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RAPID COMMUNICATION

ASIALOOROSOMUCOID HEPATOBILIARY TRANSPORT IS UNALTERED BY THE LOSS OF LIVER ASIALOGLYCOPROTEIN RECEPTORS IN AGED RATS*

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ABSTRACT. The hepatobiliary transport of asialoorosomucoid (ASOR) was examined in aging male Fischer 344 rats. The time course of transport of ^{125}I-ASOR from blood to bile was identical in both senescent and young adult rats. Peak secretion occurred at approximately 35 minutes after injection via the femoral vein. Total secretion of radiolabeled ASOR (3.6% of injected dose), bile secretion and rate of secretion of radiolabeled ligand (approximately 2% of administered dose/hr/gm bile/liver) were not significantly different for the two age groups. Determination of the binding capacity for ^{125}I-ASOR with liver plasma membrane-enriched preparations showed the membranes from old animals capable of binding approximately 50% less radiolabeled ligand as the young adult animals. Analysis of the distribution of ^{125}I-ASOR autoradiographic grains along the liver lobule indicated extensive uptake of ligand in Zone 2 and 3 cells in senescent animals, whereas uptake in young rats was essentially limited to Zone 1 parenchymal cells. These results indicate that, contrary to the age-related loss of hepatic receptors for dimeric IgA and the concomitant reduction in hepatobiliary secretion of IgA, loss of ASOR binding capacity on liver plasma membranes from old animals is not reflected in diminished hepatobiliary secretion of ASOR. The loss of ASOR binding capacity is offset by the recruitment of Zone 2 and 3 hepatocytes along the liver lobule. This result suggests that hepatic metabolism and hepatobiliary secretion of macromolecules which exhibit a lobular gradient of uptake (e.g. ASOR) will be relatively less affected by loss of receptors compared to ligands which do not display such a gradient (e.g. IgA).

INTRODUCTION. Hepatocytes endocytose many proteins via receptor-mediated mechanisms. Glycoproteins, such as asialofetuin and asialoorosomucoid (ASOR), which have lost their terminal sialic acid residues, bind to a galactose-specific asialoglycoprotein (ASGP) receptor (1,2), are endocytosed and transported intracellularly to the lysosomal compartment for ligand degradation (1,3). The ASGP receptor is not degraded, rather it is recycled to the sinusoidal surface of the hepatocyte (4). Dimeric immunoglobulin A (dIgA) is processed differently by hepatocytes. The polymeric IgA (pIgA) receptor, a membrane-bound glycoprotein of approximately 120,000 Daltons is localized to the sinusoidal surface (5-8). Following binding to the receptor, the pIgA-receptor complex is endocytosed into small vesicles and transported via a microtubule-dependent mechanism to the bile canaliculus (9-11). Secretory IgA (sIgA), the polymeric immunoglobulin covalently bound to an 80 kDalton fragment of the receptor molecule is exocytosed into bile (7,8). We have reported a 6-fold, age-related decline in the hepatobiliary secretion of dIgA in the rat (12) and a concomitant 4-fold loss of dIgA receptors from the liver plasma membranes (LPMs) of senescent Fischer rats (13).

We report here, a similar age-related loss of ASGP receptors from plasma membrane-enriched fractions from rat liver. However, there is no alteration in the secretion of ^{125}I-labeled ASOR into bile. Rather, more hepatocytes further toward the lobule periphery are recruited to bind and take-up ASOR, thus offsetting the apparent concomitant loss of ASGP binding capacity on LPM.

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METHODS. Male Fischer 344 rats (Harlan Sprague/Dawley), 3-6 months (young adult) or 24-29 months (senescent) of age were used in this study. Animals were housed briefly at the Animal Care Facility, Veterans Administration Medical Center, San Francisco, in barrier cages and were maintained on lab chow and water ad libitum. Liver plasma membranes were prepared as previously described (13). Orosomucoid (α_1-acid glycoprotein) and agarose-linked neuraminidase (Clostridium perfringens) were purchased from Sigma. Orosomucoid was desialylated by incubation of a 5 mg/ml solution with 0.5 Units/ml of agarose-linked neuraminidase in 0.1 M sodium acetate (pH 5.6) for 24 hours at 37°C. Sialic acid release was determined by the thiobarbituric acid method (14). Protein was determined by the method of Lowry (15). ASOR was labeled with ^{125}I (Amersham, Arlington Heights, IL; 15.4 mCi/mg) to a specific acitivity of 12 mCi/mg using a modified monochloride technique (16). Labeled protein was 95% TCA precipitable. Radioactivity (^{125}I) was measured in a Gamma 8500 gamma scintillation counter (Beckman Instruments, Fullerton, CA).

To examine the effect of aging on the transport of ASOR from blood to bile, three rats in each age group were anesthetized with Nembutal (5 mg/100 gm body wt., i.p.) and polyethylene cannulas inserted and secured in their common bile ducts. Animals were maintained under anesthesia for the duration of the experiments. ASOR labeled with ^{125}I (430 ng) was injected via the femoral vein and bile collected at 10 min. intervals during the subsequent 90 min. The amount of ^{125}I recovered in bile, bile weight, and trichloroacetic acid precipitable radioactivity was determined for each serial sample. The amount of radioactive label remaining in liver and blood were measured at the end of each experiment.

To evaluate the effect of animal age on the lobular gradient of ASOR uptake, two animals in each age group were anesthetized as described above, ^{125}I-ASOR (43 µg) injected via the femoral vein and 10 minutes after injection of radiolabeled protein the livers were perfused with 2.5% glutaraldehyde/0.8% paraformaldehyde in 0.1 M sodium bicarbonate, rinsed in buffer overnight, postfixed in osmium tetroxide, and prepared for light microscopic autoradiography as described previously (12).

RESULTS. The transport of femorally injected ^{125}I-ASOR from blood to bile in young and senescent animals is shown in Figure 1. Both age groups showed essentially identical hepatobiliary transport curves. Appearance of radioactivity peaked at approximately 35 minutes after injection. Table I shows that neither bile secretion, the total amount of ^{125}I-ASOR which was transported to bile (as a percentage of the injected dose), nor the rate of ^{125}I-ASOR secretion into bile over the 90' experiment differed between the

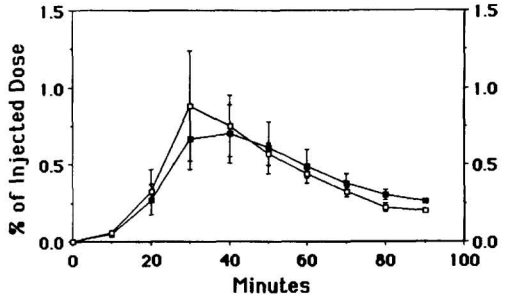

Figure 1. Effect of animal age on blood to bile transport of ^{125}I-ASOR. ASOR labeled with ^{125}iodine was injected via the femoral vein of young adult or senescent rats and 10 min serial bile samples were collected over a 90 min period. Protein bound ^{125}I was determined by precipitation in 10% trichloroacetic acid. Total counts in all bile samples were 85-95% TCA precipitable. Values are plotted as the mean TCA precipitable counts as a % of the injected dose of ^{125}I-ASOR versus time after injection and SEM for 3 animals per age group.

two age groups. At the end of 90 minute experiments 3 to 5% of the injected dose of radiolabel remained in the livers of these animals. The amount of radioactivity which was released back into the blood as degradation products was significantly greater for the young animals. This result suggests that lysosomal degradation may be more efficient in young animals or possibly the transport of endocytosed ASOR to lysosomes may be impaired in senescent animals. At least part of the age-related decline in hepatobiliary secretion of ^{125}I-dIgA in these rats is postulated to be due to age-related changes in the efficiency of the transport pathway (12). However, the present data cannot distinguish between these two possibilities.

The capacity of hepatocytes to bind ASOR was examined using isolated plasma-membrane-enriched (LPM) preparations from the livers of young and senescent rats. The binding of ^{125}I-ASOR to LPM was rapid at 4° (coming to equilibrium after approximately 60 minutes and remaining stable for at least 24 hrs). It also was saturable, dependent on both protein and ligand concentration, inhibited by excess unlabeled ASOR but not native orosomucoid and dependent on Ca^{++} (data not shown). The effect of animal age on ASOR binding is shown in Figure 2. There was approximately 50% less specific binding of ^{125}I-ASOR to LPM from old animals compared to that of young rats. Nonspecific binding in the presence of 1000-fold excess of unlabeled ASOR did not differ significantly between the two age groups. As previously reported (13), membrane preparations from

TABLE I. Effect of Animal Age on Hepatic Processing of ^{125}I-Labeled ASOR[a]

	ANIMAL AGE	
	3-6 Months	24-28 Months
Bile Secretion (gm/hr/liver)[b]	0.79±0.40	1.02±0.28
% of Dose Transported[b]	3.6±1.4	3.7±1.3
Rate of Secretion[c]	2.4±0.8	1.7±0.6
CPM/ml Blood[d]	37,230±4,900	23,650±670*

a All values represent the mean ± SD for 3 animals.

b Values are expressed as that observed over a 90 min. experiment.

c Values are expressed as % of administered dose/hr/gm bile/liver.

d Values represent the amount of radioactivity remaining in blood at the end of the 90 min experiment.

*P<0.01 by Student's t-test

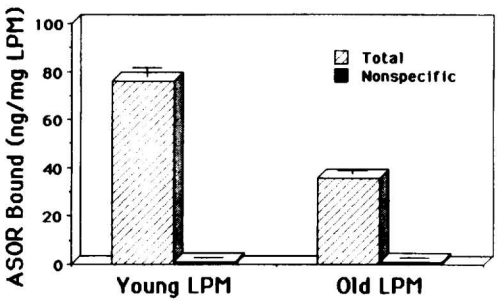

Figure 2. Binding of ^{125}I-ASOR to LPM from young and senescent rats. Isolated LPM (20 µg) were incubated for 20 hrs at 4° with 20 ng/ml ^{125}I- ASOR (0.5 nM) in a final volume of 0.2 ml Krebs Bicarbonate Ringer buffer containing 1% BSA. Nonspecific binding was determined in a set of parallel tubes by preincubating LPM with 1000-fold excess unlabeled ASOR before addition of labeled ligand. Binding was terminated by addition of 5 ml binding buffer and immediate filtration through Whatman GF/C filters under reduced pressure. Filters were washed with an additional 15 ml buffer. Blanks were generally less than 0.2% of total CPM added per tube. Values are mean±SEM for 3 different LPM preparations.

these two age-groups appear to be essentially equivalent in other characteristics including yield of LPM per gm liver, freedom from apparent contamination by other organelles as determined by electron microscopic examination and plasma membrane marker enzyme enrichment. However, the presence of silent ASGP receptors (biologically inactive but immunologically demonstratable) can not be ruled out by the current binding data.

The uptake of ^{125}I-ASOR by liver parenchymal cells along the portal to central lobule was examined qualitatively using light microscopic autoradiography. Figure 3 shows that the autoradiographic grains in the liver of young animals lie almost totally in Zone 1. Senescent animals, on the other hand, showed significant autoradiographic grains all along the the liver lobule indicating uptake of ^{125}I-ASOR by Zone 2 and 3 hepatocytes as well.

DISCUSSION. The hepatobiliary metabolism of pIgA is markedly affected by aging in the Fischer 344 rat. Transport of pIgA decreases almost 6-fold between young adult and senescent animals (12). This decreased transport is accompanied by a 4-fold loss of pIgA receptors on hepatocytes (13). Asialoglycoproteins such as ^{125}I-ASOR are avidly taken up by liver parenchymal cells. The majority of the endocytosed ligand is transported to the lysosomal compartment for degradation. Breakdown products are released back into the blood and/or bile. A small proportion

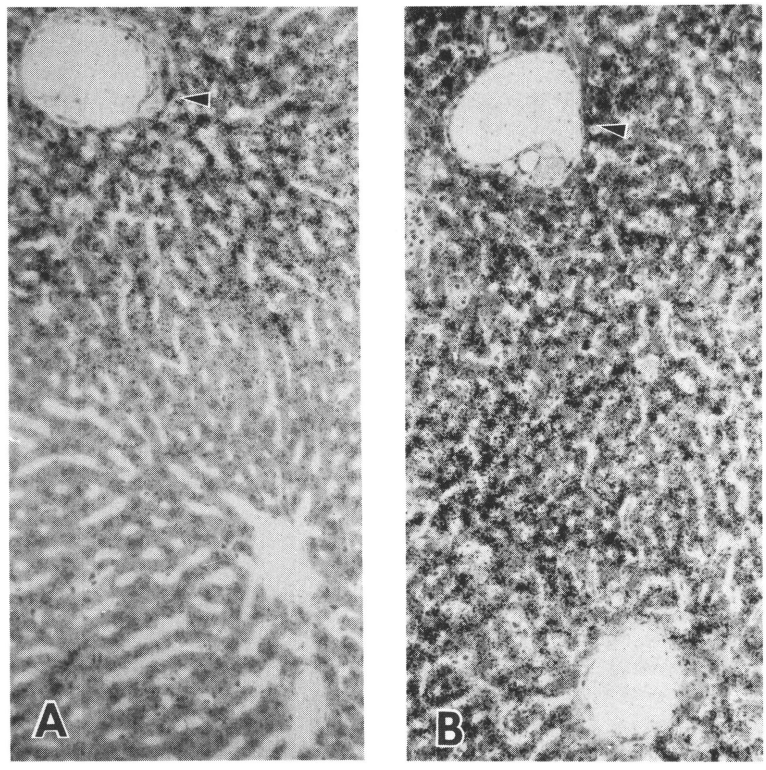

Figure 3. Light microscopic autoradiographs of liver tissue from young adult (A) and senescent (B) rats injected with ^{125}I-ASOR via the femoral vein. A portal to central vein are shown for each age group. The animals were sacrificed and the tissue prepared 10 minutes after the administration of the ligand. The distribution of autoradiographic grains in young animals are limited to portal region cells while autoradiographic grains in the senescent animals extend into Zones 2 and 3. (Arrowheads, portal veins.) X125.

(<5%) of administered ^{125}I-ASOR is secreted intact into bile. The mechanism by which this phenomenon occurs is not understood but has been hypothesized as representing missorting of receptor-bound ligand at the site of receptor sorting/uncoupling (17). It is possible that the hepatobiliary transport of intact ASOR is due to a specific subpopulation of ASGP receptors which are not altered with age in the rat. The results of the current studies suggest several conclusions. The lack of age-related effects on the hepatobiliary secretion of intact ASOR, despite a reduction in putative binding sites on plasma membranes, appears to be directly related to the recruitment of additional hepatocytes in Zones 2 and 3 of the liver lobule. Thus, hepatobiliary uptake and secretion of macromolecules such as IgA which show no lobular gradient would be severly affected by aging. If there were age-related decreases or a change in the rate-determining step of the blood to bile transport process common to both ligands one would expect reduced hepatobiliary secretion and/or a delayed secretory peak. Neither were observed. A detailed analysis of the kinetics of uptake and transport of ^{125}I-ASOR to subcellular compartments is necessary to confirm this conclusion. However, the lack of effect of aging on ASOR hepatobiliary secretion clearly demonstrates that whatever the mechanism for intracellular transport of ASOR to bile, it is unchanged with age in the rat hepatocyte. The opposite case, i.e. increased missorting, might have been predicted for senescent rats as aging generally results in decreased efficiency in biological systems.

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