Endocrine, *vol. 9, no. 3, 243–252, December 1998* 0969–711X/98/9:243–252/$10.50

Expression and Hormonal Regulation of the High-Density Lipoprotein (HDL) Receptor Scavenger Receptor Class B Type I Messenger Ribonucleic Acid in the Rat Ovary

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Since cholesterol delivery to the ovary is an essential regulated step in steroidogenesis, mRNA levels for the Scavenger Receptor Class B Type I (SR-BI), a putative high-density lipoprotein receptor (HDL-R), were examined in response to tropic hormones and the luteolytic agent prostaglandin F2α (PGF2α). For this, the rat SR-BI cDNA was isolated and cloned. The results of this investigation revealed that a single SR-BI mRNA transcript of 2.4 kb was highly expressed in the rat adrenal, ovary, and testis. The SR-BI transcript was increased (twofold) in the immature rat ovary following pregnant mare's serum gonadotropin (PMSG) administration and in the ovary, 8 d after ovulation, in response to stimulation by human chorionic gonadotropin (hCG). In the ovary 8 d following ovulation, basal ovarian SR-BI mRNA levels were elevated up to sixfold relative to the preovulatory SR-BI mRNA levels. Even with the enhanced basal level of SR-BI mRNA within the ovary, hCG administration still resulted in a 2.5- ($p < 0.025$) and sevenfold ($p < 0.01$) increase in the 2.4-kb transcript, 3 and 6 h postinjection, respectively. This increase corresponded to a 58% increase in serum progesterone. In contrast, when PGF2α was administered, SR-BI mRNA levels were significantly reduced (3.5-fold; $p < 0.01$) in concert with a fourfold reduction ($p < 0.001$) in serum progesterone secretion. Furthermore, PGF2α blocked the hCG-induced increase in SR-BI mRNA levels when administered 30 min prior to hCG injection. The results of this study demonstrate that SR-BI mRNA levels are dramatically increased following exposure to gonadotropins in the ovary, whereas PGF2α exposure significantly reduced SR-BI mRNA levels.

Received August 12, 1998; Revised September 16, 1998; Accepted September 16, 1998.

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Key Words: Ovary; corpus luteum; cholesterol; HDL receptor.

Introduction

High-density lipoproteins (HDLs) play a critical role in cholesterol metabolism by redistributing cholesterol throughout the body. HDL particles are thought to remove cholesterol from peripheral tissues and to deliver cholesteryl esters to the liver (reverse cholesterol transport) and to steroidogenic cells for the synthesis of steroid hormones. The delivery of HDL cholesterol to cells occurs through a process called selective lipid uptake *(1,2)*. This process differs from low-density lipoprotein (LDL) uptake in that cell-surface binding of HDL is not associated with endocytosis and lysosomal degradation of the lipoprotein particle. Rather, cholesteryl esters are transferred from HDL particles while docked on the cell surface by interaction with a HDL receptor (HDL-R). After cholesterol transfer, lipid-depleted HDL is released into the extracellular fluid.

A cDNA encoding a cell-surface protein that binds HDL was recently cloned and characterized in the mouse *(3–5)*. This cDNA encodes a protein that also binds modified LDL, and thus, this multiligand cell-surface receptor was named the scavenger receptor class B, type I (SR-BI) *(3–5)*. The murine SR-BI (mSR-BI) is an 82-kDA glycoprotein that appears to be clustered in caveolae *(6)*, can mediate selective HDL lipid uptake *(5)*, and in the mouse is most abundantly expressed in liver and steroidogenic tissues *(5,7,8)*. Hepatic overexpression of mSR-BI in mice dramatically reduces plasma HDL, providing indirect evidence that SR-BI is a physiologically relevant HDL-R. In rodents, HDL particles transport cholesteryl esters to the adrenal gland, ovary, and testis *(1,2,9,10)*, where selective lipid uptake appears to be especially important for transport of cholesterol to these tissues for steroid hormone biosynthesis *(9)*. Recently, estradiol was shown to regulate SR-BI expression in rodent steroidogenic tissue *(7)*.

To determine if SR-BI expression plays an important role in the rat ovary following gonadotropin or prostaglandin administration, the rat cDNA for SR-BI was isolated, characterized, and used to analyze ovarian RNA following hormone and prostaglandin treatment.

Results

A cDNA probe specific for the rat SR-BI (a putative HDL-R) was generated using polymerase chain reaction (PCR) methods. Using oligonucleotide primers specific for the mouse SR-BI and a rat ovarian cDNA library, a single PCR product was amplified. This 1579-bp PCR product (Fig. 1) was then cloned into the TA vector for sequencing and further propagation. Dideoxy chain-termination sequence analysis of the putative rat SR-BI indicated that the PCR-amplified sequence was similar to the mouse SR-BI sequence. The rat SR-BI cDNA and deduced amino acid sequences are shown in Fig. 2. To compare the rat SR-BI cDNA sequence with that of the published mouse *(5)*, hamster *(3)*, and human *(8)* cDNA sequences, the PC Gene program (IntelliGenetics, Mountain View, CA) was utilized. The rat SR-BI sequence was 91% homologous with the mouse SR-BI sequence, 88% homologous with the hamster sequence, and 68% homologous with the human SR-BI cDNA sequence. Comparisons of the deduced amino acid sequence indicate that the rat and mouse are 92% identical, the rat and hamster are 89% identical, and there is 68% identity between the rat and human SR-BI amino acid sequences.

To demonstrate that the rat cDNA probe generated was specific for SR-BI mRNA, total RNA isolated from mouse adrenal was probed and compared to the SR-BI cDNA hybridization product detected in the rat adrenal and ovary. The rat SR-BI cDNA probe hybridized to a single 2.4-kb transcript in both mouse and rat adrenal and rat ovarian RNA (Fig. 3).

To demonstrate the tissue specificity of rat SR-BI expression in the rat, RNA was isolated from several rat tissues and hybridized with the rat SR-BI cDNA. Results shown in Fig. 4 using total RNA indicate that the SR-BI transcript was detected only in the adrenal and not in any other tissue examined. When poly A+ mRNA was probed (Fig. 5), however, a single SR-BI transcript was detected in brain, liver, intestine, and kidney, in addition to being detected in the adrenal and testis. To examine the expression of SR-BI in steroidogenic tissue, total RNA from the ovary, adrenal, testis, and placenta was analyzed (Fig. 6). The results of this analysis indicated that SR-BI expression was limited to the adrenal, ovary, and testis, and that the SR-BI transcript was not as highly expressed in the placenta. In order to enhance the level of the testicular SR-BI transcript, SR-BI mRNA was induced by injecting male rats with human chorionic gonadotropin (hCG) (50 IU). Testicular SR-BI expression was examined 3 h post-hCG injection, and the transcript size was similar to that in the adrenal and ovary.

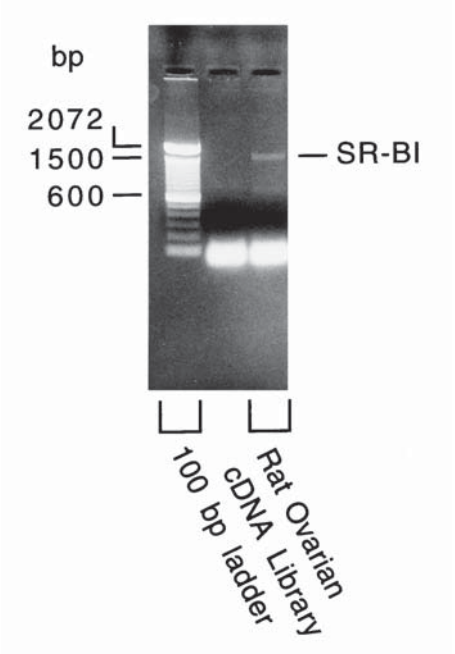

Fig. 1. PCR of the rat SR-BI cDNA. PCR products were analyzed by 1% agarose-gel electrophoresis. Lane 1 contains a 100-bp DNA ladder, lane 2 indicates the absence of a PCR product in a reaction that contained the SR-BI primers, but no template DNA (negative control), and lane 3 contained the SR-BI primers and DNA from a rat ovarian cDNA library.

To examine hormonal regulation of ovarian steady-state SR-BI mRNA levels, rats were treated with pregnant mare's serum gonadotropin (PMSG) to induce follicular development followed by hCG treatment (50 IU). The results of this experiment (Fig. 7), which examined hormonal regulation of the ovary before ovulation (preovulatory model), indicate that immature rat ovaries express very low basal SR-BI levels. Following PMSG injection, SR-BI mRNA increased 10-fold. Serum progesterone levels in these animals were increased 84% following PMSG injection. Following hCG injection, SR-BI mRNA levels were enhanced further (Fig. 7). The 2.4-kb transcript was increased 1.5-fold at 3 h post-hCG treatment. The results of this experiment indicated that ovarian SR-BI mRNA levels were increased following hCG injection, in parallel with serum progesterone levels (Table 1).

To determine whether gonadotropins can regulate ovarian SR-BI expression after ovulation, ovaries were examined on day 8 of pseudopregnancy (8 d postovulation). PMSG-primed immature rat ovaries 8 d postovulation consist mainly of corpora lutea. The SR-BI Northern blot results indicate that ovarian SR-BI expression was elevated 8 d after ovulation (Fig. 8) and that SR-BI mRNA levels were still increased twofold ($p < 0.025$) at 3 h and fourfold at 6 h post-hCG injection ($p < 0.01$). Serum progesterone levels were elevated following hCG administration

A

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   1 GTCTCCTTCA GGTCCTGAGC CCAGCGACTC TCGGCCGCGC ACGCGGACAT GGGCGTCAGC
  61 TCCAGGGCAC GCTGGGTGGC CTTGGGGCTG GGCGTTCTAG GGCTGCTGTG TGCTGCGCTC
 121 GGCGTTATCA TGATTCTCAT GGTGCCCTCG CTCATCAAGC AGCAGGTGCT CAAGAATGTC
 181 CGCATAGACC CCAGCAGCCT GTCCTTTGGG ATGTGGAAGG AGATCCCTGT TCCCTTCTAC
 241 TTGTCCGTCT ACTTTTTCGA GGTGGTCAAC CCCAGCGAGG TCCTAAATGG CCAGAAGCCA
 301 GTAGTCCGGG AGCGCGGACC CTATGTCATC AGGGAGTTCA GACAAAAGGT TAACATCACC
 361 TTCAATGACA ATGACACGGT GCCCTACATA GAGAACCGAA GCCTTCGTTT CCAGCCAGAC
 421 AGGTCCCAGG GCTCAGAGAG TGACTACATT GTACTGCCTA ACATCCTGGT CCTGGGAGGG
 481 GCAGTGATGA TGGAGGACAA GCCCCACAAGC CTGAAGCTGC TAATGACCTT GGGGTTGGTC
 541 ACCATGGGCC AGCGGGCCTT TATGAACCGC ACGGTTGGTG AGATCCTGTG GGGCTACGAA
 601 GATCCCTTTG TGAATTTCCT CAGCAAATAT TTCCCAGGCA TGTTCCCCAT CAAAGGCAAA
 661 TTTGGCCTGT TCGTTGGGAT GAACGACTCG AGTTCTGGCG TCTTCACCGT CTTCACAGGT
 721 GTCCAGAATT TCAGCAAGAT CCATCTGGTG GATAAGTGGA ACGGCCTCAG CGAGGTCAAC
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1021 GAGTCCGGCA TTCAGAATGT CAGCACCTGC AGGTTTGGTG CGCCCCTGTT TCTCTCCCAG
1081 CCCCACTTCT ACAATGCTGA CCCCGTGCTG TCAGAAGCTG TTCTTGGTCT GAACCCTGAC
1141 CCAAAGGAGC ATTCCTTGTT CCTAGACATC CACCCGGTCA CTGGGATCCC CATGAACTGT
1201 TCCGTGAAGA TGCAGCTGAG TCTGTACATC AAATCCGTCA AGGGCGTCGG GCAAACAGGG
1261 AAGATCGAGC CAGTAGTCCT GCCATTGCTG TGGTTCGAAC AGAGCGGGAT GATGGGTGGC
1321 AAGACCCTGA ACACGTTCTA CACGCAGCTG GTGCTGATGC CCCAGGTTCT TCACTACGCG
1381 CAGTATGTGC TGCTGGGGCT TGGAGGCCTC CTGCTTCTGG TGCCCATCAT TTACCAACTG
1441 CGCAGCCAGG AGAAATGCTT TTTATTTTGG AGTGGTAGTA AAAAGGGCTC GCAGGATAAG
1501 GAGGCCATGC AGGCCTACTC TGAGTCTCTG ATGTCACCAG CTGCCAAGGG CACGGTACTG
1561 CAAGAAGCCA AGCTATAGG
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B

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  1 MGVSSRARWV ALGLGVLGLL CAALGVIMIL MVPSLIKQQV LKNVRIDPSS LSFGMWKEIP
 61 VPFYLSVYFF EVVNPSEVLN GQKPVVRERG PYVIREFRQK VNITFNDNDT VPYIENRSLF
121 FQPDRSQGSE SDYIVLPNIL VLGGAVMMED KPTSLKLLMT LGLVTMGQRA GMNRTVGEIL
181 WGYEDPFVNF LSKYFPGMFP IKGKFGLFVG MNDSSSGVFT VFTGVQNFSK IHLVDKWNGL
241 SEVNYWHSEQ CNMINGTAGQ MWAPFMTPES SLEFFSPEAC RSMKLTYQES RVFEGIPTYR
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361 LNPDPKEHSL FLDIHPVTGI PMNCSVKMQL SLYIKSVKGV GQTGKIEPVV LPLLWFEQSG
421 MMGGKTLNTG YTQLVLMPQV LHYAQYVLLG LGGLLLLVPI IYQLRSQEKC FLFWSGSKKG
481 SQDKEAMQAY SESLMSPAAK GTVLQEAKL*
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Fig. 2. (A) The rat cDNA sequence for SR-BI. The rat SR-BI cDNA sequence obtained by PCR was determined to be 1579 bp. **(B)** The deduced amino acid sequence for the rat SR-BI protein. The cDNA sequence encodes a protein of 509 amino acids that is well conserved between species.

(Table 1) in parallel with the increase in SR-BI mRNA expression.

To determine whether prostaglandin F2α (PGF2α) could exert its antisteroidogenic effect on SR-BI mRNA expression, ovarian tissue was examined on day 10 of pseudopregnancy (when animals are more sensitive to prostaglandin action) before (t_0) and 4 h after PGF2α injection (250 μg). The results of this experiment indicate that ovarian SR-BI

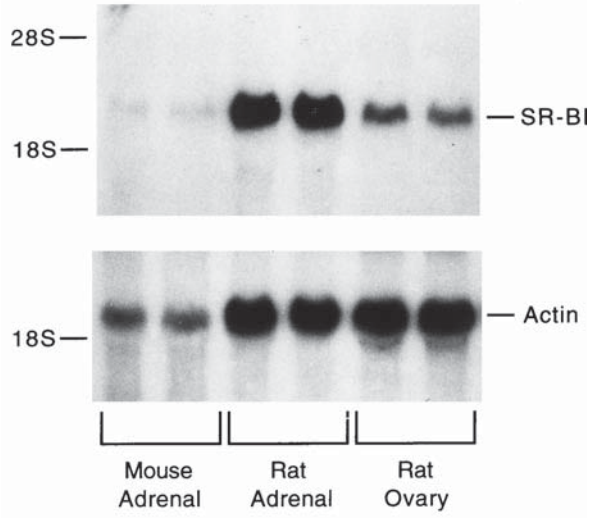

Fig. 3. *(right column)* Comparison of the SR-BI transcript in mouse and rat tissues. Northern blot containing 20 μg of total RNA isolated from the indicated tissues was probed with the rat SR-BI cDNA. Both rat and mouse transcripts migrated at 2.4 kb. This blot was stripped and probed with an actin cDNA for analysis of RNA loading.

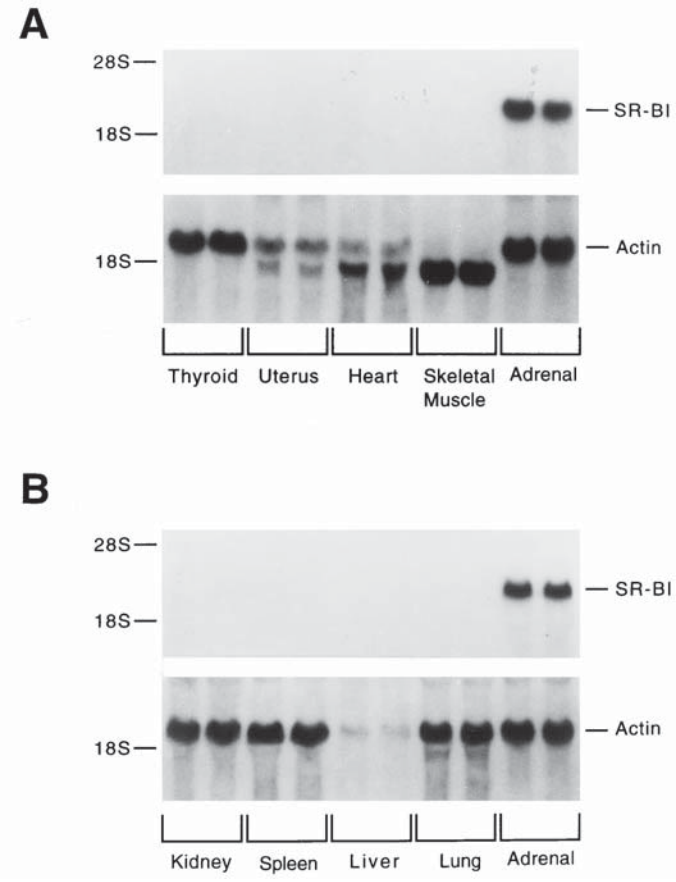

Fig. 4. Expression of SR-BI mRNA in various rat tissues. Northern blots containing 20 μg of total RNA isolated from the indicated tissues were probed sequentially with SR-BI and β-actin cDNAs. The autoradiograms were exposed for 48 and 24 h for SR-BI and β-actin, respectively.

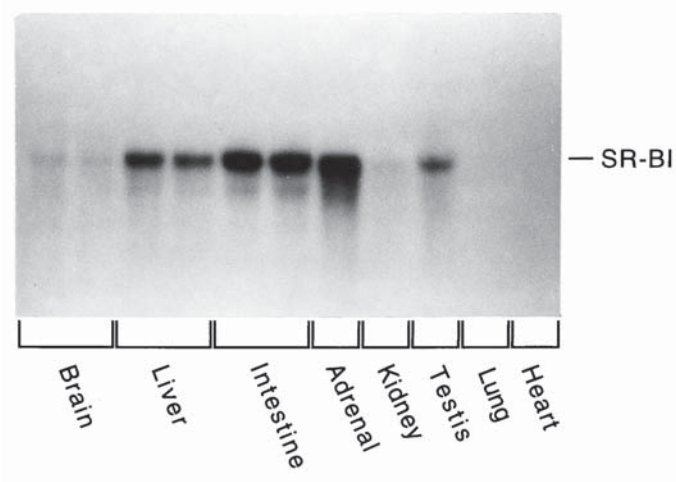

Fig. 5. Expression of SR-BI mRNA in various rat tissues using poly A+ mRNA. SR-BI was detected in all tissues, except lung and heart when poly A+ mRNA was examined by Northern blot analysis.

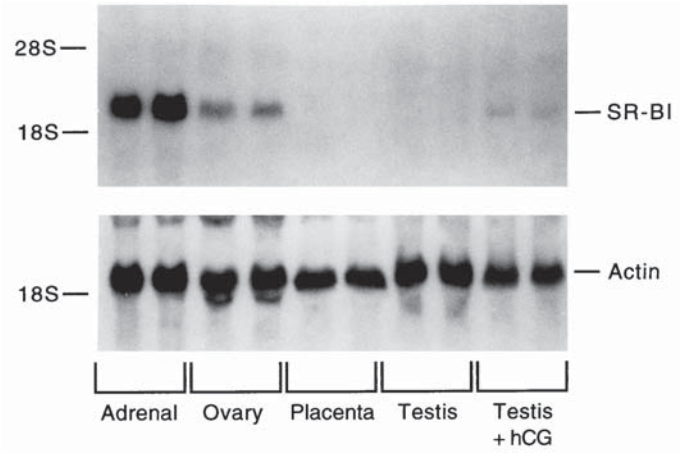

Fig. 6. Expression of SR-BI mRNA in steroidogenic tissues. Northern blots containing 20 μg of total RNA isolated from the indicated tissues were probed sequentially with SR-BI and β-actin. Expression was limited to adrenal, ovary, and testis. SR-BI expression in the testis was detected only after hCG injection.

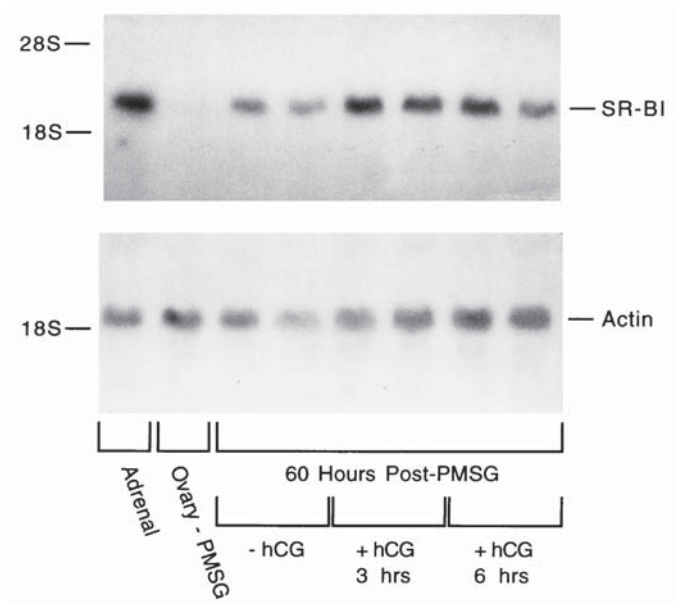

Fig. 7. Regulation of SR-BI mRNA expression in rat ovary (prior to ovulation) by hCG. Twenty-eight-day-old Sprague-Dawley rats (n = 4/time-point) were injected with 8 IU PMSG and 60 h later received an injection of hCG (50 U). Ovaries were obtained for control (t_0), 3- and 6-h time-points post-hCG injection. Northern blot analysis of SR-BI mRNA in the ovary following hCG treatment. This blot was probed sequentially for SR-BI and β-actin.

expression was downregulated (73%; $p < 0.01$) 4 h after PGF2α injection (Fig. 9). The decline in SR-BI mRNA expression paralleled a significant decrease (50%; $p < 0.001$) in serum progesterone levels (control = 127.0 ± 6.7 ng/mL; 4 h post-PGF2α = 62.9 ± 12.7 ng/mL) 4 h after PGF2α injection. To determine the time-course of reduction in SR-BI mRNA levels following PGF2α administration, ovarian tissue was examined on day 10 of pseudopregnancy before (t_0) and at 1, 2, 3, and 4 h post-PGF2α injection. The results of this experiment (Fig. 10A) indicate that SR-BI mRNA levels decreased significantly by 2 h post-PGF2α

Table 1
Serum Protesterone Levels in Pre- and Postovulatory Animals Treated with hCG[a]

Animal model	Control time = 0 (ng/mL)	Post-hCG 3 h (ng/mL)	Post-hGC 6 h (ng/mL)
Preovulation (60 h post-PMSG)	21.5 ± 0.9	35.5 ± 2.5[b]	47.5 ± 2.3[b]
Postovulation (11d post-PMSG)	79.0 ± 3.1	112.8 ± 2.8[c]	124.4 ± 3.3[c]

[a]Serum progesterone values are presented and the mean ± SEM from 6 separate animals in each treatment group.
[b]$p < 0.05$, relative to the control value.
[c]$p < 0.005$, relative to the control value.

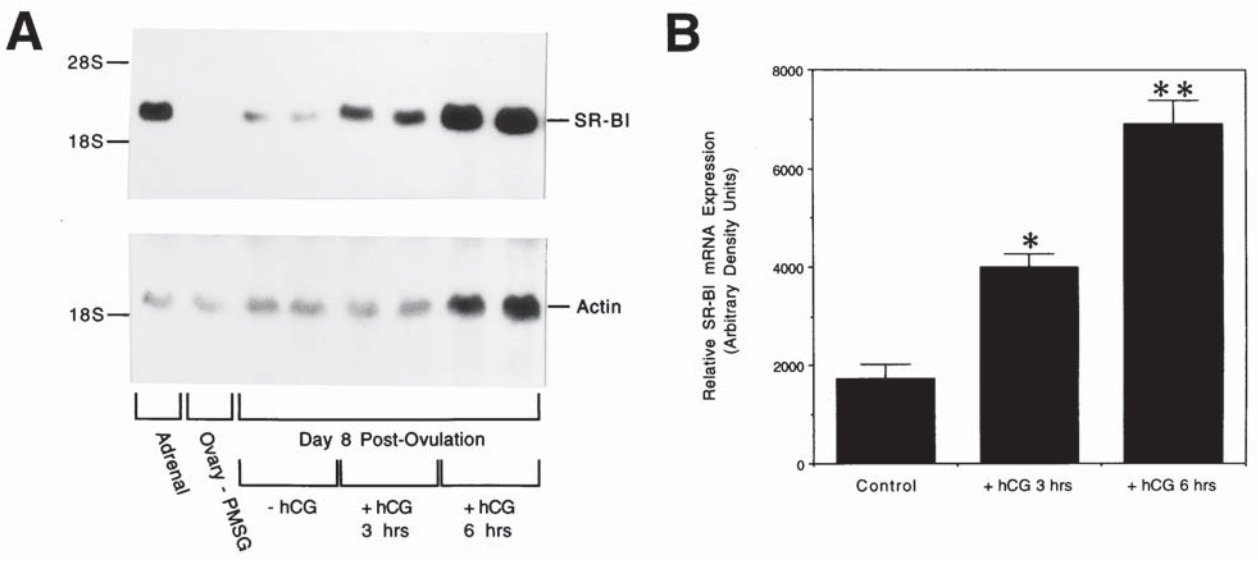

Fig. 8. Regulation of SR-BI mRNA expression in rat ovary (8 d postovulation) by hCG. Twenty-eight-day-old Sprague-Dawley rats ($n = 4$/time-point) were injected with hCG (50 U) 8 d postovulation. Ovaries were obtained for control (t_0), 3- and 6-h time-points post-hCG injection. **(A)** Northern blot analysis of SR-BI mRNA in the ovary 8 d postovulation following hCG treatment. This blot was probed sequentially for SR-BI and β-actin. **(B)** Densitometric analysis of SR-BI mRNA levels following hCG treatment. Bars represent the mean relative SR-BI mRNA levels from four separate animals expressed in arbitrary density units; $*p < 0.025$; $**p < 0.01$.

administration and remained significantly lower 4 h postinjection. Serum progesterone levels also declined during this period (Fig. 10B). By 4 h after PGF2α injection, serum progesterone was markedly reduced relative to control animals as noted in the previous experiment.

To determine what effect a gonadotropin would have on SR-BI mRNA levels after PGF2α injection, ovarian tissue was examined on day 10 of pseudopregnancy before any injections and 4 h after injection with PGF2α and hCG. The results of this experiment indicate that ovarian SR-BI mRNA induction by hCG was blocked by PGF2α (Fig. 11A). Overall, SR-BI mRNA levels were increased by hCG and decreased by PGF2α. The effect of both hCG and PGF2α on SR-BI mRNA levels indicated a small increase relative to control animals, but a large decrease relative to hCG-treated animals (Fig. 11A). The change in serum progesterone levels directly paralleled the expression of SR-BI mRNA in this experiment (Fig. 11B). As expected, serum progesterone levels were increased in response to hCG treatment (4 h post-hCG). The increase in serum progesterone was blocked, however, by pretreatment with PGF2α (4 h post-PGF2α/hCG) (Fig. 11B).

Discussion

This investigation involved the characterization of a cDNA for SR-BI, a putative HDL receptor, and its hormonal regulation within the rat ovary. The results demonstrated that the mRNA for SR-BI is expressed in several tissues involved in the metabolism of HDL with highest levels of expression being seen in steroidogenic tissues. Results of this investigation also indicate that gonadotropins enhance the expression of SR-BI, whereas PGF2α reduces the expression of its mRNA in the ovary. Whether the changes in SR-BI are owing to direct gonadotropin or prostaglandin interaction or owing to a change in intracel-

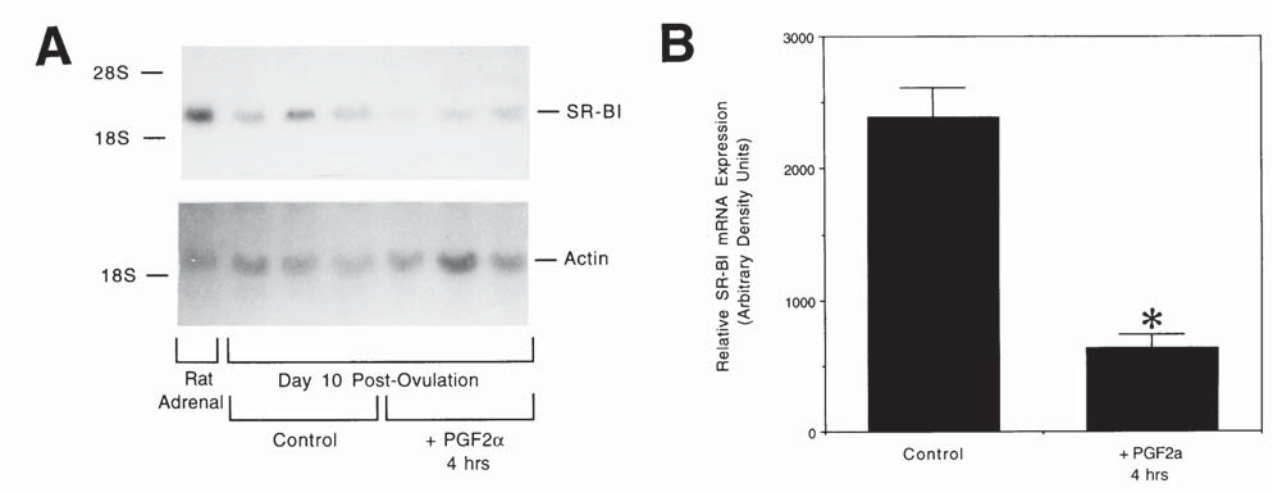

Fig. 9. Regulation of SR-BI mRNA expression in rat ovary (10 d postovulation) by PGF2α. Sprague-Dawley rats ($n = 4$/time-point) were injected 10 d postovulation with PGF2α (250 μg). **(A)** Ovarian RNA was analyzed for SR-BI expression before and 4 h following PGF2α injection. **(B)** Densitometric analysis of Northern blots of SR-BI mRNA in the ovary 10 d postovulation following PGF2α treatment. Bars represent the mean ± SEM SR-BI mRNA levels expressed in arbitrary density units from four separate animals/treatment ($*p < 0.01$).

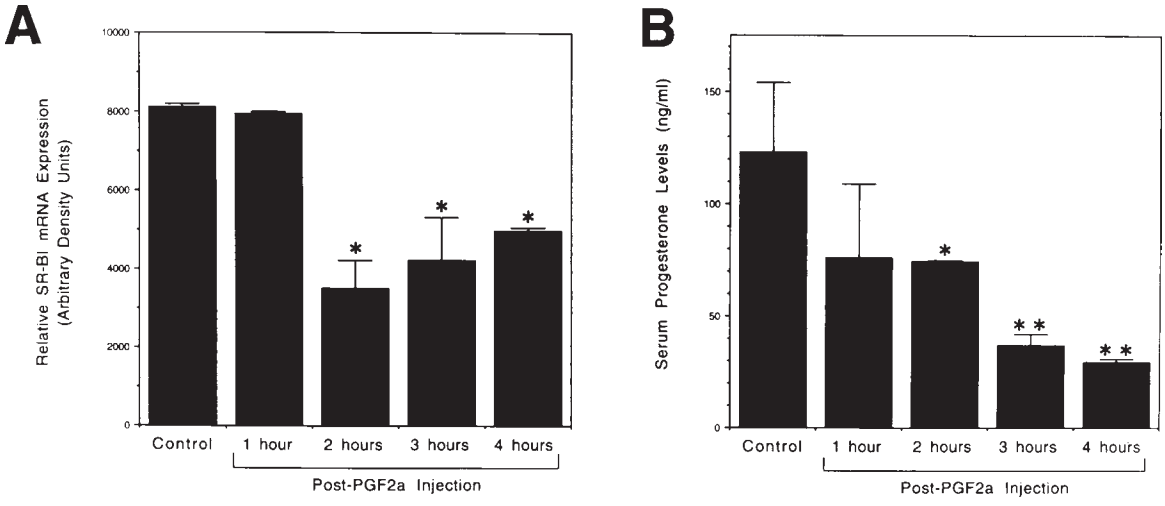

Fig. 10. Densitometric analysis of SR-BI mRNA levels and serum progesterone levels following PGF2α treatment. Sprague-Dawley rats ($n = 4$/time-point) were injected 10 d postovulation with PGF2α (250 μg). Ovaries were obtained for control (t_0), and 1- to 4-h time-points post-PGF2α injection. **(A)** Densitometric analysis of Northern blots of SR-BI mRNA in the ovary 10 d postovulation following PGF2α treatment. **(B)** Corresponding serum progesterone levels for animals injected with PGF2α. Bars represent the mean ± SEM; $*p < 0.025$; $**p < 0.01$.

lular sterol content that indirectly altered ovarian SR-BI levels was not addressed in this study. Overall, these results demonstrate that SR-BI expression in the ovary is regulated in a manner that is consistent with the stimulation of steroidogenesis in this tissue. Furthermore, SR-BI expression was reduced following prostaglandin treatment, which is consistent with PGF2α's antisteroidogenic role in the corpus luteum.

SR-BI is highly conserved between species. Recent studies *(11)* have also demonstrated similar chromosomal localization of the rat SR-BI and human CLA-1 genes to confirm representation of homologous genes. The SR-BI/CLA-1 gene encodes a protein that is thought to reside at the cellular membrane and facilitate cholesterol uptake by binding cholesterol-rich HDL particles.

Recently, Acton et al. *(5)* showed that SR-BI binds HDL and mediates the uptake of HDL cholesteryl esters. Several other groups have shown that SR-BI is expressed at all major sites of selective cholesterol uptake *(5,7,11)*. Recent studies have provided additional evidence that SR-BI is an HDL-R using a knockout mouse model *(12)*. In the SR-BI knockout mouse, serum cholesterol levels increased dramatically, but cholesterol concentration in organs that normally absorb HDL cholesterol dropped. Further support that SR-BI is an HDL-R was provided by an investigation by Kozarsky et al. *(13)* in which a modified adenovirus

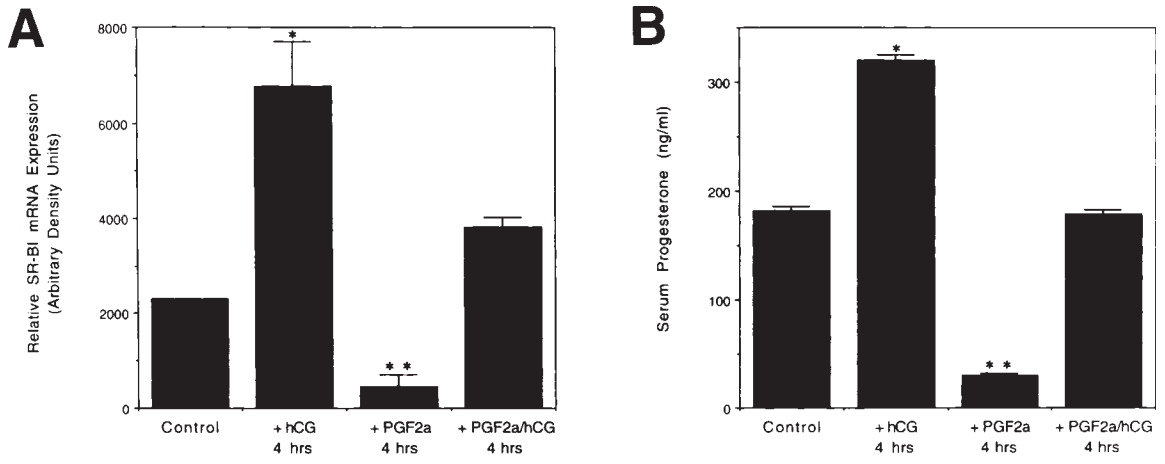

Fig. 11. Densitometric analysis of SR-BI mRNA levels **(A)** and serum progesterone levels **(B)** in the ovary following PGF2α/hCG treatment (rats were treated 10 d postovulation). Autoradiographs were scanned with a densitometer and the results presented. (B) Serum progesterone levels in the same animals were assayed and the results presented. Bars represent the mean ± SEM; $*p < 0.01$; $p < 0.025$.

carrying the SR-BI gene was overexpressed in mouse liver. In this study, serum HDL levels were significantly reduced, whereas the cholesterol content of the bile increased. Taken together, these investigations support the suggestion that SR-BI is an authentic HDL-R.

The tissue-specific expression of SR-BI in rats was similar to that in mice *(5)*. In the present study, we have extended the mouse study and conclude that SR-BI is highly expressed in rodent steroidogenic tissues. In rodents, HDL is known to transport cholesteryl esters to the adrenal gland, ovary, and testis *(1,2,9,10)* for steroidogenesis. A recent investigation by Li et al. *(14)* has demonstrated using *in situ* hybridization that SR-BI mRNA is expressed in both follicular and luteal compartments of the ovary. These data are consistent with the results of our experiment that demonstrated that SR-BI was expressed in the ovary in both the preovulatory (follicular) and postovulatory (luteal) rat models. Although it is well established that serum lipoproteins provide a source of exogenous cholesterol for several steroidogenic glands, including the human placenta, the rat differs from the human in that the major source of cholesterol for steroidogenesis appears to be from endogenously synthesized cholesterol *(15)*. Thus, the absence of detectable SR-BI mRNA in the rat placenta, as noted in our experiment, is not surprising. Several other tissues, however, such as the liver, kidney, and brain, do express the SR-BI gene product. Expression of the SR-BI gene product in nonsteroidogenic tissues may be important for maintaining cholesterol homeostasis (reverse cholesterol transport).

The findings of Landschulz *(7)* indicate that the administration of hCG to male rats induced a dramatic increase in Leydig cell SR-BI expression. These investigators have also shown that SR-BI expression is upregulated in steroidogenic tissues by estrogen *(7)*. Similar regulation of SR-BI expression in steroidogenic tissues was shown by Rigotti et al. *(16)*, who demonstrated that ACTH regulates SR-BI in the mouse adrenal gland. Consistent with the results previously reported, rat ovarian SR-BI expression was subject to regulation by gonadotropins during both follicular and luteal phases. The time-course for the increase in SR-BI mRNA was rapid, with a significant increase in SR-BI mRNA expression within 30 min after hCG administration (data not shown). In contrast to the results reported by Johnson et al. *(11)*, administration of PMSG alone was sufficient to induce expression of the SR-BI gene. When hCG was administered to PMSG-primed rats, the levels of SR-BI mRNA expression were significantly increased by 3 h relative to control rats. The difference in our results with gonadotropin stimulation may be related to the difference in the time frame following hCG injection. The acute effects of gonadotropin stimulation on SR-BI mRNA may be overlooked by 24 h postinjection. Our results indicate that the increase in SR-BI is relatively rapid, with a significant elevation in ovarian SR-BI mRNA levels being noted within 30 min of gonadotropin treatment. This rapid increase in SR-BI is consistent with the enhanced requirement for cholesterol to maintain steroid production in the ovary.

Although our study demonstrates that gonadotropins can increase SR-BI mRNA levels, we cannot rule out the possibility that enhanced mobilization of cholesterol stores, rather than direct gonadotropin action on the SR-BI gene, may play a role in enhancing SR-BI gene expression. Hormonal stimulation is associated with a depletion of ovarian cholesteryl ester content and an increase in cell-surface HDL binding *(17,18)*. As cholesterol esters are depleted in

ovarian cells to accommodate the enhanced level of cholesterol utilization for steroidogenesis, the SR-BI gene could be activated to replenish the cell's cholesterol stores in order to maintain cell steroid production. A recent study by Wang et al. *(19)* demonstrates that SR-BI expression can be upregulated when cholesterol stores are depleted. This occurred independent of whether cholesterol was depleted by reduced sterol uptake or increased cholesterol utilization. Future analysis of the SR-BI promoter region may provide clues to the molecular mechanisms that acutely regulate SR-BI gene expression for steroidogenesis compared to those mechanisms that maintain cell cholesterol homeostasis.

In pregnant and pseudopregnant rats, a high correlation between luteal PGF2α content and the demise of luteal function has been reported, clearly demonstrating a role for luteal PGF2α in rat luteolysis *(20–23)*. Uterine PGF2α is the primary luteolytic agent in the rodent *(24,25)*; however, prostaglandins are also produced within ovarian cells *(26,27)*. Corpus luteum regression is characterized by functional and structural alterations that result in the loss of steroid production by luteal cells *(28)*. PGF2α has been shown to depress cAMP accumulation and serum progesterone *(23)*, and to antagonize luteinizing hormone (LH)-stimulated steroid production in luteal tissue *(23)*. The loss of SR-BI mRNA following PGF2α is consistent with the luteolytic actions of this prostaglandin in the ovary. Alternatively, the results obtained when PGF2α and hCG were administered to the same animal suggest the possibility that hCG may reverse or prevent the loss of SR-BI expression induced by this prostaglandin. The actual mechanism through which hCG and PGF2α exert their action on SR-BI expression will require additional study. Following PGF2α-induced luteolysis in sheep and cows, corpora lutea accumulate substantial stores of lipids *(29,30)*. The enhanced level of lipid in ovarian cells may downregulate SR-BI to reduce cholesterol uptake. Whether PGF2α acts directly on the SR-BI gene in the ovary or indirectly through some other mechanism remains to be determined.

We conclude that a single SR-BI mRNA transcript of 2.4 kb was highly expressed in the rat adrenal, ovary, and testis. Within the ovary, SR-BI mRNA levels were significantly increased by PMSG and hCG. This increase corresponded to a significant increase in serum progesterone. In contrast, when PGF2α was administered, SR-BI mRNA levels were significantly reduced in concert with a reduction in serum progesterone secretion. Furthermore, PGF2α blocked the hCG-induced increase in SR-BI mRNA levels when administered 30 min prior to hCG injection. The results of this study demonstrate that SR-BI mRNA levels are dramatically increased by gonadotropins in the ovary, whereas PGF2α significantly and rapidly reduced SR-BI mRNA levels.

Materials and Methods

Chemicals and Complementary DNA (cDNA) Probes

Oligo nucleotides were obtained from Integrated DNA Technologies, Inc. (Coralville, IA). A rat ovarian cDNA library was obtained from Stratagene (La Jolla, CA). The reagents for PCR were purchased from Perkin Elmer (Norwalk, CT), and the TA cloning kit was purchased from Invitrogen (San Diego, CA). The Sephaglas BandPrep kit was obtained from Pharmacia Biotech (Piscataway, NJ). hCG and PGF2α were purchased from Sigma Chemical Co. (St. Louis, MO). PMSG was purchased from Diosynth (Chicago, IL). 1,2,6,7-^{3}H*(N)*-progesterone (104.1 Ci/mmol) and [^{35}S] deoxy-ATP (1348 Ci/mmol) were purchased from DuPont-New England Nuclear (Wilmington, DE). [α^{32}P] deoxy-CTP (3000 Ci/mmol) and the Sequenase DNA sequencing kit were obtained from the Amersham Corp. (Arlington Heights, IL). Nylon membrane was obtained from Schleicher and Schuell (Keene, NH), and BioMax film was purchased from Eastman Kodak (Rochester, NY). TRI-Reagent, Background Quencher, Formazol, Microcarrier Gel-Tit, and High-Efficiency Hybridization solution were obtained from Molecular Research Center (Cincinnati, OH). The random-primed DNA labeling kit and all restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN). The Wizard Mini- and Mega-prep DNA purification systems were purchased from Promega (Madison, WI). An RNA mol-wt marker was purchased from Gibco-BRL (Grand Island, NY). All other chemicals were reagent-grade, and were obtained from Fisher Scientific (Norcross, GA) or Sigma Chemical Co.

Isolation and Characterization of the Rat SR-BI (HDL-R) cDNA Probe

The isolation and characterization of the rat SR-BI complementary DNA (cDNA) probe was carried out by engineering primers from the mouse sequence *(5)*. The 5' primer (GTC TCC TTC AGG TCC TGA GC) spanned bases 2–22 of the mouse cDNA and the 3' primer (CCT ATA GCT TGG CTT CTT GC) spanned bases 1562–1581 *(5)*. Both primers were used to carry out PCR from a rat ovarian cDNA library (Stratagene). The conditions for PCR were denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min. The 1579-bp fragment obtained by PCR was then cloned into the TA vector (Invitrogen, San Diego, CA). The rat SR-BI cDNA was sequenced using the dideoxy chain-termination method *(31)* with ^{35}S-dATP and the Sequenase 2.0 protocol (Amersham).

Animals

Twenty-eight-day-old Sprague-Dawley rats were purchased from Harlan Industries of Madison, WI. All proce-

dures for hormone and prostaglandin treatment and the methods for tissue and blood sampling were approved by the University of South Florida Animal Care Committee. Throughout the experiment, animals had free access to food and water, and were housed under a 12-h dark, 12-h light cycle. Follicular development and ovulation were induced in rats by injection of 8 IU PMSG (im). Rats ovulate approx 72 h following treatment with 8 IU PMSG *(32)*. At 60 h post-PMSG treatment, one set of rats was injected with 50 U of hCG (iv). Ovaries were removed prior to hCG injection (t_0) and at 3 and 6 h post-hCG treatment. In a second experiment, rats were treated with PMSG as indicated above, followed by hCG injection (50 U; iv) on day 8 postovulation. Ovaries, which consist mainly of luteal tissue using this protocol *(32)*, were removed prior to hCG injection (t_0) and at 3 and 6 h post-hCG treatment. Tissue was immediately frozen in liquid nitrogen. Serum samples were obtained by cardiac puncture at the time of tissue removal and serum was stored at –20°C until assayed for progesterone.

In a third experiment, rats were treated with 8 IU of PMSG as indicated above. Ten days following ovulation, the rats were treated with either a single PGF2α injection (250 μg) or injection with PGF2α (250 μg) followed by hCG (50 U) 30 min later. With the animals under ether anesthesia, ovaries were removed prior to the PGF2α/hCG injection protocol (t_0) and at 4 h post-PGF2α treatment.

Serum Progesterone Assay

Progesterone was measured by radioimmunoassay (RIA) using 1,2,6,7-^{3}H*(N)*-progesterone. This assay followed the methods previously described *(33)* and used the progesterone antibody GDN 337, which was kindly provided by G. D. Niswender (Colorado State University, Fort Collins, CO). The specificity, validity, and reliability of this RIA have been reported previously *(34)*.

RNA Isolation and Electrophoresis

RNA was prepared from ovaries using a modification of the Chomczynski and Sacchi method *(35)* (TRI-Reagent Method, Molecular Research Center; Cincinnati, OH). This method consistently yields 5–8 μg RNA/mg tissue. Tissue (<200 mg) was homogenized in 3 mL of TRI Reagent with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY), and centrifuged at 12,000*g* for 15 min at 4°C. RNA was precipitated from the aqueous phase with isopropanol, and the RNA pellet was washed in 75% ethanol and resuspended in Formazol. RNA was quantified by absorbance at 260 nm in a Pharmacia GeneQuant (Piscataway, NJ).

For Northern blot analysis, total RNA (20 μg) or poly A+ (10 μg) was denatured at 65°C (15 min) and loaded onto 1% agarose gels containing 3% formaldehyde. Following size fractionation, RNA was blotted onto a nylon membrane (0.45-μm pore size) by capillary transfer, and RNA was fixed to the membrane by UV crosslinking (0.3 J/cm^2). Ethidium bromide staining of the gel confirmed that the ribosomal RNAs (18S and 28S subunits) were intact and determined whether equal amounts of RNA were loaded in each lane.

Northern Blot Analysis

Northern blot hybridizations were performed using a 1579-bp rat SR-BI cDNA and a 2.0-kb chicken β-actin cDNA. The cDNA inserts were labeled with [α^{32}P] deoxy-CTP using the random-primed DNA-labeling method *(36)*. Northern blots were prehybridized at 62°C for at least 3 h in a 1 *M* NaCl, 1% SDS solution containing Background Quencher (Molecular Research Center, Cincinnati, OH). Hybridization was completed in a High Efficiency Hybridization Solution (Molecular Research Center) containing the ^{32}P-labeled probe (1×10^6 dpm/mL; SA = 2×10^8 dpm/μg DNA) at 62°C for at least 16 h. Blots were washed three times at room temperature (5 min) in 1X SSC/1% SDS and three times at room temperature (10 min) in 0.1X SSC/0.1% SDS. RNA:cDNA hybrids were visualized on BioMax film using two intensifying screens and a 12- to 48-h exposure period. The RNA transcript size was determined by comparison to an RNA mol-wt marker run adjacent to the sample RNA lanes. Blots were stripped and reprobed with the actin cDNA. Densitometric analysis was performed on the 2.0-kb β-actin transcript for the standardization of RNA loading.

Data Analysis

The Northern blot results were quantitatively analyzed using a Hoefer Scanning Densitometer (Hoefer Instruments, San Francisco, CA). Minor variations in RNA loading were corrected for using the β-actin cDNA. Serum progesterone was expressed as the mean ± SEM. Data from these individual parameters were compared by analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple-comparison test when applicable *(37)*. All analysis was completed using the Statview program with graphics (Abacus Concepts, Berkeley, CA) on a Macintosh 6400/200 computer. A $p < 0.05$ was considered significant for all tests.

Acknowledgment

This work was supported by grants HD31644 and HD35163 from the National Institute of Health.

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