rapid communication

SR-BI is required for microvillar channel formation and the localization of HDL particles to the surface of adrenocortical cells in vivo

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Abstract **Scavenger receptor class B type I (SR-BI) mediates the selective uptake of HDL cholesteryl ester into liver and steroidogenic tissues. In steroidogenic cells, juxtaposed microvilli, or microvilli snuggled against the plasma membrane create microvillar channels that fill with HDL. Microvillar membranes contain SR-BI and are believed to be the site of HDL cholesteryl ester uptake. A recent study showed that SR-BI expression in insect cells elicits membrane structures that contain SR-BI, bind HDL, and closely resemble the ultrastructure of microvillar channels. In the present study we compared the ultrastructure of adrenal gland microvillar membranes in *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$ mice to test whether SR-BI is required for the formation of microvillar channels. The results show that SR-BI is absolutely required for microvillar channel formation and that the microvillar membranes of *Srb1*$^{-/-}$ mice are 17% thinner than in *Srb1*$^{+/+}$ mice. We conclude that SR-BI has a major influence on plasma membrane ultrastructure and organization in vivo.**—Williams, D. L., J. S. Wong, and R. L. Hamilton. **SR-BI is required for microvillar channel formation and the localization of HDL particles to the surface of adrenocortical cells in vivo. *J. Lipid Res.* 2002.** 43: **544–549.**

Supplementary key words cholesterol • lipoprotein • steroidogenesis • plasma membrane

Scavenger receptor class B type I (SR-BI) is an HDL receptor that mediates the selective uptake of HDL cholesteryl ester into liver and steroidogenic tissues (1–3). In addition to its effects on cholesteryl ester uptake, SR-BI has significant effects on free cholesterol flux and plasma membrane properties. In transfected cells SR-BI stimulates the bi-directional flux of free cholesterol between cells and HDL (4–6). This activity alters cholesterol homeostasis leading to increased plasma membrane free cholesterol and phospholipid under normal growth conditions (7, 8). Altered plasma membrane properties in SR-BI-expressing cells include an increased pool of membrane free cholesterol that is sensitive to oxidation by exogenous cholesterol oxidase (4, 9). This difference holds even when SR-BI-expressing and control cells have the same free cholesterol content (8), suggesting that SR-BI-dependent cholesterol oxidase sensitivity reflects the organization of plasma membrane domains rich in free cholesterol. Furthermore, SR-BI enlarges the fast kinetic pool of free cholesterol that effluxes to cyclodextrin acceptors (9). SR-BI has also been shown to mediate HDL-dependent activation of endothelial nitric oxide synthase and endothelium-dependent relaxation of aortic rings (10). Taken together, these findings indicate that SR-BI has significant effects on membrane function and the organization of free cholesterol in plasma membrane microdomains.

A recent study by Reaven et al. showed that SR-BI expression in insect Sf9 cells elicits the formation of double membrane structures that contain SR-BI, bind HDL, and resemble the double membrane microvillar channels observed in steroidogenic cells (11). Microvillar membranes are rich in SR-BI and are believed to be the site of HDL cholesteryl ester selective uptake (12–15). In the present study we have tested the hypothesis that SR-BI is required for the formation of microvillar channels in the adrenal gland of the mouse. Comparison of *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$ mice shows that SR-BI is absolutely required for microvillar channel formation and demonstrates that SR-BI has a major influence on plasma membrane ultrastructure and organization in vivo.

MATERIALS AND METHODS

Animals and tissue preparation

Srb1$^{+/-}$ mice on a mixed 129 × C57Bl/6 background were generously provided by Monty Krieger (16). Heterozygotes were

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

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crossed to generate *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$ mice that were maintained on a 12 h light/12 h dark cycle with a standard chow diet ad libitum until sacrifice at 4 to 6 months of age. Genotypes were determined by polymerase chain reaction as described (16). Protocols were approved by the Stony Brook Institutional Animal Care and Use Committee. Three female mice of each genotype were anesthetized with 0.05 ml ketamine-xylazine (3 mg/0.02 mg) and perfused through the left ventricle at constant pressure (110 mm Hg) with 0.5 ml of 0.1 M sodium cacodylate, pH 7.4, followed by 25 ml of freshly prepared 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.4. After maintenance overnight in fixative, a 1 mm disk was cut from the center of the adrenal gland and pie-shaped wedges were cut with the point in the medulla to facilitate orientation.

Microscopy

Adrenal wedges were post-fixed with reduced osmium, block stained with uranyl acetate, dehydrated with acetone, embedded in epon, sectioned, and stained with lead citrate as described (17, 18). Sections were examined on a Siemens Elmiskop 101 electron microscope, and micrographs were taken in the center of the zona (z.) fasciculata at ×12,000, ×16,000, ×20,000, and ×40,000 and enlarged three times. The frequencies of various microvillar configurations (see Results) were estimated by counting the number of such structures per field. Measurements of microvillar membrane thickness were made on micrographs taken at ×20,000. Student's *t*-test was used for statistical comparisons.

RESULTS

To test whether SR-BI alters plasma membrane properties in vivo, we examined the ultrastructure of microvilli and microvillar channels in the adrenal gland z. fasciculata cells of *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$ mice. The microvillar compartment in adrenocortical cells is found primarily in two locations: in the subendothelial space on the sinusoidal face of the cell and in the junctional region where two parenchymal cells meet (15, 17). **Figure 1A** shows the typical organization of microvilli in the subendothelial space in *Srb1*$^{+/+}$ mice. We have arbitrarily defined the configuration of microvilli as shown in **Fig. 2**. Microvilli nestled against the cell surface or apparently buried in the cytoplasm are referred to as snuggled microvilli (arrows). Microvilli that are stacked with other microvilli are referred to as juxtaposed microvilli (asterisks). Microvilli that form channels containing discernable HDL particles formed against the cell surface or between juxtaposed microvilli are referred to as microvillar channels (arrowheads). We have previously shown that the width of the microvillar channel (and therefore the diameter of the discernable HDL) in the mouse adrenal gland is 15 ± 2.2 nm when observed in thin sections of tissue postfixed with reduced osmium and stained with uranyl acetate and lead citrate (15). In *Srb1*$^{+/+}$ mice these configurations were common and seen in every field examined (Fig. 2).

In *Srb1*$^{-/-}$ mice, snuggled or juxtaposed microvilli were observed much less often on the sinusoidal face (Fig. 1B) and in the junctional region (data not shown) between parenchymal cells. **Figure 3A** and **C** shows examples of microvillar channels containing discernable HDL particles in *Srb1*$^{+/+}$ mice (arrowheads). Similar channels containing HDL were not observed in *Srb1*$^{-/-}$ mice even when microvilli were juxtaposed with approximately the same distance between opposed membranes as seen in *Srb1*$^{+/+}$ mice (Fig. 3B and D). The frequencies of these microvillar configurations for snuggled (**Fig. 4A**) and juxtaposed (Fig. 4B) microvilli were markedly reduced in *Srb1*$^{-/-}$ mice, and microvillar channels (Fig. 4C) were virtually absent. Note, however, that the frequency of microvilli was essentially the same in *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$ mice (Fig. 4D).

The microvillar plasma membrane of *Srb1*$^{-/-}$ mice

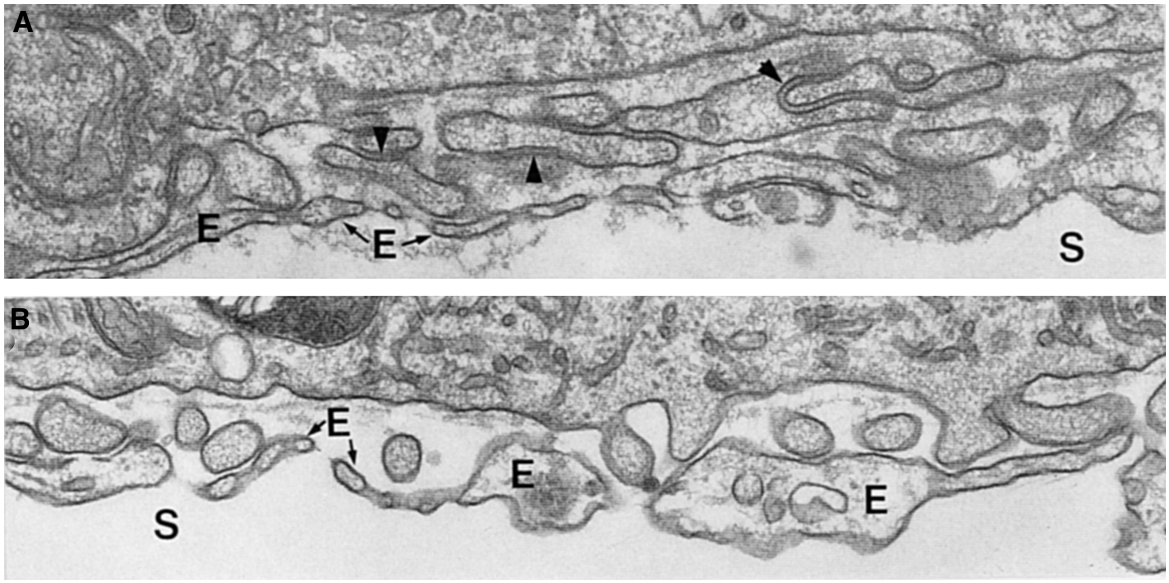

Fig. 1. Microvilli on z. fasciculata cells. Images of the sinusoidal face of the z. fasciculata cell showing microvillar channels (arrowheads) in the subendothelial space of *Srb1*$^{+/+}$ mice (A) and the absence of microvillar channels in *Srb1*$^{-/-}$ mice (B). E, endothelial cell; S, sinusoid. ×36,000.

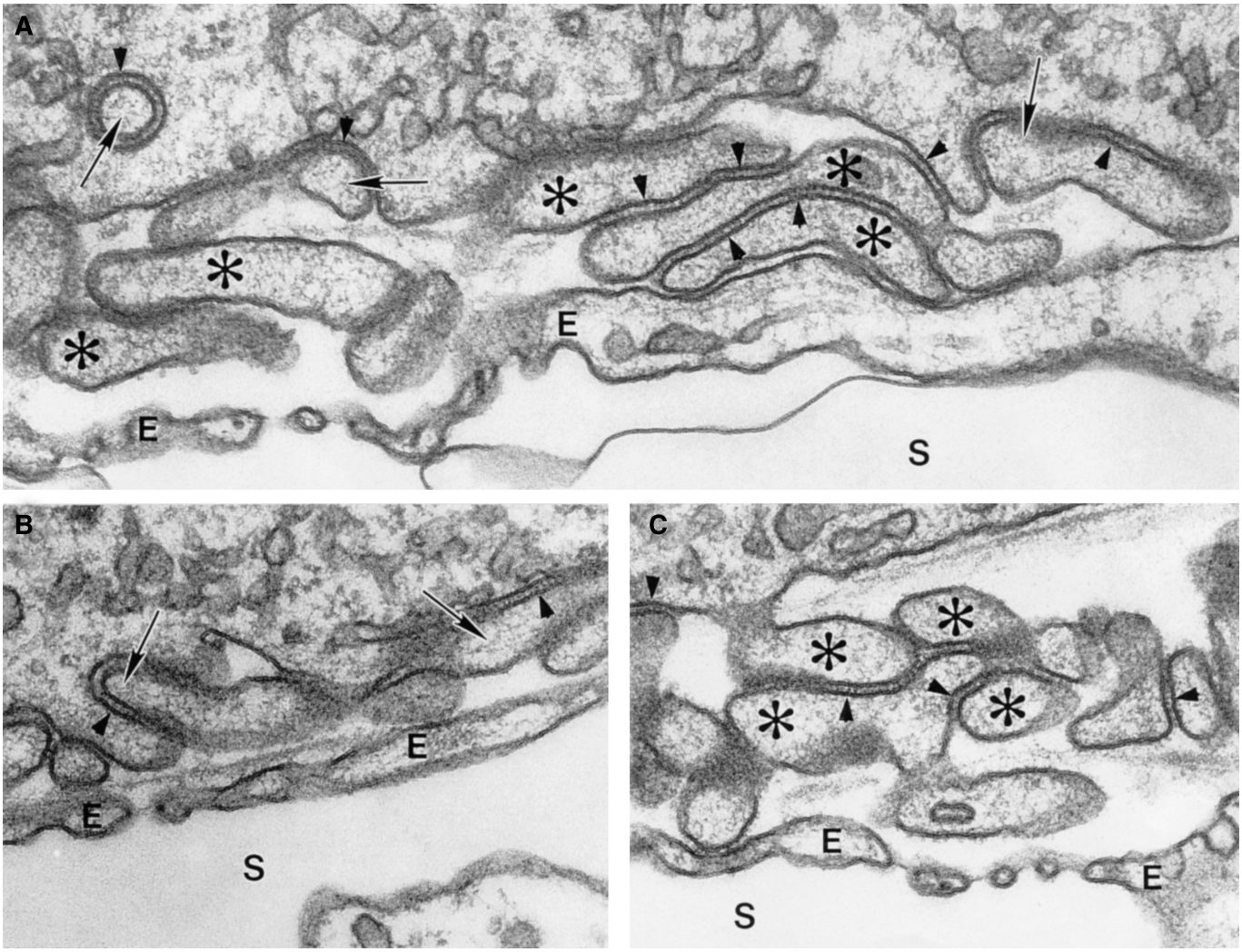

Fig. 2. Microvillar configurations on *Srb1*$^{+/+}$ z. fasciculata cells. Panels show snuggled (arrows), and juxtaposed (asterisks) microvilli and microvillar channels (arrowheads) with discernable HDL particles. See text for definitions of microvillar configurations. E, endothelial cell; S, sinusoid. ×60,000.

(Fig. 3D and **Fig. 5B**) appears to be narrower due to a reduction of the width of the light central region (the hydrophobic lipid-rich domain) of the membrane trilaminar structure (compare with the *Srb1*$^{+/+}$ microvilli, Figs. 3C and 5A). Measurements of microvillar membrane thickness showed the *Srb1*$^{-/-}$ membranes to be 1.8 nm thinner than those in the *Srb1*$^{+/+}$ mice (8.8 ± 0.9 nm vs. 10.6 ± 0.9 nm; n = 24; $P < 0.0001$).

DISCUSSION

This analysis shows disorganization of the normal configurations of microvilli on adrenocortical cells of the *Srb1*$^{-/-}$ mouse. In *Srb1*$^{-/-}$ z. fasciculata cells, snuggled microvilli were seen at 14% and juxtaposed microvilli at 38% of the frequencies observed in *Srb1*$^{+/+}$ mice, and microvillar channels were not observed in *Srb1*$^{-/-}$ mice. These results indicate that SR-BI is absolutely necessary for the formation of microvillar channels as well as to concentrate HDL particles on the surface of adrenal z. fasciculata cells in vivo.

SR-BI is localized to membrane microdomains in cultured cells, shows partial co-localization with caveolin-1, and can be isolated in a caveolin-rich membrane fraction (19). These findings suggest that SR-BI occurs in caveloae or related microdomains. In transfected cells SR-BI-expression increases free cholesterol content and alters the organization of free cholesterol in the plasma membrane (4, 7–9). SR-BI mediates the HDL-dependent enhancement of the activity of endothelial nitric oxide synthase, an enzyme found in caveolae (10). SR-BI expression in insect cells elicits the formation of double membrane structures resembling the microvillar channels of steroidogenic cells (11). These findings indicate that SR-BI has substantial effects on membrane structure, lipid organization, and physiological activities in plasma membrane microdomains in transfected cells.

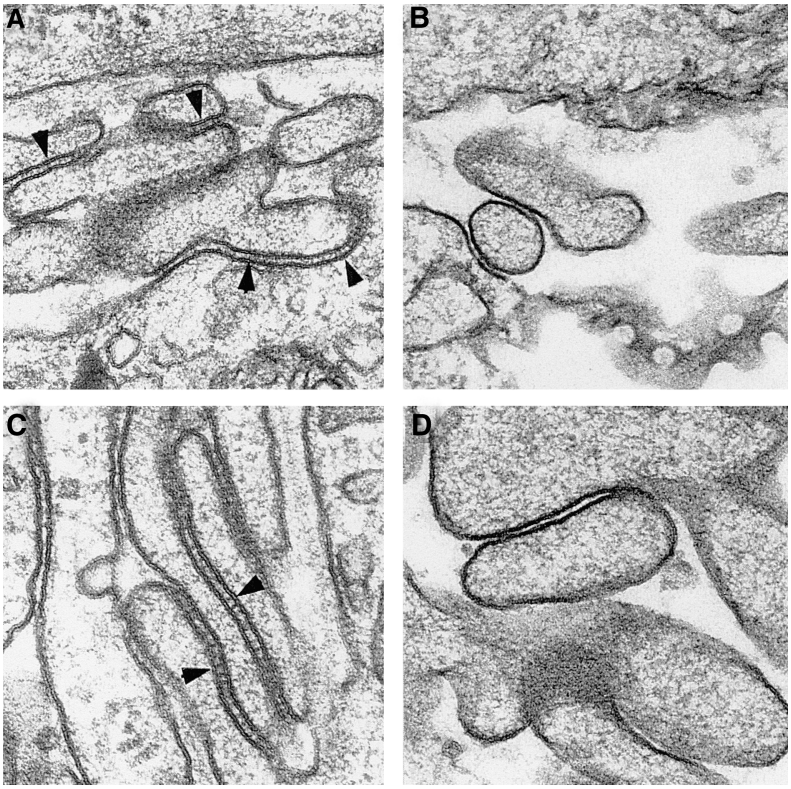

Fig. 3. Microvillar channels on z. fasciculata cells. Panels A and C show juxtaposed microvilli on *Srb1*$^{+/+}$ z. fasciculata cells with the formation of HDL-filled microvillar channels (arrowheads). Panels B and D show that juxtaposed microvilli do not form HDL-filled microvillar channels on *Srb1*$^{-/-}$ z. fasciculata cells. Panels C and D also illustrate a thinner microvillar plasma membrane on *Srb1*$^{-/-}$ (right), versus *Srb1*$^{+/+}$ cells (left). Panels A and B, ×48,000; panels C and D, ×60,000.

Adrenocortical cells in vivo show no or few classical caveolae (15, 18) but have an extensive cell surface compartment of microvillar channels, a membrane microdomain that serves to sequester HDL particles for CE selective uptake (15). Although the basis for the key role of SR-BI in microvillar channel organization is unclear, one possibility is that SR-BI-mediated changes in the cholesterol and/or phospholipid composition of microvillar membranes provokes the forma-

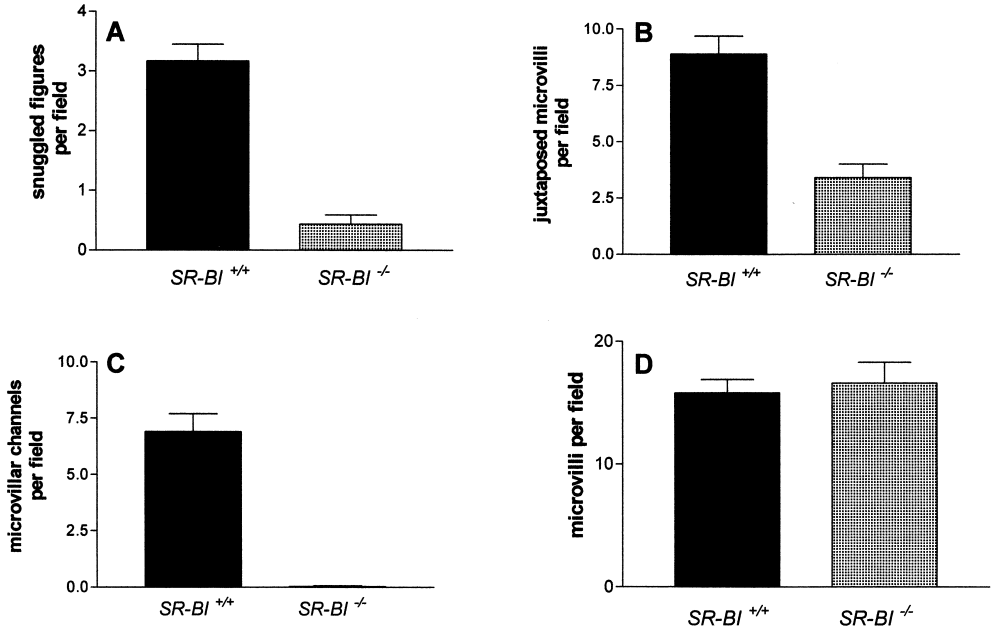

Fig. 4. Frequencies of microvillar configurations. A: Snuggled microvilli on the sinusoidal face and in junctional regions counted in 32 and 27 micrographic fields for *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$, respectively. B: Juxtaposed microvilli on the sinusoidal face counted in 29 and 23 micrographic fields for *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$, respectively. C: Microvillar channels on the sinusoidal face and in junctional regions counted in 32 and 27 micrographic fields for *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$, respectively. D: Microvilli on the sinusoidal face and in junctional regions counted in 32 and 27 micrographic fields for *Srb1*$^{+/+}$ and *Srb1*$^{-/-}$, respectively. All values shown as mean ± SEM. In panels A, B, and C, $P < 0.0001$ for *Srb1*$^{+/+}$ versus *Srb1*$^{-/-}$; $P = 0.69$ in panel D.

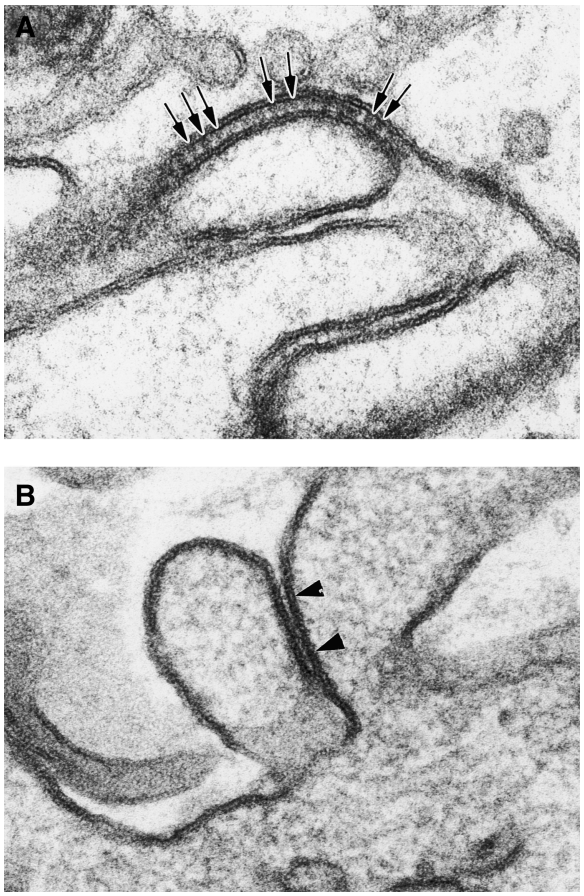

Fig. 5. Ultrastructure of microvillar channels on z. fasciculata cells A: Ultrastructure of *Srb1*$^{+/+}$ z. fasciculata cell showing HDL particles (arrows) aligned in a microvillar channel. B: Contrasted to closely juxtaposed microvilli (arrowheads) lacking HDL particles in *Srb1*$^{-/-}$ z. fasciculata cell. (×120,000).

tion of specific lipid rafts that are necessary for microvillar channel formation. Consistent with this idea, SR-BI expression in cultured cells alters the content and distribution of plasma membrane free cholesterol (4, 7–9). Additionally, the reduced thickness of the microvillar membrane in the *Srb1*$^{-/-}$ mouse is consistent with the idea of an altered lipid composition. Major determinants of membrane thickness include the free cholesterol and sphingolipid content as well as the length of the phospholipid acyl tails (20, 21), factors believed to be responsible for the greater thickness of the cholesterol-rich plasma membrane compared with the cholesterol-poor membrane of the endoplasmic reticulum (22).

Another possible role of SR-BI in microvillar channel formation could occur via interaction with HDL, which is potentially multivalent for interaction with SR-BI due to multiple apolipoprotein A-I molecules per particle. Thus, HDL could bridge SR-BI molecules on adjacent microvilli, bringing the membranes together to form the microvillar channel. This scenario does not appear likely because well-defined microvillar channels are present on adrenocortical cells of *apoA-I*$^{-/-}$ mice (17). Importantly, the microvillar channels of *apoA-I*$^{-/-}$ mice do not contain HDL particles and the width of the channel is reduced from 15 nm to 7.8 nm (17), a dimension characteristic of microvillar channels from which HDL has been removed by in situ perfusion (15). Thus, HDL binding to SR-BI does not appear necessary for microvillar channel formation. This does not eliminate the possibility that another ligand of SR-BI could serve as a bridge in microvillar channel formation.

In summary, the present results show that SR-BI has a major influence on adrenocortical plasma membrane structure and organization in vivo. Whether such effects occur in other cell types is unknown and under current investigation. Further analyses of microvillar membranes may shed light on the mechanism by which SR-BI alters plasma membrane structure and function.

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