Apo B100–containing Lipoproteins Are Secreted by the Heart

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Abstract

The apo B gene is expressed in the human heart and in the hearts of human apo B transgenic mice generated with large genomic clones spanning the human apo B gene. [^{35}S]Methionine metabolic labeling experiments demonstrated that apo B100–containing lipoproteins are secreted by human heart tissue and by human apo B transgenic and nontransgenic mouse heart tissue. Density gradient analysis revealed that most of the secreted heart lipoproteins were LDLs, even when the labeling experiments were performed in the presence of tetrahydrolipstatin, an inhibitor of lipoprotein lipase. Western blots with a microsomal triglyceride transfer protein) (MTP)-specific antiserum demonstrated that the microsomes of the heart contain the 97-kD subunit of MTP (the subunit involved in the transfer of lipids and assembly of lipoproteins). Metabolic labeling of mouse heart tissue in the presence of BMS-192951, an MTP inhibitor, abolished lipoprotein secretion by the heart but resulted in the secretion of two apo B proteolytic fragments (80 and 120 kD), which were found in the bottom fraction of the density gradient. These studies reveal that the heart, and not just the liver and intestine, secretes apo B–containing lipoproteins. We speculate that lipoprotein secretion by the heart represents a mechanism for removing excess lipids from the heart. (*J. Clin. Invest.* 1998. 101:1197–1202.) Key words: lipoproteins • microsomal triglyceride transfer protein • myocardial lipids • transgenic mice • fatty acids

Introduction

The B apolipoproteins, apo B48 and apo B100, have central roles in the assembly of triglyceride-rich lipoproteins and in the regulation of lipid metabolism plasma (1). The intestine produces apo B48, which is essential for the assembly of chylomicrons. The larger apo B protein, apo B100, is synthesized in the liver, where it is essential for the assembly of VLDL. Apo B–containing lipoproteins are also synthesized in the visceral endoderm cells of the yolk sac during embryonic development (2). In the mouse, lipoprotein synthesis by the yolk sac appears to be important for the delivery of lipid nutrients to the developing embryo and for the proper development of the central nervous system (3).

In recent years, our laboratory (4) and others (5) have used a variety of large genomic clones from P1 bacteriophages and bacterial artificial chromosomes (BAC)[1] to generate many lines of human apo B transgenic mice. In characterizing those transgenic animals, we observed that the human apo B transgene was expressed at high levels in the liver. Unexpectedly, the transgene was also expressed in the heart. The level of human apo B gene expression in the heart was less than in the liver but nevertheless could be detected easily on an RNA slot blot after a short exposure (4). Quantitative analysis of RNase protection studies has shown that the levels of the human apo B mRNA in the heart of various lines of human apo B transgenic mice range from 1 to 8% of the levels in the liver.[2] In situ hybridization studies revealed that the human apo B transgene was expressed in cardiac myocytes.[2] We considered the possibility that the human apo B expression in the hearts of transgenic mice was an artifact resulting from the expression of a foreign fragment of DNA that had been incorporated into a random site within the chromosomal DNA. However, additional experiments demonstrated that the endogenous mouse apo B gene was also expressed in the hearts of nontransgenic mice, and the human apo B gene was expressed in samples of human heart.[2]

Apo B's principal function is to serve as a structural protein for the assembly of lipoproteins (1). Thus, finding apo B gene expression in the heart suggested an intriguing and provocative hypothesis: that the heart is a lipoprotein-secreting organ. This hypothesis was exciting because it suggested an entirely new way in which the heart might be involved in lipoprotein metabolism. The heart has long been recognized to be one of the principal sites for lipoprotein lipase (LPL) gene expression (6, 7), and the LPL-mediated hydrolysis of triglycerides in chylomicrons and VLDL is known to provide fatty acids for mitochondrial β-oxidation in the heart (8, 9). More recently, it has been shown that the VLDL receptor is expressed within the capillary endothelium of the heart, where it might be active in taking up apo E–containing lipoproteins (10). However, to our

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1. *Abbreviations used in this paper:* BAC, bacterial artificial chromosomes; LPL, lipoprotein lipase; MTP, microsomal triglyceride transfer protein.

2. Nielsen, L.B., M. Véniant, J. Borén, M. Raabe, J.S. Wong, C. Tam, L. Flynn, T. Vanni-Reyes, M.D. Gunn, I. Goldberg, et al., manuscript submitted for publication.

knowledge, no one has even considered the possibility that the heart might synthesize and secrete apo B–containing lipoproteins.

In this study, we tested the hypothesis that the heart is a lipoprotein-secreting organ. Here we describe the results of metabolic labeling studies of fresh human heart tissue and heart tissue from a variety of human apo B transgenic mice generated with large fragments of human genomic DNA.

Methods

Human apo B transgenic mice. Three different lines of human apo B transgenic mice were used in these studies. One line was generated with a P1 bacteriophage clone (p158) that spanned the human apo B gene and contained 19 kb of 5′ flanking sequences and 17.5 kb of 3′ flanking sequences (4). A second line expressing human apo B80 was generated with a mutant p158 construct containing a stop codon at the apo B100 codon 3620 (11). A third line was generated with a 145-kb BAC clone that spanned the human apo B gene and contained 70 kb of 5′ flanking sequences and 22 kb of 3′ flanking sequences (12). In the transgenic line generated with the 145-kb BAC, the human apo B transgene was expressed at high levels in both the intestine and liver. In the lines generated with p158, the human apo B transgene was expressed at high levels in the liver, but expression was absent in the intestine. (The sequences controlling expression of the apo B gene in the intestine are located between −33 and −70 kb 5′ to the apo B gene and are not contained within p158 [12].) The genetic background of each of the human apo B transgenic lines was mixed (∼ 50% C57BL/6 and ∼ 50% SJL). Transgenic mice were identified at the time of weaning (21 d) by screening mouse plasma with a human apo B100–specific radioimmunoassay (4). The metabolic labeling studies were performed on the hearts of 3-mo-old nontransgenic or hemizygous transgenic mice. Mice were housed in a pathogen-free barrier facility operating on a 12-h light/12-h dark cycle and were fed rodent chow containing 4.5% fat (Ralston Purina Co., St. Louis, MO).

Human heart biopsies. Specimens of human left ventricle (∼ 0.5 cm^3) were obtained from the explanted hearts of patients with end-stage cardiomyopathies undergoing cardiac transplantation procedures. The protocol for these studies was approved by the Institutional Review Board of California Pacific Medical Center. The biopsies were performed before cross-clamping of the aorta.

Metabolic labeling of human and mouse hearts. Human apo B transgenic mice (and nontransgenic control mice) were killed by cervical dislocation. The hearts were dissected free from the thoracic cavity, opened longitudinally, and flushed with ice-cold incubation medium (methionine- and cysteine-free DME [D-0422; Sigma Chemical Co., St. Louis, MO] supplemented with 7% FCS, 1.6 mM glutamate, and 1.6 mM sodium pyruvate). For the metabolic labeling experiments, one mouse heart (∼ 0.3 g) and the human heart tissue (∼ 0.3 g) were minced with a razor blade into pieces (∼ 0.5 mm^3). The tissue was placed in a 1.6-ml Eppendorf tube and washed twice with 1.0 ml of the incubation medium. A total of 1.0 ml of labeling medium (incubation medium containing 1 mCi [^{35}S]Promix [Amersham Corp., Arlington Heights, IL]) was then added to the tube. The tube was placed in a 37°C heating block and mixed gently every 30 min. After a total incubation time of 2.5 h, the heart tissue was pelleted by centrifugation (12,000 *g* for 1 min), and the medium was collected. The metabolic labeling experiments on mouse heart tissues started immediately after dissection of the heart tissue from the thoracic cavity; for logistic reasons, the metabolic labeling of human heart tissue was delayed for ∼ 30 min after the biopsy.

Analysis of labeled lipoproteins in the incubation medium. The [^{35}S]Promix-labeling medium was subjected to discontinuous sucrose gradient ultracentrifugation as described previously (13), except that gradients were formed in 5.0-ml polyallomer tubes (model 326819; Beckman, Palo Alto, CA) rather than 12-ml tubes. The gradients were subjected to ultracentrifugation in a rotor (model SW55; Beckman) at 35,000 rpm for 65 h at 12°C and then unloaded into 6–7 fractions from the bottom of the tube with tube piercer (Isco 184; Brandel, Gaithersburg, MD). Apo B was immunoprecipitated from each of the fractions with rabbit antiserum specific for human, mouse, or rat apo B (13), as described previously (14). The immunoprecipitated proteins were size-fractionated on 3–15% polyacrylamide gels containing 0.1% SDS. The gels were then dried and autoradiographs were obtained.

Inhibition of lipase activity. The heart expresses LPL (6, 7), which conceivably could modify the size and density of lipoproteins secreted by the heart. Therefore, metabolic labeling experiments were performed in [^{35}S]Promix-labeling medium containing tetrahydrolipstatin, a drug that inhibits LDL activity (15). Tetrahydrolipstatin was generously provided by Dr. Gunilla Olivecrona (Department of Medical Biochemistry and Biophysics, University of Umeå, Sweden). Human or mouse heart tissue was prepared as described above and divided into two aliquots. One aliquot was incubated with the [^{35}S]Promix-labeling medium and tetrahydrolipstatin (final concentration 10 μM or 0.05 μg/ml). The other aliquot (control) was incubated with the [^{35}S]Promix-labeling medium alone. Tetrahydrolipstatin was prepared at a concentration of 10 mg/ml in DMSO and was added to the medium 10 min before the addition of [^{35}S]Promix. Fresh tetrahydrolipstatin was added to the medium every 60 min. The control aliquot was supplemented with identical amounts of DMSO.

Control experiments were performed to determine if tetrahydrolipstatin was active under the conditions of our metabolic labeling experiment. We reasoned that the density of the triglyceride-rich lipoproteins secreted by McA-RH7777 rat hepatoma cells might change when the cells were incubated with fresh mouse heart tissue for 2.5 h at 37°C (as a result of heart-derived LPL). We suspected that the lipoproteins would be more buoyant in the presence of tetrahydrolipstatin than in its absence. To test this possibility, McA-RH7777 cells cultured as described previously (13) were metabolically labeled by incubating one 28-cm^2 petri dish of cells for 1.0 h with 4.0 ml of the same medium used to label the heart tissues, except that 100 μCi of [^{35}S]Promix was used and the medium was supplemented with 360 μM oleic acid. The medium from the McA-RH7777 cells was then incubated with mouse heart tissue for 2.5 h in the presence or absence of tetrahydrolipstatin. The [^{35}S]-labeled lipoproteins were then analyzed by sucrose density gradient ultracentrifugation, immunoprecipitation, SDS-PAGE, and autoradiography, as described previously.

Microsomal triglyceride transfer protein (MTP) Western blots. To determine if MTP protein is present in heart tissue, hearts from five nontransgenic mice were dissected, washed with ice-cold homogenization buffer (250 mM sucrose, 10 mM Hepes, pH 7.4), and minced into pieces ∼ 0.5 mm^3 with a razor blade. The pieces were homogenized with a 5-ml Dounce homogenizer in ice-cold homogenization buffer (3 ml/g tissue). The homogenate was centrifuged at 1,000 *g* for 10 min at 4°C. The supernatant fluid was removed and centrifuged again at 15,000 *g* for 20 min. The postmitochondrial supernatant fluid was removed and centrifuged at 105,000 *g* for 1 h. The pellet, representing the microsomal vesicles, was resuspended in fresh homogenization buffer (final protein concentration, 30 μg/ml). The luminal contents of the vesicles were separated from the membranes by sodium carbonate as described by Borén et al. (13) and were precipitated with 30% trichloroacetic acid. The luminal proteins were size-fractionated on a 10% polyacrylamide/SDS gel (150 μg of protein per lane); the separated proteins were then electrophoretically transferred to a nitrocellulose membrane. The Western blot was probed with a rabbit antiserum against bovine MTP (a gift of Dr. David Gordon, Bristol-Myers Squibb, Princeton, NJ). Preimmune serum from the same rabbit was used as control. The sera (1:750 dilution) were incubated with the nitrocellulose membranes in SuperBlock blocking buffer (model 37517; Pierce Chemical Co., Rockford, IL) supplemented with 5% dried milk at 4°C for 8 h. After the primary antibody incubation, the blots were washed with SuperBlock blocking buffer, incubated for 1 h with horseradish peroxidase–conju-

gated goat anti–rabbit IgG (Amersham Corp.), developed with the ECL chemiluminescence system (Amersham Corp.), and exposed to x-ray film.

Inactivation of MTP with a photoactivatible inhibitor drug. To examine the importance of MTP for the secretion of apo B–containing lipoproteins from the heart, the MTP in the cardiac tissue was inactivated before metabolic labeling experiments by preincubating the heart tissue with an MTP inhibitor, BMS-192951 (provided by Dr. David Gordon, Bristol-Myers Squibb). Hearts from two p158-human apo B100 transgenic mice were washed in labeling medium and cut into small pieces (~ 0.5 mm^3). Heart tissue from two mice was pooled and then divided into two equal aliquots. One aliquot was incubated with incubation medium containing the MTP inhibitor BMS-192951 (final concentration, 10 μM) and 0.5% DMSO. The other aliquot was incubated with incubation medium lacking BMS-192951. After BMS-192951 was added to the incubation medium, all subsequent manipulations were performed in the dark because BMS-192951 is a photoactivatible inhibitor that can be covalently cross-linked to the active site of MTP by ultraviolet light. After incubating in the dark for 1 h at 37°C, each aliquot of heart tissue was exposed to ultraviolet light (365 nm) for 15 min at 4°C, washed twice with incubation medium, and metabolically labeled in [^{35}S]Promix-labeling medium for 2.5 h at 37°C. The labeled lipoproteins were then analyzed by sucrose density gradient ultracentrifugation, SDS-PAGE, and autoradiography, as described previously.

Results

During the characterization of human apo B transgenic mouse lines generated with a variety of large genomic clones (4), we noted an intriguing and unexpected finding: the human apo B transgene was invariably expressed in the hearts of transgenic mice.[2] In this study, we began by testing whether the hearts of human apo B transgenic mice secrete apo B–containing lipoproteins. Hearts from three different lines of human apo B transgenic mice were metabolically labeled with [^{35}S]methionine/cysteine, and the media were fractionated by sucrose density gradient ultracentrifugation. Apo B was immunoprecipitated from each density fraction and examined on SDS-polyacrylamide gels. Notably, hearts from all three lines of human apo B transgenic mice secreted apo B–containing lipoproteins (Fig. 1). No apo B48 was produced, a finding that is consistent with the fact that human apo B mRNA in the hearts of transgenic mice is not edited (16). Most of the metabolically labeled lipoproteins were in the LDL density fraction (d = 1.05 g/ml).

Human hearts and nontransgenic mouse hearts synthesize and secrete apo B–containing lipoproteins. The finding that human apo B–containing lipoproteins were secreted by the human apo B transgenic mouse hearts was intriguing. To test whether this was an artifact resulting from the expression of the human apo B transgene in the mouse, we performed [^{35}S]methionine/cysteine metabolic labeling experiments on human hearts and on the hearts of nontransgenic mice. In both cases, we documented the synthesis and secretion of apo B100–containing lipoproteins (Fig. 2). Once again, the labeled lipoproteins in the media were predominantly LDL. The human hearts appeared to secrete more apo B–containing lipoproteins than the transgenic or nontransgenic mouse hearts. The autoradiographs from human hearts (Fig. 2) were exposed for

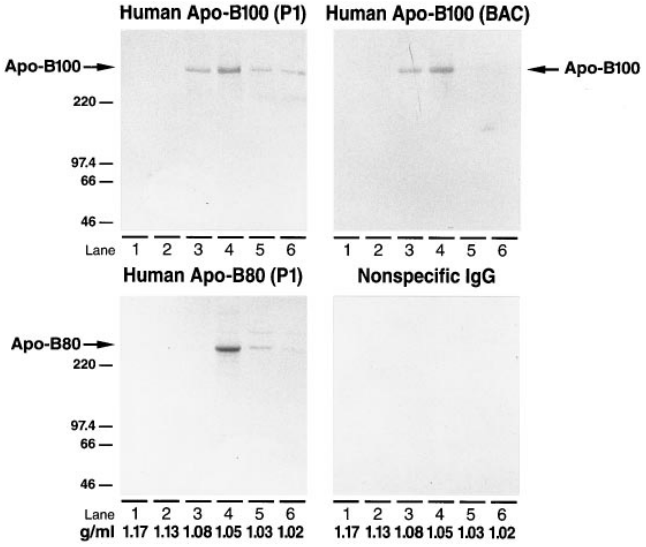

Figure 1. Metabolic labeling of hearts from human apo B transgenic mice. Heart tissue from human apo B transgenic mice was metabolically labeled with [^{35}S]methionine/cysteine, and the media were subjected to sucrose density gradient ultracentrifugation. Apo B was recovered from each fraction by immunoprecipitation and analyzed by SDS-PAGE and autoradiography. Two of the transgenic lines expressed human apo B100 and were generated with either a P1 clone (p158) or a BAC clone spanning the human apo B gene. Another transgenic line (generated with a mutant p158 clone) expressed human apo B80. Also shown is a control experiment in which nonspecific IgG was used to immunoprecipitate apo B100 from the heart of a p158-human apo B transgenic mouse. Molecular weights are indicated on the left.

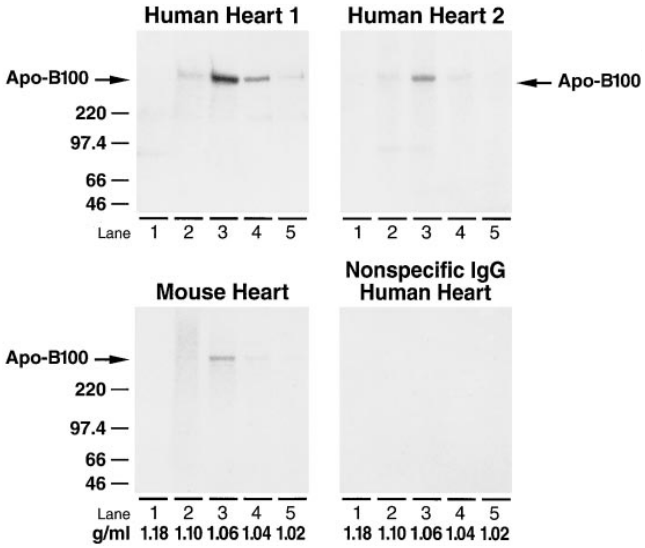

Figure 2. Metabolic labeling of human hearts and a nontransgenic mouse heart. Biopsies of two human hearts and heart tissue from a nontransgenic mouse were metabolically labeled with [^{35}S]methionine/cysteine. The media were then fractionated by sucrose density gradient ultracentrifugation. Apo B was recovered from each fraction by immunoprecipitation with polyclonal antisera against human or mouse apo B and analyzed by SDS-PAGE and autoradiography. Also shown is a control experiment in which nonspecific IgG was used for immunoprecipitating lipoproteins from human heart 1. Molecular weights are indicated on the left.

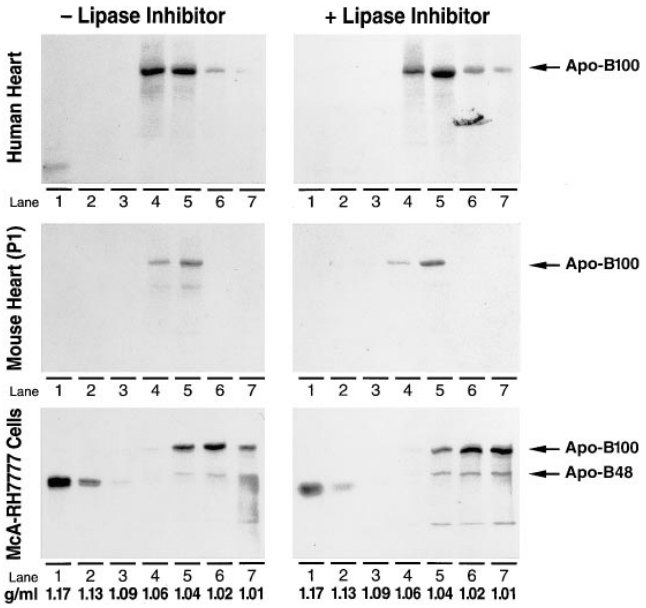

Figure 3. Density gradient ultracentrifugation of apo B–containing lipoproteins secreted in the presence or absence of a lipase inhibitor. Heart tissue from a third patient and from a human apo B100 transgenic mouse generated with p158 were metabolically labeled with [^{35}S]methionine/cysteine in the presence or absence of tetrahydrolipstatin, a lipase inhibitor. Medium from metabolically labeled McA-RH7777 cells was also incubated with mouse heart tissue in the presence or absence of tetrahydrolipstatin. The media from all incubations were subjected to sucrose density gradient ultracentrifugation, immunoprecipitation, SDS-PAGE, and autoradiography.

2 d, and the autoradiographs from transgenic and nontransgenic mouse hearts were exposed for 4 and 10 d, respectively.

Analysis of the density of lipoproteins secreted by the hearts of human apo B transgenic mice. Most of the apo B100–containing lipoproteins secreted by mouse primary hepatocytes are relatively buoyant particles (VLDL and intermediate density lipoproteins, $d < 1.019$ g/ml) (data not shown). In contrast, hearts of apo B transgenic mice secreted mainly LDL, at least under the experimental conditions that we used. Because heart tissue expresses high levels of LPL, we considered the possibility that the hearts actually secreted VLDL that were processed to LDL by the action of LPL. To examine this possibility, human heart tissue and human apo B transgenic mouse heart tissue were metabolically labeled in the presence and absence of tetrahydrolipstatin, a lipase inhibitor. These experiments revealed that the heart tissue from humans and the human apo B transgenic mice secreted largely LDL and that lipase inhibition had no detectable effect on lipoprotein density (Fig. 3). To examine whether the lipase inhibitor was truly capable of blocking lipase activity under the experimental conditions we used, McA-RH7777 (rat hepatoma) cells were incubated with [^{35}S]Promix-labeling medium supplemented with 360 μM oleic acid for 1 h. Under these conditions, McA-RH7777 cells secrete both apo B48- and apo B100–containing lipoproteins with intermediate density lipoprotein–VLDL density (13). After metabolic labeling for 1 h, the McA-RH7777 medium was incubated with heart tissue from the human apo B transgenic mice in the presence or absence of tetrahydrolipstatin. In the presence of tetrahydrolipstatin, the majority of the labeled lipoproteins were found in the most buoyant fractions of the sucrose gradient. In the absence of tetrahydrolipstatin, the lipoproteins were denser, reflecting lipase-mediated hydrolysis of core lipids. Thus, tetrahydrolipstatin was active under the experimental conditions that we used. However, only a slight difference could be detected in the absence of LPL inhibitor. Based on these experiments, we could conclude that the relatively high density of heart lipoproteins did not result from lipase activity in the medium.

MTP is expressed in the mouse heart. In the liver and intestine, lipoprotein assembly and secretion depend upon the expression of a luminal microsomal protein, MTP (17, 18). In the human disease abetalipoproteinemia, MTP is absent, leading to the virtual absence of apo B–containing lipoproteins in the plasma (18). To assess whether MTP protein is present within the microsomes of the heart, we isolated the luminal contents of microsomes from five mouse hearts and analyzed them by Western analysis with an MTP-specific antiserum. A single band with a molecular mass of ~ 97 kD (the expected molecular mass of MTP) was detected; this band was not observed in a control blot performed with a preimmune serum (Fig. 4).

Inhibition of MTP blocks the secretion of apo B–containing lipoproteins. Even though MTP is essential for efficient secretion of lipoproteins from the liver and intestine, its role in lipoprotein secretion by other tissues is not well established. For example, Herscovitz et al. (19) found that human apo B–containing lipoproteins were secreted from mouse mammary tumor cell lines transfected with an apo B cDNA construct, even though MTP activity and MTP mRNA were absent. Therefore, we sought to determine whether the secretion of apo B–containing lipoproteins by the heart was dependent on MTP activity. For these studies, heart tissue from human apo B transgenic mice was metabolically labeled with [^{35}S]methionine/cysteine in the presence or absence of 10-μM BMS-192951, an MTP inhibitor. The conditions used to inhibit MTP were identical to those used previously to inactivate MTP in hepatoma cell cultures (20). After metabolic labeling, the medium was analyzed by sucrose density gradient ultracentrifugation, immunoprecipitation, SDS-PAGE, and autoradiography. These experiments revealed that MTP inhibition completely abolished the secretion of apo B100–containing lipoproteins from the heart (Fig. 5). In the presence of the MTP inhibitor, two smaller apo B proteins with estimated molecular masses of 80 and 120 kD were detected in the bottom fraction of the gradient ($d = 1.17$ g/ml). These apo B fragments were never observed in the absence of the MTP inhibitor.

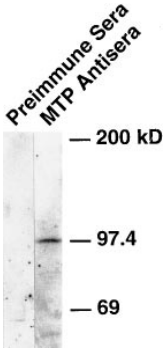

Figure 4. Western blot analysis of heart tissue with an MTP-specific antiserum. The luminal contents of microsomes from mouse hearts were size-fractionated on a 3–15% polyacrylamide-SDS gel. The separated proteins were then electrophoretically transferred to a nitrocellulose membrane for Western blotting with an MTP-specific rabbit antiserum. As a control, we used a preimmune serum from the same rabbit.

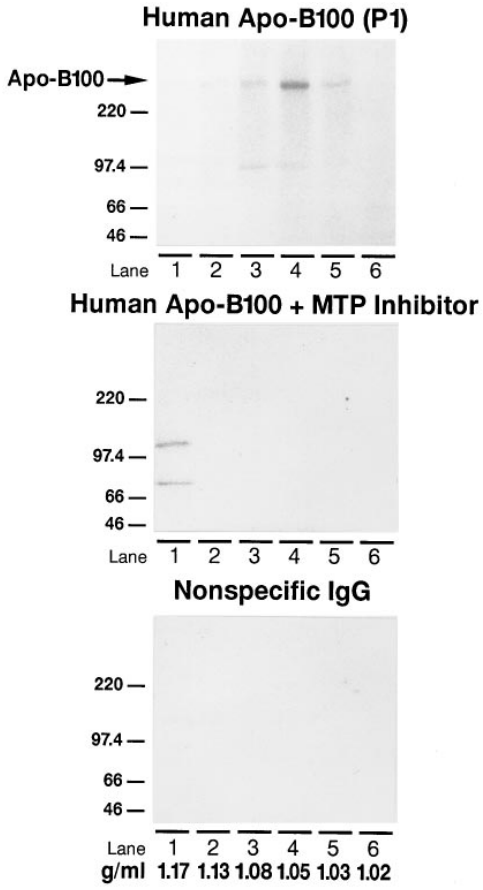

Figure 5. Inhibition of heart lipoprotein secretion with BMS-192951, an MTP inhibitor. Heart tissue from the p158-human apo B100 transgenic mice was metabolically labeled in the presence or absence of BMS-192951. After exposure to ultraviolet light to cross-link the inhibitor drug to MTP, the heart tissues were labeled with [^{35}S]methionine/cysteine. After metabolic labeling, the apo B–containing lipoproteins secreted from the heart were analyzed by sucrose density gradient ultracentrifugation, immunoprecipitation, SDS-PAGE, and autoradiography. Also shown is a control experiment in which the medium from a human apo B100 transgenic mouse heart + MTP inhibitor drug was immunoprecipitated with a nonspecific IgG.

Discussion

In this study, we report a novel and provocative finding: the heart assembles and secretes apo B–containing lipoproteins. Human apo B–containing lipoproteins were secreted by both human hearts and the hearts of human apo B transgenic mice generated with large genomic clones spanning the human apo B gene. Apo B–containing lipoproteins were also secreted by the hearts of nontransgenic mice, indicating that lipoprotein assembly and secretion is not a peculiarity of the human apo B gene.

The secretion of apo B–containing lipoproteins by the heart was documented in [^{35}S]methionine/cysteine metabolic labeling experiments on minced heart tissue. The advantage of a metabolic labeling approach is that it provides definitive data regarding whether the heart truly synthesizes apo B. Potentially, one might consider examining heart apo B expression by performing Western blots on extracts of mouse hearts that had been extensively perfused with PBS. However, we avoided that approach because it would have been virtually impossible to be sure whether the apo B on a Western blot was synthesized locally or represented hepatogenous lipoproteins that were merely trapped within the extracellular spaces of the heart. Another advantage of metabolic labeling is that it enabled us to assess the density distribution of the secreted lipoproteins. The minced heart tissue clearly secreted mainly LDL, even when the LPL activity was blocked by a specific inhibitor. However, even though the results of the density gradient experiments were consistent and unequivocal, we believe that these studies should be interpreted with caution. It might be a mistake to jump to the conclusion that a perfused, beating heart within an intact animal would secrete only LDL. For example, a greater availability of triglycerides in the beating heart of an intact animal might make it possible for the heart to secrete more buoyant lipoproteins.[3]

In the liver and intestine, MTP is essential for the assembly and secretion of apo B–containing lipoproteins. In the current experiments, we demonstrated that the MTP protein is present in cardiac tissue. In addition, our metabolic labeling experiments in the presence of an MTP inhibitor yielded two important findings. First, the secretion of lipoproteins by the heart is critically dependent on MTP, as is the case in the intestine and liver. Second, inhibition of MTP activity leads to the secretion of $\sim$ 80- and $\sim$ 120-kD apo B fragments, which are found in the bottom fraction of the density gradient. These fragments are very similar to the amino-terminal apo B proteolytic fragments in the plasma of humans with MTP deficiency (21). It has been hypothesized that these proteolytic fragments are cleaved from the apo B molecule as a direct consequence of impaired lipid delivery to apo B while it is being translated on the ribosome (17, 21).

The conservation of apo B secretion in the hearts of both humans and mice suggests that heart lipoprotein secretion might be important for heart physiology. But what is the precise role for lipoprotein secretion by the heart? Although we do not have a firm answer to that question, we are attracted to a relatively simple hypothesis: lipoprotein secretion by the heart is a mechanism for unloading excess cellular lipids back into the bloodstream. This hypothesis is attractive considering the overall function of apo B and the role of lipids in myocardial energy metabolism. Apo B serves as a structural protein in the assembly of triglyceride-rich lipoproteins. The heart is one of the principal sites for the uptake of albumin-bound fatty acids and for LPL-mediated hydrolysis of triglyceride-rich lipoproteins. Fatty acids taken up by the heart undergo β-oxidation in the mitochondria, a process that provides most of the energy for adult hearts (9). Although the activity of LPL and the concentration of fatty acids in the blood are tightly regulated on multiple levels (22, 23), it is easy to imagine that

3. The finding that the heart, under the experimental conditions used, secretes apo B–containing lipoproteins with LDL density could be analogous to how hepatoma cells behave when cultured in minimal medium. These cells normally secrete triglyceride-rich apo B–containing lipoproteins with LDL and not VLDL density. However, they can be induced to produce VLDL particles by supplementing oleic acid to the medium providing increased substrate for triglyceride synthesis (13). Thus, hepatoma cells maintain the ability to assemble VLDL even if they normally secrete LDL (13).

changing metabolic conditions might occasionally lead to an accumulation of surplus fatty acids. High intracellular levels of fatty acids (and acylcarnitines) within myocytes would be undesirable because both are thought to be toxic (9). Myocytes have the biochemical machinery to convert fatty acids to triglycerides, but their capacity for triglyceride storage is limited (24). Thus, the ability to secrete lipoproteins might provide a useful mechanism for unloading potentially toxic fatty acids and for limiting local storage of triglycerides. It is also possible that lipoprotein secretion could play a role in reverse cholesterol transport by exporting excess phospholipids and cholesterol.

Aside from defining the physiologic role for lipoprotein secretion in the heart, future experiments will need to address lipoprotein secretion in different portions of the heart and lipoprotein secretion under different metabolic conditions and in health and disease. In situ hybridization studies of human apo B transgenic mouse hearts suggested that myocytes express the human apo B gene at higher levels in the atrium than in the ventricle.[2] This possibility needs to be investigated. Heart lipoprotein synthesis during fasting and fed states needs to be examined, as does lipoprotein secretion in various forms of heart disease. Failing hearts (such as the human hearts that we studied) are defective in using fatty acids for fuel and rely to a large extent on glycolysis (8, 9). It would be interesting to determine whether the defect in the ability to use lipids for fuel might cause the failing heart to secrete more numerous (or more buoyant) lipoproteins than normal heart tissue. In the future, it will be possible to examine the overall function of apo B in the heart as well as these other issues by making use of the human apo B transgenic mice and the metabolic labeling techniques described in this paper.

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