Scavenger Receptor BI (SR-BI) Is Up-regulated in Adrenal Gland in Apolipoprotein A-I and Hepatic Lipase Knock-out Mice as a Response to Depletion of Cholesterol Stores

IN VIVO EVIDENCE THAT SR-BI IS A FUNCTIONAL HIGH DENSITY LIPOPROTEIN RECEPTOR UNDER FEEDBACK CONTROL

(Received for publication, July 1, 1996)

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Scavenger receptor BI (SR-BI), a putative high density lipoprotein (HDL) receptor, mediates the selective uptake of HDL cholesteryl ester into cells and is highly expressed in adrenal gland (Acton, S., Rigotti, A., Lande, J. et al. J. Clin. Invest. 97, 2660–2671). We now show up-regulation of adrenal SR-BI mRNA and protein in apoA-I mice, but not in apoA-10, LDL receptor 0, apoE 0, or cholesteryl ester transfer protein transgenic mice. Adrenal SR-BI mRNA and protein are also increased and cholesterol stores decreased in female mice with knock-out of hepatic lipase, an enzyme previously shown to increase selective uptake in cell culture. SR-BI mRNA is increased in stressed wild type mice and in Y1 adrenal cells treated with adrenocorticotropic hormone; the latter effect is inhibited by HDL. These findings provide in vivo evidence showing SR-BI is a functional HDL receptor under feedback control. The action of hepatic lipase on apoA-I-containing lipoproteins may facilitate the SR-BI-mediated uptake of HDL lipid.

High density protein (HDL) metabolism plays a pivotal role in cholesterol homeostasis and development of atherosclerosis.

*This work was supported by National Institutes of Health Grants HL54591 and HL22682. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: HDL, high density lipoprotein; apoA-10, apolipoprotein A-I knock-out; apoA-I, apolipoprotein A-I; LDLR0, low density lipoprotein receptor knock-out; apoA-I, apolipoprotein A-I; HL0, hepatic lipase knock-out; HL, hepatic lipase; apoA-I, apolipoprotein A-I; SR-BI, scavenger receptor BI; ACTH, adrenocorticotropic hormone; PIPES, 1,4-piperazinediethanesulfonic acid.

² A. S. Plump, T. Hayek, A. Walsh, and J. L. Breslow, submitted for publication.

³ W. Weng, T. Hayek, and J. L. Breslow, manuscript in preparation.

Plasma HDL cholesterol levels show a general inverse relationship with coronary heart disease (1). The normal function of HDL and the mechanisms underlying the HDL–coronary heart disease relationship are poorly understood. There is evidence in rodents that HDL provides cholesterol for adrenal steroid hormone synthesis via selective cholesteryl uptake, a putative receptor-mediated process for delivery of cholesteryl ester into the cells without degradation of HDL protein (2). Although long suspected, the molecular identification of a functional HDL receptor has proven to be elusive. In a major breakthrough, Acton et al. (3) recently demonstrated that murine scavenger receptor SR-BI, when expressed in transfected cells, binds HDL and mediates selective uptake of HDL cholesteryl ester. SR-BI protein is abundant in adrenal gland, ovary, testis, and, to a lesser extent, liver, precisely the tissues actively involved in selective uptake (2, 4). Therefore, SR-BI appears to be an authentic HDL receptor mediating selective uptake. We now provide in vivo evidence showing that adrenal SR-BI is a functional receptor for HDL under feedback regulation in response to changes of cellular cholesterol stores.

EXPERIMENTAL PROCEDURES

Animals—All animals used were between 3 and 4 months old. HL0 mice and wild type C57BL/6 mice were purchased from Jackson Laboratory (Maine). HL0 mice backcrossed with C57BL/6 mice were kindly provided by Dr. Nobuyo Maeda, University of North Carolina. ApoA-10 and ApoA-I0 mice were created by gene targeting in embryonic stem cells and detailed characterization will be presented elsewhere.

Ribonuclease Protection Assay—Reverse transcriptase-polymerase chain reaction was used to obtain murine SR-BI cDNA from the adrenal gland. 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. The RNase protection assay was described in detail previously (5). In brief, 20 μg of liver total RNA or 5 μg of adrenal gland total RNA were hybridized with 5×10⁶cpm SR-BI and β-actin riboprobes at 48 °C overnight in 50 μl of a buffer consisting of 40 mM Pipes, pH 6.4, 400 mM NaCl, 1 mM EDTA, and 80% formamide. The hybridization mixture was digested with 20 units of T7 ribonuclease at 37 °C for 1 h, extracted with phenol/chloroform, precipitated with ethanol, and dissolved in 5 μl of RNA loading buffer. The protected RNA hybrid fragments were resolved on a 5% polyacrylamide/urea gel and subjected to autoradiography.

Other Assays and Reagents—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. Western analysis was performed with the adrenal membrane preparation and equal quantity of membrane protein (50 μg of protein/lane) was subjected to 7.5% reducing SDS-polyacrylamide gel. SR-BI protein immunoreactivity was identified at its authentic molecular size (~82 kDa) (3). Tissue cholesterol and cholesteryl ester content were determined as described (6) using chloroform/methanol extraction and cholesterol C11 and free cholesterol C kit (Wako, Japan). Cholesteryl ester content was determined by subtracting free cholesterol from total cholesterol. Rat ACTH was purchased from Sigma. Human HDL was prepared by preparative ultracentrifugation between d 1.063 and 1.210 g/ml as described (7). For experiments with murine adrenal Y1 cells, the cells, obtained from ATCC, were maintained in Ham F-12 media plus 20% horse serum. On the day of experiments, the cells were treated for 8 h with or without horse serum. When indicated,
RESULTS

The tissue distribution pattern of SR-BI mRNA in wild type mice (C57BL/6) was determined by ribonuclease protection assay and is shown in Fig. 1. Adrenal gland was the richest source of SR-BI mRNA. Ovary and testis also had relatively abundant SR-BI mRNA. Liver had the highest SR-BI mRNA content in nonsteroidogenic tissues. Quantitation of SR-BI mRNA by phosphorimager indicated that hepatic SR-BI mRNA abundance was about 1/10 of that in the adrenal gland. These SR-BI mRNA distribution patterns are generally comparable with the SR-BI protein distribution patterns (3) and are consistent with the order of selective cholesterol uptake from HDL by different rodent organs (4). We hypothesized that adrenal SR-BI expression might be under feedback control in response to changes in cellular cholesterol stores. Plump et al. (8) recently show that apolipoprotein A-I knock-out (apoA-10) mice have decreased HDL cholesterol, depleted adrenal cholesterol stores, and impaired corticosteroid synthesis. Thus, we measured SR-BI mRNA in adrenal gland and liver of apoA-10 mice and, as shown in Fig. 2, compared the results with mice with induced mutations in a variety of other genes affecting lipoprotein metabolism including apolipoprotein A-I (apoA-1) transgenic, apolipoprotein A-II knock-out (apoA-II0), apolipoprotein E knock-out (apoE0), LDL receptor knock-out (LDLR0), hepatic lipase knock-out (HLO), and cholesteryl ester transfer protein transgenic mice. In contrast to apoA-10 mice, the first three strains of induced mutant mice have relatively normal adrenal cholesterol stores (8). Consistent with our hypothesis, there was a 3.5-fold increase in adrenal SR-BI mRNA in apoA-10 mice, but no change in apoA-II0, apoE0, or LDLR0 mice. Analysis of adrenal SR-BI protein by Western analysis in wild type and apoA-10 mice gave analogous results to the mRNA data, i.e. highest expression in the adrenal gland and 3-fold up-regulation of adrenal SR-BI protein in apoA-10 mice (data not shown).

DISCUSSION

Our data show that adrenal SR-BI expression is up-regulated as a response to depletion of cholesterol stores, whether wild type male and female mice (Fig. 3A). In parallel with these findings, adrenal cholesteryl ester and free cholesterol stores were significantly decreased (by ~60% and ~20% respectively) in female HLO mice (Fig. 3B). On the other hand, male HLO mice showed no change in adrenal cholesteryl content (Fig. 3C). In contrast to the findings in HLO mice, apoA-10 mice of both sexes showed up-regulation of adrenal SR-BI mRNA (Fig. 2 shows female mice and data not shown for male mice), which is consistent with the depletion of adrenal cholesterol store in both male and female apoA-10 mice (8).

As an additional test of the hypothesis that SR-BI expression is under feedback control, mice were stressed by the cold swim test (11), which is known to stimulate ACTH release and corticosteroid synthesis and to deplete adrenal cholesteryl stores (8, 11). In response to the stress test, mice showed a significant 2-fold up-regulation of adrenal SR-BI mRNA (p < 0.01, n = 4). Next, murine adrenal Y1 cells, which are known to show selective uptake of HDL cholesteryl ester (10), were grown in low serum medium and treated with ACTH with or without HDL in medium. ACTH treatment resulted in a significant increase in SR-BI mRNA expression, which was completely prevented by inclusion of 100 μg of protein/ml HDL in medium (Fig. 4).
resulting from decreased uptake of cholesterol (apoA-I0 and HL0 mice) or increased cholesterol utilization for corticosteroid synthesis (stress or ACTH treatment). This suggests a feedback loop that controls SR-BI expression and thereby helps to maintain adrenal cholesterol stores and corticosteroid biosynthesis (Fig. 5). Together with the findings of Acton et al. (3) and Plump et al. (8), these results imply that HDL containing apoA-I is a physiological ligand for SR-BI and that SR-BI functions to provide free and esterified cholesterol to maintain adrenal cholesterol stores. Moreover, up-regulation of SR-BI in HL0 mice suggests that the action of HL on HDL is required for efficient selective uptake in the adrenal gland.

The evidence for the proposed feedback control of SR-BI expression (Fig. 5) is based on the inverse relationship between adrenal SR-BI mRNA levels and cholesterol stores in various induced mutant mouse models, as well as HDL-inhibited up-regulation of SR-BI mRNA by ACTH in adrenal Y1 cells. Thus, in apoA-I0 mice and female HL0 mice SR-BI mRNA was increased and cholesterol stores were markedly decreased. By contrast, in male HL0 mice and in all of the other induced mutant mouse strains tested, adrenal cholesterol stores and SR-BI mRNA levels were essentially normal. There was no evidence that increased HDL levels due to apoA-I overexpression in transgenic mice or HDL addition to basal cell culture medium resulted in down-regulation of SR-BI mRNA. Thus, the feedback loop may operate in times of increased cholesterol need in response to augmented corticosteroid synthesis.

The sex difference in the up-regulation of SR-BI mRNA in HL0 mice could indicate an effect of sex steroid hormones on SR-BI gene expression or the HDL ligand, or, more likely, increased cholesterol demand for corticosteroid synthesis in female HL0 mice. In female mice, plasma corticosteroid levels are twice as high as in male mice and the cholesterol storage defect is more severe in female than male apoA-I0 mice (8). The cholesterol storage defect in female HL0 mice was not as severe as in apoA-I0 mice of either sex (8). This is consistent with the idea that HL may act on apoA-I-containing HDL particles to optimize the selective uptake process in the adrenal gland (Fig. 5). A defect in cholesterol storage results from both suboptimal delivery of cholesterol by selective uptake as well as higher utilization in female mice.

Our data suggesting that HL activity is required for optimal selective uptake of HDL free cholesterol and/or cholesteryl ester by the adrenal are consistent with earlier studies of selective uptake in cell culture (9, 10). HL enhances the selective uptake of both free cholesterol and cholesteryl ester by hepatocytes in vitro, with a major effect on free cholesterol and a much smaller effect on cholesteryl ester. HL action also is required for the conversion of large HDL to smaller, more dense HDL particles (12, 13). The more dense HDL-3 species, which are deficient in HL0 mice (14), are the optimal substrates for selective uptake in rodents (15). Further studies will be required to differentiate whether the action of HL primarily alters the ligand binding properties of HDL to SR-BI, or acts to enhance selective uptake after binding has occurred.

The present data suggest that SR-BI is involved in the delivery of HDL cholesterol to the adrenal and perhaps other...
steroidogenic tissues and that a feedback loop governing SR-BI expression helps to increase the delivery of HDL cholesterol in response to increased need. The lower expression of SR-BI in the liver and the lack of up-regulation of SR-BI or change of hepatic cholesterol stores in apoA-I0 mice indicates that SR-BI plays a less important role in hepatic cholesterol homeostasis than in the adrenal. However, in apoA-I0 mice bile salt synthesis appears to be decreased, perhaps acting as a compensatory mechanism to maintain hepatic cholesterol stores. Our data imply that increased hepatic SR-BI expression by interruption of the feedback loop controlling SR-BI expression in the liver could result in enhanced reverse cholesterol transport. However, just as selective uptake in cell culture is down-regulated by cholesterol loading (16), our studies show an inverse relationship between SR-BI expression and cellular cholesterol pools. Thus, it appears unlikely that SR-BI would be up-regulated in peripheral tissues or arterial wall foam cells as a response to cholesterol loading.

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A. S. Plump, N. Azrolan, H. Odaka, L. Wu, X. Jiang, A. Tall, S. Eisenberg, and J. L. Breslow, submitted for publication.
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J. Biol. Chem. 1996, 271:21001-21004.
doi: 10.1074/jbc.271.35.21001

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