Regulation of adrenal scavenger receptor-BI expression by ACTH and cellular cholesterol pools

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Abstract **Scavenger receptor BI (SR-BI) mediates selective uptake of high density lipoprotein (HDL) cholesteryl ester in the liver and adrenal gland. Adrenal SR-BI is increased both in adrenocorticotropic hormone (ACTH)-treated mice and also in apolipoprotein A-I knock-out (apoA-I0) mice which have depleted adrenal cholesterol stores. The goal of the present study was to determine whether adrenal cholesterol stores and ACTH have independent effects on SR-BI expression in adrenal gland. Adrenal SR-BI levels were 5-fold higher in apoA-I0 than wild-type mice when killed under low stress condition, and plasma ACTH levels were similar in both strains. After male apoA-I0 or wild-type mice were treated with dexamethasone to suppress ACTH release, adrenal SR-BI protein levels were decreased in both groups but remained 13-fold higher in apoA-I0 than in wild-type mice. By contrast, uncontrolled stress or supplemental ACTH treatment increased SR-BI levels but narrowed the difference in SR-BI expression between apoA-I0 and wild-type. Cholesterol depletion by β-cyclodextrin in cultured Y1-BS1 adrenal cells also led to a rapid 2- to 3-fold increase in SR-BI mRNA and protein levels, in association with a significant depletion of cellular free cholesterol. These results indicate that depletion of adrenal cholesterol stores can act independently from ACTH to increase SR-BI expression, but in vivo this effect is diminished under high ACTH conditions. Both stimuli may increase selective uptake via increased SR-BI as a means of replenishing cholesterol stores for steroid hormone synthesis.**—Sun, Y., N. Wang, and A. R. Tall. **Regulation of adrenal scavenger receptor-BI expression by ACTH and cellular cholesterol pools.** ***J. Lipid Res.*** **1999.** 40: **1799–1805.**

Supplementary key words scavenger receptor-BI • high density lipoprotein • adrenal gland • ACTH • cholesterol

Scavenger receptor BI (SR-BI) is an 82-kDa membrane protein, newly identified as an HDL receptor mediating selective cholesterol uptake (1). SR-BI is highly expressed in steroidogenic tissues, which show high levels of HDL cholesteryl ester selective uptake. Expression of SR-BI in cultured Chinese hamster ovary (CHO) cells also resulted in increased efflux of cellular cholesterol to HDL in the medium (2). Wang et al. (3) showed that SR-BI is up-regulated in the adrenal gland of apoA-I knock-out (apoA-I0) mice and in hepatic lipase knock-out mice in association with depletion of adrenal cholesterol stores, and that stress or ACTH also increases SR-BI expression. LCAT knock-out mice were also found to have depleted adrenal cholesterol stores and increased adrenal SR-BI expression (4). Others have also shown a role of ACTH in stimulating adrenal SR-BI expression (5). Intraperitoneal injection of ACTH increased adrenal SR-BI, while suppression of ACTH by dexamethasone treatment (which inhibits the hypothalamic–pituitary axis and decreases ACTH release) markedly decreased SR-BI levels in adrenal gland. In the rat, high-dose estrogen treatment reduced SR-BI levels in the liver and increased SR-BI in the adrenal gland and corpus luteum cells of the ovary; this treatment also markedly reduces plasma HDL cholesterol levels and thus may result in depletion of adrenal cholesterol stores (6). The administration of human chorionic gonadotropin (HCG) induced a dramatic increase in SR-BI in the steroidogenic Leydig cells of rat testes. In the above examples, changes in SR-BI mRNA were matched by alterations in SR-BI protein levels.

Thus, a variety of hormonal stimuli alter SR-BI gene expression in steroidogenic tissues. In the case of ACTH and HCG, increased SR-BI expression occurs in parallel with increased steroid hormone production. These findings could indicate that a variety of hormones directly increase expression of the SR-BI gene. Increased SR-BI expression could also occur as a response to depletion of cellular sterol stores as suggested in the various knock-out mice where increased SR-BI is associated with depleted adrenal cholesterol stores. However, apoA-I0 mice have modestly decreased plasma corticosteroid levels (7), raising the possibility that these animals could have increased plasma ACTH levels as a result of disinhibition of the hypothalamic–

Abbreviations: SR-BI, scavenger receptor-BI; ACTH, adrenocorticotropic hormone; HDL, high density lipoprotein; dex, dexamethasone; apo, apolipoprotein; apoA-I0, apolipoprotein A-I knock-out; WT, wild-type; TC, total cholesterol; CE, cholesteryl ester; FC, free cholesterol; F, female; M, male; LCAT, lethicin:cholesterol acyltransferase; HCG, human chorionic gonadotropin; CHOL, cholesterol; CD, 2-OH-propyl-β-cyclodextrin.

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pituitary feedback suppression of ACTH release. In the present study we have attempted to dissociate the effects of cholesterol depletion from increased plasma ACTH levels by measuring adrenal SR-BI after dexamethasone suppression of plasma ACTH levels, or under carefully controlled, low stress conditions. Under these conditions, we found that SR-BI levels were much higher in apoA-I0 mice than in wild-type controls, suggesting there are effects of adrenal cholesterol stores on SR-BI levels, independent of ACTH. To examine this relationship more directly, we also measured SR-BI expression in cultured Y1-BS1 cells grown under cholesterol depletion conditions and the findings confirmed that both cellular cholesterol pool and ACTH can regulate SR-BI expression.

MATERIALS AND METHODS

Animals and experimental treatments

Male and female C57BL/6 mice or C57BL/6-apoA-I0 mice were purchased from Jackson Laboratories (Bar Harbor, ME). They received standard mouse chow and water ad libitum and were maintained on a 12-h light/12-h dark cycle. Experiments were initiated 3 h into the light cycle. For experiments shown in Fig. 1, mice received intraperitoneal injections of 0.5 ml of saline containing either 50 μg of ACTH 1–24 (Sigma) or 40 μg of dexamethasone–water-soluble (Sigma), or phosphate-buffered saline at time 0, 12 h, and 24 h. Four hours after the last injection, the mice were anesthetized with isoflurane. Blood was drawn from the inferior vena cava. After perfusing the animal through the heart, tissues were removed and frozen for immunoblot analysis or cholesterol measurement. In order to produce low stress conditions (Fig. 2), mice were single caged for 1 week after they were shipped from Jackson Laboratories. They were allowed to become accustomed to the environment for a week, then killed by cervical dislocation, perfused through the portal vein, and quickly exanguinated from the heart.

Cell culture

Murine Y1-BS1 cells were maintained in a 37°C, humidified, 95% air, 5% CO_2 incubator in medium A (Ham's F-10 medium supplemented with 12.5% heat-inactivated horse serum and 2.5% heat-inactivated fetal bovine serum with 2 mm glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate). On the day of the experiment, the cells were switched to serum-free medium and treated with the indicated amounts of 2-OH-propyl-β-cyclodextrin or 100 nm ACTH for the indicated times. The cells were washed twice with phosphate-buffered saline and collected by scraping.

Immunoblotting of adrenal glands and Y1-BS1 cell lysates

Anti-SR-BI antisera were prepared by immunization of rabbits with a recombinant murine SR-BI fragment (amino acid 315–412) that was expressed in a bacterial expression system and purified. Whole adrenal glands were homogenized in 10 mm Tris-HCl (pH 7.5) with protease inhibitors. The homogenates were centrifuged at 1000 *g* for 10 min at 4°C and supernatants were saved. Y1-BS1 postnuclear lysates were prepared as described previously (5). Protein concentration was determined by the method of Lowry et al. (8). Equal quantities of protein (30 μg) were subjected to 4–20% gradient SDS-polyacrylamide gel electrophoresis prior to transfer to nitrocellulose (Trans-Blot, Bio-Rad). Western blot was performed as described previously using anti-SR-BI antibody (1:500) (3). After incubation with the primary antibody, the bound IgG was detected using horseradish peroxidase-coupled donkey anti-rabbit IgG and the enhanced chemiluminescence (ECL) system (Amersham Pharmacia Biotech). The filters were exposed to Kodak film at room temperature for 1 min. The relative intensities of the bands were determined by densitometry (Molecular Dynamics, Model 300A).

Ribonuclease protection assay

Murine SR-BI and β-actin antisense riboprobes were prepared by in vitro transcription using murine SR-BI and β-actin cDNA plasmid constructs. The protected hybrid fragments for SR-BI and β-actin were 290 and 160 bp, respectively. RNase protection assay was performed as described (3, 9). In brief, 10 μg of adrenal gland total RNA was hybridized with 5×10^5 cpm SR-BI and β-actin riboprobes at 48°C overnight in 30 μl of a buffer consisting of 40 mm PIPES, pH 6.0, 400 mm NaCl, 1 mm EDTA, and 80% formamide. The hybridization mixture was digested with 40 units T_2 ribonuclease (Gibco) at 37°C for 1 h, extracted with phenol–chloroform, precipitated with ethanol, and dissolved in 5 μl RNA loading buffer. The protected RNA hybrid fragments were resolved on a 5% polyacrylamide/urea gel and the gels were exposed to phosphor screen (Molecular Dynamics). The protected bands were detected by a Storm 800 scanner (Molecular Dynamics) and quantitated by ImageQuant for Macintosh (Molecular Dynamics, version 1.2).

Preparation of cyclodextrin: cholesterol complexes

The protocol used to make 2-OH-*p*-β-CD: cholesterol solution was described by Christian et al. (10). In brief, a cholesterol stock solution (50 mg/ml, in chloroform–methanol 1:1 (v:v)) was added to a glass tube; the solvent was evaporated under a gentle stream of nitrogen and 10 ml of 25 mm cyclodextrin solution was added. The tube was vortexed to bring the dried cholesterol off the wall of the tube and then sonicated in a bath sonicator for 3 min. The solution was incubated in a rotating water bath at 37°C overnight. Immediately before use, the solution was filtered through a 0.45-μm syringe filter (Millipore) to remove excess cholesterol crystals.

Other assays and reagents

Tissue and cellular cholesterol and cholesteryl ester content were determined as described using chloroform–methanol extraction and cholesterol CII and free cholesterol C kit (Wako, Japan) (3). Plasma ACTH levels were determined by a radioimmuno assay kit (ICN).

Statistical analysis

Data were compared by using two-tailed Student's *t* test.

RESULTS

In an attempt to dissociate the role of plasma ACTH and adrenal cholesterol stores in determining adrenal SR-BI expression, apoA-I0 or wild-type mice were treated with dexamethasone (dex) to suppress pituitary ACTH release, or were administered supplemental ACTH. Adrenal SR-BI levels were 2.9-fold higher in male apoA-I0 mice than in wild-type mice under basal conditions ($P < 0.05$, **Fig. 1**), and 2.0-fold higher in female apoA-I0 than in wild-type mice ($P < 0.05$, data not shown). SR-BI levels remained significantly higher in apoA-I0 than wild-type mice after dexamethasone suppression treatment (13-fold), or ACTH supplementation (2-fold), treatments which tended to de-

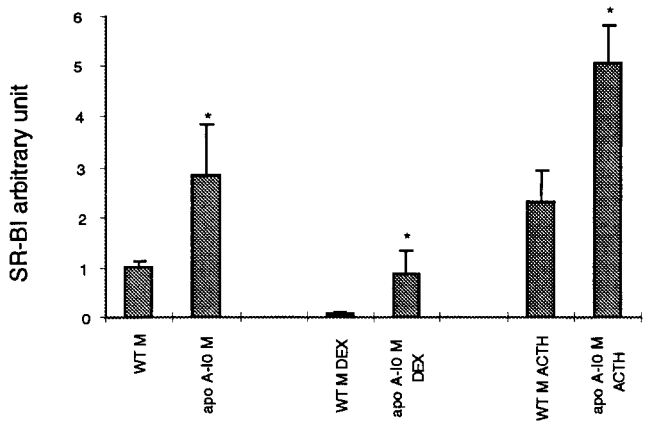

Fig. 1. Adrenal SR-BI protein expression in apoA-I0 and wild-type mice after injection with saline, dexamethasone, or ACTH. SR-BI protein levels were determined by Western blotting (30 μg total protein loaded) with specific antiserum (see Methods). Results are expressed in comparison to the mean value for wild-type males (untreated) arbitrarily designated as 1. * Denotes statistical significance ($P < 0.05$) within same treatment group between apoA-I0 and wild-type mice. Both ACTH and dexamethasone treatments changed SR-BI expression levels significantly ($P < 0.05$, compared with untreated) in apoA-I0 and wild-type mice.

crease or increase SR-BI expression in both groups. Plasma ACTH levels were already elevated and variable under basal conditions, and were significantly higher in apoA-I0 mice than in control mice (**Table 1**, untreated). Nevertheless, dexamethasone suppression successfully reduced ACTH levels in both apoA-I0 and wild-type mice to the same range (Table 1), suggesting that there is an effect of the apoA-I knock-out on SR-BI levels independent of ACTH levels. In female mice, there was also a trend to higher SR-BI levels in apoA-I0 versus wild-type mice after dexamethasone suppression, but suppression was less effective than in males and there was greater variability in ACTH and SR-BI levels than in males (data not shown); ACTH supplementation abolished the difference in SR-BI expression between female apoA-I0 and wild-type mice.

Although we had attempted to minimize stress in the previous experiment, ACTH measurements revealed that we had not been successful. Undoubtedly, there was stress related to regular injections, removal from the animal facility, and anesthesia. Therefore, additional precautions were taken: mice were single caged, allowed to become accustomed to the animal facility for at least 1 week before the experiment, not given any treatments, and quickly killed by cervical dislocation in a separate room without removal from the facility. When mice were maintained and killed under low stress conditions, there was a 5-fold increase in SR-BI protein levels in apoA-I0 mice, compared to wild-type, with a similar response in both sexes (**Fig. 2**). Under these conditions, there was relatively little variability within groups and the differences of means were highly significant ($P < 0.001$). Plasma ACTH measurements confirmed that ACTH levels were much lower than in the initial experiment and were similar in apoA-I0 and wild-type mice (Table 1).

TABLE 1. Plasma ACTH levels in C57BL/6 wild-type or apoA-I0 mice before killing

	Untreated	Dexamethasone Suppression	ACTH Supplementation	Untreated (Low Stress)
	ng/dl		*ng/dl*	
WT M	358 ± 161 (4)	24 ± 16 (6)	489 ± 98 (7)	99 ± 101 (4)
apoA-I0	597 ± 109 (5)	35 ± 18 (6)	667 ± 140 (7)	105 ± 82 (4)

The values are expressed as ng/dl and shown as means ± SD. The number of animals in each group is in parentheses after each value; WT, wild-type; M, male.

Adrenal cholesterol levels were also measured in the experiment shown in Fig. 1 and are shown in **Table 2**. As reported previously (7), apoA-I0 mice had lower total, free, and esterified cholesterol levels in the untreated state compared to wild-type. There were also pronounced significant differences in both free and esterified cholesterol levels between male apoA-I0 and wild-type mice after dexamethasone and ACTH treatments. Thus, in the case of dexamethasone suppression, SR-BI was inversely related to cholesterol stores, in the presence of similar low ACTH levels. Under low stress conditions, SR-BI was inversely related to both adrenal free cholesterol and cholesteryl ester (Fig. 2C, 2D), but had no relationship with plasma ACTH levels (Fig. 2E).

The in vivo data suggest there is an inverse relationship between SR-BI levels and cholesterol stores in adrenal cells. To further examine this relationship, we measured SR-BI expression in an adrenal cell line, Y1-BS1, after treatment with β-cyclodextrin (CD) to deplete cholesterol stores. Y1-BS1 cells were treated with 50 mm cyclodextrin, ACTH, or ACTH plus cyclodextrin. The 1-h treatment resulted in a significant increase in cellular SR-BI expression levels in response to cyclodextrin but no significant change in response to ACTH (**Fig. 3A**). With longer treatment (8 h) ACTH caused a significant increase in SR-BI levels. Fifty mm cyclodextrin treatment could not be continued for 8 h due to cellular toxicity as shown previously (11). However, at lower doses of cyclodextrin, treatment with cyclodextrin and ACTH for 6 to 8 h failed to show additive effects of the two treatments (data not shown). Measurements of cellular cholesterol content showed a marked depletion in response to cyclodextrin and a more moderate effect of ACTH (Fig. 3B). To exclude the possibility that the increase in SR-BI expression in response to cyclodextrin treatment is due to cyclodextrin itself but not cholesterol depletion, we treated Y1-BS1 cells with cyclodextrin:cholesterol complex (125:1, mol:mol) so that there was no net change of cellular cholesterol (10). There was a 3-fold increase of SR-BI protein with the cyclodextrin alone treatment, but no change of SR-BI expression in response to CD:cholesterol complexes (which did not alter cellular cholesterol stress) (**Fig. 4**). This experiment strongly suggests that the increase of SR-BI is related to cellular cholesterol depletion. We measured both total cholesterol and free cholesterol in Y1-BS1 cells and found that almost 80% of cellular cholesterol content was free cholesterol (**Table 3**). Cyclodextrin significantly decreased free cholesterol content but did not

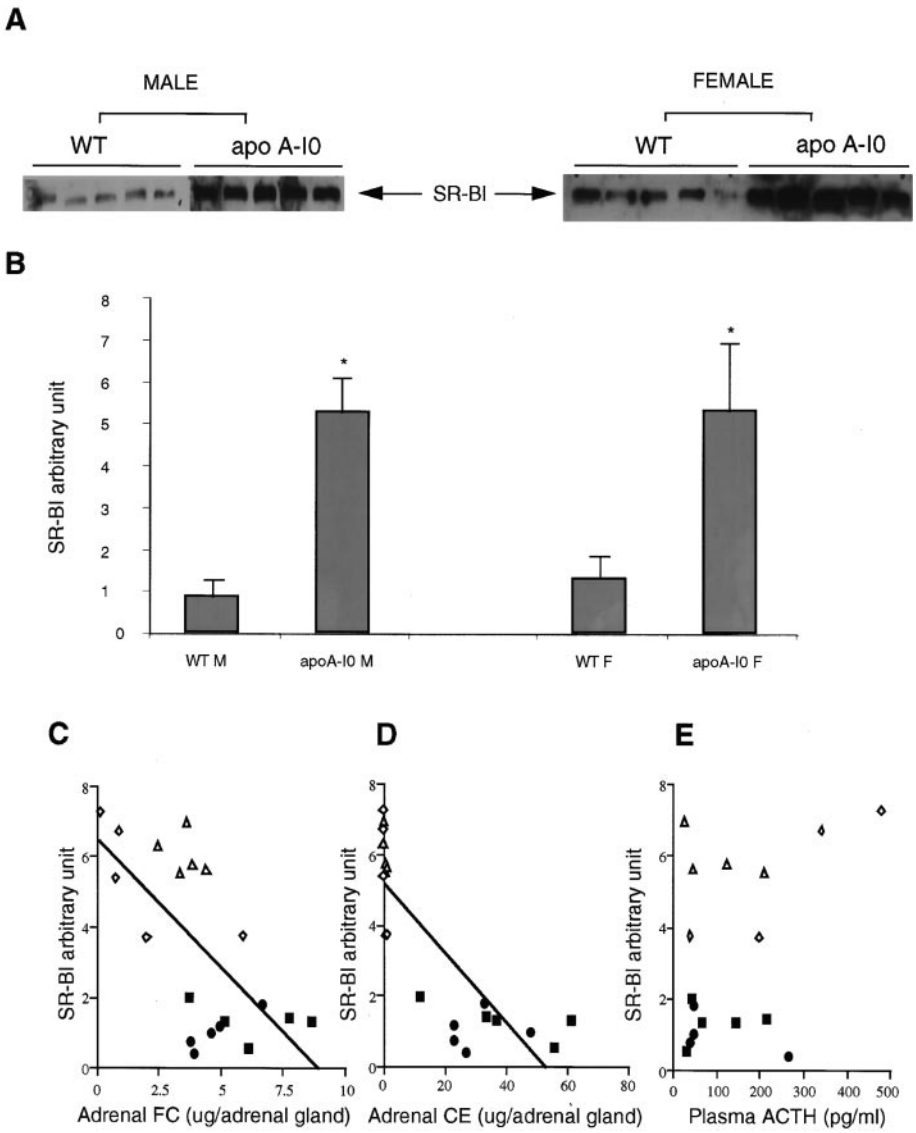

Fig. 2. Adrenal SR-BI protein expression levels in apoA-I0 and wild-type mice under low stress condition A: Western blot of adrenal lysates (30 μg total protein per lane). B: Relative fold change in SR-BI protein level in apoA-I0 mice compared to wild-type mice. SR-BI levels are expressed in comparison to wild-type male arbitrarily designated as 1. * Denotes statistical significance ($P < 0.001$) between apoA-I0 and wild-type mice. C: The relationship between SR-BI and adrenal free cholesterol levels in individual mice; $r = 0.659$, $P = 0.002$. D: The relationship between SR-BI levels and adrenal cholesteryl ester levels in individual mice; $r = 0.822$, $P < 0.001$. E: The relationship between adrenal SR-BI levels and plasma ACTH level in individual mice; $r = 0.425$, $P = 0.09$. ■ Represents wild-type female; ● represents wild-type male; ◇ represents apoA-I0 female; △ represents apoA-I0 male.

significantly change cholesteryl ester level. In cells treated with cyclodextrin:cholesterol, neither free cholesterol nor cholesteryl ester was significantly changed.

To further characterize the response to cholesterol depletion by cyclodextrin, we did a time course experiment using a lower concentration of cyclodextrin and measured both SR-BI protein and mRNA responses. As reported previously (12), cyclodextrin treatment resulted in a rapid decrease in cellular cholesterol stores (**Fig. 5C**). There was a rapid increase in SR-BI mRNA levels (within 2 h), while the response in SR-BI protein levels was more gradual and did not reach significance until 4 h (Fig. 5).

DISCUSSION

Plump et al. (7) showed that apoA-I0 mice have depleted adrenal cholesterol stores and reduced plasma corticosteroid levels, showing an essential role of HDL in providing cholesterol to the adrenal gland for steroid hormone biosynthesis. The demonstration of increased adrenal SR-BI in apoA-I0 mice provided early in vivo evidence for a physiological role of SR-BI in HDL cholesterol uptake by the adrenal gland and suggested that SR-BI expression was increased as a response to depletion of adrenal cholesterol stores (3, 4, 6). However, these studies did

TABLE 2. Adrenal total cholesterol, cholesteryl ester, and free cholesterol contents in male C57BL/6 wild-type and apolipoprotein A-I knock-out mice

Treatment	TC	CE	FC
WT-saline (5)	41.2 ± 8.9	31.8 ± 7.0	9.4 ± 5.2
ApoA-I0 untreated (5)	8.3 ± 2.4[a]	2.2 ± 0.2[a]	6.6 ± 2.4
WT DEX	49.9 ± 8.0	32.1 ± 11	17.8 ± 4.8
ApoA-I0 DEX	9.1 ± 4.8[a]	4.5 ± 1.9[a]	10.5 ± 0.7[a]
WT ACTH	23.9 ± 3.2	15.4 ± 3.4	8.5 ± 1.5
ApoA-I0 ACTH	5.7 ± 1.0[a]	1.3 ± 0.4[a]	4.5 ± 0.7[a]

Mice were injected intraperitoneally with saline, dexamethasone, or ACTH; adrenal glands were collected and SR-BI protein level, cholesteryl ester, and free cholesterol contents were measured as described in Materials and Methods. The values are expressed as means ± SD. n equals 4 per group. WT, wild type; DEX, dexamethasone; TC, total cholesterol; CE, cholesteryl ester; FC, free cholesterol.

[a] Denotes statistical significance ($P < 0.05$) within same treatment group between wild-type and apoA-I0 mice.

not establish whether increased SR-BI was a proximate response to depletion of cholesterol stores or, alternatively, could be explained by increased plasma ACTH levels that might be present in these animals as a result of lower plasma corticosteroid levels. Indeed, we found that apoA-I0 mice have higher plasma ACTH levels than wild-type mice under stressful conditions (Table 1). However, we managed to dissociate the effects of plasma ACTH levels and adrenal cholesterol stores by showing higher adrenal SR-BI protein levels in apoA-I0 than wild-type controls after dexamethasone suppression (Fig. 1), or under carefully controlled, low stress conditions (Fig. 2). Under these conditions ACTH levels were shown to be low and similar in apoA-I0 and control mice. Surprisingly, the difference in SR-BI expression between apoA-I0 mice and control mice was more pronounced under these conditions (Figs. 1, 2), and was substantially diminished by poorly controlled stress or by ACTH supplementation. In fact, in female mice, there was no difference between apoA-I0 mice and wild-type mice with ACTH supplementation. This could be because the marked increase in SR-BI at high ACTH masked the effect of cholesterol depletion, or because sterol depletion and ACTH result in convergent or mutually exclusive signals affecting SR-BI gene expression. The latter suggestion is consistent with our inability

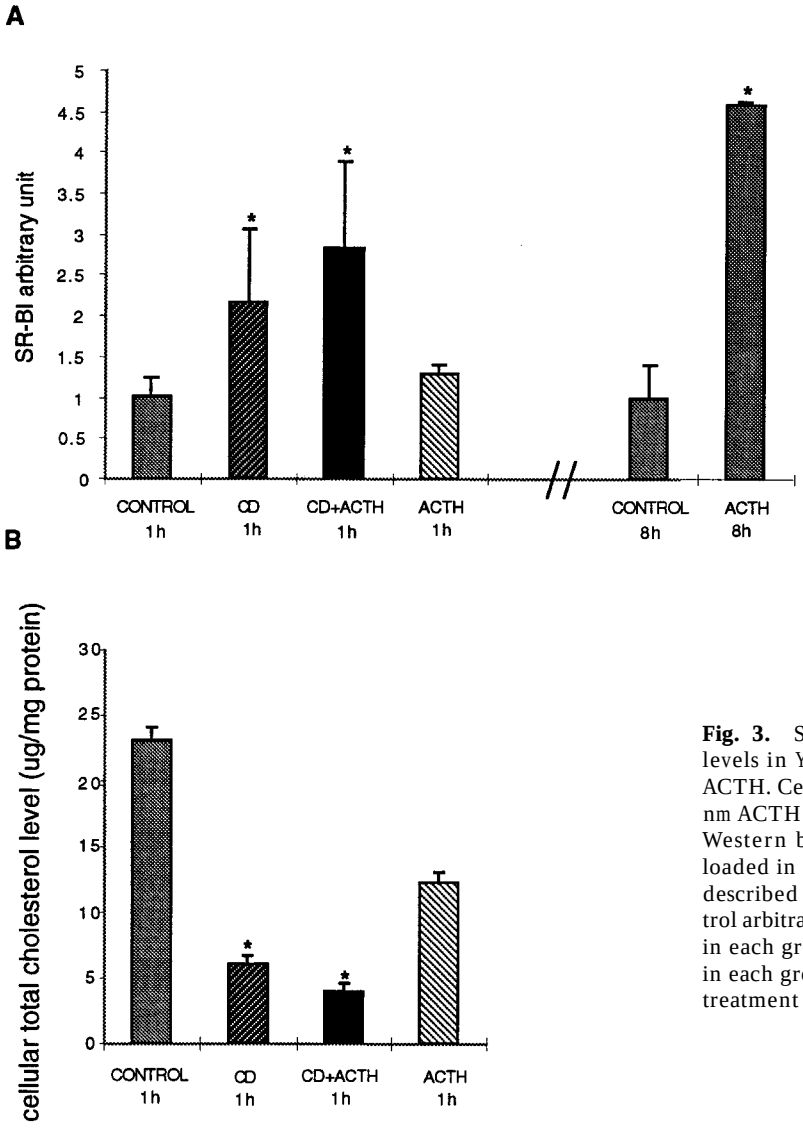

Fig. 3. SR-BI protein expression levels and cellular cholesterol levels in Y1-BS1 cells in response to cholesterol depletion and ACTH. Cells were treated with 50 mm 2-OH-*p*-β-cyclodextrin or 100 nm ACTH for indicated times. A: SR-BI levels were determined by Western blotting (30 μg postnuclear supernatant protein was loaded in each sample). The relative amounts were determined as described in Fig. 1. Results are expressed in comparison with control arbitrarily designated as 1. B: The cellular total cholesterol levels in each group. In all cell culture experiments, there are triplicates in each group; * denotes statistical significance ($P < 0.05$) between treatment group and control.

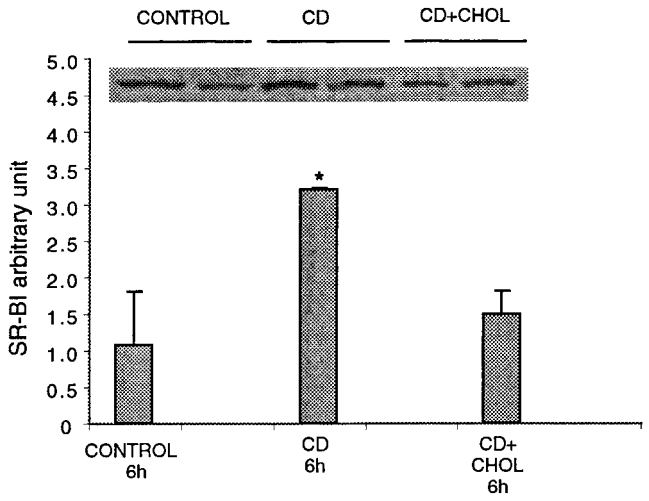

Fig. 4. SR-BI protein levels in Y1-BS1 cells in response to β-cyclodextrin or β-cyclodextrin:cholesterol complex. Cells (n = 5) were treated with 25 mm 2-OH-*p*-β-cyclodextrin or 25 mm cyclodextrin:0.2 mm cholesterol complex (125:1) for 6 h. SR-BI protein levels determined by Western blotting. The inset shows the original blots for 2 representative samples per group.

to produce additive effects of cyclodextrin and ACTH in cell culture.

One of the problems we encountered in this study is that mice are easily disturbed during the experimental procedures, even with low stress precautions. This was especially true in female mice, where two animals still showed high ACTH levels (Fig. 2E). This probably arose immediately before killing, and this transient increase in ACTH would probably not affect adrenal SR-BI expression as mice were killed within a minute.

Spady et al. (13) did not find any difference in adrenal SR-BI expression between apoA-I0 mice and wild-type controls. In their experiment, adrenal glands were collected 3 days after acute adenovirus infection. It is possible that their mice were stressed by the hepatic inflammatory response that is known to result from such treatments. This may have led to high ACTH levels and abrogated the difference between apoA-I0 and control mice. Others have also reported that SR-BI expression is increased in the adrenal gland under conditions of cholesterol depletion. Ng et al. (4) showed that adrenal SR-BI expression is similarly

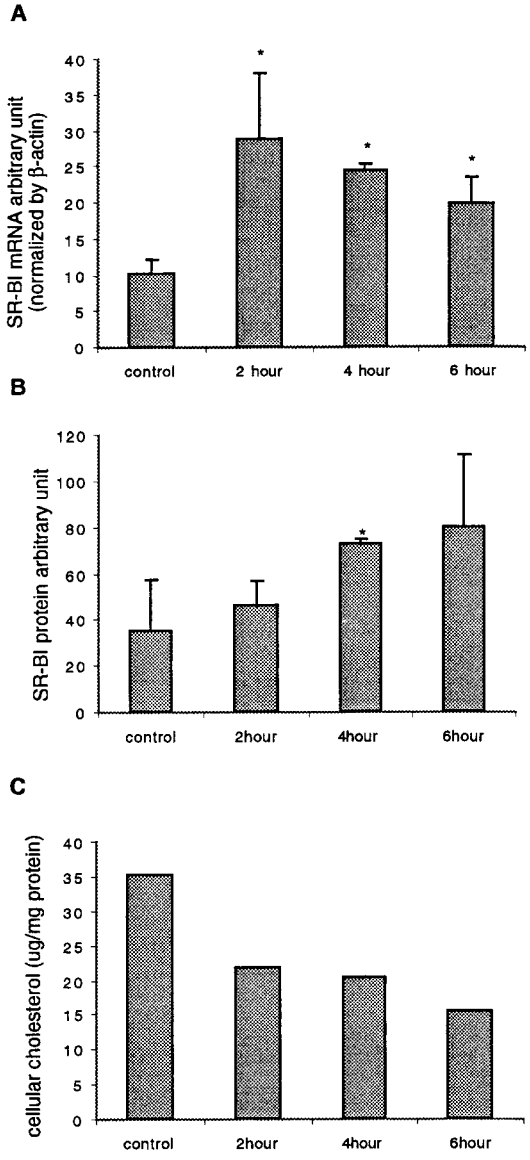

Fig. 5. Time course of changes in SR-BI mRNA and protein levels in response to cholesterol depletion. Cells were treated with 25 mm 2-OH-*p*-β-cyclodextrin for times shown. A: SR-BI mRNA levels were determined by RNase protection assay, and normalized to β-actin mRNA levels. B: SR-BI protein levels determined by Western blotting (30 μg total protein was run at each time point). C: Cellular total cholesterol level, n = 2. * Denotes statistical significance ($P < 0.05$) between treatment and control.

TABLE 3. Cellular cholesterol content in Y1-BS1 cells after different treatment

	TC	FC	CE
Control	30.2 ± 11.6	23.7 ± 5.8	6.5 ± 6.4
CD	13.1 ± 8.6[a]	10.9 ± 5.9[b]	2.3 ± 3.1
CD + cholesterol	27.2 ± 4.3	21.7 ± 4.3	6.1 ± 1.4

Cells were treated with 25 mm 2-OH-*p*-β-cyclodextrin or 25 mm cyclodextrin: 0.2 mm cholesterol complex (125:1) for 6 h. Cellular total cholesterol, free cholesterol, and cholesteryl ester contents were measured as described in Materials and Methods. The values are expressed as means ± SD; n equals 5 per group. TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester; CD, 2-OH-*p*-β-cyclodextrin.

[a] Denotes $P = 0.03$.

[b] Denotes $P = 0.009$ compared with control.

up-regulated in LCAT knock-out mice, which also have a depleted adrenal cholesterol pool. Wang et al. (3) showed increased SR-BI in hepatic lipase knock-out mice with a depleted adrenal sterol pool and Van Bruggen et al. (14) showed that adrenal SR-BI expression is induced in vivo by acute inhibition of hepatic lipase.

The experiments in Y1-BS1 cells provide further evidence that SR-BI expression can be up-regulated by cellular cholesterol depletion. β-Cyclodextrins are cyclic oligosaccharides consisting of 7-β(1–4)-glucopyranose units (15). Their hydrophilic surface and hydrophobic cavity structure make them good acceptors of sterols. Earlier studies have shown that there is an initial rapid efflux of cholesterol from the

plasma membrane in response to β-cyclodextrin (12), as also found in our experiments. The change in SR-BI mRNA expression is also rapid (within 1 h) while the increase in protein was more gradual. Our studies also showed that the major form of cholesterol inside Y1-BS1 cell is free cholesterol (Table 3), and the depletion caused by cyclodextrin treatment was only significant for free cholesterol. This finding suggested that free cholesterol or a related metabolite might be the signal for SR-BI regulation. In vivo, there was also an inverse relationship between SR-BI and cholesterol stores, but this effect was primarily due to the difference between groups (apoA-I0 vs. wild-type). Thus, it is uncertain whether subtle changes in cellular cholesterol that might occur physiologically would affect SR-BI expression.

Cao et al. (16) cloned the human SR-BI promoter and identified a SF1 site and two E-boxes (SRE like elements) (17) on the promoter. It is tempting to suggest that the SF1 site mediates the response to ACTH and the E-boxes mediate the response to cholesterol depletion. However, in one experiment, we compared SR-BI and LDL receptor mRNA responses during cholesterol depletion (data not shown). We found that SR-BI and LDL receptor up-regulation did not follow exactly the same time course, raising the possibility of a different mechanism. Martin et al. (18) recently showed that both the SR-BI and LDL receptor genes are expressed in the human adrenal cortex and coordinately regulated by activators of PKA pathway, such as cAMP. The contributions of HDL and LDL cholesterol to adrenal steroidogenesis may be different in humans and rodents (19). In rodents, the SR-BI-mediated pathway may be the major source of adrenal steroidogenesis, and the LDL receptor pathway is a backup. However, in humans, the situation is probably reversed.

Suppression of hepatocyte SR-BI expression has been found in response to a high cholesterol diet (20), suggesting that cellular cholesterol stores might influence SR-BI expression in tissues other than adrenal gland. However, this response is complex, as SR-BI was up-regulated in Kupffer cells in response to the diet. In the adrenal gland, it is tempting to speculate that both ACTH and depleted cellular cholesterol stores represent independent stimuli to increase SR-BI expression and therefore to increase cholesterol and cholesteryl ester uptake in order to maintain adequate cholesterol stores for steroid hormone synthesis.

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