Hyperglycemia suppresses hepatic scavenger receptor class B type I expression

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Murao K, Yu X, Imachi H, Cao WM, Chen K, Matsumoto K, Nishiuchi T, Wong NC, Ishida T. Hyperglycemia suppresses hepatic scavenger receptor class B type I expression. Am J Physiol Endocrinol Metab 294: E78–E87, 2008. First published October 23, 2007; doi:10.1152/ajpendo.00023.2007.—Hyperglycemia is a major risk factor for atherosclerotic disease. Hepatic scavenger receptor class B type I (SR-BI) binds HDL particles that mediate reverse cholesterol transport and thus lowers the risk of atherosclerosis. Here we examined glucose regulation of SR-BI gene expression in both HepG2 cells and whole animals. Results showed that hepatic SR-BI mRNA, protein, and uptake of cholesterol from HDL were halved following 48 h of exposure to 22.4 vs. 5.6 mM glucose. As in the case of the cell culture model, hepatic expression of SR-BI was lower in diabetic rats than in euglycemic rats. Transcriptional activity of the human SR-BI promoter paralleled endogenous expression of the gene, and this activity was dependent upon the dose of glucose. Next, we used inhibitors of select signal transduction pathways to demonstrate that glucose suppression of SR-BI was sensitive to the p38 MAPK inhibitor. Expression of a constitutively active p38 MAPK inhibited SR-BI promoter activity in the presence or absence of glucose. A dominant-negative p38 MAPK abolished the inhibitory effect of glucose on promoter activity. Deletional analysis located a 50-bp fragment of the promoter that mediated the effects of glucose. Within this DNA fragment there were several specificity protein-1 (Sp1) binding sites, and cellular knockdown of Sp1 abrogated its suppression by glucose. Together, these results indicate that the glucose suppression of SR-BI expression is partially mediated by the activation of the p38 MAPK–Sp1 pathway and raise the possibility that the inhibition of hepatic SR-BI expression under high-glucose conditions provides a mechanism for accelerated atherosclerosis in diabetics.

high-density lipoprotein; diabetes; atherosclerosis

Epidemiological data from the Framingham Study (34) and the Multiple Risk Factor Intervention Trial (50) indicate that the risk for cardiovascular death is increased two- to threefold in patients with type 2 diabetes mellitus (DM). Hyperglycemia is a major risk factor for cerebrovascular disease (43), and selected key pathways, factors, and mechanisms have been implicated, such as oxidant stress (4), advanced glycation end products (9), aldose reductase (23), reductive stress (24), carbonyl stress (5), and protein kinase C (PKC) activity (31), including the p38 mitogen-activated protein kinase (MAPK) pathway. The p38 MAPK protein is a member of the mitogen-activated serine/threonine protein kinase family that is activated following dual phosphorylation of Thr180 and Tyr182 by an upstream MAPK kinase. In endothelial cells, this pathway is activated by stress-inducing stimuli, including reactive oxygen species, hyperglycemia, and proinflammatory cytokines, such as tumor necrosis factor (TNF) (51). Activation of p38 MAPK regulates genes that mediate inflammation and the recruitment of other inflammatory signaling pathways such as nuclear factor-κB and arachidonate-signaling (13). Thus, activation of the p38 MAPK pathway by hyperglycemia could contribute significantly to the development of the vascular complications of diabetes (45).

Mouse scavenger receptor class B type I (SR-BI) mediates selective uptake of the high-density lipoprotein (HDL) cholesterol ester (CE) into transfected chines hamster ovary cells. This finding (1, 33, 35) provides an important link between a specific cell surface receptor and the uptake of HDL. Our previous report (25–29, 40) shows that the human homolog of SR-BI (hSR-BI), CD36 and LIMP II analogous-1 (CLA-1), like the mouse homologue, functions as a receptor for HDL. Overexpression of SR-BI in mouse liver dramatically decreases plasma HDL (35, 56) and increases hepatic, gallbladder, and biliary cholesterol concentrations (35, 48). SR-BI is a well-characterized HDL receptor that is highly expressed in the liver and in steroidogenic tissues in rodents (54). Its human orthologue CLA-1 (hSR-BI) has also been shown (40) as a receptor for HDL. Despite the fact that hSR-BI has not been studied as extensively as rodent SR-BI, the physiological role of hSR-BI is generally assumed to be similar to that of rodent SR-BI.

Several studies (3, 16, 53) have established the role of high plasma cholesterol and triglyceride levels, and of low plasma HDL levels, as risk factors for atherosclerosis in DM. HDL plays a critical role in cholesterol metabolism because it mediates a normal physiological process, namely reverse cholesterol transport (20, 52). In this process, HDL particles shuttle cholesterol from extrahepatic tissues to the liver for further metabolism and excretion (20). Several studies (36) have indicated that hSR-BI/CLA-1 stimulates hepatic uptake of HDL free cholesterol and its transport into bile. This information prompted us to speculate whether glucose further regulates hSR-BI. We expect that answers to this query may shed more light on the high risk of atherosclerotic disease in the presence of DM.

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MATERIALS AND METHODS

Materials. Wortmannin, LY-294002, bisindolylmaleimide I, and SB-203580 were purchased from Calbiochem. α-Amanitin was purchased from Sigma (St. Louis, MO) and used at 2 μg/ml.

Cell culture. Human hepatoma HepG2 cells (obtained from Riken Cell Bank, Ibaragi, Japan) were grown in DMEM (Life Technologies, Tokyo, Japan) supplemented with 10% heat-inactivated fetaled bovine serum (Dainippon Pharmaceutical, Osaka, Japan).

Western blot analysis. An antibody directed against the extracellular domain of hSR-BI between amino acid residues 185 and 300 of the reported sequence of the isomer containing 509 amino acid residues (10) was generated. The corresponding cDNA fragment was amplified from THP-1 cDNA by PCR. The amplified fragment was inserted into a pGEX-2T vector (Pharmacia) and sequenced, and the protein was expressed in Escherichia coli. The fusion protein was isolated with glutathione-Sepharose 4B beads (Pharmacia) and used to generate an antisera in guinea pigs as described previously (40). The membranes were blocked with 0.1% Tween-20 in PBS containing anti-hSR-BI antibody (diluted 1/3,000 from whole antiserum) (40) or anti-GAPDH antibody (diluted 1/1,000; Biomol Research, Plymouth Meeting, PA).

Northern blot analysis. A full-length cDNA of hSR-BI was synthesized by PCR using reverse-transcribed RNA from HepG2 cells and labeled with [32P]dCTP (3,000 Ci/mmol) by the random priming method (41). Electrophoresis and hybridization were performed as described (41). Blots were also probed with human GAPDH to ensure equal loading of the RNA samples (41).

Real-time reverse transcriptase-polymerase chain reaction. Polymerase chain reactions (PCR) were performed in LightCycler (Roche, Mannheim, Germany) glass capillaries. The reaction mixture consisted of 2 μl of LightCycler-FastStart DNA Master SYBR Green I (Roche), 2 μl of the cDNA template for each gene of interest, and 1 μl of 10 μm each primer. The sequences of the forward and reverse hSR-BI primers were 5′-TGAGGCGGGGATGTGAG; synthesized by Life Technologies) (41). The membranes were blocked with 0.1% Tween-20 in PBS containing anti-hSR-BI antibody (diluted 1/3,000 from whole antiserum) (40) or anti-GAPDH antibody (diluted 1/1,000; Biomol Research, Plymouth Meeting, PA).

Electrophoretic mobility shift analysis. An expression vector encoding a constitutively active p38 MAPK (α, β, γ) and a dominant-negative mutant of p38 MAPK β (p38-DN) were kindly provided by Dr. Z. Wu (Hong Kong University of Science & Technology). An expression vector encoding specificity protein-1 (Sp1) was described previously (47).

Protocol for diabetic animals. Four-week-old male Long-Evans Tokushima Otsuka (LETO) rats (n = 4) and Otsuka Long-Evans Tokushima Fatty (OLETF) rats (n = 4) were supplied by Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). All rats were kept in specific pathogen-free conditions at Kagawa University Animal Center in rooms controlled for both temperature and light (on from 8:00 AM to 8:00 PM). The experiments were approved by the local animal ethics committee. Some rats (OLET n = 4, OLETF n = 4) were provided with sucrose (20% sucrose solution as drinking water) for 4–20 wk of age. In OLETF rats (n = 4), food intake was restricted to 70% of average daily consumption for 8–16 wk of age. Otherwise, the rats had free access to tap water and a diet of standard chow (Clea Japan, Tokyo, Japan).

RESULTS

Effects of high-glucose conditions on hSR-BI expression in HepG2 cells. We measured the endogenous expression of SR-BI in HepG2 cells by Western blot analysis to determine whether glucose affected hSR-BI expression. There was an inverse correlation between hSR-BI expression and the concentration of glucose to which the cells were exposed. hSR-BI in the cells treated for 24 h with 22.4 mM glucose decreased to approximately one-half of that in the cells treated with the control medium containing 5.6 mM glucose (Fig. 1A); however, no such effect was observed in the cells treated with the osmotic control, i.e., mannitol.

To further confirm this observation, we used Northern blot analysis to assess hSR-BI CLA-1 mRNA in the cells. Results (Fig. 1B) showed a marked decrease in the abundance of hSR-BI mRNA following treatment with 22.4 mM glucose (Fig. 1B); however, GAPDH expression remained unaltered.
examined in the absence or presence of high glucose concentrations after mRNA synthesis had been blocked by α-amanitin, an RNA polymerase II inhibitor. High glucose concentrations did not affect hSR-BI mRNA stability, indicating that downregulation of hSR-BI mRNA is not due to accelerated mRNA degradation. Previous reports, including reports from our laboratory (40, 42), showed that cells take up CE from HDL by a selective nonendocytotic pathway. To test whether high glucose concentration-mediated inhibition of hSR-BI expression affected this process, we measured the kinetics of CE uptake using doubly labeled HDL. Results showed that cellular HDL-CE uptake (Fig. 2) following high-glucose treatment was decreased compared with the control cells. These findings support the idea that high-glucose treatment inhibits the surface expression and function of hSR-BI in hepatocytes.

Since glucose lowered the abundance of both hSR-BI protein and mRNA in HepG2 cells, we speculated whether glucose regulated transcriptional activity of the hSR-BI promoter in HepG2 cells. For these studies, we measured luciferase activity in the HepG2 cells transfected with pCLA-LUC and exposed to varying concentrations of glucose (Fig. 3A). In agreement with the protein and mRNA levels, high-glucose concentration-mediated decrease in hSR-BI promoter activity is significant at 11.2 and 22.4 mM glucose concentrations. Each data point shows the mean ± SE of 3 separate experiments.

Fig. 2. Selective uptake of HDL cholesterol ester (CE). The cells treated with the indicated glucose concentrations were incubated at 37°C for 1.5 h with 10 μg/ml of [125I,3H]HDL, followed by processing to determine HDL CE selective uptake. Values represent the mean of triplicate determinations. *Significant difference (P < 0.01).

Fig. 1. Effects of glucose on human homolog of scavenger receptor class B type I (hSR-BI) expression in HepG2 cells. A: dose-dependent suppression of hSR-BI protein by glucose. HepG2 cells were seeded in 6-well plates and exposed to the indicated amounts of glucose for 24 h. hSR-BI in the total cell lysate was detected by Western blot analysis probed with an anti-hSR-BI antibody. Abundance of GAPDH served as the control and is shown at the bottom of each lane. The hSR-BI/GAPDH ratio is shown as %control in the figure. Each data point shows the mean ± SE of 3 separate experiments. *Significant difference (P < 0.01). B: hyperglycemia decreases hSR-BI mRNA expression. hSR-BI mRNA was quantified by Northern blot analysis and normalized to GAPDH mRNA. A graph showing the mean ± SE of 3 experiments for each treatment group is shown. *Significant difference (P < 0.01). C: hSR-BI mRNA stability under high-glucose treatment and the effect of high glucose on the rate of decay of hSR-BI mRNA in HepG2 cells. HepG2 cells were incubated in the medium with 5.6 mM glucose for 24 h (starting time 0 h) followed by incubation in 5.6 or 22.4 mM glucose for the indicated periods of time in the presence of 2 μg/ml of α-amanitin. RNA at each point of time was analyzed by real-time PCR methods, and relative hSR-BI mRNA abundance (normalized to β-actin mRNA) was expressed as %hSR-BI mRNA present at 0 (%maximum value), 6, 12, and 24 h. •, 5.6 mM glucose + α-amanitin; ○, 22.4 mM glucose + α-amanitin. Each data point shows the mean ± SE of 3 separate experiments.
The inhibition of promoter activity by glucose was observed in HepG2 cells. This inhibition was mediated by p38 MAPK signaling. To further investigate the mechanism of glucose suppression on hSR-BI gene activity, various signaling inhibitors were used. The results showed that glucose suppression persisted in the presence of PI3K and PKC inhibitors but was blocked by SB203580, a p38 MAPK inhibitor. This suggests that p38 MAPK may mediate high glucose-induced suppression of hSR-BI promoter activity.

Kinetics of p38 MAPK phosphorylation by high-glucose treatment. To study the kinetics of p38 MAPK phosphorylation, HepG2 cells were exposed to 22.4 mM glucose for different time points. Results showed that p38 MAPK phosphorylation was evident within 15 min and peaked at 30 min following exposure to high glucose. These findings support the notion that glucose induces p38 MAPK catalytic activity.

p38 MAPK regulates hSR-BI promoter activity. The use of constitutively active forms of p38 MAPK was tested to mimic the effects of glucose. This approach showed that p38 MAPK activity is essential for the inhibitory effects of glucose on hSR-BI.

Fig. 3. Effect of hyperglycemia on hSR-BI transcriptional activity in HepG2 cells. A: hyperglycemia decreases hSR-BI gene transcription. B: a p38 MAPK inhibitor blocks the actions of hyperglycemia; effects of the phosphatidylinositol 3-kinase inhibitors Wortmannin (WM) or LY-294002 (LY), the PKC inhibitor bisindolylmaleimide I (BIS), and the p38 MAPK inhibitor SB-203580 (SB) with 5.6 or 22.4 mM glucose on hSR-BI transcriptional activity in HepG2 cells. Vehicle: 0.1% DMSO. Each data point shows the mean ± SEM of 3 separate transfections that were performed on separate days. *Significant difference (P < 0.05).

Fig. 4. Hyperglycemia stimulates the phosphorylation of p38 MAPK. Cells were exposed to 22.4 mM glucose for 0, 5, 15, 30, 60, and 120 min. Abundance of phosphorylated p38 MAPK was detected by Western blot analysis of total cell protein using a phosphospecific p38 MAPK antibody (phosph-p38; top). To show equal loading of the protein in each lane, the same blot was probed a second time with a p38 MAPK-specific antibody. The phospho-p38 MAPK/p38 MAPK ratio is shown as %basal in the figure. Each data point shows the mean ± SEM of 3 separate experiments.
test this hypothesis, we expressed constitutively active forms of p38 MAPK (p38α, -β, and -γ) in HepG2 cells carrying pCLA-LUC. Results showed that the constitutively active form of p38 MAPK suppressed hSR-BI promoter activity in the HepG2 cells (Fig. 5A). The p38 MAPK β-isoform appeared to most potently suppress hSR-BI promoter activity. The converse of this study would be on a dominant-negative mutant of p38 MAPK (p38-DN) to block the actions of glucose on hSR-BI promoter activity (Fig. 5B). Consistent with previous results, high-glucose treatment inhibited hSR-BI promoter activity; however, the expression of p38-DN masked the ability of high glucose to suppress hSR-BI promoter activity. Together, these findings support the idea that the p38 MAPK pathway is required for glucose suppression of hSR-BI promoter activity in HepG2 cells.

A DNA motif mediated glucose suppression of hSR-BI promoter activity. Since the preceding data point to the hSR-BI promoter as the site of action of the p38 MAPK cascade, we searched for a cis-acting site(s) within the DNA that may mediate the effects of high glucose. Therefore, templates were created by serial deletions of the hSR-BI promoter contained within pCLA-LUC. Constructs arising from the removal of 250, 725, and 992 base pairs from pCLA-LUC yielded the templates pCLA2-LUC, pCLA4-LUC, and pCLA6-LUC, respectively. Glucose suppression of the activity of pCLA-LUC, and of the pCLA2-LUC, and pCLA4-LUC deletion constructs was indistinguishable at 40 ± 4, 50 ± 7, and 55 ± 5%, respectively (Fig. 6). However, glucose could not suppress the activity of the pCLA6-LUC deletion construct (−209 to +2). Together, these observations show that glucose suppression of hSR-BI promoter activity requires the −476 to −210 fragment of the promoter.

Sp1 mediated high glucose-induced suppression of hSR-BI gene expression. Previous studies (47) have shown that the transcription factor Sp1 may mediate the actions of glucose on hSR-BI promoter activity. Therefore, we conducted a DNA homology search that revealed the presence of six putative Sp1 binding sites within the hSR-BI promoter. To determine whether Sp1 mediates the actions of glucose on hSR-BI pro-
Fig. 7. Transcriptional factor specificity protein-1 (Sp1) is involved in high-glucose treatment-induced suppression of hSR-BI transcriptional activity. 

A: dose-dependent induction of Sp1 protein in nuclear protein by glucose. HepG2 cells were exposed to the indicated amounts of glucose for 24 h. Sp1 in nuclear protein was detected by Western blot analysis probed with an anti-Sp1 antibody. Abundance of TFIIID served as the control and is shown at the bottom of each lane. The Sp1/TFIID ratio is shown as %control in the figure. Each data point shows the mean \pm SE of 3 separate experiments. *Significant difference (P < 0.01).

B: effects of glucose on Sp1 binding to the Sp1 probe. Cells were exposed to varying concentrations of glucose as indicated (lane 1, only the probe; lane 2, 5.6 mM glucose; lane 3, 11.2 mM glucose; lane 4, 22.4 mM glucose) for 24 h before the nuclear extracts were prepared. The binding activity of Sp1 was examined by EMSA, as described in MATERIALS AND METHODS. Abundance of OCT1 served as the control and is shown at the bottom of each lane. An identical experiment that was independently performed yielded similar results.

C: deletion of the −476 to −210 fragment of the hSR-BI promoter abolished the response to Sp1. HepG2 cells were transfected with 1 μg of several constructs (vector, CLA, CLA2, CLA4, or CLA6) and cotransfected with the Sp1 expression vector. All assays were corrected for β-galactosidase activity, and the total amount of protein in every reaction was identical. The results were expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean \pm SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01).

D: effects of Sp1 knockdown on hSR-BI promoter activity in HepG2 cells. siRNA for Sp1 or scramble siRNA (sc) was transfected into HepG2 cells with the pCLA-LUC plasmid for 24 h prior to cell harvesting. Top: efficiency of siRNA on Sp1 expression by Western blot analysis. All assays were corrected for β-galactosidase activity, and the total amount of protein in every reaction was identical. The results are expressed as relative luciferase activity compared with the control cells arbitrarily set at 100. Each data point shows the mean \pm SE of 4 separate transfections that were performed on separate days. *Significant difference (P < 0.01).
moter activity, we began by measuring the abundance of the Sp1 protein in HepG2 cells by Western blot analysis following treatment with varying concentrations of glucose. Results showed that glucose induced the abundance of Sp1 in a dose-dependent manner (Fig. 7). In contrast, basal transcriptional factor TFIID was affected to a very small extent by glucose (Fig. 7A).

To confirm this observation, electrophoretic mobility shift analysis (EMSA) with a specific Sp1 binding motif was performed. The Sp1 binding activities were assayed in the nuclear extracts prepared from the HepG2 cells exposed to 5.6, 11.2, or 22.4 mM glucose for 12 h. Results showed that glucose stimulated Sp1 DNA binding activity (Fig. 7B). In contrast, the DNA binding activity of the transcription factor OCT1 to its motif was not affected by glucose.

To determine whether Sp1 affects the transcriptional activity of the hSR-BI promoter and to locate the site that mediates the actions of Sp1, we cotransfected the above deletion constructs into the HepG2 cells along with the Sp1 expression vector. Results (Fig. 7C) showed that Sp1 expression inhibited the activity of pCLA-LUC, pCLA2-LUC, and pCLA4-LUC but not of pCLA6-LUC. To further explore the role of Sp1 in glucose suppression of hSR-BI promoter activity, we used siRNA to block Sp1 expression. Results showed that Sp1 expression was attenuated by a specific Sp1 siRNA but not by a scrambled siRNA (Fig. 7D). Next, the HepG2 cells were exposed to Sp1-specific or scrambled siRNA and then treated with 22.4 mM glucose. Results (Fig. 7D) showed that hSR-BI promoter activity was inhibited in the cells exposed to the control siRNA following treatment with 22.4 mM glucose. However, the inhibitory activity of 22.4 mM glucose was attenuated in the cells carrying Sp1 siRNA. These findings are in accordance with the idea that the glucose suppression of hSR-BI gene expression requires Sp1.

**Effect of high glucose on hSR-BI expression in vivo.** Although in vitro experiments on cultured cells, as mentioned above, have helped to elucidate the action of glucose, it remains unknown whether glucose has the same effect on an in vivo model. To answer this question, we used OLETF rats, an animal model of DM, and their lean nondiabetic counter parts, LETO rats. Sucrose feeding markedly increased blood glucose levels in OLETF rats (18.2 ± 1.2 mmol/l) compared with that in LETO rats (6.8 ± 0.4 mmol/l), as previously reported (32). We speculated whether hyperglycemia in the sucrose-fed animals would decrease hSR-BI mRNA and protein, as observed in the in vitro model. First, we measured the levels of endogenous SR-BI mRNA expression in the liver of these animals by a real-time PCR method. As expected, hepatic SR-BI mRNA in OLETF rats was decreased compared with that in the euglycemic controls (Fig. 8A). Furthermore, the abundance of the SR-BI protein also decreased in OLETF rats (Fig. 8B). These findings show that, in rats, hyperglycemia decreases hepatic SR-BI expression, and this finding is similar to the observations of the in vitro study on cultured HepG2 cells.

**DISCUSSION**

In this report, we summarized the results of the studies on glucose suppression of hSR-BI gene transcription. The suppressive effects of glucose on hSR-BI promoter activity in the HepG2 cells require the participation of the p38 MAPK cascade and the transcription factor Sp1. More importantly, the observations in vitro were extended to an in vivo diabetic rat model to demonstrate that hyperglycemia suppresses hSR-BI mRNA expression. Controversially, Milliat et al. (38), had reported that early and strong enhancement of hepatic SR-BI expression was observed in streptozotocin-induced diabetes in hypercholesterolemic rats. The discrepancy between our findings and those of that previous report may be due to the differences in the experimental methods and the rat species used. Further investigations will be needed to clarify the regulation of SR-BI by glucose in vivo.

Recent reports show that the p38 MAPK pathway is activated in response to hyperglycemia and in models of DM. In vascular smooth muscle cells, exposure to high glucose concentrations induces the activation of p38 MAPK (6). Another report (30) indicated that high glucose causes a fourfold increase in p38 MAPK in rat aortic smooth muscle cells. p38 MAPK activity was increased in the glomeruli of diabetic rats compared with the controls. In the same cells, the investigator observed increased phosphorylation of heat shock protein 25, a downstream substrate of p38 MAPK (15). Although the published findings pointed toward the involvement of the p38 MAPK cascade in mediating the actions of glucose, more
direct and detailed evidence was needed to further define its role. In the current study, we demonstrate clearly the intermediary role of p38 MAPK in glucose suppression of hSR-BI promoter activity by using both constitutively active and dominant-negative forms of p38 MAPK on hSR-BI promoter activity (Fig. 5). Our data support the model that hyperglycemia activates p38 MAPK, which, in turn, triggers a series of steps to suppress hSR-BI promoter activity. In agreement with this hypothesis, the constitutively active form of p38 MAPK mimics the inhibitory action of hyperglycemia on hSR-BI promoter activity, whereas its dominant-negative mutant blocks the effect of glucose. Although we have pointed out the role of p38 MAPK on hSR-BI expression, a recent report (49) indicated that PI3K activation posttranslationally stimulates hepatic SR-BI function by regulating subcellular localization of SR-BI in a PI3K-dependent manner. Further studies will be needed to determine the detailed regulatory mechanisms of hepatic SR-BI expression.

Sp1 plays a vital role in the regulation of transcription from TATA-less promoters that commonly encode housekeeping genes (12). Cellular Sp1 activity and concentration are regulated during development, cellular proliferation, apoptosis, and other cellular processes (8, 17). Sp1 is involved in mediating responses to various stimuli, including induction of the transforming growth factor-β receptor gene (7), epidermal growth factor-mediated expression of the gastrin gene (13), and cAMP-dependent induction of the CYP11A gene (2). A recent report (37) showed that p38 MAPK participates in the LPS-induced activation of Sp1, which, in turn, regulates the transcription of the hIL-10 gene. Another report detailed the relationship between p38 MAPK and Sp1 in that the activation of the p38 MAPK cascade enhances Sp1 phosphorylation and mediates the interaction of Sp1 with p38 MAPK. These processes are essential for force-induced filamin expression in fibroblasts (14).

On the basis of several reports that have detailed the connection between p38 MAPK and Sp1, we speculated whether p38 MAPK and Sp1 were components of the pathway leading to glucose suppression of hSR-BI promoter activity. Deletional studies on the hSR-BI promoter led to the identification of a 266-bp DNA fragment (−476 to −210) that is required for the effects of p38 MAPK, and embedded within this sequence were six putative Sp1 binding sites. This finding stands in contrast with the feature of other cellular genes, i.e., the fact that they are regulated by multiple transcription factors. Our studies do not exclude the participation of other factors that may cooperate with Sp1 to regulate hSR-BI transcription. Such factors may remain masked in transient transfections because this approach is known to generate high plasmid copy numbers and bring about the accumulation of aberrant chromatin structure in these cells.

Efflux of cellular free cholesterol from peripheral cells to the acceptor HDL particles is the first step in reverse cholesterol transport and requires the binding of HDL to the hepatocyte membrane. Several reports (1, 35) have suggested that rodent SR-BI is functionally related to hSR-BI, and this protein is likely to be the receptor that selectively takes up the HDL cholesterol ester. SR-BI is believed to act as a docking receptor for HDL (52).

Hepatic overexpression of SR-BI causes increased biliary secretion of cholesterol without a concomitant increase in the secretion of phospholipids or bile salts (48). SR-BI enhances the exchange of cholesterol between the surface of HDL and the cell. SR-BI deficiency did not affect the expression of the key regulators of hepatic cholesterol homeostasis, including HMG-CoA reductase, the low-density lipoprotein (LDL) receptor, and cholesterol 7α-hydroxylase (18). However, SR-BI deficiency reduced the cholesterol content in bile by ~40%. Rigotti et al. (44) generated mice with a targeted null mutation in the SR-BI gene and showed that plasma cholesterol concentrations were increased because of the formation of large apolipoprotein A-I containing particles in heterozygous and homozygous mutants relative to the wild-type controls. The plasma concentration of apolipoprotein A-I, the major protein in HDL, remained unchanged in the mutants. These data, in conjunction with the increased lipoprotein size, suggested that the higher plasma cholesterol concentration in the mutants was due to decreased selective cholesterol uptake. These results suggest that SR-BI may stimulate hepatic uptake of HDL free cholesterol and its transport into bile.

Several potential links between diabetes and atherosclerosis have been identified, and many clinical observations point toward the correlation between the risk of vascular complications in diabetes and poor glycemic control. A primarily important step in the development of atherosclerosis is the uptake of modified plasma LDL by monocyte-derived macrophages in the vascular wall (55). This process is largely mediated by the cell surface scavenger receptor CD36. Griffin et al. (21) recently reported a fivefold increase in macrophage CD36 expression after prolonged exposure to high glucose concentrations (11 and 33 mmol/l) in vitro, leading to increased translational efficiency of CD36 mRNA. It is possible that the increased monocyte CD36 expression observed in DM could be due to this mechanism. A previous report (46) indicated a significant increase in the baseline expression of the monocyte-macrophage LDL scavenger receptor CD36 on peripheral venous monocytes of patients with type 2 DM. Both good “HDL” and bad “modified LDL” metabolism may be affected by a hyperglycemic state, such as in DM.

In summary, the results of this study show that hyperglycemia inhibits hepatic endogenous hSR-BI expression. This inhibitory effect of hyperglycemia on hSR-BI promoter activity is mediated by the p38 MAPK/Sp1 signaling cascade. These findings raise the possibility that hyperglycemia may affect reverse cholesterol transport by controlling hSR-BI expression in diabetic patients.

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