17β-Estradiol promotes the up-regulation of SR-BII in HepG2 cells and in rat livers

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Abstract The scavenger receptor class B type I (SR-BI) binds to HDL and mediates the selective uptake of cholesterol esters from HDL to cells. SR-BII is an alternatively spliced product of the SR-BI gene that only differs in the C-terminal cytoplasmic domain. Previous studies with male mice demonstrated that SR-BII comprises about 12% of the total SR-BI/SR-BII present in liver. In the current studies we used a liver cell line, HepG2, and a rat estrogen replacement model to examine the effects of estrogen on the expression of SR-BII. HepG2 cells express SR-BI but not SR-BII. SR-BI/SR-BII-blocking antibodies demonstrated that HepG2 cells selectively internalize cholesterol esters in a SR-BI-dependent manner. Incubation of HepG2 cells with 10 pM of 17β-estradiol for 12 h eliminated the expression of SR-BI and promoted the up-regulation of SR-BII. Radiolabeled HDL-binding studies demonstrated that 17β-estradiol increased the number of HDL binding sites by 3-fold in HepG2 cells. However, 17β-estradiol-treated cell internalized approximately 25% less cholesterol ester than vehicle-only-treated cells. The livers obtained from male rats and ovariectomized female rats contained SR-BI and a small amount of SR-BII. In contrast, the livers obtained from intact female rats and ovariectomized female rats receiving estrogen replacement contained SR-BII and a small amount of SR-BI. The amount of SR-BI and SR-BII in adrenal tissue was not affected by any of the experimental treatments. We conclude that estrogen up-regulates SR-BII in HepG2 cells and rat liver.—Graf G. A., K. L. Roswell, and E. J. Smart. **17β-Estradiol promotes the up-regulation of SR-BII in HepG2 cells and in rat livers.** ***J. Lipid Res.*** **2001.** 42: **1444–1449.**

Supplementary key words HDL • cholesterol ester • scavenger receptor • selective uptake

The scavenger receptor class B type I (SR-BI) is a physiological HDL receptor involved in the selective uptake of HDL cholesterol ester (1). Selective uptake refers to the internalization of HDL cholesterol esters without the internalization of HDL apoproteins (2, 3). Webb et al. (4, 5) described a splice variant of SR-BI called SR-BII that is expressed in rodents and humans. The amino acid sequences of SR-BI and SR-BII are identical except for the C-terminal cytoplasmic tail (4, 5). Despite the remarkable amino acid similarity to SR-BI, SR-BII is approximately 4-fold less efficient at selective cholesterol ester uptake than SR-BI. SR-BII accounts for only about 12% of the total SR-BI/SR-BII found in mouse liver even though the mRNA for SR-BII comprises about 40% of the total SR-BI/SR-BII mRNA in liver (4, 5). It is unclear if SR-BII plays a significant role in selective cholesterol ester uptake in vivo.

The expression of SR-BI appears to be coordinately regulated with steroidgenesis in the adrenal gland, ovary, and testis. In mice, adrenocorticotropic hormone increases SR-BI expression, whereas glucocorticoids suppress expression in the adrenal cortex (6). In rats, luteinization of the ovary with human chorionic gondadotropin or treatment of granulosa cells with follicle-stimulating hormone induces expression of SR-BI and uptake of HDL cholesterol esters (7). Similarly, human chorionic gondadotropin stimulated SR-BI expression and uptake of DiI-labeled HDL in the testis (8). It is interesting to note that the human SR-BI promoter contains the recognition sequence (Ad4BP) for the orphan receptor steroidogenic factor-1, which induces the expression of several genes involved in steroidogeneis and modulates SR-BI promoter activity in adrenal cells (9).

Supraphysiological doses of a synthetic estrogen, 17α-ethinyl estradiol, decrease hepatic expression of SR-BI in rats, suggesting a role for estrogens in modulating SR-BI expression (8). However, high-dose estrogen also increases hepatic LDL-receptor activity (10, 11). Therefore, decreased hepatic expression of SR-BI may have been due to elevated cholesterol content, rather than a direct effect of estrogen. Alternatively, Landschulz et al. (8) noted that hepatic and adrenal expression of SR-BI were lower in female rats relative to males, suggesting that endogenous estrogens may also modulate SR-BI.

Little is known concerning the hormonal regulation of SR-BII. The goal of this study was to determine if physiological levels of 17β-estradiol modulated the levels of SR-BII. We found that 17β-estradiol promoted the down-regulation of SR-BI and the up-regulation of SR-BII in HepG2 cells and in the liver of a rat estrogen replacement model. The in vivo consequences of this switch between receptors will require additional studies.

Abbreviations: SR-BI/SR-BII, scavenger receptor class B type I/type II.

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MATERIALS AND METHODS

Materials

MEM Eagles with Earle salts (phenol red minus), fetal bovine serum (non-heat inactive), L-glutamine, trypsin-EDTA, and penicillin/streptomycin were from Life Technologies, Inc. (Grand Island, NY). Percoll, 17β-estradiol, PVDF membrane, and Tween 20 were from Sigma (St. Louis, MO). BioRad Dc assay was from BioRad (Hercules, CA). Rabbit serum directed against SR-BI and SR-BII were a generous gift from Dr. Deneys van der Westhuyzen (UK). The SR-BI/SR-BII-blocking antibody was generated by Quality Control Biochemicals. Horseradish peroxidase conjugated IgGs were supplied by Cappel (West Chester, PA). Super Signal® chemiluminescent substrate was purchased from Pierce (Rockford, IL). 1α, 2α (n) [^{3}H]cholesteryl-oleate (37 Ci/mmol) was supplied by Amersham (Arlington Heights, IL). ^{125}I-Na (1 mCi/ml) was purchased from NEN (Boston, MA).

Buffers

Lysis buffer consisted of 25 mM MES (pH 6.5), 0.15 M NaCl, 1% (v/v) Triton X-100, and 60 mM octylglucoside. Sample buffer (5×) consisted of 0.31 M Tris (pH 6.8), 2.5% (w/v) SDS, 50% (v/v) glycerol, and 0.125% (w/v) bromophenol blue. Tris-buffered saline (TBS) consisted of 20 mM Tris (pH 7.6) and 137 mM NaCl. Blotting buffer consisted of TBS plus 0.5% Tween 20 and 5% dry milk. Wash buffer consisted of TBS plus 0.5% Tween 20 and 0.2% dry milk. Tris-saline consisted of 50 mM Tris (pH 7.4) and 150 mM NaCl.

Cell culture

HepG2 cells were cultured in a monolayer and plated in a standard format. On day 0, approximately 5×10^5 cells were seeded into 100-mm dishes with 10 ml of MEM eagles (phenol red minus) with Earle salts supplemented with 100 unit/ml penicillin/streptomycin, 0.5% (v/v) L-glutamine, and 10% (v/v) fetal bovine serum (non-heat inactive). The cells were used on day 4.

Isolation and labeling of lipoproteins

HDL (d=1.063–1.21 g/ml) was isolated from fresh human plasma by density gradient ultracentrifugation as described previously (12). The HDL_3 subfraction (d=1.13–1.18 g/ml) was isolated from other HDL subfractions by ultracentrifugation. HDL_3 apolipoproteins were then iodinated with iodine monochloride (13) to a specific radioactivity of 400–600 cpm/ng protein. 1α,2α(n) [^{3}H]cholesteryl-oleate was then incorporated into ^{125}I-labeled HDL_3 as described previously (14). The specific radioactivity of [^{3}H]cholesterol ester in the dually labeled particles ranged from 32–35 dpm/ng cholesterol.

Ligand binding and uptake assays

The selective uptake of cholesterol esters from HDL into cells was determined using dual-labeled HDL_3 as described previously (15). Cells were grown to 90% confluency in 12 well plates, then rinsed twice with PBS (37°C for uptake assays and 4°C for binding assays). Medium containing 5% human lipoprotein-deficient serum and 10 μg/ml ^{125}I-[^{3}H]cholesteryl-oleate-HDL was added to the cells for the indicated intervals. Following the incubation, uptake was terminated by aspirating the medium and washing the cell monolayers four times with Tris-saline (4°C). Cholesterol ester was extracted from the cells with 3:2 hexane–isopropanol (v/v), and the cells were solubilized in 0.6 ml of 0.5 M NaOH. The total HDL_3 associated with the cells was determined by counting aliquots of the 0.5 M NaOH homogenate. The total cholesterol ester associated with the cells was determined by liquid scintillation counting of the organic extracts. The amount of HDL_3 degraded was determined by measuring the amount of non-TCA precipitatable ^{125}I in the cell medium. To compare cell-associated ^{125}I-HDL with [^{3}H]cholesterol ester HDL, [^{3}H]cholesterol ester uptake was expressed as apparent HDL protein uptake, assuming that uptake resulted from whole HDL particle uptake as described by Knecht and Pittman (16).

Animals and treatments

Female Sprague-Dawley rats were housed on a 14:10 h light:dark cycle with unrestricted access to food and water. Rats were bilaterally ovariectomized under isoflurane anesthesia at 8 weeks of age. For 17β-estradiol studies, Silastic capsules (3.8 mm diameter, 30 mm length) containing either sesame oil (control) or 17β-estradiol in sesame oil (1 mg/ml) were implanted subcutaneously at the time of surgery as previously described (17). Implants were replaced after 7 days and rats were killed after 14 days of treatment. Organs were harvested, snap frozen on dry ice, and stored at −80°C. Trunk blood was collected and serum harvested for determination of total cholesterol and fractionation by FPLC as described previously (18). Estradiol concentrations were determined by radioimmunoassay as described (19). All surgical procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

SDS-PAGE and immunoblotting

Tissues (0.1 g) were homogenized in 1.0 ml of lysis buffer with a Teflon-coated homogenizer on ice. Homogenates were incubated on ice for 20 min and centrifuged at 12,000 *g* for 15 min at 4°C. Supernatants were collected and protein concentration determined by Dc assay. Proteins were diluted in 1× sample buffer plus 1.2% (v/v) β-mercaptoethanol and heated to 95°C for 5 min immediately prior to loading. Proteins were separated on a 12.5% polyacrylamide gel at 50 mA (constant current) and subsequently transferred to PVDF membrane at 50 V (constant voltage) for 2 h. Membranes were blocked with blotting buffer for 60 min at 22°C. Primary antibodies were diluted in blotting buffer and incubated with blocked membranes for 60 min at 22°C. Membranes were washed four times for 10 min in wash buffer. Horseradish peroxidase conjugated IgGs directed against the appropriate host IgGs were diluted and incubated with membranes as described for primary antibodies. Membranes were washed four times for 10 min in wash buffer and visualized using chemoluminescence. The relative signal intensities were determined by densitometry. Increasing amounts of a single sample were analyzed to verify that signal intensity varied linearly with protein loading.

Nuclease protection assay

Rat liver total RNA was used to reverse transcribe 120 nucleotide fragments corresponding to the C-terminal regions of SR-BI or SR-BII. The C-terminal regions of SR-BI and SR-BII are unique. An RNA Polymerase Promoter Addition Kit, Ribonuclease Protection Assay Kit, and Nonisotopic Detection Kit from Ambion, Inc., were used to conduct the nuclease protection assay. The assay was performed according to the manufacturer's instructions.

RESULTS

17β-Estradiol alters the expression of SR-BI and SR-BII in HepG2 cells

We first determined if HepG2 cells contained SR-BI and/or SR-BII by immunoblotting cell lysates with SR-BI and SR-BII specific antibodies (4). **Figure 1** demonstrates that

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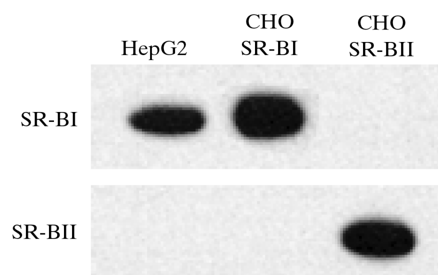

Fig. 1. HepG2 cells express SR-BI but not SR-BII. HepG2 cells were cultured for four days, then collected, and a cell lysate generated. 20 μg of protein was resolved by SDS-PAGE and transferred to nylon. As controls for SR-BI and SR-BII, 1 μg of cell lysate protein generated from CHO cells transfected with SR-BI or transfected with SR-BII were also resolved by SDS-PAGE and transferred to nylon. SR-BI and SR-BII specific antiserum (4) were used to probe the membranes. The immunoblots were developed by the method of chemoluminescence (30 s exposures). Longer exposure times (20 min) did not allow the detection of SR-BII in HepG2 cells (data not shown). The data are representative of three independent experiments.

HepG2 cells contained immunodetectable levels of SR-BI but not SR-BII. However, SR-BI and SR-BII were detected in cell lysates prepared from CHO cells transfected with either SR-BI or SR-BII.

We next determined if HepG2 cells were capable of SR-BI-dependent selective cholesterol ester uptake. HDL particles, dually labeled with [^{3}H]cholesterol ester and ^{125}I-apoprotein, were incubated with HepG2 cells (**Fig. 2**) or with HepG2 cells in the presence of 50 μg/ml of SR-BI/SR-BII blocking antibody (Fig. 2) for the indicated times and the amount of selective cholesterol ester uptake determined. HepG2 cells selectively internalized a relatively large amount of cholesterol ester. The selective uptake of cholesterol ester was abolished in the presence of a SR-BI/SR-BII blocking antibody. Non-specific IgG did not affect the selective uptake of cholesterol ester (data not shown). Less than 0.3% of the total added radiolabeled HDL particles were degraded during the time course of the experiment.

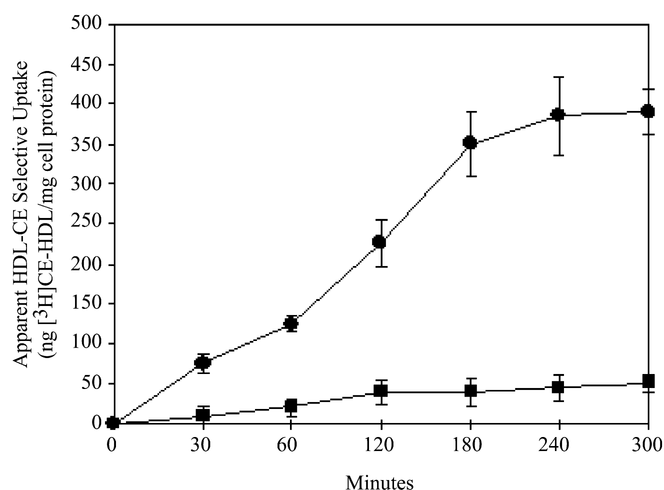

Fig. 2. HepG2 cells selectively internalize cholesterol ester in a SR-BI-dependent manner. HepG2 cells (●) or HepG2 cells plus 50 μg/ml of SR-BI/SR-BII blocking antibody (■) were incubated with 10 μg/ml of ^{125}I and [^{3}H]cholesterol ester labeled HDL for the indicated times. The cells were then washed and the amount of cell associated ^{125}I-HDL determined with a gamma counter, and the amount of [^{3}H]cholesterol ester quantified with a beta counter. Selective uptake was determined by subtracting the values obtained for ^{125}I from the values obtained for the [^{3}H]cholesterol ester (CE). Less than 0.3% of the total added radiolabeled HDL particles were degraded during the course of the experiment. The data are from four independent experiments, mean ± S.E.M, n = 3.

To determine the effect of 17β-estradiol on SR-BI and SR-BII expression, HepG2 cells were cultured in 10 pM 17β-estradiol for the indicated times (phenol red minus medium). At the end of the incubation, cell lysates were generated, resolved by SDS-PAGE and immunoblotted with SR-BI- and SR-BII-specific antibodies (**Fig. 3**). 17β-Estradiol induced a time-dependent decrease in SR-BI levels and increase in SR-BII levels. SR-BI was completely down-regulated by 12 h and SR-BII was maximally up-regulated by 20 h of 17β-estradiol treatment. Quantification measurements done with an antibody that recognizes both SR-BI and SR-BII (4) demonstrated that SR-BII was increased 3.2-fold more than the starting level of SR-BI (data not shown).

We next determined if the alterations in SR-BI and SR-BII levels affected the ability of HDL to associate with HepG2 cells. Cells were incubated with 10 pM 17β-estradiol for the indicated times. The cells were then chilled to 4°C and incubated with ^{125}I-HDL for 60 min. The cells were washed and processed to determine the extent of ^{125}I-HDL cell association and degradation. **Figure 4A** demonstrates that the amount of associated ^{125}I-HDL declines for the first 8 h of 17β-estradiol treatment. After 20 h of 17β-estradiol treatment the extent of ^{125}I-HDL that associated with the cells was approximately 3-fold greater than untreated cells. Less than 0.3% of the total added radiolabeled HDL particles were degraded during the course of the experiment.

To determine if alterations in SR-BI and SR-BII levels affected the ability of HepG2 cells to take up cholesterol ester selectively, cells were incubated with 10 pM 17β-estradiol for

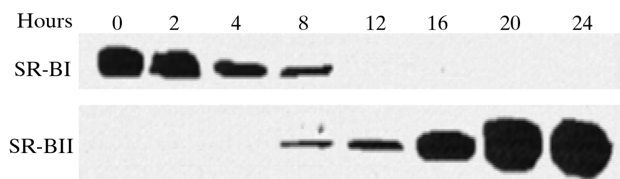

Fig. 3. 17β-Estradiol down-regulates SR-BI and up-regulates SR-BII in HepG2 cells. HepG2 cells were cultured for four days and then incubated for the indicated times (hours) with 10 pM 17β-estradiol. At the end of the incubation, cell lysates were generated and 20 μg of protein was resolved by SDS-PAGE, transferred to nylon and immunoblotted with SR-BI and SR-BII antiserum. The immunoblots were developed by the method of chemoluminescence (30 s exposures). Longer exposure times (20 min) did not significantly alter the data (data not shown). The data are representative of five independent experiments.

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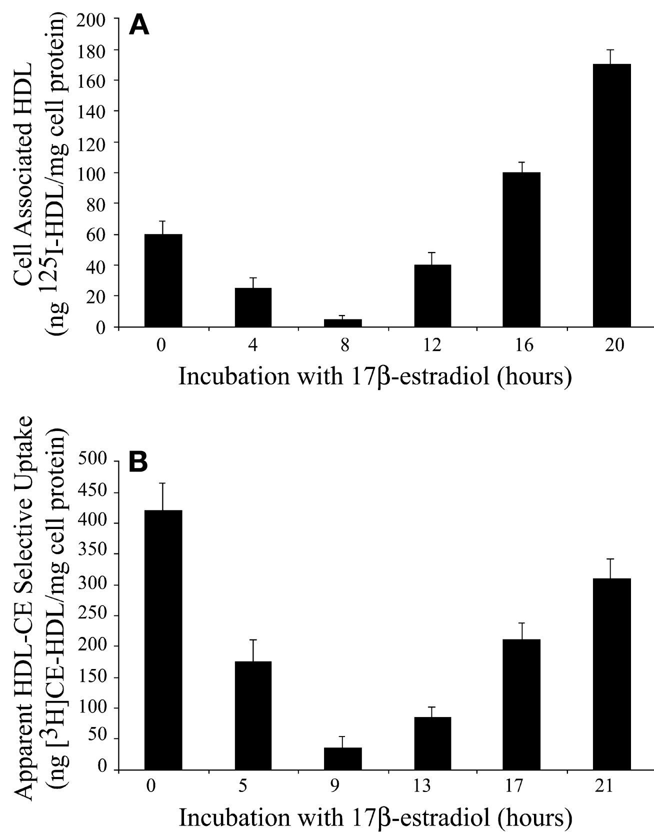

Fig. 4. The effects of 17β-estradiol on HDL cell association and selective cholesterol ester uptake. HepG2 cells were cultured for four days and then incubated for the indicated times with 10 pM 17β-estradiol. A: At the indicated times of 17β-estradiol treatment, the cells were chilled to 4°C and then incubated with 10 μg/ml of dually radiolabeled HDL for 1 h. The cells were washed and processed to determine the extent of HDL cell association and degradation. Radiolabeled HDL-binding was prevented with 50-fold excess unlabeled HDL or in the presence of 50 μg/ml blocking antibody (data not shown). The data are from six independent experiments, mean ± S.E.M, n = 3. B: Three hours before the indicated times of 17β-estradiol treatment, 10 μg/ml of dually radiolabeled HDL was added (2, 6, 10, 14, 21 h respectively). At the indicated times of 17β-estradiol treatment, the cells were then washed and the amount of cell associated ^{125}I-HDL determined with a gamma counter, and the amount of [^{3}H]cholesterol ester quantified with a beta counter. Selective uptake was determined by subtracting the values obtained for ^{125}I from the values obtained for the [^{3}H]cholesterol ester (CE). Less than 0.3% of the total added radiolabeled HDL particles were degraded during the course of the experiment. Selective cholesterol ester uptake was inhibited with 50-fold excess unlabeled HDL or in the presence of 50 μg/ml blocking antibody (data not shown). The data are from six independent experiments, means ± S.E.M, n = 3.

the indicated times (Fig. 4B). Three hours before each measurement, 10 μg/ml of dually radiolabeled HDL was added to the cells. At the indicated times the cells were processed to determine the extent of selective cholesterol ester uptake. The amount of selective cholesterol ester internalized initially decreased with minimal uptake occurring at 9 h of 17β-estradiol treatment. After 24 h of 17β-estradiol treatment the amount of cholesterol ester internalized was approximately 75% of that obtained in the absence of 17β-estradiol.

The effect of 17β-estradiol on the expression of SR-BI and SR-BII in rat liver

To determine if 17β-estradiol influences the expression of SR-BII in vivo, we used an established rat estrogen replacement model (17). Twelve rats were ovariectomized on day 0 of the study and immediately given implants containing either 17β-estradiol in sesame oil or vehicle only. Proestrus estradiol concentrations in intact cycling rats range from 40 to 80 pg/ml (20). The mean serum estradiol concentration in the ovariectomized rats was 6.9 ± 0.5 pg/ml. Estrogen replacement increased estradiol concentrations to 97.7 ± 7.4 pg/ml. Four male rats and four intact cycling female rats were also studied for comparison. On day 14 the animals were sacrificed, and the livers and the adrenal glands were harvested and immunoblotted with SR-BI- and SR-BII-specific antibodies. **Figure 5** demonstrates that none of the treatments altered the amount of SR-BI and SR-BII associated with adrenal glands. However, the experimental groups had different amounts of SR-BI and SR-BII associated with the livers. Livers from male rats and ovariectomized female rats contained predominantly SR-BI and a small amount of SR-BII. In contrast, livers from intact cycling female rats and ovariectomized rats receiving estrogen replacement had significantly less SR-BI and a dramatic increase in the amount of SR-BII. Because different antibodies were used to detect SR-BI and SR-BII, it is not possible to compare the relative mass amounts of SR-BI to SR-BII in Fig. 5.

To determine if the changes in hepatic SR-BI and SR-BII protein levels correlated with changes in mRNA levels, total RNA was isolated from hepatic tissues and the relative levels of SR-BI and SR-BII message quantified by a ribonuclease protection assay (**Fig. 6**). C-terminal specific nucleotide fragments were used so that SR-BI and SR-BII could be distinguished. In parallel, expression of GAPDH

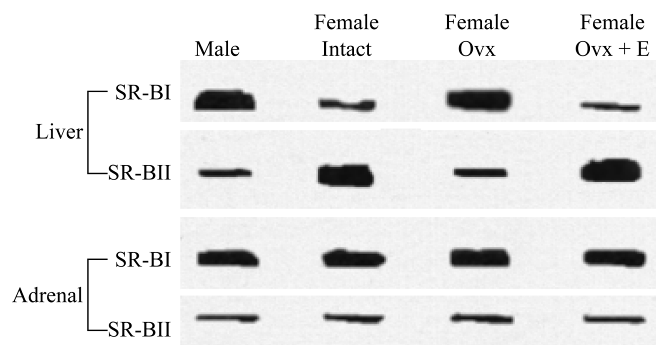

Fig. 5. Effects of ovariectomy and ovariectomy with estrogen replacement on the levels of liver SR-BI and SR-BII protein. Livers and adrenals from male rats, intact cycling female rats, and ovariectomized rats receiving a subcutaneous Silastic implant containing sesame oil or 17β-estradiol (1 mg/ml) for 14 days were harvested. Cell lysates were generated and resolved by SDS-PAGE, transferred to nylon, and immunoblotted with SR-BI and SR-BII specific antiserum. 45 μg of liver lysate and 10 μg of adrenal lysate were used. The immunoblots were developed by the method of chemoluminescence (60-sec exposures). Data from one animal in each experimental group is shown. All of the animals had nearly identical SR-BI and SR-BII profiles. Ovx, ovariectomy; E, 17β-estradiol.

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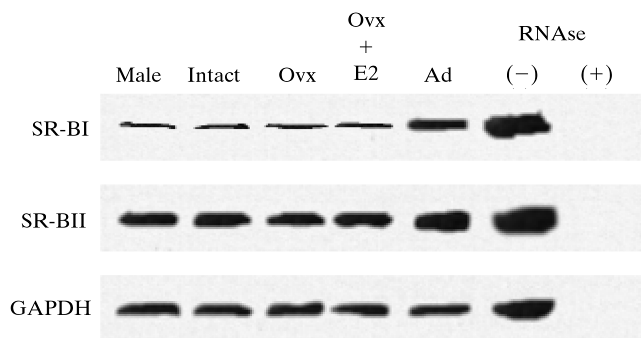

Fig. 6. Effects of ovariectomy and ovariectomy with estrogen replacement on the levels of liver SR-BI and SR-BII mRNA. Fifty micrograms of total RNA from male rats (Male), intact cycling female rats (Intact), and ovariectomized rats receiving a subcutaneous Silastic implant containing sesame oil (Ovx) or 17β-estradiol (Ovx + E_2) (1 mg/ml) for 14 days were hybridized with an antisense 120 nucleotide fragment of SR-BI or SR-BII and digested with RNAse A/T1. Adrenal RNA (Ad) (25 μg) was used as a positive control for SR-BI and SR-BII. The antisense fragment of SR-BI or SR-BII in the absence of cellular RNA was also included with (+) or without (−) treatment with RNAse A/T1. In parallel, levels of GADPH mRNA were determined to verify equal loading.

was determined to validate equality of RNA loading. The relative abundance of SR-BI and SR-BII mRNA did not change in the presence of 17β-estradiol.

DISCUSSION

SR-BI and SR-BII are two isoforms of a class B scavenger receptor that differ only in the C-terminal cytoplasmic tail (4, 5). Estrogen has been reported to induce the down-regulation of hepatic SR-BI (8, 21). In the present study we demonstrated that as little as 10 pM of 17β-estradiol could induce the complete loss of SR-BI and the dramatic up-regulation of SR-BII in HepG2 cells, a liver-derived cell line. Significantly, the amount of SR-BI and SR-BII correlated with the extent of ^{125}I-HDL that associated with the cells. The 17β-estradiol-induced switch from SR-BI to SR-BII did not inhibit selective cholesterol ester uptake although uptake did decrease by approximately 25%. Similar to other studies we observed less SR-BI in intact cycling female rats compared with male rats (8). However, we report for the first time that intact cycling female rats contain considerably more SR-BII than male rats. Ovariectomized female rats contained as much SR-BI as male rats and down-regulated SR-BII to levels comparable to male rats. Ovariectomized female rats receiving estrogen replacement contained SR-BI and SR-BII protein levels comparable to intact cycling females.

The functions of SR-BI and SR-BII have been studied in transfected cell lines. SR-BI and SR-BII both bind HDL with similar affinities and both receptors mediate the selective uptake of cholesterol ester from HDL (4). However, SR-BII is much less efficient at selectively internalizing HDL-derived cholesterol ester (4). Webb et al. (4) have estimated that SR-BII is approximately 4-fold less efficient at selective uptake than SR-BI. This estimate of SR-BII efficiency matches well with our current data. If SR-BII is 4-fold less efficient than SR-BI than a 4-fold increase in SR-BII would be required to obtained the same efficiency of selective cholesterol ester uptake. The final amount of 17β-estradiol induced SR-BII up-regulation in HepG2 cells was about 3-fold more than the starting SR-BI levels. Significantly, the amount of HDL-derived cholesterol ester internalized in 3 h was 75% of that obtained by SR-BI.

The mechanism by which class B scavenger receptors mediate the selective uptake of HDL-derived cholesterol ester is not understood. SR-BI and SR-BII are identical at the amino acid level with the exception of the C-terminal cytosolic tail. Both proteins are enriched in caveolae in transfected CHO cells (4, 22) and both proteins are acylated (4, 22). The extracellular domain and C-terminal cytosolic tail of SR-BI have been postulated to be critical domains involved in selective uptake (23). How the C-terminal cytosolic tail of SR-BII decreases the efficiency of selective cholesterol ester uptake is not known. One possibility is that the cytoplasmic tail of SR-BII interacts with a cellular protein that regulates selective uptake. This speculative mechanism has not been tested.

We have demonstrated that 17β-estradiol induces the loss of SR-BI and the accumulation of SR-BII in HepG2 cells. These observations confirm previous reports of an estrogen-induced decline in hepatic SR-BI expression and are the first to demonstrate an estrogen-induced up-regulation of SR-BII. The mechanism of how 17β-estradiol causes a switch from SR-BI to SR-BII is unclear. SR-BI and SR-BII are encoded by the same gene (5). The precursor mRNA is alternatively spliced such that a 129-nucleotide exon is either included or removed to yield SR-BI or SR-BII transcripts (5). Previous studies with adrenocorticotropic hormone demonstrated that this hormone did not regulate alternative splicing. The data presented here suggest that 17β-estradiol may regulate the splicing of the SR-BI/SR-BII transcript, however, RNase protection experiments did not detect significant changes in the amount of SR-BI and SR-BII specific transcripts. Alternatively, 17β-estradiol may affect the stability of the proteins. Both apoAI and apoE can be post-transcriptionally modified in an estrogen-dependent process (24). Indeed, apoE mRNA shifts to the fast translating polysomal pool in response to estrogen treatment (25).

In conclusion, the data demonstrate that 17β-estradiol can induce the down-regulation of SR-BI and the up-regulation of SR-BII in HepG2 cells. Despite the lower efficiency of SR-BII in selective cholesterol ester uptake, the large increase in SR-BII allowed the cells to internalize about 75% of the cholesterol ester that SR-BI internalized in 3 h. We have provided data demonstrating that in a rat estrogen replacement model SR-BII is up-regulated and SR-BI is down-regulated in the liver. This in vivo model will allow future studies to examine the impact of 17β-estradiol on the metabolism of HDL.

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