Scavenger Receptor Class B Type I-mediated Reverse Cholesterol Transport Is Inhibited by Advanced Glycation End Products*

Received for publication, December 22, 2000, and in revised form, January 16, 2001
Published, JBC Papers in Press, January 17, 2001, DOI 10.1074/jbc.M011613200

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Cellular interactions of advanced glycation end products (AGE) are mediated by AGE receptors. We demonstrated previously that class A scavenger receptor types I and II (SR-A) and CD36, a member of class B scavenger receptor family, serve as the AGE receptors. In this study, we investigated whether scavenger receptor class B type I (SR-BI), another receptor belonging to class B scavenger receptor family, was also an AGE receptor. We used Chinese hamster ovary (CHO) cells overexpressed hamster SR-BI (CHO-SR-BI cells). 125I-AGE-bovine serum albumin (AGE-BSA) was endocytosed in a dose-dependent fashion and underwent lysosomal degradation by CHO-SR-BI cells. 125I-AGE-BSA exhibited saturable binding to CHO-SR-BI cells (Kd = 8.3 μg/ml). Endocytic uptake of 125I-AGE-BSA by CHO-SR-BI cells was completely inhibited by oxidized low density lipoprotein (LDL) and acetylated LDL, whereas LDL exerted only a weak inhibitory effect (<20%). Cross-competition experiments showed that AGE-BSA had no effect on HDL binding to these cells and vice versa. Interestingly, however, SR-BI-mediated selective uptake of HDL-CE was completely inhibited by AGE-BSA in a dose-dependent manner (IC50 < 10 μg/ml). Furthermore, AGE-BSA partially inhibited (by <30%) the selective uptake of HDL-CE in human hepatocarcinoma HepG2 cells (IC50 < 30 μg/ml). In addition, [3H]cholesterol efflux from CHO-SR-BI cells to HDL was significantly inhibited by AGE-BSA in a dose-dependent manner (IC50 < 30 μg/ml). Our results indicate that AGE proteins, as ligands for SR-BI, effectively inhibit both SR-BI-mediated selective uptake of HDL-CE and cholesterol efflux from peripheral cells to HDL, suggesting that AGE proteins might modulate SR-BI-mediated cholesterol metabolism in vivo.

In the Maillard reaction, proteins react with glucose to form Schiff base and Amadori products. After long term incubation, these early products are converted to advanced glycation end products (AGE), which are characterized physicochemically by fluorescence, brown color, and intra- or inter-molecular cross-linking (1, 2), and biologically by specific recognition by AGE receptors. The presence of AGE in several human tissues suggests that they may be involved in the aging process, diabetic complications, and atherosclerosis (3–11).

The physiological significance of AGE has been analyzed primarily using AGE structure(s) expressed in vivo and AGE-binding proteins or AGE receptors, through which AGE are believed to elicit several biological phenomena in monocytes/macrophages (12–17), endothelial cells (18, 19), and mesangial cells (20, 21). Several AGE receptors have been characterized (22–25), one of which is a novel 35-kDa protein (called RAGE) from bovine lung endothelium that belongs to the immunoglobulin superfamily (23). Two AGE-binding proteins of 60- and 90-kDa (called p60 and p90) were also identified from the rat liver (24). Recently, galectin-3, a lectin-like protein with a high binding affinity for galactose-containing glycoproteins, was identified as a component of p90 (25). We have recently shown that the macrophage scavenger receptor class AI/AII (SR-A), which is known as a receptor for oxidized low density lipoprotein (Ox-LDL) (26), mediates the endocytic uptake and degradation of AGE-BSA by macrophages (26, 27).

In contrast, although functionally related to SR-A, the class B receptors differ significantly in structure. CD36, the defining member of this class, binds Ox-LDL, fatty acids, and the proteins collagen and thrombospondin (28–32). CD36 has a broad ligand specificity, and its multiple potential roles have been proposed. We recently discovered that the class B scavenger family member CD36 also serves as a receptor for AGE-BSA (33). Our study provided novel information that in addition to SR-A the class B scavenger receptor family seems to serve as AGE receptors in vivo and therefore might participate in the pathogenesis of diabetic macrovascular complications (33). The scavenger receptor class B type I (SR-BI), a member of the class B scavenger receptor family, was first identified as a high density lipoprotein (HDL) receptor and was shown to mediate selective uptake of cholesteryl esters from HDL (HDL-CE) in vitro (34). A recent study demonstrated that SR-BI-mediated selective uptake of HDL-CE was much more efficient than CD36-mediated uptake, despite the high similarity in structure of SR-BI and CD36 (35). Immunohistochemical anal-

* This work was supported in part by Grants-in-aid for Scientific Research 09470513, 12557220 (to H. N.), 10044305, and 11557081 (to H. N.) from the Ministry of Education, Science, Sports and Cultures of Japan and the Fugaku Trust for Medicinal Research (to H. N.).

The abbreviations used are: AGE(s), advanced glycation end products; BSA, bovine serum albumin; LDL, low density lipoprotein; HDL, high density lipoprotein; Ox-LDL, oxidized LDL; acetyl-LDL, acetylated LDL; CE, cholesteryl oleoyl ether; SR-A, scavenger receptor class AI/ AII; SR-BI, scavenger receptor class B type I; PHS, phosphate-buffered saline; CHO, Chinese hamster ovary; PPARγ, peroxisome proliferator-activated receptor γ; VLDL, very low density lipoprotein.

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yse in rodents revealed abundant SR-BI expression in the liver and steroidogenic tissues such as the adrenal glands and ovaries (34), where a selective uptake of HDL-CE is known to predominate (36–39). Adenovirus-mediated overexpression of SR-BI in mouse liver is associated with reduced plasma HDL levels and increased cholesterol secretion into bile (40). Recent studies (40–43) provided further solid evidence for a crucial role of SR-BI in selective uptake of HDL-CE by hepatocytes in vitro and in vivo.

In the present study, we investigated whether AGE-proteins could be recognized as ligands by SR-BI. Our results suggest that AGE proteins generated in vivo could affect SR-BI-mediated cholesterol metabolism.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Materials—**Penicillin G (1,650 IU/mg), streptomycin sulfate (750 IU/mg), G418, Dulbecco’s modified Eagle’s medium (DMEM), and Ham’s F-12 medium were purchased from Life Technologies, Inc. Na[125I] (3.7 GBq/m), 1.2-3Hcholeryleoyl ether (1 mc/ ml), and 1.2-Hcholesterol (1 mc/ml) were purchased from Amersham Pharmacia Biotech. Rabbit polyclonal anti-mouse SR-BI antibody was purchased from Novus Biologicals. Other chemicals were of the best grade available from commercial sources.

**Ligand Preparation and Iodination—**AGE-BSA was prepared as described previously (33). Briefly, 2.0 g of BSA was dissolved in 10 ml of 0.5 M sodium phosphate buffer (pH 7.4) with 3.0 g of D-glucose. Each sample was sterilized by ultrafiltration, incubated at 37 °C for 40 weeks, and dialyzed against phosphate-buffered saline (pH 7.4) (PBS). The extent of lysine modification was 71% for AGE-BSA. The N\(^{-}\)(carboxymethyl) lysine content of AGE-BSA was 7.7 mol/mol of BSA (33). Nonglycated BSA was prepared as described previously (44). Briefly, 1.0 g of BSA was incubated with phenyl boronic acid resin (PBA-60, Amicon, Beverly, MA) in 800 ml of 0.5 M glycine/NaOH buffer (pH 8.5) for 2 h at room temperature. The filtrate through a glass filter was refiltered with another portion of the same resin. The final solution was dialyzed against PBS and used for the experiments. Human LDL (d = 1.019–1.063 g/ml) (33) and HDL (d = 1.063–1.21 g/ml) (45) were isolated by sequential ultracentrifugation of human plasma from normolipidemic subjects after overnight fasting and dialyzed against 0.15 M NaCl and 1 mM EDTA (pH 7.4) (33). Traces of apoA-I and -E were removed from HDL by a heparin–agarose column. Acetylated LDL (acetyl-LDL) was prepared by chemical modification of LDL with acetic anhydride as described previously (46). To prepare Ox-LDL, LDL was dialyzed against PBS to remove EDTA. LDL (0.1 mg/ml) was then incubated for 16 h at 37 °C with 5 \(\mu\)M CuSO\(_4\), followed by addition of 1 mM EDTA and cooling (46). Electrophoretic mobility of AGE-BSA preparations toward the anode was 1.4 times higher than native LDL. LDL with acetic anhydride as described previously (46). To prepare Ox-LDL, LDL was dialyzed against PBS to remove EDTA. LDL (0.1 mg/ml) was then incubated for 16 h at 37 °C with 5 \(\mu\)M CuSO\(_4\), followed by addition of 1 mM EDTA and cooling (46). Electrophoretic mobility of AGE-BSA preparations toward the anode was 1.4 times higher than native LDL. LDL with acetic anhydride as described previously (46). To prepare Ox-LDL, LDL was dialyzed against PBS to remove EDTA. LDL (0.1 mg/ml) was then incubated for 16 h at 37 °C with 5 \(\mu\)M CuSO\(_4\), followed by addition of 1 mM EDTA and cooling (46). Electrophoretic mobility of AGE-BSA preparations toward the anode was 1.4 times higher than native LDL.

**Preparation of 125I-HDL-[3H]Cholesteryl Oleyl Ether—**Human HDL (d = 1.063–1.21 g/ml) labeled with \[\text{125I}\]-cholesteryl oleyl ether (CE), a nonhydrolysable cholesteryl ester analogue, was prepared by a modification of the procedure of Miyazaki et al. (50). Briefly, 250 \(\mu\)Ci of \[\text{3H}\]CE in toluene was evaporated under a gentle stream of \(N_2\) at room temperature (49). \[\text{3H}\]CE was then added to 20 mg of human lipoprotein-deficient serum (20–40 mg/ml) with gentle stirring under a gentle stream of \(N_2\) at room temperature for 1 h. Human HDL (15 mg) was then added to the mixture with gentle stirring at room temperature for 30 min. After incubation and adjustment of the mixture density with KBr (d = 1.21 g/ml), the labeled HDL was collected by ultracentrifugation, followed by addition of 0.15 \(\mu\)l of cholesteryl esterase (52). HDL-[\text{3H}]CE was then labeled with \[\text{3H}\]CE as described above. The specific activity for 125I-HDL-\[\text{3H}\]CE was 158.8 cpm/ng of protein for 125I and 11.8 dpm/ng of protein for \[\text{3H}\].

**Selective Uptake of HDL-CE by CHO-SR-BI Cells and HepG2 Cells—**Cells (8 \(\times\) 10\(^4\)) were seeded in a 24-well plate and cultured for 2 days in 1.0 ml of medium B (for CHO-SR-BI and CHO-mock cells) or medium C (for HepG2 cells), which was then replaced by medium D. After culture for 1 h, each well received 0.5 ml of medium C containing various concentrations of 125I-HDL-[\text{3H}]CE in the presence (nonnullspecific uptake) or absence (total uptake) of 10-fold excess amounts of the unlabeled ligand to be tested. After incubation for the indicated times, the cells were washed three times with 1 ml of medium D and then three more times with PBS. The washed cells were lysed with 1 ml of 0.1 M NaOH for 30 min at 37 °C. After incubation, 125I radioactivity of 0.1 n NaOH-soluble protein was determined (as the cell association of 125I-HDL or 125I-AGE-BSA), and cellular proteins were determined using BCA protein assay reagent (Bio-Rad). For the binding study, cells that had been seeded in each well as described above were incubated for 90 min at 4 °C in 0.5 ml of medium D with various concentrations of 125I-HDL or 125I-AGE-BSA in the presence or absence of 20-fold excess amounts of the unlabeled ligands. Each well was washed with ice-cold PBS containing 3% BSA and PBS. The cells were lysed, and the cell-bound radioactivity and cellular proteins were determined as described above.
mined by measuring radioactivity released from cells into the medium upon the addition of HDL.

Statistical Analysis—All data were expressed as mean ± S.D. Differences between groups were examined for statistical significance using the Student’s t test. A p value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Expression of SR-BI in CHO Cells Overexpressing Hamster SR-BI—CHO-SR-BI cells that stably expressed hamster SR-BI were established. Immunoblotting with a polyclonal anti-SR-BI antibody yielded no bands in CHO-mock cells, whereas CHO-SR-BI cells exhibited a distinctive band. Parallel control experiments demonstrated that rat liver parenchymal cells expressing high levels of SR-BI exhibited a positive band with molecular weight indistinguishable from that obtained from CHO-SR-BI cells as reported previously (47) (Fig. 1). We further examined whether SR-BI expressed on the cells was functional; CHO-SR-BI cells exhibited cellular binding of 125I-HDL with high affinity (Kd = 6.5 μg/ml, Bmax = 72.3 μg/mg cell protein) but no significant capacity for endocytic uptake of 125I-HDL (data not shown), as reported previously (34). Selective uptake of HDL-CE by CHO-SR-BI cells was 4-fold higher, at least, than that of CHO-mock cells (data not shown), as reported previously (47). These results indicated that SR-BI expressed on CHO-SR-BI cells functioned as the HDL receptor.

Endocytic Uptake and Degradation of 125I-AGE-BSA by CHO-SR-BI Cells—We determined the endocytic uptake and degradation of 125I-AGE-BSA by CHO-SR-BI cells at 37 °C. The amount of cell association of 125I-AGE-BSA with CHO-SR-BI cells increased in a dose-dependent manner and was almost competed away by a 20-fold excess of unlabeled AGE-BSA (Fig. 2A). The specific binding exhibited a dose-dependent saturation pattern with a plateau level of 230 ng/mg cell protein; the apparent Kd for degradation was 3.1 μg/ml, and the maximal ligand degradation was 297 ng/mg cell protein, whereas CHO-mock cells did not degrade 125I-AGE-BSA under the same conditions (Fig. 2B). This result indicates that SR-BI mediates the cell association of AGE-BSA and its endocytic degradation.

Binding of 125I-AGE-BSA to CHO-SR-BI Cells—We next determined the cellular binding of 125I-AGE-BSA to CHO-SR-BI cells at 4 °C. Total binding of 125I-AGE-BSA was inhibited by 70% by an excess amount of the same ligand. The specific binding, obtained by subtracting nonspecific binding from the total binding, yielded a saturation pattern. Scatchard analysis of the specific binding disclosed a binding site with an apparent Kd of 8.3 μg/ml and maximal binding of 85.7 ng/mg cell protein, indicating that CHO-SR-BI cells possess a high affinity binding site for AGE-BSA (Fig. 3). Since the parallel binding experiment of 125I-AGE-BSA to CHO-mock cells failed to yield specific binding (data not shown), it is likely that the specific binding site for AGE-BSA on CHO-SR-BI cells is identical to SR-BI.

Effect of Modified LDL on Cellular Binding of 125I-AGE-BSA to CHO-SR-BI Cells—To examine whether the recognition site for AGE-BSA in CHO-SR-BI cells was identical to that for Ox-LDL, acetyl-LDL, LDL, and HDL, we determined the effect of these lipoproteins on cellular binding of 125I-AGE-BSA to CHO-SR-BI cells. Cellular binding of 125I-AGE-BSA to CHO-SR-BI cells was effectively (>62%) replaced by unlabeled Ox-LDL and acetyl-LDL and by unlabeled AGE-BSA, whereas unlabeled LDL and HDL had a slightly weaker effect (<20%) (Fig. 4A). Non-glycated BSA, a negative control, had no effect
on this process (Fig. 4A). Parallel experiments demonstrated that cellular binding of $^{125}$I-HDL to CHO-SR-BI cells was effectively (60%) replaced by unlabeled HDL, whereas AGE-BSA had little effect (<15%) (Fig. 4B). These results suggest that the binding site of SR-BI for AGE-BSA might overlap with that for Ox-LDL and acetyl-LDL but not that for LDL and HDL.

**Effect of Modified LDL on Endocytic Uptake of $^{125}$I-AGE-BSA by CHO-SR-BI Cells**—We determined the effect of Ox-LDL, acetyl-LDL, LDL, and HDL on endocytic uptake of $^{125}$I-AGE-BSA by CHO-SR-BI cells. The cell association of $^{125}$I-AGE-BSA was effectively (>85%) replaced by unlabeled Ox-LDL and acetyl-LDL and by unlabeled AGE-BSA, whereas the effect of unlabeled LDL and HDL was almost negligible (<20%), and nonglycated BSA, a negative control, had no effect on this process (Fig. 5A). Similarly, the endocytic degradation of $^{125}$I-AGE-BSA was effectively inhibited (>85%) by the presence of unlabeled Ox-LDL, acetyl-LDL, and unlabeled AGE-BSA, whereas unlabeled LDL and HDL also exerted a much weaker effect (<25%) (Fig. 5B). The results of endocytic experiments are consistent with those of the binding experiments (Fig. 4).

**Effect of AGE-BSA on Selective Uptake of HDL-CE by CHO-SR-BI Cells**—We next examined the effects of AGE-BSA on the selective uptake of HDL-CE by CHO-SR-BI cells. AGE-BSA inhibited the selective uptake of HDL-CE by CHO-SR-BI cells in a dose-dependent manner, whereas non-glycated BSA had no effect (Fig. 6). SR-BI-mediated selective uptake of HDL-CE, which was obtained by subtracting the amount of selective uptake by CHO-mock cells from that by CHO-SR-BI cells, showed that it was inhibited by AGE-BSA dose-dependently with 50% inhibition at <30 µg/ml of AGE-BSA, whereas non-glycated BSA had no effect (Fig. 7). Taken together, these results suggest that AGE-BSA inhibits SR-BI-mediated HDL-dependent cholesterol efflux.

**DISCUSSION**

Our previous studies using CHO cells overexpressing the class A scavenger receptor (SR-A) (27) and peritoneal macrophages from SR-A knockout mice (26) and our recent study using CHO cells overexpressing the class B scavenger receptor CD36 (33) demonstrated that AGE ligands are effectively taken up by these scavenger receptors by a typical receptor-mediated endocytosis mechanism. These findings raised the general hypothesis that the scavenger receptor as the AGE receptor is not confined to the class A but also class B scavenger receptor family (33). We have now demonstrated this to be the case with another class B scavenger receptor, SR-BI, using CHO cells overexpressing hamster SR-BI. The unique function of SR-BI (not found in other scavenger receptors such as SR-A and CD36) is its major role as the HDL receptor in reverse cholesterol transport, from cholesterol efflux from peripheral cells into HDL particles to selective uptake of HDL-CE by the liver. Therefore, the novel findings of our present study were that SR-BI mediated the endocytic degradation of AGE ligands in a manner similar to SR-A and CD36, and also that the selective uptake of HDL-CE and cholesterol efflux mediated uniquely by SR-BI as an HDL receptor was inhibited by AGE ligands. These findings suggest a novel idea in AGE research that AGE proteins generated in situ could regulate SR-BI-mediated cholesterol metabolism in vivo.

SR-BI was first identified as an HDL receptor by Acton et al. (34). Immunohistochemical analyses of SR-BI in rodents showed its major sites of expression to be the liver and in steroidogenic cells such as adrenal glands and ovaries (34), where selective uptake of HDL-CE is significant (36–39). Al-
though belonging to the class B scavenger receptor family, SR-BI is functionally characterized by mediating selective uptake of HDL-CE in vitro (34) and in vivo (40–43). The present study showed that this phenomenon mediated by CHO-SR-BI cells was effectively (almost completely) inhibited by AGE-BSA (Fig. 6), suggesting that AGE ligands could inhibit selective CE uptake in the liver and steroidogenic tissues. This finding in CHO-SR-BI cells was supported by results obtained from HepG2 cells, model cells for human hepatocytes; selective uptake of HDL-CE by these cells was partially but significantly inhibited by AGE-BSA (Fig. 7). Although immunoblot analysis showed that SR-BI was positively expressed in these cells (data not shown), the extent of contribution of human SR-BI to selective uptake of HDL-CE by HepG2 cells is unknown at present. Rhains et al. (54) reported that SR-BI and/or CD36 of HepG2 cells are involved, at least in part, in this process. Therefore, the extent of inhibition by AGE-BSA of selective uptake of HDL-CE by HepG2 cells could be lower than estimated. A recent study using transiently expressing cells demonstrated that selective uptake of HDL-CE by SR-BI is of more functional significance than that by CD36 (35). Therefore, it is likely that the extent of inhibition by AGE-BSA of selective uptake of HDL-CE by HepG2 cells could be significant. Thus, it is possible that AGE proteins generated in vivo may inhibit SR-BI-mediated selective uptake of HDL-CE, the final step of reverse cholesterol transport, implicating AGE ligands in regulation of plasma cholesterol levels.

In addition to selective uptake of HDL-CE by SR-BI, this receptor is also involved in cholesterol efflux from peripheral cells to HDL, the first step of reverse cholesterol transport (52). The present study using CHO-SR-BI cells showed that AGE-BSA effectively inhibited [3H]cholesterol efflux from CHO-SR-BI cells to HDL (Fig. 8). Although Hiran et al. (55) reported that human SR-BI is expressed and markedly up-regulated in differentiated human macrophages, the lack of in vivo experimental results means that the functional involvement of SR-BI in cholesterol efflux remains uncertain. It has been proposed, however, that SR-BI facilitates bidirectional cholesterol flux depending on the cholesterol gradient between HDL particles and the cell plasma membrane (52, 56). There-
cells (open circle) were incubated with 10 μg/ml 125I-HDL-[3H]cholesteryl oleate ether in the presence or absence of increasing concentrations of unlabeled HDL (closed squares), AGE-BSA (closed circle), or non-glycated BSA (closed triangles). Under the identical conditions, CHO-mock containing 10⁶, HepG2 cells were incubated for 5 h in 0.5 ml of 3% BSA in DMEM containing 10 μg/ml 125I-HDL-[3H]cholesteryl oleate ether in the absence of competitors. Selective uptake of HDL-CE was determined as described under “Experimental Procedures.” Data represent the means of three separate experiments. Error bars represent S.D.

Fig. 7. Effect of AGE-BSA on the selective uptake of HDL-CE by HepG2 cells. Under identical conditions to those described in Fig. 6, HepG2 cells were incubated for 5 h in 0.5 ml of 3% BSA in DMEM containing 10 μg/ml 125I-HDL-[3H]cholesteryl oleate ether in the presence or absence of increasing concentrations of unlabeled HDL (closed circle), AGE-BSA (closed square), or non-glycated BSA (closed triangles). Selective uptake of HDL-CE was determined as described under “Experimental Procedures.” Data represent the means of three separate experiments. Error bars represent S.D.

Furthermore, the present results suggest that AGE proteins inhibit both selective CE uptake and cholesterol efflux. Recent studies using genetically engineered mice, however, have shown that SR-BI plays an important role in the control of biliary cholesterol secretion (40, 41). This notion was supported by the fact that SR-BI was expressed on the canalicular membrane of mouse hepatocytes overexpressing SR-BI (40, 58). In addition, the immunohistochemical study by Ling et al. (59) using a monoclonal antibody against fluorolink, one of the AGE structures, demonstrated that AGE proteins are localized intracellularly and extracellularly in human hepatocytes. Therefore, it is possible that AGE proteins formed in hepatocytes may inhibit SR-BI-mediated biliary cholesterol secretion in vivo.

SR-BI is known to be a multifunctional molecule that recognizes many ligands of different structures including acetyl-LDL, Ox-LDL, LDL, maleylated BSA (60), HDL (34), VLDL (61), anionic phospholipids (62), and apoptotic cells (63). These findings do not provide a clear picture of the molecular basis for the recognition of these ligands by SR-BI. However, among these ligands, modified LDLs have in common a negatively charged nature, suggesting that SR-BI recognizes the negative charge of AGE proteins. Further studies are necessary to determine the AGE structure(s) required for recognition by SR-BI.

Several attempts were made to characterize the ligand binding domain of SR-BI. Temel et al. (64) prepared anti-SR-BI antisera raised against the extracellular domain of mouse SR-BI (amino acid residues 174–356) and demonstrated that it not only inhibited HDL binding and selective lipid uptake but also inhibited LDL binding and selective lipid uptake (65). Gu et al. (57) reported that a double substitution of arginine for glutamine at positions 402 and 418 (Q402R/Q418R) of mouse SR-BI led to a loss of ability to bind HDL but not LDL. Another mutant of mouse SR-BI with a Met-to-Arg substitution at position 158 (M158R) exhibited diminished binding ability to HDL and LDL, but no change in its binding capacity to acetylated LDL (57). Furthermore, Acton et al. (60) demonstrated that the binding of 125I-acetyl-LDL to COS cells transfected with hamster SR-BI was completely inhibited by Ox-LDL. Similarly, Calvo et al. (61) also showed that Ox-LDL and LDL effectively replaced binding of Dil-HDL to Sf9 cells transfected with human SR-BI. Taken together, these results support the notion that the region corresponding to amino acids 174–356 of the extracellular domain of SR-BI is necessary for its HDL binding and selective CE uptake, and that the binding domain of Ox-LDL and acetyl-LDL to SR-BI might overlap with amino acids 174–356 of its extracellular domain. In addition, it is likely that amino acids 402 and 418 in the extracellular domain of mouse SR-BI is important for binding HDL but not LDL.

In the present study, Ox-LDL and acetyl-LDL completely inhibited endocytic uptake of 125I-AGE-BSA by CHO-SR-BI cells, indicating that the binding domain of AGE-BSA to SR-BI might overlap with amino acids 174–356 of its extracellular domain (Figs. 4 and 5). However, the extent of inhibition of HDL and LDL for 125I-AGE-BSA binding was weak (Figs. 4 and 5), suggesting that the main binding site of AGE-BSA to SR-BI would be within amino acids 174–356 of its extracellular domain but not its binding domain to HDL and LDL. The most novel finding in the present study was that AGE-BSA completely (>90%) inhibited selective uptake of HDL-CE by CHO-SR-BI cells (Fig. 6). In addition, AGE-BSA exhibited a significant inhibitory effect on HDL-induced cholesterol efflux from these cells (Fig. 8). It is not clear whether the HDL binding domain of SR-BI is involved in selective CE uptake. Williams and coworkers (66, 67) recently proposed that SR-BI may form “a lipid channel” that facilitates transfer of lipid between cells and lipoproteins. Therefore, it is possible that AGE-BSA could inhibit SR-BI-mediated CE uptake by binding to the lipid channel rather than by binding to the HDL binding domain. Further studies are needed to elucidate the binding domain of SR-BI to AGE ligands.

Recently, it has been reported that CD36 and SR-BI are localized in caveolae of the plasma membrane and that Ox-LDL binding to CD36 or HDL binding to SR-BI affects the lipid composition of caveolae, which in turn modulates activation of endothelial nitric-oxide synthase in human microvascular endothelial cells (68). Stitt et al. (69) also reported that the R1 (OST-48), R2 (80K-H), and R3 (galectin-3) components of the AGE receptor complex were localized in caveolae of the plasma membrane and that Ox-LDL and acetyl-LDL to SR-BI might overlap with amino acids 174–356 of its extracellular domain. In addition, it is likely that amino acids 402 and 418 in the extracellular domain of mouse SR-BI is important for binding HDL but not LDL. In the present study, Ox-LDL and acetyl-LDL completely inhibited endocytic uptake of 125I-AGE-BSA by CHO-SR-BI cells, indicating that the binding domain of AGE-BSA to SR-BI might overlap with amino acids 174–356 of its extracellular domain (Figs. 4 and 5). However, the extent of inhibition of HDL and LDL for 125I-AGE-BSA binding was weak (Figs. 4 and 5), suggesting that the main binding site of AGE-BSA to SR-BI would be within amino acids 174–356 of its extracellular domain but not its binding domain to HDL and LDL. The most novel finding in the present study was that AGE-BSA completely (>90%) inhibited selective uptake of HDL-CE by CHO-SR-BI cells (Fig. 6). In addition, AGE-BSA exhibited a significant inhibitory effect on HDL-induced cholesterol efflux from these cells (Fig. 8). It is not clear whether the HDL binding domain of SR-BI is involved in selective CE uptake. Williams and coworkers (66, 67) recently proposed that SR-BI may form “a lipid channel” that facilitates transfer of lipid between cells and lipoproteins. Therefore, it is possible that AGE-BSA could inhibit SR-BI-mediated CE uptake by binding to the lipid channel rather than by binding to the HDL binding domain. Further studies are needed to elucidate the binding domain of SR-BI to AGE ligands.
AGE Inhibits SR-BI-mediated Cholesterol Transfer

Fig. 8. Effect of AGE-BSA on $[^3]H$cholesterol efflux to HDL from CHO-SR-BI cells. A, time course of $[^3]H$cholesterol efflux from CHO-SR-BI, CHO-SR-BI cells were labeled overnight with $[^3]H$cholesterol (1 μCi/ml), incubated for 1 day in 0.5 ml of 0.5% BSA in DMEM, washed, and incubated with HDL (50 μg/ml) (closed circle) in the presence or absence of AGE-BSA (100 μg/ml) (open triangles) or nonglycated BSA (100 μg/ml) (open squares), or incubated without HDL (open circle). Efflux values are expressed as $[^3]H$cholesterol released to the medium. Data represent the means of three separate experiments. Error bars represent S.D.

Chimenti et al. (70) recently showed that CLA-1/SR-BI expressed on macrophages is induced by ligands for PPARγ and PPARα in human atherosclerotic lesions. Recent reverse transcription-polymerase chain reaction analyses by Iwashima et al. (71) demonstrated that AGE-BSA induced PPARγ expression and activation in cultured mesangial cells, and that SR-BI, SR-A, CD36, and Lox-1 were also up-regulated by AGE-proteins through activation of PPARγ in phorbol 12-myristate 13-acetate-treated THP-1 cells (72).

In conclusion, our finding that SR-BI-mediated functions were inhibited by AGE proteins suggests a potential link between SR-BI-mediated cholesterol metabolism and development of diabetic vascular complications.

Acknowledgments—We are grateful to Dr. Akihiko Kuniyasu of our laboratory for his contribution to this study in the Department of Biochemistry, Kumamoto University School of Medicine, for helpful discussions.

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