The publication of this article was made possible by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Cyclosporin-induced dyslipoproteinemia is associated with selective activation of SREBP-2

JINMEI WU, YONG HONG ZHU, AND SHAILENDRA B. PATEL
Division of Endocrinology, Diabetes, and Medical Genetics, Medical University of South Carolina, Charleston, South Carolina 29425-2222

Wu, J inimei, Yong Hong Zhu, and Shailendra B. Patel.
Cyclosporin-induced dyslipoproteinemia is associated with selective activation of SREBP-2. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E1087–E1094, 1999.—The use of cyclosporin A has contributed greatly to the success of organ transplantation. However, cyclosporin-associated side effects of hypertension, nephrotoxicity, and dyslipoproteinemia have tempered these benefits. Cyclosporin-induced dyslipoproteinemia may be an important risk factor for the accelerated atherosclerosis observed posttransplantation. Using a mouse model, we treated Swiss-Webster mice for 6 days with a daily dose of 20 μg/g body wt of cyclosporin and observed significant elevations of plasma cholesterol, triglyceride, and apolipoprotein B (apoB) levels relative to vehicle-alone treated control animals. Measurement of the rate of secretion of very low-density lipoprotein (VLDL) by the liver in vivo showed that cyclosporin treatment led to a significant increase in the rate of hepatic VLDL triglyceride secretion. Total apoB secretion was unaffected. Northern analysis showed that cyclosporin A treatment increased the abundance of hepatic mRNA levels for a number of key genes involved in cholesterol biosynthesis relative to vehicle-alone treated animals. Two key transcriptional factors, sterol regulatory element-binding protein (SREBP)-1 and SREBP-2, also showed differential expression; SREBP-2 expression was increased at the mRNA level, and there was an increase in the active nuclear form, whereas the mRNA and the nuclear form of SREBP-1 were reduced. These results show that the molecular mechanisms by which cyclosporin causes dyslipoproteinemia may, in part, be mediated by selective activation of SREBP-2, leading to enhanced expression of lipid metabolism genes and hepatic secretion of VLDL triglyceride.

very low-density lipoprotein; sterol regulatory element-binding protein; immunotherapy; heart disease; transcription; liver

Cyclosporin A treatment for the prevention of organ rejection has revolutionized the success of transplantation therapy (18). However, this success is associated with an increase in three important side effects, namely hypertension, nephrotoxicity, and dyslipoproteinemia (17, 27, 37). The subsequent increased morbidity from cardiovascular disease is of major concern (19). We have investigated the mechanism by which cyclosporin use may be responsible for one of these risk factors, namely dyslipoproteinemia (2, 9, 13, 14, 19, 25). This pathological change may be one of the many reasons for the increased cardiovascular morbidity and mortality associated with solid organ transplantation. Elucidation of the mechanism of cyclosporin-induced dyslipoproteinemia may allow the identification of pathways that are inappropriately activated by cyclosporin, and this knowledge may allow the development of better immunosuppressive agents. In humans, cyclosporin treatment is associated with an increase in both plasma low-density lipoprotein (LDL) cholesterol as well as triglycerides (2, 27). Although the concomitant use of other drug therapies, such as steroid use, complicates the issue of whether cyclosporin per se is responsible for causing the dyslipoproteinemia (10, 27), the majority of the clinical evidence strongly implicates cyclosporin as the causative agent. Clearly either an increase in production or a reduction in the clearance of lipoprotein particles from the plasma, or a combination of both, may be responsible for the dyslipoproteinemia. Studies of lipoprotein particle clearance in rats treated with cyclosporin show that the fractional catabolic rate of LDL was reduced by drug treatment, as was calculated LDL production rates (24). Interestingly, in the same study, in vitro analysis of LDL-receptor-mediated clearance of LDL isolated from cyclosporin-treated animals by cultured normal fibroblasts showed it to be increased, not reduced, suggesting that LDL containing cyclosporin was cleared at a faster rate (24). In vitro studies with Hep G2 cells show that cyclosporin can reduce LDL-receptor activity in a dose-dependent manner, although the precise mechanism may not involve reduced 27-hydroxycholesterol levels (1, 43). Because most LDL particles are cleared by the liver in vivo, and not by fibroblasts, the above observations are compatible.

Cyclosporin has also been shown to inhibit apolipoprotein B (apoB) secretion from permeabilized Hep G2 cells (26) and has been implicated in causing cholestasis, with reduced bile acid synthesis, based on in vitro studies of inhibition of sterol 27-hydroxylase (2, 21, 31). Furthermore, LDL is produced in the plasma from very low-density lipoprotein (VLDL) as a result of metabolic cascades, and it is unclear whether any overproduction of VLDL is caused by cyclosporin. If cyclosporin inhibits apoB secretion (26), the reduced VLDL secretion should lead to an amelioration of any loss of LDL-receptor activity.

To investigate the mechanisms involved in cyclosporin-induced dyslipoproteinemia, we treated Swiss-Webster mice with cyclosporin or vehicle for 6 days. Cyclosporin A-treated mice showed significantly higher fasting plasma levels of cholesterol, triglyceride, and apoB. Measurement of the hepatic rates of secretion of VLDL triglyceride and apoB, as well as mRNAs for a number of hepatic genes involved in lipoprotein synthet-
sis and secretion, implicated a transcriptional mechanism as the cause of the dyslipoproteinemia.

**METHODS**

**Materials.** Cyclosporin was a kind gift from Dr. David Weinstein (Sandoz, NJ). Tyloxapol (WR1339) was obtained from Sigma (St. Louis, MO). All other reagents were purchased from commercial sources and were of reagent grade or better.

**Measurement of hepatic VLDL secretion in vivo.** All animals used in this study were fasted for 2 h and measurement of hepatic VLDL secretion rates of VLDL with tyloxapol injection was performed as previously described in detail (22, 23). Briefly, tyloxapol was injected at a dose of 400–600 mg/kg, and blood was sampled preinjection and at various time points thereafter (30, 90, 150, and 210 min) for triglyceride determination. At the end of 5 h, animals were euthanized, and blood was obtained by exanguination and portions of livers were flash-frozen in liquid nitrogen and stored at −80°C for subsequent RNA analyses. VLDL was isolated from plasma of each animal obtained at the 5-h time point. Loss of VLDL during ultracentrifugation and harvesting was corrected for by determining the plasma triglyceride before centrifugation and the ultracentrifugation and harvesting was corrected for by determining the plasma triglyceride before centrifugation and the serum triglyceride was measured enzymatically as previously described (22). Aliquots of VLDL obtained from each animal were analyzed from frozen or freshly harvested liver, with Trizol (GIBCO-BRL, Bethesda, MD), according to the conditions of the manufacturer. Ten micrograms of RNA were denatured and separated by size on 1% formaldehyde-agarose gels and transferred to Hybond-N with standard techniques. Northern analysis was performed with radiolabeled probes and quantitated by phosphorimager analysis (Molecular Dynamics, Sunnyvale, CA). All data were normalized to the signal obtained with a glyceraldehyde-3-phosphate dehydrogenase probe. Mouse cDNA probes for hydroxymethylglutaryl (HMG)-CoA reductase, HMG-CoA synthase, LDL receptor, squalene synthase, sterol regulatory element-binding protein (SREBP)-1 and -2, acetyl-CoA carboxylase, and fatty acid synthase have been previously described (36) and were gifts from Drs. Hitoshi Shimano and Jay Horton (University of Texas Southwestern Medical Center, Dallas, TX). Murine microsomal triglyceride transfer protein (MTP) cDNA was a gift from Dr. Narayan Hariharan (Bristol-Myers Squibb, Princeton, NJ), and the murine hepatic lipase probe was kindly provided by Dr. J imin Gao (University of Texas Southwestern Medical Center).

**Western blotting.** Extractions of mouse hepatic membranes and nuclear fractions for the detection of SREBPs were performed as previously described (16, 36), except that ~100–mg portions of fresh liver from five control or five cyclosporin A-treated animals were pooled and homogenized in buffer A, containing protease inhibitors. Aliquots of liver were also taken for subsequent RNA analysis. Nuclear or membrane fractions were prepared as described, and the protein concentration was determined with a bicinchoninic acid kit (Pierce Chemical, Rockford, IL). Aliquots of nuclear or membrane extracts (30 µg) from control and cyclosporin A-treated groups were separated by 8% SDS-PAGE, blotted onto Hybond-C membranes, and stained with amido black for verification of protein loading. Blotted membranes were incubated with rabbit polyclonal antibodies to SREBP-1 or -2, and washed, and bound antibodies were detected with horseradish-peroxidase-labeled donkey anti-rabbit antibody and enhanced chemiluminescence detection (Amersham, Arlington Heights, IL). For detection of SREBP cleavage-activating protein (SCAP), membrane fractions were blotted and incubated with primary antibody to SCAP as previously described (35).

**Statistical analysis.** Statistical analysis between control and treated groups was performed with unpaired two-tailed t-test and Prizm software (GraphPad Software, San Diego, CA) and expressed as means ± SD. Significance was set at P < 0.05.

**RESULTS**

Cyclosporin treatment induces a combined hyperlipidemia. Table 1 shows the baseline characteristics of the control and cyclosporin-treated animals after 6 days of

Table 1. Baseline characteristics of animals used in this study

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Body Wt, g</th>
<th>Liver Wt, g</th>
<th>Plasma Triglyceride, mg/dl</th>
<th>Plasma Cholesterol, mg/dl</th>
<th>Plasma ApoB-100, pmol/l*</th>
<th>Plasma ApoB-48, pmol/l*</th>
<th>Total ApoB, pmol/l*</th>
<th>Ratio of ApoB-48/100*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated (n = 14)</td>
<td>8</td>
<td>23.8 ± 2.0</td>
<td>1.19 ± 0.12</td>
<td>116 ± 27</td>
<td>77 ± 11</td>
<td>1.4 ± 0.5</td>
<td>8.5 ± 1.4</td>
<td>9.9 ± 1.7</td>
</tr>
<tr>
<td>Cyclosporin treated (n = 13)</td>
<td>8</td>
<td>24.4 ± 1.8</td>
<td>1.19 ± 0.14</td>
<td>175 ± 56</td>
<td>107 ± 17</td>
<td>3.4 ± 0.7</td>
<td>16.5 ± 5.2</td>
<td>19.9 ± 5.8</td>
</tr>
</tbody>
</table>

*P value | 0.40 | 0.95 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.091

Values are means ± SE; n = no. of animals. ApoB, apolipoprotein B. Age-matched female Swiss-Webster mice were treated with vehicle alone (control) or cyclosporin in vehicle (treated) as described in METHODS. Fasting plasma triglyceride, cholesterol, and apoB levels were determined at end of treatment period. Significant differences in plasma triglyceride, cholesterol, and apoB were noted in treated group, relative to control. *Four animals per group were used to determine apoB levels.
treatment. No significant differences were observed in the body weights or liver weights between control and treated animals. Cyclosporin-treated animals showed a significant increase in fasting triglyceride (175 ± 56 vs. 116 ± 27 mg/dl, \( P < 0.05 \)) and cholesterol (107 ± 17 vs. 77 ± 11 mg/dl, \( P < 0.05 \)) levels. Additionally, baseline plasma apoB-48 (16.5 ± 5.2 vs. 8.5 ± 1.4 pmol/l, \( P < 0.05 \)) and apoB-100 concentrations (3.4 ± 0.7 vs. 1.37 ± 0.5 pmol/l, \( P < 0.05 \)) were also higher. An increase in the proportion of apoB-100 relative to apoB-48 was also detected in the treatment group. Hence, treatment of mice with cyclosporin resulted in dyslipoproteinemia, characterized by increased cholesterol, triglyceride, and apoB levels.

Cyclosporin results in an increased hepatic VLDL triglyceride secretion rate. The Triton method was used to measure hepatic VLDL triglyceride secretion rate in vivo (22). Figure 1 shows the rates of VLDL triglyceride secretion between control and cyclosporin-treated animals. Cyclosporin treatment (open bar, Fig. 1), relative to vehicle alone, resulted in a statistically significant increase in hepatic secretion of VLDL triglyceride (6.65 ± 0.84 vs. 5.22 ± 0.34 mg·h⁻¹·g liver⁻¹, \( P < 0.05 \)). No difference in liver or body weights between the two groups of animals was present (see Table 1); thus triglyceride secretion rate, whether expressed per body weight or per animal, shows significantly higher secretion of VLDL triglyceride in the treated group (data not shown).

Cyclosporin causes a change in the proportions of apoB-48 and apoB-100 secreted by the liver. The murine liver secretes both apoB-48 and apoB-100, a functional consequence of apoB mRNA editing (6, 12). However, each VLDL particle secreted contains only one apoB protein, either apoB-48 or apoB-100. Hence, measurement of apoB secretion rates allows an estimation of the number of VLDL particles secreted. Figure 2 shows the rates of secretion of apoB-100 and apoB-48. Although apoB-100 was significantly increased by cyclosporin treatment (open bars) relative to vehicle alone (32.6 ± 7.3 vs. 26.5 ± 4.9 pmol·h⁻¹·g liver⁻¹, \( P < 0.05 \)), apoB-48 was unchanged relative to vehicle alone (212 ± 37 vs. 238 ± 52 pmol·h⁻¹·g liver⁻¹, \( P = 0.22 \)). Because the mouse liver secretes predominantly apoB-48-containing VLDL (22), this increase in apoB-100 represents a change in the type of the VLDL particle secreted (Fig. 3A), as the total amount of apoB secreted was not significantly different (Fig. 3B). Because there is only one apoB molecule per VLDL particle, these results imply that the total number of VLDL particles secreted by the liver is unchanged with treatment with cyclosporin A but more apoB-100 is secreted. Although there is a 23% increase in apoB-100 secretion by cyclosporin treatment, the total apoB rates are relatively unaffected by this increase (Fig. 3B) because murine hepatic secretion of apoB-100 represents between 9 and 12% of total apoB secreted, the predominant form secreted being apoB-48 (12, 22). The method used in the current study does not allow the determination of whether the increased triglyceride is accounted for by the increased apoB-100 secretion or whether there is an increase in triglyceride content of all of the individual particles.

Cyclosporin treatment causes differential regulation of genes for sterol and fatty acid biosynthesis and metabolism. To further characterize the mechanism underlying the increased hepatic triglyceride secretion, we examined mRNA levels by Northern analysis for a number of key genes in the cholesterol biosynthesis pathway. The mRNA for the rate-limiting enzyme, HMG-CoA reductase, as well as LDL receptor, steraryl-CoA desaturase, and squalene synthase mRNAs, was increased in abundance in the cyclosporin-treated group (Fig. 4). However, the abundance of MTP mRNA was not increased. MTP-mediated transfer of triglyceride to the nascent apoB-containing lipoprotein in the endoplasmic reticulum is thought to be a rate-limiting step in VLDL secretion (11, 42). The abundance of hepatic triglyceride lipase mRNA was also not affected by cyclosporin treatment (Fig. 4). Northern analysis for two genes in the fatty acid biosynthesis pathway, acetyl
CoA carboxylase (×0.93-fold) and fatty acid synthase (×2.4-fold), showed that only the fatty acid synthase mRNA was increased (Fig. 4). There was no change in the mRNA abundance for cholesterol 7β-hydroxylase, the rate-limiting enzyme for bile acid synthesis (Fig. 4).

Two transcriptional factors that regulate cholesterol and fatty acid biosynthetic pathways (4), SREBPs, were also analyzed. SREBP-1 mRNA was reduced in abundance (×0.45-fold), and mRNA for SREBP-2 was increased (×1.6-fold) in the cyclosporin-treated group (Fig. 4). SREBPs are activated from membrane-bound precursor forms by proteolytic cleavage and nuclear translocation. To establish that the increased abundance of the various mRNAs was mediated by increased SREBP activation, we performed semiquantitative Western blot analysis of SREBP-1 and -2 in liver homogenates from control and treated animals (Fig. 5).

Fig. 3. Effect of cyclosporin A treatment on hepatic apoB secretion. Mouse liver secretes both apoB-48 and apoB-100. Molar ratio of apoB-48 to apoB-100 indicates proportions of these 2 secreted by the liver. A: effects of cyclosporin on this ratio. Cyclosporin A increased the proportion of apoB-100 secreted relative to apoB-48 (compare with Fig. 2) with a consequent reduction in this ratio (6.6 ± 1.7, open bars, n = 10) compared with vehicle alone (9.1 ± 1.6, n = 9). Note that total apoB secreted was unchanged, control: 264 ± 55 vs. cyclosporin A: 244 ± 39 pmol·h⁻¹·g liver⁻¹ (B, see text for discussion). Error bars are means ± SD. *P < 0.05.

Fig. 4. Effect of cyclosporin treatment on abundance of various hepatic mRNAs. Hepatic total RNA was isolated and analyzed by Northern blotting as described in Methods. Data from a representative set is shown. Each track is RNA isolated from livers of different animals. RNA from 2 control and 2 cyclosporin-treated animals are shown. Bottom: results obtained with a probe for GAPDH for normalization of loading and blotting. mRNA for hydroxymethylglutaryl (HMG)-CoA reductase, low-density lipoprotein (LDL) receptor, stearoyl CoA desaturase and squalene synthase were all increased in abundance in livers from cyclosporin-treated animals. No significant change in MTP or hepatic lipase were observed. However, the mRNA for sterol regulatory element-binding protein (SREBP)-2 was increased 1.6-fold but that for SREBP-1 was reduced by 0.45-fold by cyclosporin A treatment.

Fig. 5. Effect of cyclosporin A treatment on abundance of SREBPs. A: active forms (nuclear extract, Fig. 5A) appeared to be marginally reduced by cyclosporin treatment. In contrast, there was an increase in the active form of SREBP-2 in the nuclear extracts from the cyclosporin-treated livers (Fig. 5B). The protein detected at ~84 kDa in Fig. 5A is an intermediate cleaved form of SREBP that has been previously described (39). One of the key proteins that has been identified as responsible for activation of cleavage of membrane-bound SREBPs is SCAP (5). Western blot analysis showed that the amounts of this protein were not significantly altered by cyclosporin treatment (Fig. 5C).
DISCUSSION

The clinical use of cyclosporin A as a powerful immunosuppressive agent has contributed greatly to the success of transplantation as a therapy, a success that is associated with an increase in cardiovascular morbidity and mortality observed in patients treated with cyclosporin.

We have examined the mechanism of how cyclosporin may cause dyslipoproteinemia, with a mouse model. Cyclosporin A treatment of Swiss-Webster mice for 6 days resulted in a significant elevation of plasma cholesterol, triglyceride, and apoB levels, resulting in a dyslipoproteinemia. Cyclosporin treatment can be hypothesized to cause this dyslipoproteinemia by 1) increased hepatic secretion of lipoproteins, 2) decreased clearance of lipoproteins, or 3) a combination of both. Our study shows that a combination of both overproduction of triglyceride and reduced clearance is present in cyclosporin-treated animals. Measurement of the rate of hepatic VLDL secretion showed that cyclosporin treatment caused a 27% increase in triglyceride secretion but no increase in the amount of total apoB secreted. Because each VLDL particle contains only one apoB molecule, the number of VLDL particles secreted does not appear to be increased by cyclosporin treatment. However, plasma levels of apoB are increased by cyclosporin treatment, suggesting that the dyslipoproteinemia induced by cyclosporin is a combination of both overproduction of triglyceride and reduced clearance of apoB-containing lipoproteins. This interpretation is complicated in that the mouse liver secretes predominantly apoB-48 (~90%) and only a small amount of apoB-100 (~10%) (12). Hence, although cyclosporin treatment resulted in a 23% increase in hepatic apoB-100 secretion, this increased contribution is somewhat attenuated by the disproportionate secretion of apoB-48 and apoB-100. Because human liver secretes only apoB-100 (12), it is possible that cyclosporin may result in overproduction of apoB in humans. Our data suggest that in the mouse, the cyclosporin-mediated dyslipoproteinemia is complex, as both an element of overproduction (measured directly) and a defect in clearance (inferred) are present.

To investigate the molecular mechanism(s) by which cyclosporin causes an increase in secretion of triglyceride and apoB-100 by the mouse liver, we examined the mRNA levels for MTP. MTP, in a tight complex with protein disulfide isomerase, shuttles triglyceride to the nascent apoB, and this activity is essential for normal VLDL secretion (41, 42). Mutations of MTP that inactivate lipid transfer prevent secretion of VLDL both in vivo (40) and in vitro (11, 33). Hence, an increase in MTP could account for the increased triglyceride and apoB-100 secretion seen in cyclosporin-treated animals. However, Northern analysis did not support this hypothesis; the mRNA for MTP was unchanged, if not slightly reduced by cyclosporin treatment (Fig. 4). Instead, a number of mRNAs encoding cholesterol biosynthesis enzymes, including the rate-limiting enzyme, HMG-CoA reductase, were significantly elevated by cyclosporin treatment, suggesting that this pathway was upregulated. To explore these results further, we examined mRNAs for two transcriptional factors known to regulate the transcription of many genes involved in lipid metabolism, namely SREBP-1 and SREBP-2. SREBP-1 mRNA was reduced in abundance, and this decline was further reflected in a reduction of the active, nuclear form of the protein. In contrast, both SREBP-2 mRNA and its active nuclear form were increased by cyclosporin treatment (Fig. 5). SREBP-1 and -2 are potent transcriptional activators of genes along the cholesterol and fatty acid biosynthesis path-
ways (4). In mice that transgenically overexpress the active forms of either SREBP-1 or SREBP-2, hepatic synthesis of cholesterol and fatty acids is increased, as is triglyceride storage, although there are differences in the resultant gene expression patterns between SREBP-1 and SREBP-2 transgenic mice (16, 36). SREBP-2 transgenic animals had a greater induction of the sterol biosynthesis pathway relative to the SREBP-1 transgenic mice, which induce the fatty acid biosynthesis pathway (16). More recently, the same investigators have now shown that there is increased hepatic cholesterol and fatty acid secretion in the SREBP-1a transgenic mice when unmasked on a background of LDL-receptor deficiency (15). Thus increased SREBP-2 active form in cyclosporin-treated animals observed in the present study is likely to lead to increased free fatty acid and triglyceride synthesis and secretion. The precise mechanism by which SREBP-2 activation leads to increased VLDL secretion has not been characterized. Although MTP contains sterol regulatory elements (29), in our study we observed no changes in MTP mRNA abundance, although activity for this protein was not examined. Pathways other than direct MTP involvement may be important for the link between SREBP and VLDL secretion.

Much of our understanding of the molecular mechanisms by which cyclosporin mediates its biological actions stems from characterization of its actions in activated T cells. Cyclosporin specifically binds to a ubiquitous cytoplasmic protein, cyclophilin A (an immunophilin), which in turn inhibits calcineurin, a serine-threonine protein phosphatase (20, 32, 34). The latter protein is required for the activation of a number of transcriptional pathways. One well-characterized pathway is the dephosphorylation and activation of the nuclear factor of activated T cells, after stimulation by calmodulin and increases in intracellular calcium, mediated by cell surface receptor activation (32). The actions of cyclosporin on tissues other than lymphoid cells are also now beginning to be unraveled (3, 28). Although the expression of the nuclear factor of activated T cells is thought to be confined mainly to lymphoid cells, tissues other than those involved in immune regulation may also express this factor. Another mechanism by which cyclosporin may express biological activity is by modulation of the peptidyl-prolyl cis/trans isomerase activity of cyclophilins (34). Although all of the immunophilins possess this activity, inhibition of this activity by cyclosporin has not yet been shown to lead to a disruption of a biological pathway.

Finally, cyclosporin A has also been shown to inhibit directly an enzyme in the bile acid synthesis pathway, sterol 27-hydroxylase, both in rat liver and a human hepatoma cell line (7, 21, 31). Sterol 27-hydroxylase is a multifunctional enzyme, catalyzing oxidation steps of a variety of sterol substrates. Cyclosporin A inhibits 27-hydroxylation of cholesterol but not hydroxylation of 5β-cholastane-3α, 7α, 12α-triol. The latter substrate is an intermediate in the formation of cholic acid, whereas in some species, including humans, the former is an intermediate in chenodeoxycholic acid synthesis. Inhibition of 27-hydroxylation cholesterol is therefore predicted to lead to a decrease in the cellular levels of the oxysterol 27-hydroxycholesterol. One possibility is that

![Fig. 6. Possible sites of cyclosporin action to account for increased SREBP-2 activation. Three speculative sites for cyclosporin-mediated action are proposed. If SREBP-2 is phosphorylated and its dephosphorylation, mediated by calcineurin, is a signal for rapid degradation (indicated by site 1 protease (SIP)), then inhibition at this site could lead to increased SREBP-2 active forms (A). Another possibility is that cyclophilin-cyclosporin A complex could directly stimulate activation by cleavage of SREBP-2 (indicated by SIP, B). This activation could involve direct stimulation of SCAP, or site 1 protease or both. A third possibility (C) is that cyclosporin A directly inhibits sterol 27-hydroxylase, leading to a fall in intracellular concentration of 27-hydroxycholesterol. If 27-hydroxycholesterol were a physiological inhibitor of SCAP, the fall in concentration of 27-hydroxycholesterol would lead to a derepression and activation of SCAP and increased cleavage of SREBP-2.](http://ajpendo.physiology.org/)
27-hydroxycholesterol is a potent inhibitor of SREBP cleavage; thus a fall in its intracellular concentration, in response to cyclosporin treatment, may lead to an activation of SREBP.

We propose three speculative mechanisms by which cyclosporin may increase SREBP-2 activation: 1) If activation of the nuclear form of SREBP-2 was also dependent on phosphorylation of SREBP-2, and its inactivation, by dephosphorylation mediated by calcineurin, then cyclosporin-mediated inhibition of this pathway would lead to an increase in active SREBP-2, which is also known to stimulate its own transcription (Fig. 6A); 2) that cyclosporin leads to increased cleavage activation of SREBP-2 (perhaps mediated by a phosphorylation-dephosphorylation cycle, Fig. 6B); and 3) cyclosporin causes a fall in the concentration of a regulatory molecule, such as 27-hydroxycholesterol via inhibition of the sterol 27-hydroxylase, leading to activation of SREBP-2 (Fig. 6C). We note that the effect of cyclosporin in vivo is to selectively activate SREBP-2 but not SREBP-1. Hence, it is likely that the mechanism leading to this selective activation may involve more than the explanations put forward above.

We thank the Goldstein and Brown labs, and in particular, Dr. Yuriv Bashmakov, for the help with the Western blot analysis for SREBPs, and Drs. Hitoshi Shimano and J ay Horton and J inmn Gao for the generous gifts of the cDNA probes. We also thank Dr. Harirhan Narayan for the mouse MTP cDNA, Dr. David Weinstein at Sandz (now Novartis) for the gift of cyclosporin A, A nih Whitaker for technical assistance, and Dr. David Russell for critical reading of the manuscript.

This work was initiated at University of Texas Southwestern Medical Center, Dallas and was supported by a Grant-in-Aid from the American Heart Association, Texas Affiliate, Inc. and by unrestricted grants from Merck & Co., Inc., West Point, PA, Bristol-Myers Squibb, New Brunswick, NJ, the Southwestern Medical Foundation, Dallas, TX, and the Moss Heart Foundation, Dallas, TX.

Address for reprint requests and other correspondence: S. B. Patel, Medical Univ of South Carolina, Division of Endocrinology, Diabetes and Medical Genetics, STR RM. 541, 114 Doughty St., Charleston, SC 29403 (E-mail: patelsb@musc.edu).

Received 26 March 1999; accepted in final form 13 July 1999.

REFERENCES


