Rapid Publication

Adenovirus-Mediated Overexpression of Sterol Regulatory Element Binding Protein-1c Mimics Insulin Effects on Hepatic Gene Expression and Glucose Homeostasis in Diabetic Mice

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In vitro, the transcription factor sterol regulatory element binding protein-1c (SREBP-1c) mimics the positive effects of insulin on hepatic genes involved in glucose utilization, such as glucokinase (GK) and enzymes of the lipogenic pathway, suggesting that it is a key factor in the control of hepatic glucose metabolism. Decreased glucose utilization and increased glucose production by the liver play an important role in the development of the hyperglycemia in diabetic states. We thus reasoned that if SREBP-1c is indeed a mediator of hepatic insulin action, a hepatic targeted overexpression of SREBP-1c should greatly improve glucose homeostasis in diabetic mice. This was achieved by injecting streptozotocin-induced diabetic mice with a recombinant adenovirus containing the cDNA of the mature, transcriptionally active form of SREBP-1c. We show here that overexpressing SREBP-1c specifically in the liver of diabetic mice induces GK and lipogenic enzyme gene expression and represses the expression of phosphoenolpyruvate carboxykinase, a key enzyme of the gluconeogenic pathway. This in turn increases glycogen and triglyceride hepatic content and leads to a marked decrease in hyperglycemia in diabetic mice. We conclude that SREBP-1c has a major role in vivo in the long-term control of glucose homeostasis by insulin. Diabetes 50:2425–2430, 2001
type 2 diabetes (14). In contrast, overexpression of hepatic GK in diabetic mice improves glucose homeostasis (15).

We then reasoned that if SREBP-1c is really mimicking the positive effects of insulin on gene expression, a hepatic targeted overexpression of this factor should greatly improve glucose homeostasis in diabetic mice. This was achieved by injecting streptozotocin (STZ)-induced diabetic mice with a recombinant adenovirus containing the cDNA of the mature, transcriptionally active form of SREBP-1c. We show here that overexpression of SREBP-1c in the liver of diabetic mice induces GK and lipogenic enzyme gene expression, represses PEPCK expression, and leads to a dramatic decrease in hyperglycemia, thus mimicking insulin action.

RESEARCH DESIGN AND METHODS

All procedures were carried out according to the French guidelines for the care and use of experimental animals. Male 10-week-old CBA mice (Janvier Breeding Laboratories, Le Genest-St-Isle, France) were housed at 20°C with light from 0700 to 1900, with free access to water and food (UAR, Villemonois sur Orge, France). Diabetes was induced by a single intraperitoneal injection of STZ (250 mg/kg body wt; Sigma, St. Louis, MO). STZ was dissolved in 50 mM dilute HCl. The mixture was immediately injected intraperitoneally at a dose of 50 mg/kg body weight (16). Diabetes was assessed by both blood sampling from the tail vein and plasma glucose as well as by the presence of overt glycosuria.

Recombinant adenoviruses. The adenovirus vector containing the transcriptionally active NH₂-terminal fragment (amino acids 1–403) of SREBP-1c was constructed according to He et al. (16). Briefly, the cDNA of the transcriptionally active fragment of SREBP-1c was subcloned into the shuttle vector pAdTrack-CMV. The recombinant plasmid was linearized by the restriction endonuclease Pmel and cotransformed with the supercoiled adenoviral vector pAd-Easy1 into Eschericia coli strain BJ5183. Recombinants were selected by kanamycin resistance and screened by restriction endonuclease digestion. Then, the recombinant adenoviral construct was cleaved with Pmel and transfected into the packaging cell line 293. The adenovirus vector containing the major late promoter with no exogenous gene (null adenovirus) was used as a control. The adenoviral vectors were propagated in 250 cells, purified by cesium chloride density centrifugation, and stored as previously described (9).

Injection of recombinant adenovirus and insulin administration. STZ-induced diabetic mice were treated with either the recombinant adenovirus or insulin. Recombinant adenoviruses were injected to mice anesthetized with halothane (Abbott Laboratoires, Orge, France). Recombinant adenoviruses (1.4 × 10⁹ particles in a final volume of 200 μl sterile phosphate-buffered saline) were injected intravenously into the tail vein of the mouse. The adenovirus was suspended in phosphate-buffered saline (pH 7.4) immediately before administration. Diabetes was assessed by blood sampling from the tail vein and plasma glucose as well as by the presence of overt glycosuria.

RESULTS

Establishment of diabetes in CBA mice. After STZ injection, mice were checked daily for glycosuria and plasma glucose concentration. Once their hyperglycemia was stable (i.e., after ~1 week), mice were divided into two groups of similar hyperglycemia: 470 ± 50 mg/dl (n = 15) for the group injected with null adenovirus and 460 ± 60 mg/dl (n = 15) for the group injected with SREBP-1c adenovirus. The weight of nondiabetic control mice was 28.5 ± 0.7 g (n = 5), and the weight of mice 1 week after the onset of diabetes was 22.5 ± 0.2 g (n = 16). Diabetic mice were hyperketonemic, as shown by β-hydroxybutyrate measurements of 0.447 ± 0.25 mmol/l (n = 8) vs. 0.095 ± 0.24 mmol/l (n = 5) in nondiabetic mice.

At 48 h after the injection with null adenovirus or SREBP-1c adenovirus, we found that in the two groups (n = 8 in each group), body weight (22.2 ± 0.2 and 23 ± 0.5 g, respectively) and liver weight (1.1 ± 0.1 and 1.30 ± 0.06 g) were similar.

Tissue expression of recombinant SREBP-1c mRNA and protein. Previous studies in rodents indicated that systemic infusion of recombinant adenovirus results in a preferential targeting of the transgene to the liver (23–25). To verify that the expression of recombinant SREBP-1c was indeed restricted to the liver, we performed Northern blots with total RNA extracted from different tissues of mice injected with the null or SREBP-1c adenovirus (Fig. 1A). Injection of the SREBP-1c adenovirus resulted in a marked expression of the transgene in the liver, whereas its expression remained undetectable in other tissues (Fig. 1A), even after overexposure of the blots (not shown). Nuclear extracts from the livers of SREBP-1c adenovirus–injected mice showed high amounts of recombinant SREBP-1c (Fig. 1B). As previously described in diabetic rats (5), endogenous SREBP-1c was not detected in nuclear extracts from STZ-induced diabetic mice.

Effect of adenovirus-mediated expression of SREBP-1c on hepatic gene expression and enzyme activities: comparison with insulin injection. Insulin injection increased the mRNA content of endogenous SREBP-1c in diabetic mice (Fig. 2), as shown previously in diabetic rats (5) and in agreement with in vitro studies in cultured hepatocytes (4). As expected, insulin increased the hepatic expression of known insulin-inducible genes, GK, FAS, and S14. The expression of the LDL receptor was also increased by insulin treatment, as described previously in

U.K.). FAS, GK, PEPCK, SREBP-1c, and S14 cDNAs were obtained as previously described (9). Mouse LDL receptor cDNA was obtained by reverse transcriptase–polymerase chain reaction using the following primers: sense, 5′-GAGGAACTGGCGGCTGAA-3′; anti-sense, 5′-GTGGCGTGGAGGGAGGT CT-3′. Northern blots were hybridized with a ribosomal 18S probe to verify that equivalent amounts of total RNA were loaded in each lane.

Immunoblotting. Nuclear extracts of mouse liver were prepared according to Gorski et al. (22). A volume of 30 μg nuclear extract was mixed with SDS loading buffer and subjected to SDS/PAGE on a 12% acrylamide gel. Proteins were electrotransferred on to a Hybond C nitrocellulose membrane (Amer sham Pharmacia Biotech). Recombinant SREBP-1c was detected with a mouse monoclonal antibody (lgG-2A4) raised against amino acids 301–407 of human SREBP-1. Primary antibodies against SREBP-1c were used at 4 μg/ml, and detection of signals was performed using an enhanced chemilluminescence Western blot detection kit (Amer sham Pharmacia Biotech) with anti-mouse horseradish peroxidase–conjugated IgG as second antibody.

Statistical analysis. Results are expressed as the means ± SE. Statistical analysis was performed with Student’s t test for unpaired data. Differences were considered significant at P < 0.05.
Finally, insulin repressed the expression of PEPCK (Fig. 2A). Forced expression of the mature form of SREBP-1c in the liver of diabetic mice markedly stimulated the expression of GK, lipogenesis-related proteins, FAS, and S14 as well as the LDL receptor (Fig. 2B). Surprisingly, it also led to a marked decrease in the expression of PEPCK, which was then nearly undetectable at the mRNA level (Fig. 2B). Thus, the presence of an active form of SREBP-1c in the liver mimics both inductive and repressive effects of insulin and results in a pattern of hepatic gene expression nearly identical to that of insulin injection.

**Functional consequences of adenovirus-mediated expression of SREBP-1c on hepatic metabolism and glucose homeostasis.** The consequences of SREBP-1c overexpression at the gene level were also apparent when enzyme activities were measured. GK and FAS activities were increased 1.8- and 2.1-fold, respectively (Table 1), whereas PEPCK activity was decreased nearly fourfold. In the liver, this led to a fourfold increase in liver glycogen concentration and a doubling of triglyceride concentration (Fig. 3), clearly underlying the increased capacity for glucose utilization in this organ. In contrast, the circulating concentration of triglycerides was decreased (Fig. 3). A striking feature of SREBP-1c hepatic overexpression was the marked decrease in the blood glucose of diabetic mice. Indeed, their blood glucose was already lower after 18 h when compared with diabetic mice injected with the null adenovirus (not shown) and nearly normalized 48 h after the injection, whereas the glycemia of diabetic mice injected with the null adenovirus remained high (Fig. 3).

**DISCUSSION**

**SREBP-1c, a key factor of the regulation of glucose homeostasis.** The transcription factors of the SREBP family SREBP-2, -1a, and -1c have been involved in the control of the expression of genes related to the metabolism of specific nutrients. The comprehensive work of Brown and Goldstein (26) has demonstrated the involvement of SREBP-2 and -1a in the control of genes involved in cholesterol synthesis and uptake. In vitro and in vivo studies on SREBP-1c first related this isoform to the upregulation of genes of the lipogenic pathway (9,27–29), a condition encountered during high carbohydrate intake.
Later, it was found that SREBP-1c transcriptional activity could be regulated by insulin at the gene level (4,5,8) and possibly by post-translational mechanisms (30), thus bridging insulin signaling and the well-known ability of this hormone to enhance long-term lipogenic capacity.

Finally, the discovery that GK, a key enzyme of hepatic glucose utilization regulated by insulin at the gene level, was a target of SREBP-1c (9) has extended the potential glucose utilization regulated by insulin at the gene level, this hormone to enhance long-term lipogenic capacity.

**Hypoglycemic effects of SREBP-1c hepatic overexpression.** What are the mechanisms that can explain the strong hypoglycemic effects of SREBP-1c? Induction of GK by SREBP-1c is likely to have an important role in the hypoglycemic action of this transcription factor. Indeed, in transgenic diabetic mice overexpressing GK in the liver (15), in transgenic mice with additional GK gene copy (31), and in rats injected with a recombinant adenovirus expressing GK (24), blood glucose was decreased. The glycemia of diabetic mice was even nearly normalized by hepatic GK overexpression (15). Increased GK activity can in turn enhance hepatic glucose flux into glycogen synthesis (15,24,31) (Fig. 3) and in the lipogenic pathway, as shown by the increased hepatic triglyceride content (24) (Fig. 3). This latter event would be favored by the concomitant enhanced expression of lipogenic enzymes such as FAS because their gene expression is sensitive to both insulin and increased glucose metabolism (32). Interestingly enough, and in contrast with previous findings in rats overexpressing hepatic GK (24), the increased hepatic triglyceride content was not paralleled with an increased serum triglyceride concentration, but rather with a decreased concentration. The same kind of result was found in transgenic mice overexpressing SREBP-1c (27). This was attributed to the induction by this transcription factor of the hepatic LDL receptor, which could mediate the degradation of VLDL particles before their secretion. Indeed, when transgenic mice overexpressing SREBP-1a were mated with mice in which the LDL receptor had been disrupted, a considerable increase in plasma lipids was revealed (33). This explanation could also hold in the present study, because the expression of the LDL receptor was enhanced in the liver of diabetic mice injected with the SREBP-1c adenovirus.

In addition to decreased glucose utilization, diabetes is characterized by excessive hepatic glucose production that is linked to active gluconeogenesis. Because of a lack of insulin or insulin resistance, insulin is unable to repress the transcription of one of the key enzymes of this pathway, cytosolic PEPCK, which is regulated at the transcriptional level by this hormone. One of the striking findings of this study is that overexpressed SREBP-1c also strongly represses the expression and activity of PEPCK and thus should induce a marked decrease of endogenous glucose production. It is therefore important to assess whether this is a direct effect of SREBP-1c on the PEPCK promoter. It has been already noted that the overexpression of GK can decrease PEPCK expression in diabetic mice (15). This was attributed to an inhibitory effect of a GK-mediated increased glucose metabolism, because glucose or one of its metabolites represses PEPCK promoter activity (34,35). In the present study, because of the increased GK activity, a glucose effect could certainly contribute to the strong decrease of PEPCK expression and activity. However, 1) the repression caused by SREBP-1c overexpression is equally or even more potent compared with that induced by insulin injection, and 2) recent studies performed in vitro and using transfection experiments have shown that SREBP-1c is a potent repressor of PEPCK promoter activity (36). STZ-induced diabetes is characterized by a rise in glucagon concentration. It is interesting to emphasize that SREBP-1c overexpression is thus able, like insulin, to fully antagonize the effects of glucagon on PEPCK expression, as seen in this study and those of Chakravarty et al. (36) and Sasaki et al. (37).

In summary, the hypoglycemic effect of SREBP-1c, which mimics the known action of insulin, is probably the consequence of both an increase in glucose utilization and a decrease in glucose production by the liver. Given the
results of this study, it would certainly be of interest to assess, under various nutritional conditions, the glucose homeostasis of existing models of either mice deleted for the SREBP-1 gene (38) or transgenic mice overexpressing SREBP-1 isoforms, although in the latter case, the situation might be confounded by the fact that in order to confer hepatic specificity to the transgene, the SREBP-1 gene was driven by a PECK promoter (27,39).

In conclusion, peroxisome proliferator-activated receptor-γ was the first transcription factor involved (by a still unknown mechanism) in the control of insulin sensitivity and was used as a therapeutic target in type 2 diabetes. Our studies point to another transcription factor, SREBP-1c, as 1) having a major role in the long-term control of glucose homeostasis by insulin; 2) being a potential causal factor in a number of diseases related to insulin resistance and blood glucose disturbances, such as obesity, syndrome X, and type 2 diabetes; and 3) being a potential therapeutic target in these diseases.

ACKNOWLEDGMENTS

This work was supported by a grant from ALFEDIAM/ Roche. D.B. is the recipient of a doctoral fellowship from the Ministère de l’Enseignement Supérieur et de la Recherche. P.F is supported by the Centre National de la Recherche Scientifique.

We thank the vector core of the University Hospital of Nantes, which is supported by the Association Française contre les Myopathies (AFM), for production of the SREBP-1c adenovirus. We thank Drs. I. Dugail, J. Girard, P. Lema
crand, and S. Darqui for helpful discussions and advice.

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crand, and S. Darqui for helpful discussions and advice.


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