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# CHOLESTEROL UPTAKE IN ADRENAL AND GONADAL TISSUES: THE SR-BI AND 'SELECTIVE' PATHWAY CONNECTION

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## TABLE OF CONTENTS

*1. Abstract*
*2. Introduction*
*3. Lipoprotein cholesterol: transport, intracellular processing and utilization for steroidogenesis*
- *3.1. Steroidogenesis: an overview*
- *3.2. Evidence that lipoprotein cholesterol is a primary precursor for steroid biosynthesis: a historical perspective*
- *3.3. Uptake of lipoprotein cholesterol via the LDL (B/E)-receptor mediated 'endocytic' pathway*
- *3.4. Uptake of lipoprotein cholesterol via the 'selective' pathway*

*4. Scavenger receptor class B, type I (SR-BI)*
- *4.1. Molecular characteristics*
- *4.2. Ligands for SR-BI*
- *4.3. SR-BI-mediated selective cholesteryl ester uptake and other SR-BI-associated functions*
- *4.4. Potential mechanisms involved in SR-BI-mediated selective CE uptake*
- *4.5. Are there other receptor mechanisms for HDL-CE internalization?*
- *4.6. Tissue distribution of SR-BI expression*
- *4.7. Cellular regulation of SR-BI*
  - *4.7.1. Adrenal gland*
  - *4.7.2. Ovary*
  - *4.7.3. Testis*
  - *4.7.4. Liver*
  - *4.7.5. Macrophages and other tissues and cells*

*5. Conclusions*
*6. Acknowledgement*
*7. References*

## 1. ABSTRACT

A constant supply of cholesterol is needed as a substrate for steroid hormone synthesis in steroidogenic tissues. Although there are three potential sources, which could contribute to the 'cholesterol pool', needed for steroidogenesis (i.e., *de novo* synthesis, hydrolysis of stored cholesteryl esters and exogenous lipoproteins), current evidence suggests that plasma lipoproteins are the major source of cholesterol for steroid production in adrenal gland, ovary and, under certain conditions, testicular Leydig cells. In many species, steroid producing cells and tissues obtain this lipoprotein-cholesterol by a unique pathway in which circulating lipoproteins bind to the surface of the steroidogenic cells and contribute their cholesteryl esters to the cells by a 'selective' process. This is a process in which cholesterol is selectively absorbed while the lipoprotein remains at the cell surface. The discovery of a specific receptor for this process (scavenger receptor class B, type I, known as SR-BI) has revolutionized our knowledge about the selective uptake pathway. The present review summarizes the functional importance of the selective pathway as a bulk cholesterol delivery system for steroidogenesis, and attempts to detail the expression, regulation and characteristics of SR-BI as it is deployed in steroidogenic systems as a means of achieving cholesterol balance.

## 2. INTRODUCTION

Cholesterol is an essential structural component of mammalian cell membranes (1,2) and is the major regulator of numerous cellular processes including signal transduction, receptor function, membrane micro-domains (rafts and caveolae), gene expression, cell growth, and lipoprotein synthesis (3-11). It is also the sole biosynthetic precursor for steroid hormones, bile acids and vitamin D (12-15). To meet the cellular demands for product formation, cells obtain cholesterol by *de novo* synthesis from acetate in the endoplasmic reticulum and by internalization of cholesterol from plasma lipoproteins. However, since excess free cholesterol is toxic in most cells, complex mechanisms exist that tightly regulate cholesterol homeostasis through a balance of influx, synthesis, catabolism and efflux to ensure a constant supply, yet avoid excess. In addition, a second tier of safeguards is generally achieved via the sequestration of free cholesterol in cell membranes, primarily the plasma membrane (which is estimated to contain approximately 65-80% of total free cellular cholesterol), (3) and storage in lipid droplets in the form of cholesteryl esters (16).

The steroid producing tissues such as gonads, adrenal gland and placenta display a dual requirement for cholesterol as an essential architectural component of

cellular membranes and as a precursor for steroid hormone synthesis (13,17-21). These specialized tissues have the potential to obtain and utilize cholesterol for steroid biosynthesis from a number of sources including cholesterol synthesized *de novo*, cholesterol acquired from cholesterol-rich circulating low- and high-density lipoproteins, and cholesterol concentrated in the plasma membrane or stored in cytoplasmic lipid inclusions (lipid droplets) as cholesteryl esters (22-25). However, there is now overwhelming evidence to suggest that adrenal, ovarian, placental, and possibly testicular tissues and cells of many species including human, preferentially utilize lipoprotein-derived cholesterol for steroid hormone production (25). During the past two decades considerable progress has been made regarding the mechanisms and regulation of uptake of lipoprotein cholesterol by steroidogenic organs (22-25). Two major and entirely separate and physiologically regulated cholesterol transport pathways for delivering lipoprotein cholesterol have been identified and well characterized in various steroidogenic cells. These are broadly classified as low capacity 'endocytic' (26,27) and bulk delivery 'selective' (25,28,29) cholesteryl ester uptake pathways. In the former case, lipoprotein-derived cholesterol can be delivered by endocytic uptake in which human LDL, or other apolipoprotein B- or apolipoprotein E- containing lipoproteins bind to the LDL (B/E) receptor, located predominantly within the clathrin coated pits on the cell surface and the entire LDL-receptor complex is rapidly internalized by endocytosis (26,27). Subsequently, endocytosed cholesterol moves to the late endocytic compartment for its release into the cell interior (26,27). In contrast, the bulk delivery selective pathway differs from endocytic pathway in that exogenous circulating lipoproteins (such as HDL) contribute their cholesteryl esters to cells without internalization of the intact particle (25,28-30). Thus, in selective cholesterol uptake process, lipoprotein lipids enter cells unaccompanied by apolipoproteins. In this review, we summarize the major events involved in lipid transport into the gonads and adrenal gland with particular emphasis on the selective pathway and its receptor, scavenger receptor class B, type I (SR-BI).

## 3. LIPOPROTEIN CHOLESTEROL: TRANSPORT, INTRACELLULAR PROCESSING AND UTILIZATION IN STEROIDOGENESIS

### 3.1. Steroidogenesis: an overview

Although the focus of this review is the mechanism and regulation of cholesterol transport in adrenals and gonads, in order to familiarize the readers, a brief overview regarding the critical events involved in steroid hormone biosynthesis is warranted. Steroidogenesis is under the control of many players. Tissue-specific peptide hormones mainly regulate the rate of steroidogenesis, but other agents also control steroidogenesis (31-33). Thus, adrenocorticotropic hormone (ACTH) increases glucocorticoid (cortisol/corticosterone) synthesis in adrenal cortex fasiculata-reticularis cells; ACTH or angiotensin II increases mineralocorticoid aldosterone synthesis in adrenal glomerulosa cells; human chorionic gonadotropin (hCG) or luteinizing hormone (LH) increases progestin synthesis in luteinized granulosa/luteal cells; follicle stimulating hormone (FSH) stimulates progestin and estrogen synthesis; LH regulates androgen synthesis in theca cells; and LH increases androgen (testosterone) synthesis in testicular Leydig cells (13,33). Although the final steroid product differs among these cell types, the first committed step in the biosynthesis of steroids begins with the cleavage of a six-carbon unit from the 27carbon cholesterol molecule to form the common steroid precursor, 21 carbon pregnenolone (13,33,34). This NADPH dependent reaction is catalyzed by the side chain cleavage (SCC) cytochrome P450 (P450scc) complex, which is comprised of a flavoprotein (NADH-adrenodoxin reductase), a $Fe_2$-$S_2$* type iron—sulfur protein (adrenodoxin) and a hemoprotein CYP11A1 (cytochrome P450scc) localized in the inner mitochondrial membranes (34). As in many metabolic pathways, this initial reaction is the site of steroid hormone regulation, and its rate determines the flux through the pathway. The rate limiting nature of this step is not determined by the activity of CYP11A1 (i.e., enzymatic conversion of cholesterol to pregnenolone) but rather the delivery of cholesterol to the substrate site of CYP11A1 (i.e., translocation of cholesterol from an outer to an inner mitochondrial [steroidogenic] pool readily accessible to CYP11A1) (34,35). Trophic hormones rapidly stimulate this process by acting through a cAMP second messenger system, to facilitate mobilization of intracellular cholesterol and its transport and accumulation at the inner mitochondrial sites of the P450scc (CYP11A1) system (36,37).

The entire process of intracellular cholesterol transport to mitochondria can be broadly divided into two separate but equally important steps. In the first step of the acute steroid response, activation of neutral cholesteryl esterase by protein kinase-A (PKA)-catalyzed phosphorylation hydrolyzes stored (lipid droplet) cholesteryl esters (CE) to free cholesterol (FC) (38), which, in turn, is transported to the outer mitochondrial membrane. In addition, depending on cell type, the cAMP-PKA signaling cascade may also directly mobilize cholesterol from plasma membrane or other cellular membranes to the outer mitochondrial membrane (25). Because cholesterol is a hydrophobic molecule and diffuses poorly in an aqueous environment, a number of putative factors including cholesterol transport proteins such as sterol carrier protein 2 ($SCP_2$) (39-41) and steroidogenesis activator polypeptide (SAP) (39,40,42), cytoskeletal components/structures (43) and changes in cellular architecture (33,37,40) have been suggested to facilitate cholesterol transport to the outer mitochondrial membrane, however, their mechanisms of action have not been fully established. The second critical step is the delivery of the substrate, cholesterol, to the inner mitochondrial membrane P450scc site. This step is considered rate limiting because hydrophobic cholesterol cannot rapidly diffuse through the aqueous intermembrane space of the mitochondria to support acute steroid synthesis, and requires the de novo synthesis and participation of a labile protein (33,44). The recently characterized steroidogenic acute regulatory (StAR) protein

is an indispensable component in this process and is now considered the best candidate to fulfill the role of a putative labile protein that is essential for the translocation of cholesterol to inner mitochondrial sites of P450scc (33,45,46). Recently, the peripheral-type benzodiazepine receptor (PBR), which is present in the mitochondrial membranes of steroid producing cells, and its endogenous ligand, diazepam binding inhibitor (DBI), have also been shown to play a role in the transport of cholesterol (47). There is now evidence that StAR and PBR may heterodimerize (48) and that their physical interaction is important for efficient cholesterol transport from the outer to the inner mitochondrial membranes (49). Thus, there is likelihood that StAR, PBR/DBI and perhaps $SCP_2$, SAP and other StAR-related lipid transfer proteins (3,50,51) may function in concert to support normal transport of cholesterol from the outer to the inner mitochondrial membrane.

### 3.2. Evidence that lipoprotein cholesterol is a primary precursor for steroid biosynthesis: a historical perspective

In 1945, the pioneering work of Block (22,53) provided the first direct evidence that steroid hormones are derived from cholesterol by feeding radiolabeled cholesterol to a pregnant woman and subsequently recovering labeled pregnanediol from her urine. In the succeeding 15-20 years, extensive studies were conducted to further establish a precursor role for cholesterol in the biosynthesis of steroidal products by various steroidogenic glands (53-55). Srere and collaborators (56,57) were the first group of investigators to experimentally demonstrate that slices of bovine adrenocortical and testicular tissues could incorporate radiolabeled acetate into cholesterol and transform newly synthesized cholesterol into steroid products. Hechter et al (58) and Savard et al (59) confirmed these pioneering studies *in vivo* with adrenal and testicular tissues, respectively, while Sweat et al (60) and Hellig and Savard (61) reported similar findings using the bovine corpus luteum.

Later, with the advent of improved technologies, a great majority of studies were devoted to determine the extent to which exogenous versus endogenous cholesterol contributes to steroidogenesis. In a classic experiment, Morris and Chaikoff (62) fed [$^{14}$C] cholesterol to male rats and observed that with time, the specific activity of adrenal cholesterol became equal to that of plasma. These data were interpreted to suggest that the majority of adrenal cholesterol was derived from plasma cholesterol and not from *de novo* synthesis. Similarly, Krum et al (63) reported that the relative specific activities of plasma unesterified cholesterol, adrenal unesterified cholesterol, and plasma corticosteroids were identical when dogs were fed [$^{14}$C] cholesterol for long periods. These authors also concluded that almost all of the cholesterol needed for steroid synthesis was derived from circulation. Using a similar experimental strategy, Flint and Armstrong (64) reported rapid equilibration of *in vivo* infused [$^{14}$C] cholesterol with the rat ovarian unesterified cholesterol and newly synthesized progestins, confirming that ovaries, like adrenals, preferentially utilize lipoprotein-supplied cholesterol for steroidogenesis. Further studies by Dexter et al (65) showed that ACTH treatment enhanced adrenal uptake of [$^{3}$H] cholesterol from plasma, and thus, provided the first evidence that lipoprotein-derived cholesterol uptake by adrenals is a hormonally regulated process. Finally, Borkowski et al (66,67) and Bolte et al (68,69), using complex in vivo isotopic kinetic studies, provided evidence that human adrenals, ovaries and placenta also obtain the bulk of their cholesterol from plasma-associated cholesterol.

Once it was established that adrenal and ovarian tissues preferentially utilize plasma supplied cholesterol for steroid synthesis, a great majority of follow-up studies focused attention on the problems of how hepatic and steroidogenic tissues acquire cholesterol from plasma lipoproteins and whether significant differences existed in the mode of cholesterol transport between low-density (LDL) and high-density (HDL) lipoproteins. These investigations were aided by the timely introduction of hypocholesterolemic animal models in which various lipoprotein fractions were markedly reduced with the administration of pharmacological agents such as 4-aminopyrazolo [3,4-*d*] pyrimidine (4-APP) (70,71) and 17 alpha-ethinyl estradiol (17 alpha-$E_2$) (72,73). Andersen and Dietschy were the first to utilize one of these models (4-APP) to evaluate potential regulatory actions of LDL and HDL on sterol synthesis as an indirect measure of cholesterol transport in rat ovary and adrenal gland (74). They observed that infusion of HDL markedly suppressed sterol synthesis in these two tissues. In contrast, physiological doses of LDL had no significant effect on sterol synthesis in the ovary and caused only a modest suppression in the adrenal gland (73). On the basis of these observations and other published reports in laboratory animals, these investigators raised the possibility of the existence of two transport systems; one LDL system (i.e., LDL receptor/endocytic pathway) that regulates sterol synthesis in a number of non-hepatic, nonendocrine tissues, and a second HDL system that principally regulates sterol synthesis in the ovary and adrenal gland (74). Moreover, the same investigators in another report were able to further conclude that three principal steroidogenic tissues, rat adrenal gland, testis and ovary preferentially take up and utilize HDL-derived, rather than LDL-derived, cholesterol for the production of steroid hormones (73). Finally, the use of two independent techniques designed to quantify *in vivo* uptake of lipoprotein cholesterol led to the unequivocal demonstration that HDL and LDL supply cholesterol to the adrenal gland and ovary by separate mechanisms involving two different pathways (75-78), i.e., LDL supplies cholesterol via the B/E receptor/endocytic pathway while HDL donates its cholesterol via a pathway that does not involve an endocytic process. This putative HDL pathway has now been well characterized and is commonly referred to as the 'selective' pathway (25,28-30).

### 3.3. Uptake of lipoprotein cholesterol via the LDL (B/E)-receptor mediated 'endocytic' pathway

The low-density lipoprotein (LDL) receptor (LDL-R) is a prototypic member of a rapidly enlarging

family of single transmembrane glycoproteins, generally identified as a cell surface associated endocytosis receptors, which bind and efficiently internalize extracellular ligands for proteolytic degradation by lysosomes (26,27). At present, this family consists of nine members in mammals: the LDL-R itself, a very low-density lipoprotein receptor (VLDL-R), the LDL-R-related proteins (LRP)/$\alpha_2$-macroglobulin receptor ($\alpha_2$MR) (here referred to as LRP1), LRP1B, epithelial glycoprotein 330/megalin (LRP2), LRP5, LRP6, the apolipoprotein E receptor 2 (apoER2, also called LRP8), and SorLA/LR11 (26,27,79-83). While other members of this gene family bind and internalize a variety of structurally and functionally diverse ligands and play significant roles in development, cell signaling and pathogenesis, the sole function of the LDL-receptor protein is to mediate the uptake and intracellular degradation of cholesterol-rich lipoproteins for cholesterol delivery to the cell interior. The pioneering work of Brown and Goldstein (26) led to the identification and functional characterization of the LDL-receptor and its associated endocytic pathway. The LDL-receptor is a transmembrane glycoprotein of 839 amino acids, is predominantly localized in clathrin-coated pits and undergoes constitutive endocytosis and recycling (26,27). It specifically binds apoB 100 (LDL) and apoE-containing lipoproteins (26,27). Following binding, the LDL (or apoE lipoprotein)-complex is rapidly internalized by endocytosis. Within the cell, LDL-receptor complexes rapidly dissociate as the endosomal pH falls, and the receptor recycles back to the cell surface. The resulting endosome containing the whole LDL particle is delivered to, and fuses with, lysosomes, where the lipid and protein components are degraded by acid proteases and lipases. The free cholesterol released by the hydrolysis of cholesterol esters by lysosomal acid lipase enters the cytoplasm where it can now be utilized for product formation, membrane biogenesis, or re-esterified and stored in lipid droplets.

Numerous studies carried out during the late 70s and mid 80s led to the demonstration that the adrenal, ovary, and testis from a number of animal species all express the classical LDL-receptor pathway (23-25). However, the extent of the functional expression of this pathway was shown to vary considerably with the tissue and species being examined as well as the experimental conditions employed to study its expression. For example, cultured mouse adrenal (Y-1) and bovine adrenocortical cells were shown to express significant levels of LDL receptors and internalize sufficient amounts of LDL-derived cholesterol for use in steroid hormone biosynthesis (84-86). Also, both LDL receptor activity and endocytic degradation of LDL can be upregulated following treatment of these cell types with ACTH (84-88). Likewise, angiotensin II was also shown to stimulate receptor-mediated uptake of LDL in bovine adrenal cells (89), and ACTH treatment *in vivo* or 17alpha-ethinyl estradiol-induced hypocholesterolemia enhanced the LDL receptor binding activity in isolated rat adrenal membranes (88,90,91). Strott reported that while the high affinity LDL binding capacity was comparable in the outer (glomerulosa/fasciculata) and inner (reticularis) zones of control guinea pig adrenal cortex, only the outer zone activity responded to ACTH stimulation (92). Human fetal adrenals also possess an LDL receptor pathway that supplies cholesterol for steroid synthesis and is subjected to dual hormonal and cholesterol regulation (93). Besides these studies, there are many additional reports covering various aspects of the LDL-receptor pathway and utilization of LDL-cholesterol for glucocorticoid synthesis in different adrenal systems including human (94,95), rat (96-100), hamster (99,100,101), cattle (97,102-104), sheep (105), squirrel monkey (106), rabbit (97, 107), pig (108), mouse (76,87,109), guinea pig (110,111) and dog (112).

Data concerning the *in vivo* utilization of LDL cholesterol by adrenals are also available. A number of reports from the laboratories of Dietschy and colleagues (74,75,77,78,113) and Brown and Goldstein (71,76) demonstrated that infusion of human LDL into 4-APP-treated rats restored the circulating lipoprotein levels to almost normal levels and reversed the effects of the *hypo*cholesterolemia on adrenal cholesterol homeostasis i.e., de novo cholesterol biosynthesis was suppressed toward control levels as measured by reduced incorporation of radiolabeled precursors into sterol by adrenal slices and decreased activity of the rate-limiting enzyme, the HMG-CoA reductase (71,74,75). In a follow-up study, Andersen and Dietschy (77) determined the kinetic parameters of lipoprotein-cholesterol transport by adrenals after infusing human/rat LDL or human/rat HDL into the hypocholesterolemic rats that were pre-treated with aminoglutethimide to prevent further conversion of cholesterol into steroids. The apparent half-maximal rate of uptake (Km) of human LDL was 61 mg/dl with a Vmax of 0.48 mg/pair of glands/h. Infusion of physiological concentrations of rat LDL also resulted in the accumulation of cholesterol into adrenals, and there was no difference in the rate of cholesterol uptake from LDL of rat or human origin, although the limited availability of rat LDL precluded complete kinetic comparisons between rat LDL and human LDL (75,77). [Interestingly, adrenal uptake of both human- and rat HDL-cholesterol was roughly five times more efficient than with either human or rat LDL (77,78)]. Finally, Pittman et al (114), using residualizing [$^{14}$C] sucrose-human LDL, were able to not only confirm the above findings that adrenal glands accumulate LDL-cholesterol *in vivo,* but further demonstrated that of all the tissues examined, the adrenal took up the LDL at the highest rate followed by the ovary (114).

The pathway of LDL-cholesterol transport and utilization of LDL cholesterol for steroidogenesis was studied with almost the same intensity in the ovary as in the adrenal gland and available data are indicative of a functional but variable role of the LDL receptor pathway in supplying cholesterol for gonadal hormone synthesis in various ovarian tissue and cell models. Strauss and colleagues were the first to extensively characterize LDL processing via the LDL-receptor endocytic pathway as well as utilization of LDL-cholesterol for progestin production in rat and human granulosa-luteal cells. Initially, Schuler et al (115) demonstrated that progestin secretion by cultured luteinized human granulosa cells was markedly reduced when cells were cultured in medium supplemented with

serum deficient in lipoprotein cholesterol, although the incorporation of [$^{14}$C] acetate into cellular sterols and secreted progestins was substantially increased. In contrast, addition of high concentrations of human LDL effectively suppressed the [$^{14}$C] precursor incorporation into sterols and secreted progestins, but enhanced its incorporation into fatty acids of stored cellular cholesterol esters in a dose dependent manner, thus providing the first direct evidence of a functional role of LDL-derived cholesterol in the regulation of granulosa cell cholesterol homeostasis and steroid synthesis. Later Soto et al (116) and Golos et al (117), carrying out complex kinetic studies, concluded that LDL-derived cholesterol is required for maximal steroidogenic response of luteinized human granulosa cells to tropic hormones such as LH/hCG or its second messenger, cAMP and that these agents stimulated the cellular uptake and metabolism of LDL-cholesterol and promoted the conversion of LDL-cholesterol into steroid products. Paavola et al (118,119) used electron microscopic, cytochemical and biochemical techniques to identify the critical events involved in the uptake and metabolism of LDL in human and rat granulosa cells, which require lipoprotein-cholesterol for full functional activity related to steroidogenesis. It was also reported that in granulosa cells, trophic hormones and their second messenger, cAMP, positively regulate LDL-receptor expression (120,121). At the same time, earlier work from our own laboratory demonstrated that freshly isolated rat granulosa cells express very little LDL-receptor, but show posttranslational increases in receptor activity with time in culture and also respond to hormonal and cAMP stimulation with transcriptional increases in LDL-receptor levels (121).

Utilizing freshly isolated rat luteal cells, several investigators reported enhanced lipoprotein-supported progestin synthesis and secretion (122-125). Azhar & Menon (122,123) further reported that LDL (or HDL) stimulation was most apparent when luteal cells were isolated from hypocholesterolemic (4-APP treated) rats or when cells were co-incubated with lipoprotein $\pm$ trophic hormones or cAMP analogs. Subsequent studies confirmed LDL-supported steroidogenesis in a variety of ovarian cells including mouse granulosa cells (126), bovine luteal cells (127), pig granulosa cells (128,129), monkey granulosa cells (130), bovine theca and granulosa cells (131), hamster follicles (132), porcine luteal cells (133), ovine luteal cells (134), rabbit luteal cells (135), rat theca cells (136), rat granulosa cells (137,138) and organ culture or human corpus luteum (139).

Much less information is available on the expression of the LDL-receptor pathway and LDL metabolism by testicular Leydig cells as compared with our understanding of adrenal and ovarian systems. Classical experiments of Morris and Chaikoff (62) provided the first evidence that rat testis relies primarily on endogenously synthesized cholesterol for steroidogenesis despite cholesterol feeding and excess availability of lipoprotein-derived cholesterol. In 4-APP-treated hypocholesterolemic rats, Andersen and Dietschy found little difference in the rate of cholesterol synthesis in the testis as compared with controls, whereas rates of sterol synthesis were markedly increased in adrenal gland (42-fold) and ovary (2.7-fold) (75). Also, we reported previously that human LDL did not alter the rates of basal and hCG-stimulated testosterone production by freshly isolated rat Leydig cells during short-term incubations (140). Likewise, murine Leydig cells utilize endogenous cholesterol to support steroidogenesis and exogenous mouse LDL does not exert any modulatory actions on testosterone biosynthesis (141). Thus, there seems to be a consensus that normal rodent Leydig cells preferentially utilize endogenously supplied cholesterol for testosterone production, and that exogenously supplied (LDL or HDL) cholesterol probably does not have a significant impact on androgen synthesis in these cells. However, these rodent Leydig cells can be programmed, following physiological manipulations, to effectively acquire and utilize LDL (or HDL) cholesterol for steroid synthesis. For example, Quinn et al (142) reported that the addition of human LDL to incubation medium increased testosterone synthesis by Leydig cells from rats pretreated with a desensitizing dose of human chrionic gonadotropin, while it had no effect on testosterone production by cells from control animals. Charreau et al reported very similar findings (143). Employing an alternative strategy to deplete intracellular cholesterol, Freeman and Ascoli (144) and Schreiber et al (145) reported that the presence of LDL had little or no effect on the amount of steroid products synthesized during the acute phase of stimulation of MA-10 and rat Leydig cells, respectively, but that it greatly enhanced steroid secretion during prolonged hormonal stimulation by directly providing cholesterol substrate to cells. In contrast to rodent Leydig cells, pig Leydig cells acquire >75% of their cholesterol for steroidogenesis from circulating lipoproteins (146). Moreover, it was demonstrated that addition of either human LDL or porcine LDL substantially enhanced both basal and hCG-stimulated testosterone production by pig Leydig cells (146). Human fetal Leydig cells show similar dependence on both low-density lipoprotein cholesterol and de novo synthesized cholesterol as a precursor for testosterone synthesis (147).

Although the vast evidence presented above may be indicative of the LDL-receptor pathway being a major contributor of cholesterol for steroidogenesis, in reality this may not be the case. First, the LDL-receptor pathway is a high affinity and low capacity cholesterol delivery system, and therefore, it may not be an economical and/or efficient means of delivering bulk quantity of cholesterol needed for cellular steroidogenesis. Second, given that the functional expression of the LDL-receptor is inversely related to cellular sterols levels (26) and that adrenal, ovarian and some testicular Leydig cells systems are known to store large quantities of cholesterol (23-25), which should keep this pathway dysfunctional under normal physiological conditions, also argue against a significant role of LDL-endocytic pathway in supplying cholesterol for steroidogenesis. Third, exhaustive kinetic, physiological, pharmacological, and hormonal studies performed in the past suggested that HDL was the preferred donor in providing cholesterol for steroid hormone biosynthesis (74-77,90,91,150-153). Finally, a number of combined

biochemical and morphological studies reported previously from our laboratory led to the unequivocal demonstration that adrenal and ovarian tissues take up and process the bulk of HDL or other lipoprotein-derived cholesterol by an alternative pathway, which did not require internalization and degradation of the intact particle (90,148,150,153-155). Indeed, with the recent discovery of SR-BI, extensive characterization of the SR-BI-linked 'selective' pathway and robust expression of SR-BI/selective pathway in steroidogenic tissues and cells all have resulted in the general acceptance of the selective pathway as a major route for the delivery of HDL-cholesterol esters in gonadal and adrenal cells both in vitro and in vivo (25,28-30). Based on kinetic measurements, it is estimated that this pathway roughly accounts for >90% of total cholesterol transported into steroidogenic cells for steroid synthesis or cholesteryl ester storage in lipid droplets (25,29,90,91). In the remaining sections of the review, we will focus our efforts toward describing the characteristics of the selective pathway; its contribution in the regulation of steroidogenesis, functional expression of SR-BI and the potential mechanisms involved in SR-BI mediated selective CE transport.

### 3.4. Uptake of lipoprotein cholesterol via the 'selective' pathway

The steroidogenic tissues and other high-cholesterol requiring tissues such as liver obtain much of the lipoprotein-derived cholesterol by an alternative, nonendocytic pathway commonly referred to as the "selective" pathway. This pathway is defined as a high capacity, nonendocytic, physiologically regulated bulk delivery system in which cells internalize large amounts of cholesteryl esters (and other lipids) (25,28,29) and is fundamentally distinct from the endocytic pathway used by LDL receptor wherein LDL binds to its receptor, is internalized via clathrin coated pits and vesicles and is transported to lysosomes for whole particle degradation (26,27). In the selective pathway, HDL-CE is taken up by a non-endocytic mechanism without significant degradation of the apolipoprotein component of the particle or whole particle uptake (148,150,157-162). Thereafter, cholesteryl ester molecules are irreversibly translocated into the cell interior and are hydrolyzed by a non-lysosomal route possibly involving neutral cholesteryl esterase (161,162,164,165). In steroidogenic cells, which require large quantities of internalized cholesterol for hormone production, the cholesteryl esters may be directly used for steroid synthesis or stored in lipid droplets with or without prior hydrolysis (138,161,162,167). The scavenger receptor class B type I (SR-BI) is now recognized with few exceptions, as a functional receptor linked to the 'selective' pathway to facilitate the selective transport of lipoprotein-derived CE in steroidogenic, and other high cholesterol requiring cells, both under *in vitro* and *in vivo* conditions (25,28-30).

The earliest clue about the existence of the selective pathway was provided by the work of Dietschy and his collaborating investigators (75,77). Using techniques that allowed them to compare the binding of different lipoproteins and to accurately measure the rates of uptake of lipoprotein-derived cholesterol *in vivo*, they were able to show that HDL, irrespective of its source, manifested disproportionally greater uptake to the adrenal gland and ovary (75,77). Furthermore, it was found that at physiological concentrations, both human and rat LDL were 10 times less efficient in delivering cholesterol to the adrenal gland compared with rates observed with human or rat HDL (77). Moreover, when saturating concentrations of both HDL and LDL were infused together, the rate of cholesterol uptake essentially equaled the sum of the maximal transport rates for the two lipoproteins. These data were interpreted to suggest that the rat adrenal gland and ovary take up LDL and HDL by separate mechanisms and that these two endocrine glands contain an alternative pathway that specifically transports HDL-cholesterol (75-77). Subsequently, Gwynne and Hess (157) extended the *in vitro* characterization of HDL-cholesterol transport to show that isolated rat adrenocortical cells possess a specific, saturable, ACTH-dependent mechanism for the uptake of HDL-cholesterol (157). Moreover, they found that uptake of HDL-cholesterol was at least 4-fold greater than the apoprotein and that internalization of HDL-cholesterol did not require endocytosis and lysosomal degradation of the entire HDL particle (157). Steinberg and colleagues coined the term "selective uptake" for the preferential delivery of HDL-derived CE to target cells (158). These investigators injected rats with HDL containing $^{125}$I-tyramine cellobiose-labeled apo A-1 and [$^{3}$H] cholesteryl linoleyl ether (residualizing labels for apolipoprotein and CE, respectively), and observed that high-cholesterol requiring tissues such as liver, adrenal and ovary took up the CE tracer 2 to 7-times more efficiently than the Apo A-I label (158). A similar selective uptake of HDL-cholesterol was reported *in vitro* in cultured hepatocytes and adrenocortical cells (159). These initial reports were followed by numerous investigations, which resulted in extensive characterization of the selective CE uptake pathway in various animal models, tissues and cultured cell systems (25). Our laboratory was instrumental in providing the physiological significance of lipoprotein-derived selective CE uptake (25). Indeed, our efforts led to the demonstration that adrenal gland, ovary and, under certain conditions, testicular Leydig cells, predominantly utilize this pathway to meet their cellular cholesterol demands and to support steroidogenesis (25,91,154,155,166,167).

The expression of the selective pathway is most abundant in steroidogenic tissues (adrenal gland and gonads) and liver (for a comprehensive review see # 25). In the adrenal gland, the selective pathway is functional primarily in the fasciculata-reticularis and glomerulosa cells (25). Currently, there are reports of selective cholesteryl ester uptake by adrenal tissues and cells in a number of mammalian species including mouse (160,168-172), rat (91,158-160,170), hamster (173), bovine (174,175) and humans (94,95,175). Likewise, cells of ovarian origin including mouse, rat and human granulosa cells (126,138,161,168, 176,177), rat theca interstitial cells (178) and rat luteal cells (90,148,150) also exhibit significant levels of selective uptake of lipoprotein-CE (25). In testis, normal Leydig cells show low selective

uptake while the selective pathway is constitutively active in R2C rat Leydig tumor cells, which take up substantial amounts of HDL-CE under normal physiological conditions without prior hormonal stimulation (179). This pathway is also functional in the liver of several mammalian species (173,180-183), in hepatocytes (159,160,184-187) fibroblasts (160,170), adipocytes (188,189), and macrophages (190).

Support for the steroidogenic function of the selective pathway comes mostly from studies with intact rat adrenals, adrenocortical cells, luteinized ovaries, and cultured granulosa cells. It was first reported that ACTH stimulates selective uptake several fold, in parallel with the stimulation of corticosteroid synthesis (164,174). Employing ACTH-treated primary rat adrenocortical cells, Gwynne and Mahaffee (157) reported that HDL induces substantial stimulation of steroidogenesis, which in most parts was accounted for by the quantitative conversion of selectively internalized CE into secreted steroids. Similarly in Y1-BS1 mouse adrenocortical tumor cells, selectively internalized HDL-CE is preferentially utilized for steroid hormone production (191). Additional studies from our laboratory demonstrated that *in situ* perfused rat adrenals, like adrenal gland *in vivo* (91) and adrenocortical cells in culture, are capable of internalizing lipoprotein-derived CE by the selective pathway (192). Interestingly, both rat HDL (with apoE) and affinity-purified human $HDL_3$ (without apoE) delivered similar amounts of CE to the cell interior as estimated by doubly labeled residualizing tags on HDL preparations and greater than 90% of CE from each lipoprotein was transported *via* the selective pathway. Furthermore, selectively internalized cholesterol accounted for much of the corticosterone produced during *in situ* perfusion, while the contribution from the endocytic pathway was minimal at best.

We performed similar *in situ* perfusion studies in a rather specialized steroidogenic organ, the luteinized ovary of superovulated immature rats, which requires especially large amounts of exogenous lipoprotein-derived cholesterol for progestin production (90,148,150). These studies indicated that not only HDL, but also human LDL, which is a prototypic ligand for the LDL-receptor, supplies cholesterol to the luteinized ovary by the nonendocytic pathway. Indeed, by employing combined biochemical and morphological approaches, we were able to demonstrate that both rat and human HDL and LDL bind primarily and similarly to the surface of ovarian luteal cells and selectively release cholesterol for steroidogenesis without internalization and uptake of intact particles (91,149,151,157,159). In each instance, selective cholesteryl ester uptake paralleled a dramatic increase in the steroidogenic output of the perfused ovary. What was of particular interest in this system was the observation that despite the presence of LDL-receptor protein in luteinized ovary (91), intact LDL particles were not internalized and degraded to any significant extent, instead LDL interacted with the luteal tissue atypically, and delivered cholesterol principally through the selective pathway (151). Green and Pittman subsequently confirmed these observations (196).

The situation is, however, quite different for cultured cells where functional efficiencies of endocytic and selective pathways are often dictated by the apolipoprotein composition of the lipoprotein preparations as well as the culture conditions. For example, Y1 mouse adrenocortical cells, express fully functional LDL (B/E)-receptor/endocytic (22,84,87) and selective pathways (138,171,172,191) and depending on the type of lipoprotein used (human LDL vs human $HDL_3$) and culture conditions employed (i.e., cells grown in serum or lipoprotein deficient serum), the cells can utilize one of the two pathways and provide cholesterol for maximal steroid production. Similarly, cultured rat ovarian granulosa cells can internalize lipoprotein-derived cholesteryl esters through the B/E receptor endocytic pathway or through the nonendocytic selective pathway (121,138). In hormone-stimulated granulosa cells, like Y1 adrenal cells, use of a specific cholesterol delivery pathway is dependent upon which lipoproteins are provided to the cells; our studies have shown that apoE-free human $HDL_3$ particles are exclusively processed by the selective pathway (121,138), most human LDL are processed by the endocytic pathway (though they may also be processed by the selective pathway) and rat HDL (with apoE) are processed by both pathways. It is of interest that the two cholesterol uptake pathways are equally efficient in cholesterol delivery, and in any given experimental period, human $HDL_3$ and human LDL (with equal amounts of CE to contribute) will deliver an identical mass of CE to granulosa cells and support steroidogenesis to the same extent.

In steroidogenic tissues, trophic hormones (LH/hCG, FSH, ACTH) and their common second messenger cAMP are the principal regulators of the selective uptake process *in vivo* and *in vitro*. For example, ACTH stimulation of primary rat adrenocortical cells, Y1 BS1 mouse adrenal tumor cells or bovine adrenal cells is accompanied by increased selective uptake of HDL cholesterol (164,174). Both human adrenal glomerulosa and bovine adrenal cells also respond to angiotensin II treatment with enhanced selective uptake of HDL-CE (175). Hormonal regulation of selective CE was also demonstrated in cultured granulosa cells. When cultured under basal conditions, these cells take up very little HDL-CE via the selective pathway but selective uptake of CE is exponentially increased following stimulation and luteinization of granulosa cells with FSH, stimulators of adenylate cyclase or cAMP agonists (139). Qualitatively similar stimulatory actions of cAMP analogs on the selective pathway were reported for both mouse and human granulosa cells (126,176,177). More recently we have shown that chronic *in vivo* treatment of adult rats with a desensitizing dose of hCG results in enhanced selective delivery of HDL-CE in isolated and purified testicular Leydig cells (170). Likewise, hCG-induced desensitization of pseudopregnant rats is also accompanied by a several-fold upregulation of selective CE transport in the luteinized ovary *in vivo* (166).

Besides trophic hormones, a number of other agents either directly or indirectly exert regulatory actions on the function of the selective pathway. Lipid degrading enzymes such as phospholipase $A_2$ (194), lipoprotein lipase (195,196), hepatic lipase (197-199) and salt-stimulated cholesteryl esterase (200) all enhance the selective uptake process, possibly by a mechanism which involves remodeling of the HDL particle into a species with substantially increased efficiency as a CE donor. There is some evidence that selective

uptake is downregulated by providing cholesterol to cultured cells and upregulated in certain rat tissues by lowering plasma lipoprotein-cholesterol levels (201,202). A variety of other factors such as apoE, CETP, proteoglycans and LRP/$\alpha_2$-macroglobulin receptors, fatty acids are known to modulate selective lipoprotein-CE uptake in a variety of cell systems (171, 178,189,198). Also, in order to ensure exceptionally high and continuous rates of selective CE uptake, the steroidogenic tissues have developed an exceptionally complex and flexible network of microvillar channels at their cell surface whose principal function is to trap large quantities of lipoprotein particles and to facilitate selective CE uptake (148,150,154-156).

Although the exact molecular mechanism(s) by which cells selectively internalize large quantities of cholesteryl esters has remained elusive, some aspects of the selective uptake pathway have been well characterized. First of all, selective uptake represents a saturable process involving mass transfer of CE from HDL to the cell interior. There is overwhelming biochemical and morphological evidence to suggest that endocytosis is not required (148,150,160) and, in general, there is no specific apolipoprotein requirement for the process (90,91,161,170). The initial step in selective uptake involves permissive transfer of cholesteryl esters from donor lipoprotein (HDL) into specialized microdomains or specific sites of the plasma membrane from which they are then translocated to the interior of the cell by a process that appears essentially irreversible (25,29,161,162,164). This phase of selective cholesteryl ester transport shows no energy dependence (163-165) but requires the participation of SR-BI (25,28-30). The fact that isolated plasma membrane preparations exhibit selective CE uptake suggests cell membranes can accomplish the initial phase of selective transport without participation of potential cytoplasmic factors (203-206). In contrast, the second or internalization phase of the selective process is energy dependent and may require the assistance of yet unidentified membranous/cytosolic protein factor(s). A number of different metabolic, energy and cytoskeletal disrupting agents can interfere with the transfer of CE to the cell interior (161,162). Among these, the sulfhydryl reactive reagent, *N*-ethylmaleimide (NEM) completely blocks the translocation of cholesterol from plasma membrane depots to the interior of cell (161,162,176). Performing experiments at cold temperature (4 $^{o}$C) also interferes with the transfer of CE (161,162). These studies suggest the involvement of protein-mediated energy-requiring events at the membrane/cytoplasmic interface during the internalization phase of selective uptake. The final phase of the selective pathway is comprised of non-lysosomal processing of internalized cholesteryl esters for steroid hormone synthesis, membrane biogenesis, or storage in lipid droplets (25,161,162).

## 4. SCAVENGER RECEPTOR CLASS B, TYPE I (SR-BI)

### 4.1. Molecular Characteristics

The high-density lipoprotein (HDL) receptor, scavenger receptor, class B, type I (SR-BI) binds HDL and other lipoprotein particles and facilitates selective transport of lipoprotein (HDL)-associated lipids, primarily neutral lipids such as cholesteryl esters to cells (25,28-30). SR-BI belongs to a class B scavenger receptor superfamily of proteins, which also includes cluster determinant 36 (CD36), lysosomal integral membrane protein II (LIMP II), two *Drosophila melanogaster* proteins, epithelial membrane protein (emp) and hemocyte/macrophage receptor (croquemort), a membrane protein of olfactory neurons of the silk moth, *Antheraea polyphemus* (Snmp-1), and a putative protein from *Caenorhabditis elegance* (25,28). It was independently cloned by two different laboratories; first as a human CLA-I (CD36 and LIMP II Analogous-I) by virtue of its sequence similarity to CD 36 and LIMP II (207) and later as a rodent CD 36-related class B scavenger receptor from a Chinese hamster ovary cell line (208). SR-BI is a 509-amino acid (aa), ~82 kDa integral membrane cell surface glycoprotein with a horseshoe-like membrane topology consisting of a short *N*-terminal cytoplasmic domain (9 aa residues), a transmembrane domain (22 aa residues), a large extracellular domain (408 aa residues), a second transmembrane domain (23 aa residues) and a *C*-terminal cytoplasmic tail (44 aa residues) (28,29). The protein is highly conserved among various mammalian species; the predicted protein sequences of the hamster, mouse, rat, cow and human SR-BI show ~75-80% identity over their 509-amino-acid lengths (28). An alternatively spliced form of SR-BI, termed SR-BII or SR-BI.2 in which 42 of the 45 *C*-terminal amino acid residues in *C*-terminal cytoplasmic domain of SR-BI are replaced by 40 entirely different amino acid residues (209), is also expressed in some cell types and is capable of mediating low level selective uptake of neutral lipids from HDL (210).

SR-BI is a heavily glycosylated protein and this accounts for the difference between its mass deduced from the amino acid sequence (~57 kDa) and that observed by SDS-PAGE/Western blot analysis (~82-86 kDa) (28,29,211). The extracellular domain of SR-BI is *N*-glycosylated at 11 sites of which two sites (positions 108 and 173) are essential for normal cell surface expression and efficient lipid uptake but not for HDL binding (212). Metabolic labeling of SR-BI overexpressing cells with radiolabeled fatty acids demonstrated that SR-BI undergoes fatty acylation in the form of myristoylation and palmitoylation (214). However, palmitoylation is not required for SR-BI expression and/or function, at least in SR-BI overexpressing cultured cells (28,211).

### 4.2. Ligands for SR-BI

Cell surface-associated SR-BI binds rat and human HDL with high affinity and specificity (25,28,29). This binding, at least in part, involves apo-AI and represents a saturable process insensitive to EDTA (28). In addition, SR-BI can bind human LDL, human $HDL_2$, acetylated LDL, oxidized LDL and various other native and modified lipoproteins (Table 1) (213-218). Upon binding lipoproteins, SR-BI facilitates both the selective delivery of lipoprotein CE to the cells and the bi-directional flow of unesterified (free) cholesterol (219-222). Isolated apoAI, apoAII, apoE2, apoE3, apoE4 and apoCIII, either as lipid-free proteins or as phospholipid/cholesterol complexed discoidal particles (223-226), can also interact with SR-BI,

**Table 1.** Ligands for SR-BI

| Ligand | Comments | References |
|---|---|---|
| Lipoproteins | Lipoprotein-CE transport | 25,162,213-218,223-227 |
| Human high-density lipoprotein$_2$ (hHDL$_2$) | | |
| Human high-density lipoprotein$_3$ (hHDL$_3$) | | |
| Human low-density lipoprotein (hLDL) | | |
| Rat high-density lipoprotein (rHDL) | | |
| Acetylated LDL | | |
| Oxidized LDL | | |
| Tyrosylated HDL | | |
| Hypochlorite-modified HDL | | |
| HDL-ApoAI | | |
| HDL-apoAI/apoAII | | |
| Discoidal recombinant HDL | | |
| BODIPY-CE-HDL | | |
| Phospholipase $A_2$ ($PLA_2$) modified HDL | | |
| CETP and hepatic lipase-modified HDL | | |
| CETP and hepatic lipase-modified HDL | | |
| Lipid-free or lipoprotein-associated apoAI, apoAII and apoCII | | |
| Discoidal apoE, apoE2, apoE3 and apoE4-phospholipid particles | | |
| Phospholipid/free cholesterol-rich lipoproteins | | |
| Phosphatidylserine | Phagocytosis, apoptosis | 227,232 |
| Hepatitis C virus | Viral transport | 233 |
| Apoptotic cells | Binding and phagocytosis of apoptotic cells | 229-231 |
| Miscellaneous ligands | Scavenger receptor function | 214,215,228 |
| Fucoidan | | |
| Poly (G)/poly (I) | | |
| m-BSA | | |
| mal-BSA | | |
| AGE-BSA | | |

m-BSA = maleylated bovine serum albumin (BSA); mal-BSA = malondialdehyde BSA; AGE-BSA = advanced glycation end product-conjugated-BSA

as do the lipid vesicles containing anionic phospholipids (227). In addition, as a scavenger receptor, SR-BI binds and shows high specificity towards a diverse group of non-lipoprotein and non-lipid ligands. These include m-BSA, mal-BSA, AGE-BSA, fucoidan, and poly (G)/poly (I) (214,228). Current evidence suggests that SR-BI also functions as a receptor for apoptotic cells and thus, may have a role in the phagocytosis of apoptotic cells (229-231). More recently, SR-BI has been linked to the uptake and intracellular transport of the hepatitis C virus (233). It is interesting to note that when the hepatitis C virus core protein is expressed in mammalian cells it assumes a cytoplasmic localization and associates with cellular storage lipid droplets (233). Considered together, these reports point to a potential functional role of SR-BI and cellular lipid metabolism in the viral cycle and pathogenesis (232). In summary, SR-BI represents a multi-lipoprotein, multi-apolipoprotein and multi-ligand receptor that not only facilitates selective transport of lipoprotein-derived neutral lipids to the cell but also is likely to participate in the regulation of many additional diverse metabolic processes.

### 4.3. SR-BI-mediated selective cholesteryl ester uptake and other SR-BI-associated functions

SR-BI is now recognized as an authentic, physiologically relevant HDL receptor, whose principal function is to initiate selective transport of HDL-cholesterol to the cells for the purpose of product formation or the regulation of cholesterol homeostasis (25,28-30,234). Since its original identification as a potential HDL receptor in 1996, a number of subsequent studies have revealed a tight functional association between SR-BI expression and the selective cholesteryl ester uptake pathway (25,28,29). Acton et al (208) initially showed that SR-BI-transfected Chinese hamster ovary cells bind HDL with high affinity and take up both radiolabeled ([$^3$H] cholesteryl ester or ether) or fluorescent (DiI) lipid markers, and this idea was reinforced by subsequent observations showing that SR-BI itself was specifically associated with steroidogenic tissues, liver and a variety of cell models known to use large quantities of HDL cholesterol (25,28,29,234). Direct evidence for SR-BI function was provided by studies in which antibody to the extracellular domain of mouse SR-BI blocked HDL-CE selective uptake and delivery of HDL cholesterol to the steroidogenic pathway in cultured murine adrenocortical and ovarian granulosa cells (177,191). Additional evidence for SR-BI-mediated selective uptake of HDL-associated cholesteryl esters was demonstrated in genetically modified animals. Inactivation of the functional SR-BI gene in mice increased plasma HDL cholesterol levels, reduced neutral lipid stores in the adrenal gland and ovary and decreased HDL cholesterol transport to the bile, implicating SR-BI as necessary for HDL-cholesterol uptake *in vivo* (235-237). Similarly, mice carrying an induced SR-BI mutation that reduced hepatic SR-BI expression levels by ~50% showed a similar reduction in hepatic HDL-CE selective uptake (238). These studies were complemented by studies in which hepatic SR-BI was overexpressed by either an adenovirus vector (239) or via a transgene (240,241). SR-BI overexpression reduced circulating levels of HDL and non-HDL-cholesterol, and increased selective HDL-CE delivery to hepatocytes and the bile (240-242). Taken together, these observations indicate that SR-BI plays a key role in mediating HDL-CE selective uptake in the liver and in steroidogenic cells, and in influencing the plasma levels of HDL cholesterol in mice.

Numerous studies now indicate that SR-BI is capable of mediating selective CE transport from a number of different HDL preparations including human HDL$_2$,

human $HDL_3$, rat HDL, and variously reconstituted HDL particles (25). However, since HDL size, apolipoprotein confirmation and apolipoprotein and lipid compositions may influence HDL binding to SR-BI as well as the donor efficiency of any given HDL, not all these HDL particles deliver cholesteryl esters to cells with similar efficiencies. For example, de Beer et al (216,224) have recently shown that human $HDL_2$ binds to SR-BI with far greater affinity than $HDL_3$ and a corresponding greater rate of selective CE uptake was observed when comparisons were made using sub-saturating concentrations of $HDL_2$ and $HDL_3$. Similarly, when the apparent $K_d$ values of *rec*-apoAI-rHDL were compared with those of apoAI/apoAII-*rec*-HDL, the apoAI-*rec*HDL was shown to bind with ~3 fold greater affinity to SR-BI overexpressing CHO cells than HDL containing apoAI/apoAII (216,224). In contrast, the selective cholesteryl ester uptake was not compromised, and, in fact, the efficiency of selective CE uptake of SR-BI bound apoAI/apoAII-*rec*-HDL was at least 4-5 fold greater than apoAI-*rec*HDL (227,246). Two other studies also involving apoAI-*rec*HDL and apoAI/apoAII-*rec*HDL particles, however, reached somewhat different conclusions (243,244). These studies demonstrated that ApoAI and apoAI/apoAII HDL particles differ in their abilities to selectively deliver cholesterol ester to the cell interior in that apoAI-HDL serves as a better cholesteryl ester donor than an apoAI/apoAII hybrid HDL. Also, it was shown that apoAII enrichment of HDL particles enhances their affinity for SR-BI, but inhibits selective cholesteryl ester uptake. Although the exact explanation for these conflicting findings is not known, it may have some thing to do with the fact three different SR-BI expressing cell lines were used to asses selective cholesteryl ester uptake in these studies; de Beer et al used LDL-receptor negative, SR-BI over-expressing CHO cell line (CHO-ldlA7) (216), while Rinninger et al (243) and Pilon et al (244) employed cultured hepatic cells and a human adrenal cell line (NCI-H295R), respectively, which express high levels of endogenous SR-BI. Whether these differences are due to differences in surface characteristics of these cell lines, differences in cellular cholesterol homeostasis, or differences between genetic over-expression vs high endogenous expression remains to be established.

In other studies, apolipoprotein (AI) conformation itself is suggested to markedly influence interactions between SR-BI and HDL and SR-BI mediated selective CE transport (216,245). Indeed, it was reported recently that descoidal *rec*-apoAI particles of different sizes (7.8 and 9.6 nm in diameter) and differing apoAI conformation markedly influenced apoAI recognition by SR-BI and selective CE uptake (249); the affinity of HDL binding to SR-BI was roughly 50 fold-greater for the larger (9.6 nm) than for the smaller (7.8 nm) particles (216). Based upon these observations, it was proposed that preferential binding of larger particles (with an expanded pool of CE) to SR-BI should translate into greater amount of cholesteryl ester being delivered to the cells as compared with smaller, relatively CE-poor HDL particles. Thus, it can be concluded from the these studies that HDL particle size, composition and apolipoprotein conformation all exert considerable influence on HDL binding to SR-BI and SR-BI-facilitated selective cholesterol transport.

Several recent studies have also shown that SR-BI mediates the efficient transfer of LDL-CE via the selective uptake mechanism in cells that normally express SR-BI and in SR-BI overexpressing cells (246,247). However, there is growing evidence to suggest that SR-BI-mediated selective CE-uptake from LDL may also involve the participation of accessory factors such as LRP, apoE, LPL and proteoglycans (171,172). In addition to cholesteryl esters, SR-BI can mediate cellular uptake of unesterified cholesterol (UC) (248), phospholipids (PL) (248,249) triglycerides (TG) (248,250), and vitamin E (251) from HDL with the relative selective rate constants of 1.0, 1.6, 0.7 and 0.2, for CE, UC, TG and PL, respectively.

In addition to its indispensable role in mediating the selective transport of lipids, it is now increasingly recognized that SR-BI has additional functions (Table 2). For example SR-BI is implicated in the regulation of hepatic cholesterol and bile acid homeostasis (236,239,242), in normal absorption of intestinal cholesterol (252-255) and cellular cholesterol metabolism (256-260). SR-BI has also been shown to facilitate bi-directional transfer of free cholesterol between HDL and cells, and, under certain conditions, SR-BI promotes efflux of cellular cholesterol (219-222). SR-BI may participate in remodeling of plasma membrane lipids (257-260), regulating the pool-size of plasma membrane associated cholesterol (257-260), altering the function of some membrane associated signaling proteins (261,262) and stabilizing caveolin-1 protein posttranslationally (263). Normal expression of SR-BI in mice is required for the development of red blood cells (268), female fertility (237,265), protection against atherosclerosis (266-270), and in the removal of apoptotic cells (229-232). SR-BI can activate nitric oxide synthase (271-273), accelerate apolipoprotein clearance from the circulation (239-241), promote cell adhesion (274), induce microvillar channel formation (275-277), and inhibit cholesterol efflux mediated by ABC1 transporters (278).

### 4.4. Potential mechanisms involved in SR-BI mediated selective CE uptake

Although the precise cellular mechanism by which SR-BI facilitates the selective transport of lipoprotein-derived CE to the cells has not yet been delineated, some key steps of the process have been unraveled recently. The entire process of SR-BI-mediated selective CE uptake can be broadly divided into 3 steps: the first step involves binding of donor lipoprotein particles to SR-BI, the second step proceeds via efficient transfer of CE from SR-BI bound HDL to the plasma membrane and the final step initiates internalization of plasma membrane associated CE pool into the cell interior. A number of studies now indicate that the first step in the initiation of selective uptake is the binding of donor HDL to the ~ 40 kDa extracellular glycoprotein portion of the SR-BI (25,28,29). Structure/function studies in transfected cell systems using point mutations or domain swaps with CD36

**Table 2.** Various Functions Attributed to SR-BI

| Function | Comments | References |
|---|---|---|
| Selective uptake of lipoprotein-derived cholesteryl esters | SR-BI mediates selective uptake of CE from $hHDL_2$, $hHDL_3$, rat HDL, hLDL, apoAI-HDL, apoAI/apoAII-HDL and other donor lipoproteins | 25,208,216,224,243-247 |
| Selective uptake of lipoprotein-derived other neutral lipids | In addition to CE, SR-BI also facilitates selective transport of lipids such as free cholesterol, TG, phospholipids and Vitamin E | 248-251 |
| Cholesterol efflux | SR-BI regulates bi-directional cholesterol flow and cholesterol efflux from cell surface to HDL and other acceptors | 219-222 |
| Modulation of cholesterol homeostasis | SR-BI is shown to influence both the rate of free cholesterol flux and steady state level of cellular cholesterol and phospholipid. Also, SR-BI may modulate cellular cholesterol content and cause redistribution of cholesterol to membrane domains that function to mediate the flux between cells and lipoproteins | 257-260 |
| Bile acid metabolism | Hepatic SR-BI selectively controls the utilization of HDL cholesterol for biliary secretion | 236,239,242 |
| Female fertility | SR-BI is required for oocyte development and female fertility | 237,265 |
| Inhibition of cholesterol efflux | SR-BI may function as antagonist and inhibit ABC1-mediated cholesterol efflux | 278 |
| Anti-atherogenic actions | Hepatic over-expression of SR-BI reduces atherosclerosis in the cholesterol-fed and LDL-deficient (LDL-R-/-) mice | 266,268 |
| | SR-BI can protect against early onset of atherosclerosis | 265,268 |
| | Attenuated expression of SR-BI leads to increased LDL-cholesterol levels and atherosclerosis in LDL-receptor deficient mice | 267 |
| | Low level over-expression of SR-BI in apoB transgenics attenuates development of diet-induced fatty streak lesions in apoB transgenic mice | 269 |
| | SR-BI deficiency leads to accelerated deficiency, cardiac dysfunction and premature death in apoE-deficient mice | 270 |
| Clearance of apolipoproteins | Overexpression of SR-BI promotes clearance (removal) of both HDL and non-HDL apoproteins | 240,241,269 |
| | Other studies suggest that SR-BI promotes clearance of apoAI, but not apoB | 349 |
| Promotion of microvillar channel formation | SR-BI is essential for formation and maintenance of microvillar channels | 275-277 |
| Apoptosis | SR-BI facilitates the recognition and ingestion of apoptotic thymocytes by nursing thymic epithelial cells | 231 |
| | SR-BI also mediates the recognition of apoptotic granulosa cells by theca cells of ovarian follicles, and may have a role in the remodeling of follicles to secondary interstitial cells | 230 |
| | SR-BI may function as a phosphatidylserine receptor to promote phagocytosis of apoptotic rat spermatogenic cells by Sertoli cells | 229,232 |
| Hematologic function | Red blood cells fail to mature in SR-BI-deficient mice | 264 |
| Stimulation of nitric oxide synthase | HDL activates endothelial nitric oxide synthase through a process that requires HDL interaction with SR-BI | 271-273 |
| Cell adhesion | SR-BI participates in adhesion of neonatal murine microglia to fibrillar beta amyloid | 274 |
| Mediator of dietary cholesterol absorption | SR-BI is shown to promote absorption of dietary cholesterol in intestine | 252-255 |
| Stabilization of caveolin-1 | SR-BI is shown to specifically stabilize the caveolin-1 protein levels without affecting its transcriptional regulation | 263 |

(a closely related receptor that also binds HDL with high affinity), have shown that the extracellular domain of SR-BI is essential for efficient selective uptake (279,280). This step in steroidogenic tissues is further aided by the presence of microvillar channels (275-277) that not only provide a platform for the anchoring of SR-BI but also trap large amounts of lipoprotein particles. It is presumed that such tethering of SR-BI leads to significant increases in local concentrations of donor particles, and subsequently, an accelerated rate of CE transfer to acceptor plasma membranes.

The second step in the selective uptake process involves efficient SR-BI-mediated movement of CE from SR-BI- bound donor particles to the exterior surface of the cell plasma membrane. A number of different working models have been proposed to explain the CE transfer process. Rodrigueza et al (245) proposed the first and currently most viable model; this model is based on thermodynamic and kinetic data and proposes that SR-BI contains a non-aqueous channel, which excludes water, and serves as a conduit for hydrophobic CE molecules to diffuse from SR-BI bound HDL down a concentration

gradient to the cell plasma membrane (245). Note: the Rodriqueza non-aqueous channel described here is not to be confused with the structural microvillar channel (known to trap intact lipoproteins) described above (275-277). A similar model has emerged from studies designed to evaluate the influence of HDL core lipids on the efficiency of selective CE transfer to cultured cells. According to this model, HDL docks with SR-BI, forming a hydrophobic channel in which neutral core lipids, CE and TG are translocated to the plasma membrane (250). Gu et al (280) proposed an alternative mechanism in which SR-BI-mediated docking or anchoring of lipoprotein particles to the cell surface creates an environment in which SR-BI promotes the collision-dependent transfer of CE from donor particles to the plasma membrane (280). At present, however, it is unclear if SR-BI is directly responsible for lipid-lipid CE transfers or requires the participation of other accessory factors. Since this model calls for lipid-lipid interactions between the donor lipoproteins and acceptor plasma membrane, it is likely that specific lipid types and the lipid content of lipoproteins may influence overall CE transfer efficiency. In support of this, it has been reported that the functional efficiency of HDL particles in donating CE for selective transport is dependent upon lipid composition, particle size and apoAI presence on the HDL particle (216,243-245).

The final step in selective transport is the transfer of plasma membrane-associated CE to the cell interior. Relatively much less is known about the events that may be involved in this final step. Deletion and exchange studies have demonstrated that the 45-amino acid C-terminal cytoplasmic tail of SR-BI is essential for its maximal function (279). The carboxy terminus of SR-BI may serve as a docking platform for other membrane and/or cytoplasmic protein factor(s) that are necessary for internalization of CE. Recently we reported the development of a cell-free reconstituted proteoliposome system that uses either partially purified preparations of SR-BI from rat luteal cells or purified, recombinant SR-BI preparations from baculovirus-expressed rat SR-BI (25). Using this reconstitution system, we observed that SR-BI-liposomes incubated with cytosolic extracts from luteinized ovaries (a rich source of SR-BI with high selective uptake activity) were several fold more efficient in selectively taking up cholesteryl ester from donor $^{125}I/^{3}H$-HDL, than were control SR-BI-liposomes incubated without the cytosolic extract. Currently, the identity of any specific required factor is not known, although we believe that such a factor must be heat labile, sensitive to protease treatment, nondialyzable and hydrophobic in nature. This suggests that a cytosolic protein or multiprotein complex is required for efficient internalization of SR-B-delivered selective CE from the cell surface to the cell interior.

In this regard, the role of caveolin in SR-BI dependent selective cholesteryl ester uptake has been intensively studied in the last few years. In large part, interest was sparked by the demonstration of co-localization of caveolin and SR-BI in caveolae like structures (210,211,284). To date, however, neither the co-localization of these proteins in steroidogenic cells or tissues, or the functional relationship of the two proteins in selective CE uptake systems has been satisfactorily resolved. Using electron microscopic immunochemical techniques, early studies had clearly localized caveolin to plasma membrane caveolar structures (11,285) found in adipocytes, fibroblasts and endothelial cells. However, steroidogenic cells from the adrenal, ovary, and testis, or liver parenchymal cells that prominently express SR-BI and actively carry out HDL selective cholesteryl ester uptake, do not have caveolar structures (25). In fact, many isolated cell systems, which are used in these cholesterol uptake studies, also do not express caveolae. More worrisome is the fact that many of the studies demonstrating caveolin plus SR-BI localized to the same membrane sites have used light microscopic immunofluorescent, not electron microscopic immunocytochemical techniques, and one has to assume, rather than actually visualize, that the 2 proteins are co-localized in caveolae. Our own experience in using the electron microscope for these types of studies, has been that in many cell types overexpressing SR-BI and caveolin, no caveolae are present, SR-BI is clearly expressed on the plasma membrane and in double membraned channel membranes opening to the cell surface, and that caveolin, instead, is localized nearby, but apparently associated with actin-like filaments (Reaven & Azhar, unpublished observations). The problems are similar when considering the functional relationship between caveolin and SR-BI in achieving cholesterol uptake. No direct experimental evidence of an interaction between the two molecules exists; the data are mostly correlative with a variety of results regarding either a positive (286), negative (287,288) or no (289-291) relationship between the proteins during selective cholesteryl ester uptake. These issues are complicated by several problems; first, very different types of cell systems have been studied using a variety of conditions, second, there exist three, if not more, caveolins to consider (and their localization in cells, function and relationship to each other are still not entirely clear), and, third, presumptive SR-BI related cholesterol transport in cells may proceed in different directions (i.e., as cholesteryl ester influx into cells vs. free cholesterol efflux out of cells), and the influence of caveolin on these different cell processes may differ (25,28-30). Finally, evidence from caveolin knockout mice has not been particularly helpful. The caveolin-1 knockout mouse is viable and phenotypic changes have been relatively minor (despite a dramatic reduction in caveolae expression), with no particular suggestions that SR-BI function is compromised (292). Thus the true relationship between caveolin, caveolae, SR-BI and selective cholesteryl ester uptake remains to be clarified.

Recently, another protein, a PDZ domain-containing protein called CLAMP (PDZK1/Diphor-1/CAP70/NaPi-Cap1) has been identified with SR-BI mediated selected CE uptake. CLAMP was purified from rat liver extracts by affinity chromatography using the carboxy terminus of SR-BI (293). CLAMP was shown to interact with the last 15 amino acids of SR-BI. Co-expression of CLAMP and SR-BI in Chinese hamster ovary cells led to an almost 2-fold increase in HDL-CE selective uptake that was proportional to increased SR-BI protein

levels without increased SR-BI mRNA, strongly suggesting that CLAMP functions as a scaffolding protein to protect SR-BI from proteolytic degradation (297). More recently Silver (294) provided evidence that the CLAMP-interacting domain of SR-BI is essential for surface expression of hepatic SR-BI and suggested that CLAMP or related PDZ domain proteins may play an indispensable role in the surface expression of SR-BI and SR-BI-mediated selective CE uptake (294).

Overall, it is logical to assume that there are a variety of yet unidentified SR-BI interacting proteins that participate in, and facilitate the action of selective delivery of lipoprotein-CE to the cells. With the current intense interest in this pathway, we expect to hear much on this topic in the near future.

Finally, although SR-BI is currently considered a major mediator of selective CE transport, there is growing evidence to suggest that other factors, independent of SR-BI, facilitate selective CE uptake. Thus, it has been reported recently that LRP, which transport lipids by an endocytic process, can mediate HDL-CE selective uptake in some specialized cells (281). Likewise, Swarnakar et al (171,172) reported that two different pathways mediate the selective uptake of LDL-CE (but not HDL-CE) in cultured cells; one pathway was shown to be strictly via SR-BI, while the other pathway was SR-BI-independent and required the participation of LRP and proteoglycans. Also, lipid-free Apo-E can directly bind to SR-BI and enhance selective cholesteryl ester transport from lipoprotein particles (226). Finally, lipoprotein lipase and unsaturated fatty acids have been reported to promote selective cholesteryl ester transport from LDL by a mechanism that is independent of SR-BI (282,283).

### 4.5. Are there other receptor mechanisms for HDL-CE internalization?

So far the discussion on HDL-CE uptake is based on the premise that SR-BI is a non-endocytic receptor that possibly creates a hydrophobic channel (via receptor dimerization?) to promote the transport of CE and other neutral lipids transport to the cell membrane. However, two recent reports by Silver et al (30,295) have challenged this prevailing view and instead suggest that SR-BI is an *endocytic* receptor, which mediates HDL uptake and recycling but not degradation. Using several molecular, cell biological and biochemical approaches, these investigators have concluded that HDL particles are, in fact, internalized (analogous to the process of iron delivery via the transferrin/transferrin receptor system), and enter into the endocytic recycling compartment followed by re-secretion of the majority of HDL particles in an intact form, by a process termed 'retroendocytosis' (30). Presumably, selective delivery of HDL-CE occurs during this re-cycling (30,295). While the retroendocytosis pathway may take place in certain cell types, the validity of this pathway has not been tested in more traditional, high cholesterol requiring systems such as steroidogenic tissues and isolated steroidogenic cells. At present, there is an overwhelming biochemical and morphological evidence that favors non-endocytic, SR-BI-mediated bulk selective delivery of HDL-CE in steroid synthesizing systems (25,28,29). Obviously more rigorous experimental scrutiny is required before the retroendocytic pathway gains general acceptance.

In addition, it may turn out that different tissues use different mechanisms for HDL-CE uptake. A paper currently in press (296) describes a novel GPI-anchored HDL binding protein (called GPI-HBP1), which like SR-BI mediates *selective* lipid uptake in CHO cells, but lacks HDL dependent cholesterol efflux. Transcripts for this protein were detected with highest levels in heart, and to a lesser extent in lung and liver, and the protein appears specific to cardiac muscle cells, hepatic Kupffer cells, bronchial epithelium and alveolar macrophages. Also, it turns out that an ectopic β–chain of ATP synthase may play a role in apolipoprotein A1 binding and intact HDL internalization in human hepatocytes (IHHcells) and rat liver by a process, which does not involve SR-BI (297). In this instance, it is postulated that the high affinity binding of HDL to the β chains stimulates ADP formation, which promotes HDL endocytosis. Over the years, however, many HDL receptors have been identified (296-302) (see Table 3), and, to date, only one, SR-BI has stood the test of time. Clearly, the new receptor candidates will require further investigation.

### 4.6. Tissue distribution of SR-BI expression

Mouse and rat SR-BI are strongly expressed in those tissues that exhibit the highest rates of selective cholesteryl ester uptake, namely adrenal gland, ovary, testis and liver (166,167,177,277,289,303,304). A similar distribution of SR-BI occurs in human tissues (213,305-307).

In addition, low to moderate levels of SR-BI expression have been reported in non-steroidogenic tissues/cells such as gallbladder (308), intestine (252,254,309), stomach (253,309), kidney (310), brain (311,312), eye (313), skin (314), lung (208,313), thymus (231), endometrium (316), macrophages (317-319), monocytes (317), aorta (320), adipose tissue and 3T3 cells (28,211), human choriocarcinoma cells (25), human breast carcinoma cell line (321), and mammary glands of the pregnant rats (28) (Table 4).

Immunohistochemical and immunofluorescence studies have demonstrated that SR-BI is present on the surfaces of hepatocytes and steroidogenic cells including: zona fasciculata and zona reticularis cells of the adrenal cortex, bovine adrenal glomerulosa cells, human adrenal NCI-H295 R cell line, the theca-interstitial cells of non-hormone primed ovaries, ovarian corpora luteal cells, luteinized granulosa cells, testicular Sertoli cells, and hormone-desensitized testicular Leydig cells (25,28,29). Immunolocalization at the electron microscope level in rat adrenal fasciculata, ovarian luteal and testicular Leydig cells show that SR-BI is present on microvillar membrane domains that form channels in which HDL and other lipoprotein particles are sequestered (166,167,277). These microvillar channels are believed to be the site at which selective uptake of lipoprotein-CE takes place (for review see 25,28 and 29). The expression of SR-BI is also reported

**Table 3.** List of Additional Candidate HDL-Receptors

| HDL-Receptor/HDL binding protein | References |
|---|---|
| 110 kDa HDL-binding protein (HBP/Viglin) | 298 |
| 110 kDa GPI-anchored HDL binding protein (HB2/ALCAM/BEN) | 300 |
| 80- and 130-kDa GPI-anchored HDL binding protein | 299,301 |
| 95 kDa HDL binding protein | 302 |
| Ectopic beta-chain of ATP synthase | 297 |
| 22.6 kDa GPI-anchored HDL binding protein (GPI-HBP) | 296 |

**Table 4.** Cell and Tissue Type Expression of SR-BI

| Cell/Tissues | SR-BI Expressing cells and tissues | References |
|---|---|---|
| Adrenal gland | Intact mouse, rat and human adrenal glands | 208,213,277,303-307,325 |
| Adrenal cells | Mouse Y1-BS1 adrenocortical cells | 191,304 |
| | Bovine adrenal glomerulosa cells | 175 |
| | Human adrenocortical cell line (NCI-H295 R) | 175 |
| Ovary | Normal mouse and rat ovary | 25,328,331-333 |
| | Luteinized rat ovary | 166 |
| Ovarian Cells | Mouse, rat and bovine ovarian granulosa cells | 177,289,330 |
| | Rat theca interstitial cells | 178 |
| Testis | Intact mouse and rat testis express SR-BI and SR-BII | 167,209,303 |
| | Rat Leydig cells | 167 |
| Testicular cells | Isolated Sertoli and Leydig cells | 229,232 |
| | MA-10 mouse Leydig tumor cells | 179 |
| | MLTC mouse Leydig cells (unpublished observations) | |
| | R2C rat Leydig tumor cells | 179 |
| Liver | Intact mouse, rat, hamster and human liver | 240,241,303,305, 339 |
| Hepatic cells | Isolated hepatocytes | 334,335 |
| | Isolated Kupffer Cells | 334,335 |
| | Hep3B hepatoma cells | 337 |
| | HepG2 hepatoma cells | 342 |
| Stomach/intestine | CLA/SR-BI is expressed in the brush-border membrane of human enterocytes from all parts of the elementary canal. SR-BI expression is detected in apical membrane of brush border in the rat duodenum. SR-BI is also present on both apical and basolateral surfaces of the jejunum villus. Low levels of SR-BI expression is detected in ileum | 252-254,309 |
| Intestinal cells | Rat and mouse in enterocytes | 348,350 |
| | Human colorectal carcinoma-derived cell line—CaCO-2 cells | 309 |
| Nervous system/ Neuronal cells | The presence of SR-BI is demonstrated in the parietal cortex and cerebellum | 312 |
| | Neonatal macroglia | 312 |
| | SR-BI expression is absent in microglia from normal mouse and adult human brains but astrocytes and smooth muscle cells of mouse and human brains express significant amounts of SR-BI | 311 |
| | Porcine brain capillary endothelial cells also express SR-BI | 251 |
| Aorta | Human SR-BI is detected in foam cells of aortic atherosclerotic lesions | |
| | CLA-1/SR-BI is expressed in macrophages of atherosclerotic lesion | 318,319 |
| | Endothelial cells | 320 |
| | Smooth muscle cells | 320 |
| Macrophages | Macrophage cell line J774 cells | 317-319 |
| | Macrophage-like RAW 264.7 cell line | 317-319,345 |
| Eye | Human retinal pigment epithelial cells express both SR-BI and SR-BII isoforms | 313 |
| Gallbladder | SR-BI and SR-BII mRNAs are present in columnar epithelium | 308 |
| Human choriocarcinoma cells | Abundant expression of SR-BI is observed in trophoblast-like human choriocarcinoma cells, JAr and Jeg 3 cells | 351 |
| Kidney | SR-BI is expressed in rat renal cortex and proximal tubule | 310 |
| Skin | SR-BI is present in isolated keratinocytes. Its mRNA is also expressed in murine epidermis | 314 |
| Lung | Significant amount of SR-BI expression is detected in alveolar type II cells | 208,313 |
| Human breast carcinoma cell line | Human breast carcinoma cell line, HBL-100 expresses significant amount of CLA-1/SR-BI | 321 |
| Thymus | Strong expression of SR-BI is detected in nursing thymic epithelial cells | 231 |
| Monocytes | Human THP-1 monocytes | 286 |
| | Human monocytes | 317 |
| Endometrium | SR-BI expression is localized in the glandular epithelium of endometrium | 316 |

in turtle tissues and other nonmammalian vertebrates (322) and in *Dictostelium discoideum* (323).

### 4.7. Cellular regulation of SR-BI

SR-BI is highly expressed in steroid producing tissues--and trophic hormones are the principal regulators of expression in these tissues. The expression of SR-BI is also abundant in liver and in macrophages of the vascular system, where dietary constituents and pharmacological agents are the principal regulators of expression (Table 5).

#### 4.7.1. Adrenal gland

In the rodent adrenal gland, SR-BI is localized on cell surfaces of fasiculata-reticularis cells (277,303,304). Immunoelectron microscopic studies show that SR-BI is specifically localized to the microvillar compartment including the microvillar channels (277), the latter, which are known to trap large quantities of lipoproteins to ensure efficient selective uptake of lipoprotein-derived CE (25). In rats and mice, adrenal expression of SR-BI is up-regulated in response to ACTH treatment (277,304,324). The hypocholesterolemic agent, 17 alpha-ethinyl estradiol (17α-E2) also results in increased expression of SR-BI (277,303). Interestingly, both ACTH and 17 alpha-E-2 treatments lead to a significant increase in the number of microvilli, the formation of microvillar channels, and the general complexity of the microvillar compartment (277). Conversely, use of dexamethasone (which inhibits endogenous ACTH secretion) drastically reduces SR-BI protein expression, microvillar area and microvillar channel formation (277). Likewise, genetic ablation of SR-BI is accompanied by disorganization and loss of adrenal microvillar channels (276). These studies indicate that hormonal regulation of SR-BI expression and the structural configuration of the surface of adrenocortical cells are closely linked.

Besides ACTH, adrenal glomerulosa cell lines respond to angiotensin II stimulation with increased expression of mRNA and SR-BI protein levels (175). In addition, a number of genetic manipulations have been shown to alter SR-BI expression. For example, disruption of the hepatic lipase gene was shown to up-regulate SR-BI expression 3-4 fold in female, but not male mice, in which adrenal free and esterified cholesterol levels were significantly reduced (325). Likewise, SR-BI protein and mRNA are expressed at high levels in the adrenals of mice lacking functional StAR or lecithin: cholesterol acyltransferase (LCAT) genes (327). Wang et al (325) observed a substantial increase in the expression of adrenal SR-BI protein and mRNA levels in apoAI null mice, but not in apoAII, apoE or LDL-receptor knockout mice. However, a more recent study reported normal expression of SR-BI protein and mRNA levels in apoAI knockout mice (169). Other studies have shown that adrenal SR-BI expression is also developmentally regulated; both human and rodent fetal adrenals express >50-fold more SR-BI as compared to their adult counterparts (25,28,326).

#### 4.7.2. Ovary

In rat preovulatory follicles, SR-BI mRNA and protein are expressed in theca-interna and interstitial cells but not in granulosa cells (328). Steady state mRNA levels of SR-BI in theca cells are rapidly induced following *in vivo* treatment of immature rats with hCG or insulin; and a synergistic stimulation of mRNA is observed when animals are treated simultaneously with hCG plus insulin (329). Isolated mouse, rat and bovine granulosa cells cultured under basal conditions show little or no expression of SR-BI protein and mRNA, but their expression is increased many fold during gonadotropin or cAMP-induced luteinization of the cells (177,289,330). Moderate levels of SR-BI are also detected in normal cycling mouse and rat ovary (25) and mRNA transcripts in rat ovary are further up-regulated in response to treatment with gonadotropins such as pregnant mare serum gonadotropin (PMSG), human chronic gonadotropin (hCG) and PMSG followed by hCG (328,331-333). SR-BI is highly expressed in rat corpus luteum where it is localized on microvillar domains that form channels (166) and its expression is further enhanced by treatment of the animals with a desensitizing dose of hCG (166). Likewise, treatment of normal cycling rats with 17 alpha-E2 also induces SR-BI protein expression in corpora luteal cells of the ovary (303). In contrast, prostaglandin F2alpha-induced luteolysis is accompanied by reduced expression of SR-BI in corpora lutea (331).

#### 4.7.3. Testis

In untreated rat testis, a low level of SR-BI expression is detected in Leydig and Sertoli cells (179,229). However, the protein and mRNA levels of SR-BI are dramatically induced in androgen producing Leydig cells following chronic treatment of mature rats with hCG (167). Mouse testicular Leydig MA-10 (179) and MLTC mouse Leydig tumor cells, also express significant levels of SR-BI (Azhar & Reaven, unpublished observations) and hormonal treatment further stimulates its expression in both cell types. On the other hand, cell line, R2C Leydig tumor cells, which constitutively secrete large amounts of steroids also show very high levels of SR-BI mRNA and protein under basal conditions and cAMP treatment only modestly stimulates SR-BI expression (179). Of particular interest is the demonstration that this cell line, unlike many other steroid producing cell lines, possesses surface structures resembling the microvillar components previously described in intact rat adrenal and luteal tissues, and morphological analysis of these cells suggests that SR-BI is almost exclusively localized to the microvillar channels (Reaven and Azhar unpublished observations).

#### 4.7.4. Liver

In the liver, SR-BI is expressed principally in parenchymal cells under normal physiological conditions (303). However, 17 alpha-E2-induced hypocholesterolemia, or chronic feeding of a diet rich in cholesterol, has been reported to decrease SR-BI expression in hepatocytes but increases its expression in the Kupffer cells (334,335). Feeding cholesterol does not affect SR-BI levels in hamster liver; however, hepatic SR-BI expression can be induced by feeding a diet enriched in polyunsaturated fatty acids (336). Bacterial lipopolysaccharides (LPS), TNF and IL-1 down-regulate liver SR-BI mRNA levels when animals are maintained on a high-cholesterol diet (337). A number of

**Table 5.** Regulation of SR-BI Expression

| Tissue/Cells | Hormones, cytokines, genetic, physiological or dietary manipulations | Effect/action | References |
|---|---|---|---|
| Adrenal gland | ACTH | ACTH treatment in rats results in increased SR-BI mRNA and protein levels and promotes microvillar channel formation | 277,303,304, 324 |
| | 17 Alpha-ethinyl estradiol (17 alpha-E2) | 17 Alpha-E2-induced hypocholesterolemia in rats is accompanied by enhanced expression of SR-BI and microvillar components | 277,303 |
| | SR-BI-/- mice | Genetic ablation of SR-BI leads to loss and disorganization of microvillar channels | 276 |
| | Apo AI-/- mice | Functional disruption of Apo AI gene is accompanied by increased expression of SR-BI mRNA and protein levels | 325 |
| | | However, a more recent study reported comparable expression of adrenal SR-BI between control (Apo AI+/+) and Apo AI-/- mice | 169 |
| | HL-/-, LCAT-/- and StAR-/- mice | High levels of adrenal SR-BI mRNA and/or protein levels are detected in these genetic models | 327 |
| | Development | The levels of fetal human adrenal SR-BI are at least 50-fold higher than in adult adrenal gland | 25,28,326 |
| Adrenal cells | | | |
| Mouse Y1-BS1 adrenocortical cells | ACTH and lipoproteins | Addition of ACTH stimulates and lipoproteins inhibit SR-BI expression | 25,324 |
| Rat adrenocortical cells | ACTH | Primary cultures of rat adrenal cells respond to hormone with time-dependent increases in SR-BI expression | 277 |
| Bovine adrenal zona glomerulosa cells | Angiotensin II | SR-BI levels are increased ~ 2-fold when cells are exposed to angiotensin II | 175 |
| Human adreno-cortical carcinoma cells (NCI H295R) | Angiotensin II and cAMP analogs | Both agents stimulate SR-BI expression | 175 |
| Ovary | PMSG, hCG and FSH | PMSG, hCG or PMSG + hCG treatment results in rapid stimulation of SR-BI mRNA transcripts in normal rat ovaries. FSH has no detectable effect | 328,331-333 |
| | hCG | Treatment of PMSG-hCG primed (luteinized ovaries) with a desensitizing of hCG further induces SR-BI expression | 166 |
| | 17 Alpha-E2 | Estrogen-induced hypocholesterolemia up-regulates SR-BI protein levels in the corpora luteal cells of the rat ovary | 303 |
| Ovarian cells | | | |
| Mouse, rat and bovine granulosa cells | FSH, LH or cAMP analogs | Robust expression of SR-BI is observed when cells are treated with hormones or cAMP analogs | 177,289,330 |
| Rat theca interstitial cells | Insulin and hCG | Insulin and hCG both independently and synergistically up-regulate SR-BI mRNA and protein levels | 329 |
| Testis | hCG | Chronic hCG treatment of intact rats results in a dramatic increase in SR-BI in Leydig cells | 167 |
| Testicular cells | | | |
| MA-10, MLT mouse Leydig tumor cells | cAMP analogs | Both cell types respond to cAMP analogs with a significant stimulation of mRNA and protein levels of SR-BI | 179 |
| Liver | 17 Alpha-E2 and cholesterol-rich diet | 17 alpha-E2 treatment or feeding a cholesterol rich diet decreases SR-BI expression in rat hepatocytes but increases its expression in Kupffer cells | 303,334,332 |
| | Cholesterol and polyunsaturated fatty acids | Hamster liver is insensitive to cholesterol feeding but SR-BI expression in hamster liver can be induced be feeding animals a diet rich in polyunsaturated fatty acids | 336-339 |
| | LPS, TNF and IL-1 | LPS and cytokines can reduce SR-BI mRNA levels when animals are fed a cholesterol-rich diet | 337 |
| | Fibrates, ACTH and dietary myristic acid | All these agents inhibit rodent hepatic SR-BI levels | 336-339 |
| | Vitamin E | Cellular levels of vitamin E inversely regulate rat hepatic SR-BI levels | 338 |
| Hepatic cells | | | |
| Hep3B hepatoma cells | TNF and IL-1 | Cytokines repress SR-BI expression in this cell line | 337 |
| HepG2 hepatoma cells | Testosterone, estradiol and vitamin E | Androgens up-regulate SR-BI mRNA and protein levels while estrogens specifically induce SR-BIIexpression. Vitamin E lowers SR-BI levels in a concentration dependent manner | 337,338,342 |
| Intestine | Bile duct ligation | Complete loss of SR-BI protein and roughly 50% reduction in SR-BI mRNA levels | 348 |
| | | Decreased expression of SR-BI protein but not its mRNA is detected in bile-diverted rats and Mdr2-/- and CYP 7 alpha-/- mice | 348 |
| Macrophages | Oxidized LDL and TGF-beta | Decreased expression of SR-BI | 315,344-346 |
| Human monocyte-derived macrophages | Testosterone and activators of PPAR-alpha and PPAR-gamma | Testosterone promotes SR-BI expression. | 347 |
| | | Ligand specific activation of PPAR-alpha and PPAR-gamma induce CLA-1/SR-BI protein expression in monocytes and differentiated macrophages | 319 |
| RAW 264.7 macrophage cell line | LPS | LPS is a potent negative regulator of SR-BI mRNA expression | 317 |
| Skin keratinocytes | Simvastatin and 25-hydroxycholesterol | Exposure of keratinocytes to a cholesterol synthesis inhibitor stimulates while oxysterols inhibit SR-BI expression | 314 |
| Kidney | Glomerulopathy | Experimental nephropathy can alter renal cortical SR-BI expression | 310 |

other agents including fibrates, increased amounts of dietary myristic acid, vitamin E and ACTH suppress SR-BI expression in rodent liver (336-339), while 17beta-E2 induces SR-BII protein (342). Also, a marked down-regulation of hepatic SR-BI is observed in rats with nephritic syndrome (343).

#### 4.7.5. Macrophages and other tissues and cells

Expression of CLA-1, the human homolog of SR-BI, is not detected in human monocytes, but is induced with monocyte differentiation into macrophages (316). Additional regulation studies have shown that SR-BI expression in macrophages can be regulated through peroxisome proliferator-activated receptors alpha and gamma (317), and that estrogen and dietary cholesterol induce the activation of SR-BI expression in hepatic macrophages (334,335). LPS, oxidized LDL and TGF-beta inhibit CLA-1/SR-BI expression (315, 344-346) while androgens have been shown to increase SR-BI expression in human monocyte-derived macrophages (347).

Treatment of keratinocytes with an inhibitor of cholesterol synthesis (simvastatin) results in a several fold increase in SR-BI mRNA and protein levels (312), while exposure to 25-hydroxycholesterol suppresses SR-BI levels indicating that SR-BI expression in skin keratinocytes is under negative feedback control by cholesterol. Also, calcium-induced differentiation of keratinocytes is accompanied by markedly decreased expression of SR-BI (312). In rats, bile-duct ligation leads to complete loss of intestinal SR-BI protein and ~ 50% reduction in SR-BI mRNA levels (348). Likewise, bile diverted rats and Mdr2 and Cyp7−alpha knockout mice show significantly reduced levels of SR-BI protein, while SR-BI mRNA content is not affected in these models (348).

## 5. CONCLUDING REMARKS

Many steroidogenic tissues and cells preferentially utilize a non-endocytic pathway, termed the 'selective' pathway for supplying the bulk of the cholesterol needed for steroid hormone biosynthesis. Identification of SR-BI as a functional receptor for the selective pathway has not only opened new horizons in the investigation of cholesterol and steroid metabolism, but has shown how architectural changes in the development and maintenance of certain cellular plasma membrane domains (microvillar channels) may influence lipoprotein cholesterol delivery to steroidogenic cells. This latter function of SR-BI represents a unique example of a receptor, which not only initiates a metabolic process, but is involved in the expression and maintenance of structural components for optimal functioning of the process.

In addition to the seminal role of SR-BI in facilitating selective CE uptake, it is becoming increasingly clear that this receptor protein performs many other functions; e.g., SR-BI appears to regulate processes involved in cellular cholesterol homeostasis, bi-directional cholesterol flow, membrane lipid expression, female fertility (oocyte maturation), apoptosis, and, in addition, SR-BI may act as an athero-protective agent.

Despite the abundant work cited in this review, many questions still remain regarding the mechanism of action of SR-BI in these various biological processes. A crucial area not yet explored is whether SR-BI acts alone, or requires participation of other modulating factors (both positive and negative) to facilitate selective CE transport. Future integrated biochemical, molecular, and morphological studies of SR-BI, and its family members, will be required to solve such important questions.

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