabetes mellitus; PEPCK, phosphoenolpyruvate carboxykinase; PCR, polymerase chain reaction; STZ, streptozotocin.
expectedly, FVB mice with STZ-induced IDDM fail to rectify the increased hyperglycemia, the glucose loading only exacerbating the already high glucose levels observed in the fasting state. In sharp contrast, the STZ-treated mice harboring the transgene display a marked rectification of glucose, able to reduce glucose levels significantly within 80 min of a glucose loading. Although not achieving the fasting glucose levels of untreated mice, the Q205L Giα2 mice challenged with glucose display a nearly normal pattern of glucose handling (Fig. 2), displaced to a higher fasting glucose level (Fig. 1).

Insulin sensitivity of the mice was measured by monitoring fasting glucose levels in mice challenged with increasing, graded doses of insulin, ranging from 0.05 to 0.4 IU insulin/kg body weight (Fig. 2B). Setting the initial fasting glucose level to 100%, the insulin sensitivity measurements reveal that the mice harboring the Q205L Giα2 responded much as their control FVB littermates do, although the extent of the changes were somewhat greater in the transgenic mice. STZ-induced IDDM in FVB mice was accompanied by frank insulin resistance, reflecting the pronounced hyperglycemia (8–10). Harboring the Q205L Giα2 transgene, in sharp contrast, ameliorates to
Constitutively Activated Gi2 Ameliorates Type I Diabetes

a large extent the insulin resistance characteristic of IDDM, whether induced by STZ or the result of other lesions.

The glucose tolerance and insulin sensitivity tests reveal two features about the ability of Q205L Gi2 to be insulinomimetic. First, the results obtained with STZ-treated mice demonstrate that constitutive activation of this G-protein α-subunit in liver, adipose tissue, and skeletal muscle results in increased glucose disposal in the virtual absence of endogenous insulin itself. Therefore, activation of Gi2 can be considered to be insulinomimetic in character. Second, unlike the STZ-treated control mice, the IDDM-like mice harboring the Q205L Gi2 transgene do respond to insulin although subject to mild hyperglycemia. Whether directly via signaling integration or indirectly through the reduction in fasting hyperglycemia, activated Gi2 enhances insulin action. The ability of Gi2 deficiency in skeletal muscle, liver, and adipose tissue to render mice insulin-resistant in the presence of nearly normal insulin levels lends further support to the hypothesis that Gi2 activity modulates insulin action in vivo (3).

To complement the studies of insulin action in vivo, we investigated features of insulin action directly in skeletal muscle focusing upon the activation of glycogen synthase a major insulin-dependent pathway for glucose disposal in vivo (11, 12). The ability of Q205L Gi2 to enhance glucose disposal in the absence of insulin was examined in STZ-treated and control FVB mice (Fig. 3). Skeletal muscle from fasted, STZ-treated mice displayed glycogen synthase activity ratios approximately one-third of that observed in the fasted control mice, likely reflecting the reduction in serum insulin levels. In the fed state, glycogen synthase activity is increased in control and STZ-treated FVB mice alike, the glycogen synthase activity ratio about two to three times greater in the former than the later. For the mice harboring the Q205L Gi2-expressing transgene, glycogen synthase activation of skeletal muscle is high in both the fasting and fed states (Fig. 3). Although treatment with STZ reduces glycogen synthase activation in Q205L Gi2 mice, the residual activity in the virtual absence of insulin is 70% of that of fasted, untreated FVB littermates. Glycogen synthase activation in fed, STZ-treated mice harboring the transgene is >50% greater than that of the STZ-treated FVB littermates.

The constitutive activation of glycogen synthase in the Q205L Gi2-expressing mice provides an explanation for the enhanced glucose disposal observed in these mice. Even in the virtual absence of insulin following STZ-induced IDDM, the Q205L Gi2-expressing mice display considerable glycogen synthase activation, which would provide significant rectification of the fasting hyperglycemia. The enhanced glucose tolerance observed in STZ-treated mice reflects a novel and significant capacity for Gi2-signaling to contribute to glucose disposal, i.e. Gi2 action in skeletal muscle is insulinomimetic. The ability of the transgene to rectify the insulin resistance characteristic of STZ-induced IDDM (8–10) may reflect a further capacity for Gi2 to contribute to glucose disposal through insulin action itself, or it may reflect a partial rectification of STZ-induced hyperglycemia. The ability of Gi2 deficiency to provoke insulin resistance in the face of normal serum insulin levels and responses (3) argues for a unique role for Gi2 in insulin action itself. These provocative results identify activation of Gi2 as a new therapeutic target toward ameliorating IDDM.

REFERENCES

1. Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–647
2. White, M. F., and Kahn, C. R. (1994) J. Biol. Chem. 269, 1–4
3. Moxham, C. M., and Malbon, C. C. (1996) Nature 379, 840–844
4. Chen, J. F., Gu, J. H., Moxham, C. M., Wang, H. Y., and Malbon, C. C. (1997) J. Mol. Med. 75, 283–289
5. Rakieten, N., Gordon, B. S., Beatty, A., Bates, R. W., Schein, P. S., and Standaert, F. G. (1976) Proc. Soc. Exp. Biol. Med. 151, 632–635
6. Like, A. A., Apple, M. C., Williams, R. M., and Rossini, A. A. (1978) Lab. Invest. 38, 470–486
7. Yamamoto, H., Uchigata, Y., and Okamoto, H. (1981) Nature 294, 284–286
8. Monkayashi, M., and Olefsky, J. M. (1979) Diabetes 28, 87–95
9. Karmielli, E., Hissin, P. J., Simpson, I. A., Salans L. B., and Cushman, S. W. (1981) J. Clin. Invest. 68, 811–814
10. Nishimura, H., Kuzuya, H., Okamoto, M., Yamada, K., Kosaki, A., Kakehi, T., Inoue, G., Kuno, S., and Imura, H. (1989) Am. J. Physiol. 256, E624–E630
11. Lawrence, J. C., Jr., and Roach, P. J. (1997) Diabetes 46, 541–547
12. Villar-Palasi, C., and Guinovart, J. J. (1997) FASEB J. 11, 544–558

FIG. 3. Q205L Gi2 expression enhances glycogen synthase activation in skeletal muscle of mice with STZ-induced streptozotocin. Glycogen synthase ratios (G6-P) were determined in skeletal muscle samples from the four experimental groups. The open and solid bars display the activity ratios for skeletal muscle glycogen synthase in fed and overnight fasted states, respectively. The values shown are the mean values ± S.D. (n = 6). The asterisks denote values statistically different from FVB mice (p < 0.05).