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NASCENT VERY LOW DENSITY LIPOPROTEINS FROM RAT HEPATOCYTIC GOLGI FRACTIONS ARE ENRICHED IN PHOSPHATIDYLETHANOLAMINE

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SUMMARY The phospholipid composition of nascent very low density lipoproteins (VLDL) of rat hepatocytic Golgi fractions differs greatly from that of plasma VLDL. The phospholipids of nascent VLDL contain about four times more phosphatidylethanolamine (PE) than plasma VLDL, whereas plasma VLDL contain considerably more sphingomyelin. Thus, the ratio of PE to sphingomyelin differs by a factor of about 12 between nascent Golgi VLDL and circulating plasma VLDL. It is evident from these data that the PE/sphingomyelin ratio of VLDL can be used to estimate endosomal contamination of hepatocytic Golgi fractions.

Human plasma contains two distinct very low density lipoprotein (VLDL) particles. One is enriched in phosphatidylethanolamine (PE), lacks apolipoprotein (apo) E, and has a lower free cholesterol/phospholipid ratio than the major species (1,2). The PE-enriched, apo E-deficient particles appear to be precursors of the major species that are PE-depleted and contain apo E. It has been shown that apo E binds to these VLDL particles following lipoprotein lipase modification (2). Based on these data, it was hypothesized that the PE-enriched apo E-deficient plasma VLDL represent nascent particles newly secreted from human hepatocytes (2).

We report here experiments to test this hypothesis by measuring the phospholipid composition of nascent VLDL recovered from a Golgi fraction from rat liver. We have shown previously that intact Golgi apparatus fractions, isolated by the procedure described by Morré and associates (3), contain significant amounts of remnant particles within multivesicular bodies (MVBs) (4). However, a simple modification of this procedure virtually

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eliminates MVB contamination providing the first opportunity to study truly nascent VLDL (see Methods).

METHODS

Intact Golgi fractions from livers of male (250-275 g) rats fed ad libitim were isolated as described (4) except that the white Golgi band from the 1.2 M sucrose interface was diluted 28-34-fold (instead of 5-10-fold) with iced homogenizing medium prior to the final pelleting spin of 5,000 rpm/20 min. This large dilution releases entrained MVBs, which do not sediment at this low centrifugal force with the intact Golgi membranes with attached "secretory vesicles" filled with nascent VLDL (3,4). This was shown in studies (Hamilton, R.L., and Havel, R.J., unpublished data) in which radiolabeled lipoproteins were injected intravenously to label MVBs 15 min before rat livers were homogenized, a time at which MVBs are maximally labeled (for review, see ref. 5). The contents of the intact Golgi fractions were released, after resuspension in water, by passing the fraction two times through a French pressure cell (iced) at 16,000 psi. D_2O (0.4 ml) was added to 4.0 ml of ruptured Golgi membranes to raise the density to ~1.010 g/ml in order to layer the sample below ice-cold 0.15 M sodium chloride (2 ml). Nascent VLDL were floated through the saline by a single 16-hr ultracentrifugation at 35,000 rpm in a 40.3 Beckman rotor. We routinely recover about 0.2 mg of nascent VLDL protein from about 80 g rat liver.

Lipids of VLDL and Golgi membranes (free of contents) were extracted, fractionated by chromatography, and quantified as phospholipid phosphorous as described (1,2).

RESULTS

Striking differences in phospholipid composition were found between the percent mass of the major phospholipid species of nascent Golgi VLDL and plasma VLDL (Table 1). The largest difference observed was that nascent Golgi VLDL contained 12.6 $\pm$ 0.9% PE, whereas plasma VLDL from the same rats contained only 3.5 $\pm$ 0.9%. This fourfold difference in PE content was mirrored by a reversed ratio of sphingomyelin between the two VLDL species. Nascent Golgi VLDL contained only 3.0 $\pm$ 1.8% sphingomyelin contrasted to plasma VLDL which had 9.8 $\pm$ 5.6%. Thus, the PE to sphingomyelin ratio of the two VLDL species differs by a factor of about 12.

Phosphatidylserine is a minor phospholipid in both plasma lipoproteins and nascent Golgi VLDL, representing only about 1% of total phospholipid (Table 1). The content of phosphatidylinositol was significantly greater in Golgi VLDL than in plasma VLDL. The lysophosphatidylcholine content of plasma VLDL was about threefold

Table 1. Phospholipid composition of nascent Golgi VLDL, plasma VLDL, and Golgi membranes

Lipoprotein	LPC	SPH	PC	PS	PI	PE
	weight %					
Golgi VLDL	2.2±1.3	3.0±1.8	72.9± 2.2	1.3±0.3	7.8±0.3	12.6±0.9
Plasma VLDL	6.7±3.2	9.8±5.6	73.0±10.3	1.0±0.9	6.1±1.1	3.5±0.9
Golgi membranes	2.9±0.8	3.6±0.9	60.1±.7.8	3.3±0.8	13.5±3.5	16.6±4.7

Values above are mean ± S.D. Plasma VLDL and Golgi membranes, n = 5; nascent Golgi VLDL, n = 4. LPC, lysophosphatidylcholine; SPH, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine. The difference in the PE content between nascent Golgi VLDL and plasma VLDL was significant ($p < 0.001$), as was the difference in SPH ($p < 0.025$), LPC ($p < 0.005$), and PI ($p < 0.001$). There was no significant difference between the PS and PC values. Significance between paired values was determined by t test, and a p value of <0.05 was considered significant.

higher than that of nascent Golgi VLDL, but was almost fivefold higher when VLDL was prepared from blood serum (9.7 vs. 2.2%), presumably due to phospholipase degradation of serum VLDL phosphatidylcholine during the time required for blood to clot at room temperature.

The high PE content of nascent Golgi VLDL was reflected by a similarly high content of PE in the phospholipids of the Golgi membranes (largely free of contents), the membrane compartment from which nascent VLDL were released (both in vitro and in vivo). Similarly, the Golgi membranes were found to be enriched in phosphatidylinositol (Table 1).

DISCUSSION

Nascent VLDL from hepatocytes are discharged by exocytosis into the space of Disse (6) where they become mixed with macromolecules of blood plasma including remnants of triglyceride-rich lipoproteins that are taken up into hepatocytes by receptor-mediated endocytosis (5). Although nascent VLDL are exposed to the same receptors that mediate remnant uptake, most newly secreted particles apparently escape these receptors and enter the circulating blood plasma where they undergo a cascade of modifica-

tions that transforms them into remnants which ultimately return to hepatocytes for receptor-mediated uptake and terminal catabolism in lysosomes (5).

The observations reported here probably reflect changes in the surface phospholipids that are associated with specific steps in the complex chain of events in the metabolism of triglyceride-rich particles. The high PE content of nascent Golgi VLDL could confer special surface properties, which allow the newly secreted particles to escape lipoprotein receptors on the microvilli in the space of Disse, permitting their entry into the circulating plasma. Future work will be required to determine whether or not rat plasma VLDL, like those of human plasma, contain apo B, PE-enriched particles that lack apo E (1,2). In any event, the high PE content of nascent Golgi VLDL probably reflects a common process of triglyceride-rich particle metabolism, because nascent chylomicrons are also PE enriched (7,8) and deficient in apo E (9).

Certainly, the high PE content of nascent Golgi VLDL strongly supports the idea that the PE-enriched VLDL from human plasma are a nascent form of human hepatocyte lipoprotein secretion. In fact, PE enrichment may be a characteristic of nascent triglyceride-rich particles, in general. For example, nascent chylomicrons in dogs and rats have been reported to have a high PE content (7,8). In vivo, chylomicron phospholipids are rapidly transferred to high density lipoproteins (8,10), together with apo A-I (9). The PE transferred to HDL is more rapidly cleared from the plasma than other phospholipids, and this appears to be mediated by hepatic lipase (8,11). Interestingly, the addition of PE to apo B-containing particles is a very late event in VLDL assembly by chick hepatocytes in culture (12), possibly while the nascent VLDL are in the Golgi apparatus, an organelle recently shown to contain enzymes required for PE synthesis (13). Thus, although the physiological significance of the association of PE with triglyceride-rich particles remains to be discovered, it could underlie an important early step in the metabolism of triglyceride-rich lipoproteins.

Our data on the phospholipid composition of Golgi membranes (largely free of contents) are comparable to those reported for whole Golgi fractions (with contents), also isolated by the Morré technique (14). Our data, however, differ from the reported phospholipid composition of content VLDL of rat liver Golgi fractions

isolated by a substantially different method (15). In that report, Golgi VLDL contained only 6% PE and the sphingomyelin content was higher (9.5%) than that of serum VLDL (6.4%) (15). Our data show that plasma VLDL contain about fourfold as much sphingomyelin as nascent Golgi VLDL (Table 1). The PE/ sphingomyelin ratio in nascent Golgi VLDL and plasma VLDL differed in our experiments by a factor of about 12 whereas the difference was only 1.6 in the studies reported (15), in spite of the fact that the ratio for plasma VLDL was almost the same (0.36 compared with 0.39) in both studies. The Golgi isolation method used in those studies (15) co-isolates large amounts of lipoprotein-containing endosomes (16), which we have found are MVBs filled with remnants of triglyceride-rich lipoproteins (5,17). Remnants of triglyceride-rich lipoproteins are taken up into MVBs of hepatocytes by the process of receptor-mediated endocytosis and represent the terminal particle of VLDL metabolism, even though certain properties of both nascent and remnant VLDL are similar (4).

Our demonstration of the greatly different PE/sphingomyelin ratio of nascent Golgi VLDL (4.2) and plasma VLDL (0.36) provides a benchmark for future research, which could be used to estimate the purity of hepatocytic Golgi and endosomal fractions. We would predict that the PE/sphingomyelin ratio in highly purified remnant lipoproteins recovered from MVBs isolated from hepatocytes may be even lower than that of plasma VLDL because the latter represent a complex mixture of nascent VLDL, nascent chylomicrons, and remnants of both types of triglyceride-rich particles in various stages of metabolism.

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