Degradation of Apolipoprotein B in Cultured Rat Hepatocytes Occurs in a Post-endoplasmic Reticulum Compartment*

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The site of apolipoprotein B (apoB) degradation was investigated in cultured rat hepatocytes. Brefeldin A plus nocodazole completely blocked apoB degradation suggesting the involvement of a post-endoplasmic reticulum (ER) compartment. Monensin inhibited apoB degradation by 40% implying that a post-Golgi compartment could be involved in degradation of apoB. Ammonium chloride or chloroquine inhibited partially the degradation of apoB100 and apoB48, indicating some degradation in lysosomes, or in an acidic compartment such as trans-Golgi or endosomes. The degradations of apoB100 and apoB48 were blocked completely by (25,35)-trans-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester (EST) during a chase of 90 min demonstrating that a cysteine protease was responsible for apoB degradation. Chymostatin, leupeptin, pepstatin, phenylmethylsulfonyl fluoride, and aprotinin had no significant effect on the degradation of apoB48. However, leupeptin and pepstatin decreased the degradation of apoB100 by 20–30%. Degradation of apoB100 and apoB48 occurred in isolated Golgi fractions with little degradation in heavy or light ER. Degradation of apoB in Golgi fractions was inhibited by EST and by preincubating hepatocytes with 10 μM dexamethasone. Immunofluorescent microscopy revealed that apoB accumulated in the Golgi region after EST treatment. It is concluded that a major part of apoB degradation in rat hepatocytes occurs in a post-ER compartment via the action of a cysteine protease that is regulated by glucocorticoids.

Apolipoprotein B (apoB) plays a central role in the assembly, secretion, and metabolism of triacylglycerol-rich lipoproteins (chylomicrons and VLDL) and LDL (1). There are two forms of apoB in mammals: the larger molecular weight form, apoB100, consists of 4536 amino acids, whereas the smaller form, apoB48, is the amino-terminal 48% of apoB100. Both apoB100 and apoB48 are products of the same gene. ApoB48 mRNA is produced from apoB100 mRNA mainly in the intestine by RNA editing which involves a cytidine deaminase (2–5). Although most mammalian livers produce only apoB100, rat liver synthesizes both apoB100 and apoB48. ApoB is synthesized on polyribosomes bound to the cytoplasmic surface of the ER and then translocates into ER lumen. ApoB translocation has been suggested to involve specific multiple pause-transfer sequences that temporarily arrest the translocation process (6–8). Changes in the lipid composition in microsomal membranes also diminish apoB translocation across ER membranes (9). Several studies suggested that association of apoB with the full complement of lipids occurs in ER (10–13). Some experiments, however, indicated that the majority of triacylglycerols and phospholipids are assembled into VLDL particles in the Golgi (14–16).

Pulse-chase studies suggest that a significant proportion of the apoB synthesized de novo in rat hepatocytes is degraded intracellularly (17–19). Intracellular degradation of apoB has also been observed in HepG2 cells (20–22), and the degradation may be important in the regulation of apoB secretion. Treatment of HepG2 cells with oleate increases apoB secretion by decreasing apoB degradation (22, 23), whereas n-3 fatty acids have the opposite effects (24). In primary rat hepatocytes, insulin decreases the secretion of apoB and stimulates its degradation (19). Conversely, glucocorticoids (dexamethasone) stimulate the secretion (25) and decrease apoB degradation (26). Some studies demonstrated that intracellular degradation of apoB in HepG2 cells occurred in a pre-Golgi compartment (20, 22, 27). However, considerable differences exist in the control of apoB metabolism and secretion in HepG2 cells and primary hepatocytes. Despite evidence implicating the existence of intracellular apoB degradation in rat hepatocytes (17–19, 24, 26), little is known about the pathway responsible for apoB degradation.

In the present study, we investigated the site(s) of degradation of nascent apoB in cultured rat hepatocytes and have characterized the nature of protease for apoB degradation. We used brefeldin A and monensin, inhibitors of protein transport in the secretory pathway, and nocodazole, which inhibits retrograde transport from Golgi to ER in order to identify the location of nascent apoB degradation in whole cells. A cell-free assay system was developed to assess the degradation of labeled apoB in isolated subcellular fractions. The results demonstrate that a major portion of the intracellular degradation of newly synthesized apoB occurs in a post-ER compartment, possibly the Golgi apparatus. A cysteine protease is responsible for this apoB degradation, and this protease activity is decreased by pretreating hepatocytes with dexamethasone.

EXPERIMENTAL PROCEDURES

Materials—Sheep polyclonal antibodies for human apoB, fluorescein-conjugated sheep anti-rabbit IgG antibodies, UDP-galactose, trypsin, trypsin inhibitor, chymostatin, pepstatin, and leupeptin were from Boehringer Mannheim. Texas Red-conjugated goat anti-mouse IgG antibodies were from Jackson Immunoresearch. Brefeldin A, nocodazole, monensin, EST, phenylmethylsulfonyl fluoride, aprotinin, mannose...
6-phosphate, p-nitrophenyl phosphate, puromycin, and fatty acid-poor BSA were from Sigma. All reagents for electrophoresis were from Bio-Rad Laboratories. Polyvinylidene difluoride membranes were from Millipore. Leibovitz L-15 medium, methionine-cysteine-free Dulbecco’s modified Eagle’s medium, and fetal bovine serum were from Life Technologies, Inc. UDP-[3H]galactose was purchased from DuPont NEN. [35S]Methionine was obtained from Amersham, and conjugated anti-rabbit IgG-horseradish peroxidase for immunoblotting were obtained from Amersham. Rabbit polyclonal antibodies against rat albumin for immunoprecipitation were purchased from Organon Teknika Inc. Rabbit polyclonal antibodies directed against rat apoB were provided by Dr. G. F. Gibbons (University of Oxford, United Kingdom). These anti-apoB antibodies were immunopurified for additional methods. Enzyme-linked immunosorbent assay detection reagents, and conjugated anti-rabbit IgG-horseradish peroxidase for immunoblotting were obtained from Amersham. Rabbit polyclonal antibodies against rat albumin for immunoprecipitation were purchased from Organon Teknika Inc. Rabbit polyclonal antibodies directed against rat apoB were provided by Dr. G. F. Gibbons (University of Oxford, United Kingdom). These anti-apoB antibodies were immunopurified for the Western blot analysis. Indirect Immunofluorescent Microscopy—Hepatocytes were plated on polylysine-coated coverslips and cultured overnight in Leibovitz medium containing 0.2% (w/v) fatty acid-poor BSA. Cells were treated with 100 μM puromycin in the presence or absence of 40 μg/ml EST for 2 h. Hepatocytes were washed twice with PBS and then fixed with methanol at −20°C for 10 min followed by acetone at −20°C for 5 min. Coverslips were washed with PBS to remove residual methanol or acetone, air-dried, and ethanol-precipitated cells were resuspended in 0.375 M sucrose. Half-volumes of 0.4 M sodium phosphate buffer (pH 6.5) containing 8 mM dithiothreitol were added to the fractions which were then incubated at 40°C for 3 h (36). The control fraction was incubated at 0°C for 3 h. In the case of EST, fractions were preincubated on ice for 30 min with this inhibitor before transferring to 40°C and incubating for another 3 h. The radioactivities associated with apoB and albumin were immunoprecipitated and separated by SDS-polyacrylamide gel electrophoresis. The percentage degradation of apoB at 40°C relative to that at 0°C was determined by scanning densitometry.

Results

Protein Transport from Endoplasmic Reticulum to Golgi Is Necessary for ApoB Degradation in Rat Hepatocytes—Rat hepatocytes were pulse-labeled with [35S]methionine for 15 min and then chased for 2 h in the presence or absence of protein transport inhibitors. The maximum incorporation of [35S]methionine into apoB48, which occurred at 10 min into the chase period, was about twice that into apoB100 (6652 ± 734 dpm/mg of cell protein compared to 3467 ± 400 dpm/mg of cell protein) as expected from our previous work (26). The recovery of apoB100 and apoB48 from cells plus medium after a chase of 2 h was quantitated by measuring mannose-6-phosphate phosphohydrolase activity (42). Degradation of apoB was measured on ice for 30 min with this inhibitor before acid-soluble radioactivity from radiolabeled cells and medium (43). Results are expressed as means ± S.D. for the number of independent experiments indicated.

2 T. C. Hobman and L. C. Hendricks, unpublished results.
Degradation of ApoB in Rat Hepatocytes—Ammonium chloride and chloroquine were used to examine whether acid proteases could be involved in apoB degradation. Hepatocytes were labeled with [35S]methionine for 15 min and then chased for 2 h in medium supplemented with a lysosomotropic agent. Intracellular degradation in control cells was 49% and 30% for apoB100 and apoB48, respectively. Ammonium chloride and chloroquine decreased the degradation of labeled apoB100 by 45% and 57%, respectively. The equivalent values for apoB48 were 17% and 53%, respectively (Table I). This result indicated that a portion of newly synthesized apoB could be degraded in an acidic compartment such as lysosomes, trans-Golgi or endosomes.

Seven protease inhibitors were employed to determine the nature of the enzyme responsible for apoB degradation. Cells were pretreated with protease inhibitor (except EST) for 1 h before labeling to allow time for interaction with the cells. The percentage recovery of labeled apoB from cell and medium was measured at the 2-h chase point compared to the peak of [35S]methionine incorporation at 10 min into the chase (Table I). Leupeptin (a serine and cysteine protease inhibitor) and pepstatin (an aspartic protease inhibitor) inhibited intracellular degradation of apoB100 by about 20–30%, but has no effect on apoB48 degradation. No significant inhibition of apoB degradation was observed with chymostatin (inhibitor of chymotrypsin and papain), phenylmethylsulfonyl fluoride, and aprotinin (serine protease inhibitors). The penetrations of leupeptin, chymostatin, and pepstatin into hepatocytes were confirmed by the inhibition of total cell protein degradation which was determined by the increase of acid-soluble radioactivity from cell and medium during the 2-h chase. The percentage inhibition of total protein degradation was 34 ± 11%, 47 ± 11%, and 47 ± 8% (n = 3) with leupeptin, chymostatin, and pepstatin, respectively.

Previous studies showed that apoB degradation was blocked by ALLN (cysteine protease inhibitor) in HepG2 cells (50). In our hepatocyte system, we were not able to observe a reproducible inhibition of apoB degradation by ALLN. We used, therefore, an alternative cysteine protease inhibitor, EST, to investigate apoB degradation since this agent is more readily permeable to membranes and therefore better able to enter cell compartments. A preincubation with EST seemed not to be
required. EST inhibited the intracellular degradation of both apoB100 and apoB48 by 50% after 2 h (Table I) and blocked apoB degradation completely during the first 90 min of the chase (Fig. 2). Therefore, a cysteine protease was responsible for most of the degradation of apoB100 and apoB48 in this hepatocyte system. It is possible that the action of EST on the cysteine proteases, cathepsin L and cathepsin B, within lysosomes may account for some of the inhibition of apoB degradation. To investigate this possibility, the labeled apoB that remained in the hepatocytes or was secreted into medium was investigated. To determine this possibility, the labeled apoB that remained in the hepatocytes or was secreted into medium was measured by using NADPH:cytochrome c reductase (ER marker enzyme), nanomoles of [3H]galactose incorporated/h/mg of protein for UDP-glucose: N-acetylglucosamine galactosyltransferase (Golgi marker enzyme), and nanomoles of p-nitrophenyl phosphate dephosphorylated/min/mg of protein for acid phosphatase (lysosome marker enzyme). Results are means ± S.D. from six independent preparations of cultured rat hepatocytes.

### Table II

| Marker for | Homogenate | Golgi | ERI | ERII | ERI |
|------------|------------|------|-----|------|-----|
| ER Specific activity | 3.4 ± 0.8 | 7.1 ± 2.4 | 70.0 ± 11.7 | 73.1 ± 13.6 | 2.1 |
| ER Relative enrichment | | | | | 20.6 |
| ER Recovery (%) | 100 | 2.1 ± 0.6 | 12.2 ± 2.2 | 24.3 ± 4.4 |
| Golgi Specific activity | 6.4 ± 3.6 | 478 ± 43 | 27.3 ± 10 | 18.9 ± 8.4 |
| Golgi Relative enrichment | | 74.7 | 4.3 | 3.0 |
| Golgi Recovery (%) | 100 | 32.4 ± 3.9 | 2.6 ± 1.9 | 1.9 ± 1.4 |
| Lysosomes Specific activity | 36.2 ± 15.7 | 80 ± 16.1 | 55.3 ± 1.8 | 48.1 ± 8.3 |
| Lysosomes Relative enrichment | | 2.2 | 1.5 | 1.3 |
| Lysosomes Recovery (%) | 100 | 1.1 ± 0.8 | 0.8 ± 0.3 | 1.1 ± 0.5 |

Two pools of apoB have been reported in microsomal fractions: membrane-bound apoB and luminal apoB. The membrane-bound pool of apoB was suggested to be subjected to intracellular degradation (18). Two approaches were employed to measure the distribution of apoB between membrane and lumen in purified fractions. Subcellular fractions prepared from cultured hepatocytes were incubated with trypsin and apoB and albumin were detected by immunoblotting (Fig. 3A). Trypsin treatment decreased apoB100 in ERI and ERII by about 90% in these experiments, but about 40% of the apoB100 remained in the Golgi fraction after proteolysis. By contrast, only a small portion of apoB48 was accessible to trypsin, and, in the case of ERI and ERII, a proteolytic fragment was observed. The amount of albumin was not affected significantly in the fractions by trypsin treatment, nor was the total or the latent activity of mannose 6-phosphate phosphohydrolase. The latter result indicates that the membranes remained intact after trypsin treatment. These results indicated that a large proportion of apoB100 was at the cytosolic side of the endoplasmic reticulum, and a significant amount of apoB100 was exposed on Golgi membranes. ApoB48 was located mainly inside the ER and Golgi, and, therefore, it was not degraded by external trypsin.

The second approach was to separate the membrane and luminal contents of fractions by treatment with sodium carbonate (35), and this was reflected by albumin existing only in the luminal contents (Fig. 3B). The percentage of apoB100 in the membrane fraction was 85 ± 8.7%, 78 ± 10%, and 43 ± 6.3% for Golgi was 32%. The enrichment of the respective marker enzymes in the endoplasmic reticulum and Golgi membranes was approximately 21-fold and 75-fold, respectively, compared to homogenate. The contamination by lysosomes was determined by measuring acid phosphatase activity. More than 85% of acid phosphatase activity was recovered in particulate fractions (centrifuged at 170,000 × g for 30 min) indicating that the lysosomal vesicles were intact during subcellular fractionation. The recovery of acid phosphatase in ERI, ERII, and Golgi fraction was 1.1, 0.8, and 1.1% of the total acid phosphatase which demonstrated a very low contamination by lysosomes in these fractions.

**Fig. 2. Time course effect of EST on apoB degradation.** Hepatocytes were cultured overnight and then pulse-labeled with [35S]methionine (300 μCi/35-mm dish) for 15 min. Hepatocytes were washed with chase medium and incubated in control chase medium (C) or medium containing 40 μg/ml EST (Ç) for the time indicated. The radioactivity associated with cell and medium apoB100 (A) and apoB48 (B) was quantified as described in the legend of Fig. 1. The results are means ± S.D. (where large enough to be shown) from five independent experiments.

**Fig. 3.** Subcellular Distribution of ApoB Degradation—The results from the experiments with the protein traffic inhibitors indicated that a major site of intracellular degradation of apoB is a post-ER compartment possibly in Golgi. We therefore isolated subcellular fractions from cultured hepatocytes in order to investigate this possibility. Three fractions were purified: ERI (heavy ER), ERII (light ER), and Golgi. The purities of subcellular fractions were assessed by using NADPH-cytochrome c reductase and UDP-glucose: N-acetylglucosamine galactosyltransferase and acid phosphatase as marker for ER, Golgi, and lysosomes, respectively (Table II). The cross-contamination of Golgi by the ER marker enzyme was about 10% by calculating on the basis of specific activity in either the ERI or the ERII fraction. On the same basis, contamination of the ERI and ERII fractions by the Golgi marker enzyme was approximately 4–6%. The percentage recovery of ERI and ERII was about 12% and 24%, respectively, compared to that in homogenate. The equivalent value
contrast, most of apoB48 was present in luminal content (ERI, ERII (400 μg of protein/assay), and Golgi fraction (100 μg of protein/assay) were prepared from hepatocytes cultured for 4 h. A, the fractions were incubated with, or without, trypsin on ice for 30 min, after which trypsin inhibitor was added to a final concentration of 0.4 mg/ml. Samples were centrifuged to remove trypsin and then subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis by using rabbit antiserum against rat apoB and albumin. B, the fractions were diluted 50-fold with 100 mM sodium carbonate (pH 11.5) and incubated on ice for 30 min. Samples were centrifuged at 200,000 × g for 1 h. Pellets (lanes 1, 3, and 5) and concentrated luminal contents (lanes 2, 4, and 6) were separated by SDS-polyacrylamide gel electrophoresis and then subjected to Western blot analysis. A representative photograph is shown, and the results from three independent experiments are described in the text.

Degradation of labeled apoB in subcellular fractions, which were isolated from [35S]methionine-labeled hepatocytes, was studied to determine the intracellular sites of this process. The degradation was measured by a method that was developed for the assay of cysteine protease activities. Fractions were incubated at 40°C for 3 h with 0.13 M sodium potassium phosphate buffer (pH 6.5) containing 2.7 mM dithiothreitol. Labeled apoB was immunoprecipitated and separated by SDS-polyacrylamide gel electrophoresis, and the amount of [35S]apoB was determined by scanning densitometry (Fig. 4). The percentage distribution of apoB100 in Golgi, ERII, and ERI fraction was 75 ± 5%, 14 ± 3%, and 12 ± 3.2% (n = 4), respectively. The equivalent values for apoB48 were 30 ± 6.3%, 42 ± 7.5%, and 28 ± 7.5%. The percentage degradation of apoB was calculated as the difference in radioactivity recovered from incubations at 40°C and 0°C. The degradation of apoB100 in Golgi, ERII, and ERI was 33 ± 5.9%, 6.4 ± 4.8%, and 5.2 ± 1.7%, respectively. For apoB48, the equivalent values were 26 ± 2.8%, 6.9 ± 5.5%, and 3.5 ± 4.9%. When the percentage degradation in ER was corrected for the percentage of cross-contamination with the Golgi marker enzyme (Table II), the degradation values for apoB100 and apoB48 was decreased to approximately 3.8% and 4.9% in the ERI fraction, respectively. The equivalent values for the ERII fraction were 3.3% and 2.0%, respectively. These results showed that a large portion of labeled apoB100 was associated with Golgi fraction; however, the sequence of distribution for labeled apoB48 was ERII fraction > Golgi fraction > ERII fraction. Degradation of labeled apoB occurred to a significant extent only in the Golgi-enriched fraction with little proteolysis in the ERII or ERI fractions. This result and those with protein traffic inhibitors demonstrate that the major part of apoB degradation occurs in a post-ER compartment, which is associated with a Golgi membrane fraction.

Degradation of apoB in the Golgi Fraction Was Inhibited by EST and Regulated by Dexamethasone—The results from Table I and Fig. 2 showed that EST was an effective inhibitor of the degradation of apoB100 and apoB48 in intact hepatocytes. If the degradation of apoB that is measured in the Golgi fraction is representative of degradation in whole cells, then it should also be EST-sensitive. Golgi fractions were incubated with 40 μg/ml EST on ice for 30 min and then incubated at 40°C for 3 h. EST treatment decreased the degradation of apoB100 from 33 ± 5.6% to 5.1 ± 5.3% and decreased apoB48 degradation from 24 ± 4.1% to 11 ± 2.1% (Fig. 5). Therefore, the inhibition of apoB degradation by EST in the isolated Golgi fraction was 85% for apoB100 and 55% for apoB48. Triton X-100 (final concentration of 0.1% (w/v)) was also introduced into the cell-free system used to study apoB degradation. Triton X-100 increased the extent of apoB100 and apoB48 degradation in the Golgi fraction to 85 ± 6.6% and 38 ± 0.9% (n = 3), respectively. ApoB100 and apoB48 degradation in ERI was increased to 80 ± 7.1% and 68 ± 12% by Triton X-100 treatment. The equivalent values for ERII fractions were 78 ± 3.5% and 59 ± 5.4%, respectively. However, this Triton X-100-dependent degradation was not inhibited significantly by EST.

Our previous studies demonstrated that incubating the
apoB degradation remained to be elucidated. Treatment of cells with oleate (22, 23), subcellular fractionation (27), or permeabilized cells (52) all suggested that the intracellular degradation of nascent apoB in HepG2 cells occurs mainly in the ER. In rat hepatocytes, however, the intracellular site of apoB degradation remains to be elucidated.

The degradation of apoB is an important step in the regulation of its metabolism. Treatment of cells with oleate (22, 23, 27), n-3 fatty acids (24, 51), insulin (19), or dexamethasone (26) changes the secretion of apoB and modulates its intracellular degradation. The intracellular site of apoB degradation in HepG2 cells has been studied widely. The use of protein trafficking inhibitors (20, 27), subcellular fractionation (27), or permeabilized cells (52) all suggested that the intracellular degradation of nascent apoB in HepG2 cells occurs mainly in the ER. In rat hepatocytes, however, the intracellular site of apoB degradation remains to be elucidated.

Brefeldin A plus nocodazole completely blocked apoB degradation in the Golgi fraction. ApoB degradation in Golgi fractions prepared from hepatocytes cultured for 4 h was similar to that from hepatocytes cultured overnight. ApoB degradation in Golgi fractions prepared from hepatocytes which were pretreated for 16 h with 10 mM dexamethasone was decreased by approximately 44% and 72% for apoB100 and apoB48, respectively. Therefore, the combined results from Fig. 5 demonstrated that the degradation of apoB as measured in isolated Golgi fractions has the same characters as the intracellular degradation of apoB in cultured hepatocytes.

Accumulation of ApoB Occurs in the Golgi Region after EST Treatment—Immunofluorescent microscopy was performed to investigate further the compartment where EST exerts its effect on apoB degradation. Puromycin was used to exclude the interference of continuous synthesis of apoB. ApoB accumulated in the perinuclear region in cultures treated with puromycin and EST (Fig. 6C). This morphology resembled that of the Golgi marker, α-mannosidase II (Fig. 6D) rather than the ER marker, Bip (Fig. 6E). In addition, the size and appearance of the lysosomal vacuoles recognized by IgG120 were very different from the distribution of apoB (Fig. 6, C and F). Double staining was used to determine the region of apoB accumulation (Fig. 7). ApoB again accumulated in a perinuclear region (Fig. 7, A, B, and C) which was very similar to, although not identical with, that shown for α-mannosidase II (Fig. 7D). This distribution did not resemble that for the ER marker (Fig. 7, B and E) or the lysosomal marker, 4E4.A6 (Fig. 7, C and F).

**DISCUSSION**

The degradation of apoB is an important step in the regulation of its metabolism. Treatment of cells with oleate (22, 23, 27), n-3 fatty acids (24, 51), insulin (19), or dexamethasone (26) changes the secretion of apoB and modulates its intracellular degradation. The intracellular site of apoB degradation in HepG2 cells has been studied widely. The use of protein trafficking inhibitors (20, 27), subcellular fractionation (27), or permeabilized cells (52) all suggested that the intracellular degradation of nascent apoB in HepG2 cells occurs mainly in the ER. In rat hepatocytes, however, the intracellular site of apoB degradation remains to be elucidated.

Brefeldin A plus nocodazole completely blocked apoB degradation in the rat hepatocyte system. This suggests that apoB degradation could occur in the post-ER compartment. Treatment with monensin partially inhibits apoB degradation implying that a post-Golgi compartment, or lysosomes, could also be involved in apoB degradation. Lysosomotropic agents (ammonium chloride or chloroquine) also decrease apoB degradation, and this also suggests that lysosomes may be involved in this process. However, the vesicles and cisternae of the trans-Golgi are acidic compartments (53). Weakly basic amines may therefore disrupt the acidic environment of trans-Golgi and may block the normal functions of this compartment (48, 53, 54). Thus, it is possible that monensin, or lysosomotropic agents elevate the pH value of the trans-Golgi compartment and thereby exert their inhibitory effects on the degradation of newly synthesized apoB.
The assay of apoB degradation in isolated subcellular fractions from cultured hepatocytes demonstrates that the major degradation of labeled apoB occurs in the Golgi fraction rather than in ERI or ERII fractions. This degradation can be inhibited by the cysteine protease inhibitors, EST, and by treating hepatocytes with dexamethasone. The extent of inhibition by EST and dexamethasone on apoB degradation is similar to the results obtained from intact hepatocytes (Ref. 26, Table I, Fig. 2). This suggests strongly that the degradation of apoB in a post-ER compartment accounts for a large part of intracellular degradation in cultured rat hepatocytes. Marker enzyme determinations exclude the possibility that the contamination with lysosomes accounts for the degradation of apoB in isolated Golgi fractions. Immunohistochemical studies demonstrate that the accumulation of apoB in hepatocytes incubated with puromycin and EST occurs in the Golgi region. However, there is not an exact colocalization with α-mannosidase II which in hepatocytes is found in Golgi stacks (55). Therefore, apoB may be accumulating in specific regions of the Golgi apparatus. ApoB accumulation did not coincide with that of either of the two lysosomal markers employed (Figs. 6 and 7). We cannot exclude the possibility that some apoB is shuttled to lysosomes for degradation. However, the majority of the apoB did not accumulate in lysosomes, indicating that the latter organelles are not the major sites of apoB degradation. We also cannot exclude completely that there is also an extremely rapid (less than 15 min) pathway of degradation for apoB in the ER in cultured rat hepatocytes.

Two approaches were used to investigate the distribution of apoB between membrane and luminal content in isolated fractions: the accessibility of apoB to exogenous trypsin and the separation of membrane-bound and luminal apoB by sodium carbonate treatment. Both results indicate that a large proportion of apoB100 exists in the membrane-bound pool in ER. This result is consistent with the observations from previous studies (13, 16, 18, 56, 57). Also, a significant amount of apoB100 is membrane-bound in the Golgi fraction which agrees with the results obtained by radioimmunoassay and enzyme-linked immunosassay (16, 56, 57). In contrast, most of the apoB48 is in the luminal contents of subcellular fractions.

The difference in the distribution of apoB100 and apoB48 may indicate the different fate of apoB100 and apoB48 during the secretory process: the proportion of apoB100 in the membrane is higher as is the extent of degradation of apoB100 compared to apoB48 (17, 19, 24, 26). Also, the mean diameter of secreted apoB100-containing lipoprotein is distinct from that of apoB48-containing lipoprotein. ApoB100 is associated with VLDL, whereas apoB48 distributes in both high density lipoprotein and VLDL (58, 59). The membrane-bound pool of apoB has been suggested to be degraded intracellularly (18, 21, 60). Our results agree with this proposition. The existence of apoB in the membrane-bound pool in the Golgi fraction also implies the possibility that Golgi may play a role in the assembly of apoB-containing lipoproteins (14, 15) or in the remodeling process of VLDL particles (61, 62).

The conclusions that a post-ER compartment is involved in apoB degradation differ from those obtained with HepG2 cells. Brefeldin A was used by Sato et al. (20) who concluded that the degradation pathway for apoB100 in HepG2 cells was in a pre-Golgi compartment. Results obtained by Furukawa et al. (27) by using protein trafficking inhibitors and subcellular fractionation also indicated that the degradation of nascent apoB occurred in the ER in HepG2 cells. Degradation of apoB100 in the ER fraction could be observed by adding Triton X-100 (27). We were also able to observe degradation of apoB100 and apoB48 in the ERI and ERII fractions of rat hepatocytes when Triton X-100 was added, but this degradation was not inhibited by EST. We therefore concluded that this degradation did not have the characteristics that were seen in intact rat hepatocytes. The protease responsible for apoB degradation in HepG2 cells and in CHO cells expressing apoB was ALLN-sensitive, pH-dependent, and ATP-stimulated (50, 52, 63). This degradation of apoB resembled that of 3-hydroxy-3-methylglutaryl coenzyme A reductase (64). However, we found no ATP-stimulated hydrolysis of apoB in isolated Golgi fractions or in permeabilized hepatocytes.

Recently, Davis et al. (10) showed that the proteolytic fragments of apoB were detectable in rough and smooth ER fractions and suggested that the site of apoB degradation was ER. We also observe some small fragments of apoB in the ERI and ERII fractions (Fig. 4), but the intensity of the small fragment of apoB showed no change during the degradation assay. Moreover, the results from Verkade et al. (65) showed that a decrease in the number of VLDL particles in choline-deficient rat was observed in Golgi fraction, not in ER. This result also implies that the Golgi apparatus could be a site of apoB degradation and this is consistent with our findings. This conclusion is inconsistent with the results from HepG2 cells (20, 27, 52). It is well known that there are several differences in apoB metabolism and secretion in rat hepatocytes versus HepG2 cells. The availability of triacylglycerol for VLDL secretion in HepG2 cells is much lower than that in rat hepatocytes. The low mobilization of intracellular triacylglycerol may account for the inability of HepG2 cells to recruit sufficient triacylglycerol to assemble apoB-containing lipoproteins that are triacylglycerol-rich (66), and this could lead to degradation of apoB in the ER. This effect can be blocked by adding unesterified fatty acids to the culture medium which stimulates apoB secretion (22, 23). This level of regulation is not observed with primary cultures of hepatocytes (24). Reviews of Dixon and Ginsberg (67) and Sparks and Sparks (68) also suggest that the degradation in rat hepatocytes is different from that in HepG2 cells.

There are precedents for the degradation of proteins in a post-ER and non-lysosomal compartments. This degradation is involved in controlling the amount of the protein that is secreted or destined for the cell surface. Such regulation is observed for acetylcholinesterase in cultured muscle cells (69). In HeLa cells there is a two-phase degradation of ribonuclease: the first phase is in the ER and the second phase is in a post-ER compartment (70). Furthermore, the degradation of immunoglobulin M in B lymphocytes occurs via a cysteine protease in a post-ER compartment that is distinct from lysosomes (71, 72).

It is concluded that a major part of the regulated degradation of apoB in rat hepatocytes occurs in a post-ER compartment via the activity of a cysteine protease. This activity is decreased by long term treatment with glucocorticoids and is accompanied by increased VLDL secretion (26).

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