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DISTRIBUTION OF CHYLOMICRON DEGRADATION PRODUCTS IN THE ISOLATED, PERFUSED RAT HEART

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SUMMARY Chylomicron degradation by hearts from fed and fasted rats was studied using a perfusion technique, which allows the separate collection of coronary (Q_{rv}) and interstitial effluent (Q_i). Upon perfusion with [^{3}H]-cholesterol-containing chylomicrons the tissue recovery of label was highest in the fasted state, while label recovered in Q_i was highest in the fed state. Density gradient centrifugation of Q_i indicated that the label was recovered in lipoproteins with higher densities: low density lipoproteins ($1.019<d<1.050$), high density lipoproteins ($1.050<d<1.21$) and a fraction of $d>1.21$. These particles probably represent chylomicron degradation products (remnants and "surface fragments"). Our results indicate that tissue cholesterol uptake during chylomicron degradation may be inhibited in the fed state. Furthermore, the role of the myocyte (or interstitial) lipoprotein lipase in chylomicron degradation is discussed.

Serum triacylglycerols (from VLDL or chylomicrons) are degraded by LPL (EC 3.1.1.34). This enzyme has been shown to exist at the vascular endothelial surface[1], as well as in myocytes, isolated from adult rats[2]. Part of the enzyme activity in isolated myocytes is heparin-releasable[3], leaving an intracellular pool of lipolytic activity unaffected[2,3]. Collagenase used in the preparation of myocytes from adult rats inactivates part of the myocyte lipase activity[4]. In order to prevent this and to evaluate the existence of more than one pool of LPL activity in rat heart, we employed a modified perfusion technique, developed by De Deckere and Ten Hoor[5]. Using this technique, we[6] were able to demonstrate the presence of heparin-releasable LPL activities in Q_{rv}, as well as in Q_i from in vitro perfused rat hearts. This indicated a dual localization of the enzyme, one part arising from the coronary vascular endothelium and another part from cardiac myocytes. Furthermore, it was shown that LPL activity derived from either cell type was dependent on the feeding condition of the rat, being high in Q_{rv} during fasting and high in Q_i in the

Abbreviations: BSA, bovine serum albumin; CE, cholesterol ester; FC, free cholesterol; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; LSC, liquid scintillation counting; PB, perfusion buffer; Q_i, interstitial effluent; Q_{rv}, coronary effluent; TG, triglyceride(s); VLDL, very low density lipoprotein.

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fed state. The scope of the present study was to investigate the distribution of chylomicron degradation products over different compartments of the perfused rat heart during in vitro perfusion.

MATERIALS AND METHODS

Animals Male Wistar rats (250-300 g body weight) were used throughout the study. They were fed ad libitum or fasted overnight. Some animals were injected intraperitoneally (i.p.) with the protein synthesis inhibitor cycloheximide (2 mg/kg body weight dissolved in 0.15 M NaCl) 1 h prior to perfusion.

Perfusion procedure The hearts (1.09±0.04 g mean ± SEM, n=8) were quickly excised from pentobarbital-anesthetized rats (70 mg/kg body weight i.p.) and arranged for an open, substrate-free, retrograde preperfusion period of 15 min as described previously[6-8]. During the preperfusion period the caval and pulmonary veins were ligated and the pulmonary artery canulated as described before[5,6], allowing the separate collection of Q_{rv} and Q_i. After a lipoprotein-free preperfusion, a recirculating perfusion with chylomicrons- or HDL was started. The flow rate was adjusted to 8 ml/min by a peristaltic pump. Degradation of chylomicrons was studied during a 2 h recirculating perfusion with 35 ml basic, glucose-free, PB, supplemented with [^{3}H]-cholesterol labeled chylomicrons (about 180 000 dpm; cholesterol specific radioactivity 346 000 dpm/μmol) and 5% rat serum (v/v). Total TG content in the PB ranged from 0.56-0.61 mM. In some experiments cycloheximide (3 μg/ml) was included in the PB during pre- and chylomicron-perfusion of hearts of cycloheximide-pretreated rats and heparin (5 U/ml) during the preperfusion period. In the latter case heparin was washed out (5 min) prior to chylomicron perfusion. This procedure caused a drop of LPL activity of 81±4% (n=4). HDL perfusion of rat hearts took place with PB supplemented with [^{3}H]-cholesterol labeled HDL (about 350 000 dpm; cholesterol specific radioactivity 145 000 dpm/μmol), 11.1 mM glucose and 1% defatted BSA (w/v). In the present experiments, i.e. in the presence of serum or BSA in the PB, the contribution of Q_i to total flow rates (8 ml/min) was 0.60±0.09% (n=8). We have shown earlier that during protein-free perfusion Q_i amounts to 2.5% total coronary flow rate[6]. The increase in capillary permeability in vascular beds perfused with protein-free solutions has been observed before[9]. Total radioactivity and label distribution were determined at the start in chylomicrons-containing PB, Q_{rv} and Q_i. At the end, the hearts were flushed with label-free basic PB (5 min). Radioactivity recovered in heart tissue was collected in a Packard Tri-Carb sample oxidizer and estimated by LSC. The recovery of radioactivity was 95.6±1.6% (n=8). Appropriate corrections in all calculations for this loss were made.

Preparation of [^{3}H]-labeled chylomicrons and [^{3}H]-labeled HDL Under ether anesthesia of the rats a fistula was made between the cisterna chyli and the right ureter. This surgical procedure results in the appearance of chylomicrons in the urine. A detailed description of the technique will be published elsewhere. 100 μCi [1α,2α(n)-^{3}H]-cholesterol in toluene was dried under N_2-gas and sonicated with 5 ml 10% Intralipid (Vitrum, Stockholm, Sweden). This mixture was administered at the end of the day to the rats by intragastric intubation. The animals were placed in a metabolic cage and the urine (about 20 ml) collected overnight in small beakers containing 2 ml of a 100 mM Tris-HCl buffer of pH 7.4 with 10 mM EGTA and 0.02% NaN_3. To remove protein clots the chylomicron-containing urine was filtered through glass wool, saline-containing 1% BSA (w/v) was added and the density was adjusted to 1.006 g/ml. After centrifugation for 45 min at 100 000 x g_{av} at 20°C, the floating chylomicron cake was collected by tube slicing, dispersed in saline-free BSA (1%) and again centrifuged at the same speed. This procedure was repeated once in the absence of BSA. The final chylomicron cake was suspended in, and dialyzed against saline and stored at 4°C. The final chylomicron suspension, obtained

by this procedure, contained 40-50 mg TG/ml and 0.16-0.24 mg total cholesterol/ml. The FC:CE ratio varied between 0.6-1.4. [^{3}H]-labeled HDL was prepared as described by Avignan[10], using serum from overnight fasted rats.

Density gradient centrifugation The density of the products of chylomicron degradation and HDL-perfusion was analyzed by density gradient centrifugation[11]. Five density classes were obtained by tube slicing; (1) chylomicrons, d<1.006, (2) IDL, 1.006<d<1.019, (3) LDL, 1.019<d<1.050, (4) HDL, 1.050<d<1.21 and (5) a (protein) fraction of d>1.21. Radioactivity in each density fraction was determined by LSC.

Analytical procedures Chylomicron TG were determined according to Laurell[12], total and free cholesterol were measured using a Boehringer Test-Kit combination and protein by the biuret method[13]. Lipids in PB were extracted[14]; FC and CE in the $CHCl_3$-phase were separated on Silica gel G plates developed in heptane/diethylether/acetic acid (80:20:1, v/v). After visualization of the spots by I_2-vapour they were scraped off the plates, sonicated with 1 ml H_2O and radioactivity was determined as described above. Lipolytic activity in acetone-ether powders of perfused hearts[15] was determined at 30°C as described previously[6].

RESULTS AND DISCUSSION

The present experiments were undertaken to investigate chylomicron breakdown in the rat heart under different feeding conditions. Cholesterol-labeled chylomicrons were used instead of triacylglycerol-labeled particles, since triacylglycerol hydrolysis products can be completely oxidized in contrast to cholesterol. First, it was necessary to determine whether the chylomicron preparations, obtained and isolated from rat urine, were a suitable substrate for rat heart LPL. During retrograde perfusion of hearts the rate of chylomicron TG fatty acid oxidation amounted to 25±3 nmoles.min^{-1}. g myocardial wet weight^{-1} (n=6) (W.C. Hülsmann, W.A.P. Breeman, H. Stam and W.J. Kort; unpublished). This rate compares well with that obtained by Borensztajn and Robinson[1].

The modified perfusion technique[5,16] allowed to collect Q_i and Q_{rv} from isolated hearts. Evidence has been presented that Q_i contains products derived from the myocytes, while products from the vascular epithelium are recovered in Q_{rv}[5,7,16]. This technique was used during perfusion of hearts from fed and fasted rats with [^{3}H]-cholesterol-containing chylomicrons and the distribution of radioactivity between Q_i, Q_{rv} and myocardial tissue during a 2 h perfusion was assessed. Rat hearts, depleted of functional LPL activity (by cycloheximide- and heparin-pretreatment, see Materials and Methods) were also studied. It can be seen from Table I that in this condition the disappearance of [^{3}H]-cholesterol from Q_{rv} is low (8%). With intact LPL the removal of radioactivity from Q_{rv} in both other groups is larger (20%), so that only after chylomicron breakdown the uptake of cholesterol by the heart or appearance in Q_i occurs.

TABLE I

DISTRIBUTION OF RADIOACTIVITY BETWEEN Q_{rv}, Q_i AND MYOCARDIAL TISSUE AFTER RAT HEART PERFUSION WITH [^{3}H]-CHOLESTEROL-CONTAINING CHYLOMICRONS

Condition	Cycloheximide and heparin treatment	% of total radioactivity		
		Q_{rv}	Q_i	Tissue
Fed	-	79.0(78.1;80.0)	10.4(11.5;9.2)	10.6(10.4;10.8)
Fasted	-	78.5(76.4;80.7)	3.2(2.1;4.2)	18.3(21.5;15.1)
Fed	+	91.9(91.7;92.2)	1.3(1.1;1.5)	6.8(7.2; 6.3)

Rat hearts were perfused with [^{3}H]-cholesterol containing chylomicrons. After 120 min perfusion the distribution of label in Q_{rv}, Q_i and tissue was determined and expressed as per cent of total radioactivity at the start of the experiment. The mean results of two separate experiments (brackets) are presented.

To evaluate the composition of the chylomicron degradation products from all groups of hearts, the distribution of label between different density classes was determined by gradient centrifugation of PB, Q_{rv} and Q_i and is presented in Fig. 1. In the starting PB, 70-74% of the label was associated with chylomicrons (subfraction 1). The amounts of label present in fractions

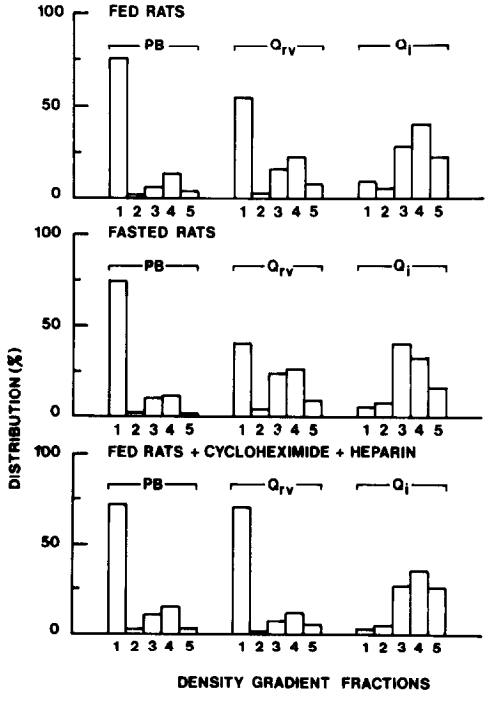

Fig. 1 Distribution of radioactivity between density gradient subfractions from PB at the start of the experiment, Qrv and Qi obtained from rat hearts of fed, fasted and cycloheximide + heparin pretreated hearts. For density ranges of the subfractions 1-5 see Materials and Methods. The mean values of two experiments are presented.

2 (IDL) and 5 (protein) were very low, while about 15-20% of total radioactivity was present in fractions 3 (LDL) and 4 (HDL), presumably representing [^{3}H]-cholesterol exchange between chylomicrons and serum lipoproteins in PB. Fig. 1 indicates that after a 2 h perfusion of hearts from fed rats 57% of the label is present in Q_{rv} subfraction 1 (chylomicrons) while in Q_{rv} of fasted rats only 38% of the radioactivity was recovered in subfraction 1. This means that chylomicron degradation is enhanced by 50% in the fasted state and confirms the higher myocardial LPL activity during fasting[1].

The chylomicron degradation products of higher densities (subfractions 2 to 5) accumulate in Q_{rv} of fasted rats more than in fed animals so that total radioactivity in Q_{rv} is identical (Table I). The higher tissue uptake of cholesterol in hearts from fasted rats parallels the higher removal of chylomicrons from Q_{rv} (Table I and Fig. 1). Q_i is poor in chylomicrons after 2 h perfusion, as may be expected from the low filtration rate of these large particles. The higher accumulation of breakdown products in Q_i in the fed state than in the fasted state (Table I), inversely corresponds with the tissue exchange or uptake of cholesterol (particularly large differences are obtained when the values are corrected for cycloheximide- and heparin-treated rats). It suggests that cholesterol exchange or uptake is inhibited during feeding. This may be of physiological importance since it indicates that not only lipoprotein-triacylglycerol uptake by the heart is low during feeding, but also cholesterol exchange or uptake. The lipoproteins of higher density probably represent products of LPL action on chylomicrons such as chylomicron-remnants and "surface fragments". This also follows from the high [^{3}H]-FC:[^{3}H]-CE ratio in Q_i during feeding and fasting which varied between 1.2 and 3.8 in contrast with this ratio in the starting PB which varied between 0.7 and 1.3 dependent on the batch of chylomicrons used.

The presence of some radioactivity associated with Q_i chylomicrons (fraction 1) in all groups of hearts can be explained by leakage of [^{3}H]-cholesterol-containing chylomicrons from the (ligated) vascular system or by passage of chylomicrons through the vascular endothelium and subsequent wash-out in Q_i without being degraded by the myocyte (or interstitial) LPL. The presence of label in lipoprotein subfractions of Q_i of the LPL-"depleted" hearts might be explained by leakage of [^{3}H]-cholesterol contained in IDL, LDL and HDL, or protein-bound, through the vascular endothelium. In any case, Q_i from chylomicron-perfused hearts of fed rats is enriched in lipoproteins of a higher density.

From the difference between the radioactivity in the subfractions at the start of the experiment and the end of the perfusion, the distribution of chylomicron degradation products between Q_i and Q_{rv} can be calculated (Table II).

TABLE II

DISTRIBUTION OF [^{3}H]-CHOLESTEROL-CONTAINING CHYLOMICRON DEGRADATION PRODUCTS BETWEEN Q_{rv} AND Q_i

Density gradient fraction	Lipoprotein class	% of label[a]			
		Fed		Fasted	
		Q_{rv}	Q_i	Q_{rv}	Q_i
1.006<d<1.019	IDL	64.9	35.1	90.1	9.9
1.019<d<1.050	LDL	63.1	36.9	84.7	15.3
1.050<d<1.21	HDL	36.6	63.4	85.2	14.8
1.21 <d	-	48.3	51.7	90.6	9.4

[^{3}H]-cholesterol-labeled chylomicrons were degraded during a 2 h recirculating perfusion. Lipoproteins in PB, Q_{rv} and Q_i were separated using gradient centrifugation. By subtracting individual subfraction radioactivity at the start of the experiment from that at the end of the perfusion (Q_{rv} + Q_i) net label recovery in the indicated lipoprotein classes could be calculated and was set at 100%. The per cent distribution between Q_{rv} and Q_i (a) is the mean of two separate experiments.

To evaluate whether the presence of chylomicron degradation products in Q_i could be explained by the transport of these particles from Q_{rv} through the vascular endothelium, we studied the distribution of label between Q_{rv}, Q_i and myocardial tissue in hearts perfused with [^{3}H]-cholesterol-containing HDL. Furthermore, assuming that [^{3}H]-CE present in HDL cannot easily exchange with non-labeled CE in heart tissues, the Q_{rv}:Q_i ratio of HDL-[^{3}H]-CE was used as an index of HDL permeability through the vascular wall and compared with the protein permeability (Table III). [^{3}H]-Cholesterol exchange between HDL and heart tissue was substantial. After 2 h of perfusion 20% of the label was recovered in the tissue, while only 8.7% of the label was recovered in Q_i. During HDL perfusion total cholesterol in the perfusate (Q_{rv} + Q_i) increased by 0.54 (0.49; 0.58) µmoles, indicating the capability of HDL to extract cholesterol from the perfused heart. At the end of the perfusion 11.2% of the HDL-[^{3}H]-CE is recovered in Q_i, while the Q_{rv}:Q_i ratio of [^{3}H]-CE (dpm/ml) amounted to 1.91. This indicates that the permeability of the HDL particle is

TABLE III

DISTRIBUTION OF TOTAL RADIOACTIVITY, [^{3}H]-CE AND PROTEIN BETWEEN Q_{rv} AND Q_i DURING RAT HEART PERFUSION WITH [^{3}H]-CHOLESTEROL-CONTAINING HDL

% of total radioactivity			HDL-[^{3}H]-CE	Protein
Qrv	Qi	Tissue	Q_{rv}/Q_i	Q_{rv}/Q_i
70.9(66.0,75.8)	8.7(6.1,11.2)	20.5(27.9,13.0)	1.91(1.99,1.82)	1.02(1.00,1.04)

Hearts from fed rats were perfused for 2 h with [^{3}H]-cholesterol-containing HDL. Protein and radioactivity recovered in Q_{rv}, Q_i and tissue were determined. After gradient centrifugation of Q_{rv} and Q_i samples, the "HDL"-density fraction was analyzed for [^{3}H]-CE. Results are the mean of two separate experiments (brackets). Q_{rv}/Q_i represent concentration ratios of indicated substances.

limited while protein of the PB permeates freely through the endothelial barrier, resulting in equal protein concentration in Q_{rv} and Q_i. Therefore, the products of the LPL reaction on chylomicrons in the fed state probably have a vectorial advantage over coronary products to pass into Q_i and the heart tissue, as was suggested by Scow et al.[17]. The relative enrichment of radioactivity in the chylomicron degradation products present in Q_i of fed rats can be explained by an inhibition of cholesterol uptake in the fed state. In addition, the contribution of myocyte LPL to the overall reaction may also be larger in the fed state, provided chylomicrons are permeable through the vascular wall. Indeed, it has been shown that fat particles, belonging to lipoproteins of d<1.006, chylomicrons and VLDL which range in diameter from 0.05 to 0.5 μ[18,19], can cross membranes[20] and vascular endothelium[21] probably by way of plasmalemmal vesicles or transendothelial channels[22,23]. Furthermore, in the capillary wall, pores of about 0.07 μ in diameter, possibly representing the transendothelial channels, have been described[9,23]. Endothelial or myocyte (and interstitial) LPL by binding to chylomicrons, may serve as receptors for chylomicrons. In LPL-"depleted" hearts no transfer of chylomicrons through the vascular bed was observed (Table I, Fig. 1). Possible evidence for interstitial degradation of chylomicrons may be the demonstration of a cardiac lymph pool of TG-rich particles, HDL and LDL in the pig heart[24]. The extrapolation of our findings during in vitro perfusion to the in vivo situation is still difficult, especially since the capillary permeability of the rat heart changes upon isolation and perfusion[9,25].

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