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The effect of β,β'-tetramethylhexadecanedioic acid (MEDICA 16) on plasma very-low-density lipoprotein metabolism in rats: role of apolipoprotein C-III

Baruch FRENKEL, Janette BISHARA-SHIEBAN and Jacob BAR-TANA*

Department of Human Nutrition and Metabolism, Hebrew University – Hadassah Faculty of Medicine, P.O. Box 1172, Jerusalem 91010, Israel

Short term treatment of rats with β,β'-tetramethylhexadecanedioic acid (MEDICA 16) results in a pronounced decrease in plasma very-low-density-lipoprotein (VLDL) cholesterol and VLDL triacylglycerol, previously ascribed to a decrease in liver VLDL production [Bar-Tana, Rose-Kahn, Frenkel, Shafer and Fainaru (1988) J. Lipid Res. 29, 431–441]. The hypolipidaemic effect of MEDICA 16 was further analysed here by monitoring plasma VLDL clearance and its hepatic uptake. VLDL triacylglycerol and VLDL apolipoprotein (apo) B fractional clearance rates were increased 7–8-fold in MEDICA 16-treated rats. The increase in the fractional clearance rate of plasma VLDL was essentially eliminated by functional hepatectomy. It was accounted for by activation of plasma VLDL uptake by the liver being completed during the first 4 min after the injection of the VLDL label and before commencement of uptake in non-treated animals. The hypolipidaemic effect of MEDICA 16 was accompanied by a 3.5-fold decrease in plasma apoC-III, but plasma apoC-III clearance remained unaffected by MEDICA 16. MEDICA 16-induced premature hepatic uptake of plasma VLDL due to suppression of apoC-III production may thus account for enhancement of plasma VLDL clearance in treated animals.

INTRODUCTION

MEDICA 16 (β,β'-tetramethylhexadecanedioic acid) is one of a series of long-chain dioic acid analogues previously reported to act as hypolipidaemic agents [1]. Short-term treatment of rats fed on either a balanced Purina chow diet or a carbohydrate-rich diet with MEDICA 16 resulted in a marked decrease in the triacylglycerol and cholesterol contents in the plasma very-low-density lipoprotein (VLDL) fraction [2,3]. The observed decrease in plasma VLDL was ascribed to inhibition of long-chain fatty acid and cholesterol synthesis due to inhibition of liver ATP citrate-lyase and acetyl-CoA carboxylase by the free MEDICA 16 acid or its CoA thioester [1,4,5]. Since the hypolipidaemic effect with respect to VLDL was sustained under conditions of starvation, where the production of VLDL was already suppressed and could not be further inhibited by MEDICA 16 treatment [3], it became of interest to study the effect of MEDICA 16 on the catabolism of plasma VLDL. Activation of VLDL catabolism by MEDICA 16 was indicated by the previously observed increase in plasma low-density lipoprotein (LDL) cholesterol in treated rats [3].

In the present paper, the hypolipidaemic effect of MEDICA 16 with respect of VLDL was studied by monitoring its plasma clearance, hepatic uptake and the role played by plasma apolipoprotein (apo) C-III in the overall effect observed.

METHODS

Animals and diet

Male albino rats of the Hebrew University strain weighing 200–250 g were kept in individual cages and fed *ad libitum* with ground Purina chow diet containing 52.5% (w/w) carbohydrate, 18.1% (w/w) protein, 4.6% (w/w) fat, 5.9% (w/w) cellulose, 8.5% (w/w) salt vitamin mixture and 10.4% (w/w) moisture. MEDICA 16 was administered for 5 days by adding 0.25% (w/w) of the finely powdered drug to the diet. Food consumption by MEDICA 16-treated and non-treated rats was similar and amounted to approx. 13 g/100 g body wt./per day. Unless otherwise stated, all experiments were carried out between 09:00 and 14:00 h with non-starved animals.

Preparation of VLDL

Plasma was obtained from non-fasted rats by aortic puncture under light ether anaesthesia, EDTA (final concn. 1 mg/ml) being used as anticoagulant. Plasma chylomicrons were removed by flotation at 30000 rev./min for 20 min in a Beckman SW-41 rotor. VLDL was then floated at 40000 rev./min for 18 h, dialysed against > 3000 vol. of 2 mM Tris/HCl buffer (pH 7.4) containing 0.15 M NaCl and 0.01% EDTA, and used within 10 h. [^{3}H]Triacylglycerol-labelled VLDL was prepared by injecting an ether-anaesthetized non-fasted rat through the jugular vein with about 2 mCi of [9,10-^{3}H]palmitate (60 Ci/mmol) in 0.1% BSA solution (pH 7.4). The rat was allowed to wake and was bled 40 min later under light ether anaesthesia, and VLDL was prepared as described above.

^{125}I-VLDL was prepared by radioiodination with Na^{125}I [6] of an undialysed pooled VLDL prepared as described above. After radioiodination, the VLDL preparation was subjected to three successive dialyses against > 3000 vol. each of 2 mM Tris/HCl buffer (pH 7.4) containing 0.15 M NaCl and 0.01% EDTA. The distribution of the label between VLDL apolipoproteins was 4.4:7.0:9.2:79.4 for apoB-100, apoB-48, apoE and apoC respectively.

Plasma apoC

Plasma apoC isoforms were determined in tail blood samples of ether-anaesthetized rats. The blood samples were allowed to clot, and serum samples were mixed and vortex-mixed with 1 vol. of water and 2 vol. of propan-2-ol, and then left for 1 h at room temperature. The mixture was then centrifuged, and a sample of the supernatant was subjected to isoelectric focusing (pI 4.0–6.0)

Abbreviations used: apo, apolipoprotein; FCR, fractional clearance rate; LDL, low-density lipoprotein; MEDICA 16, β,β'-tetramethylhexadecanedioic acid; VLDL, very-low-density lipoprotein.

* To whom correspondence should be addressed.

essentially as described in [3]. After staining with Coomassie Blue R, the four distinct bands corresponding to apoC-II, apoC-III_0, apoC-$III_{1,2}$ and apoC-III_3 could be clearly defined and were quantified by photodensitometry [3]. The reproducibility and yield of apoC focused in whole blood samples as described here was better than that described in [3] for the isolated lipoprotein preparations. The yield of focused apoC was not dependent on whether plasma was derived from MEDICA 16-treated or non-treated rats.

Plasma apoC-III mass was determined by e.l.i.s.a. for rat apoC-III. Anti-apoC-III antibodies were prepared by immunizing rabbits with apoC-III prepared from whole rat plasma by propan-2-ol treatment, followed by isoelectrofocusing as described above. Stained apoC-III bands were cut out of the gel, electroeluted, freeze-dried, and 100 μg samples were injected intradermally to rabbits in incomplete Freund adjuvant once every week for 7 weeks. The antiserum raised was absorbed on to lipoprotein-free rat plasma (> 1.21 g/ml) covalently bound to CNBr-activated Sepharose. After absorption, the purified antibody preparation was found by Western-blot analysis of whole rat plasma to react specifically with rat plasma apoC-III. For apoC-III e.l.i.s.a., flexible polystyrene microtitre plates (Nunc) were coated with 100 μl/well of apoC-III standards (0.1–5 μg) or unknown sera diluted 1:25000 in PBS (pH 7.4). Coating was carried out overnight at 4 °C, followed by washing the plates five times with PBS. The washed plates were further incubated for 2 h at room temperature with 20 μl/well 2% BSA, and subsequently were washed five times with PBS containing 0.1% (v/v) Tween 20 (polyoxyethylene sorbitan monolaurate) and 1% BSA. The washed plates were then incubated at 37 °C for 2 h with purified anti-apoC-III antiserum (100 μl/well) diluted 1:100 in PBS/1% BSA, then washed five times with PBS/Tween, followed by adding 100 μl/well of 1:2500 dilution of anti-(rabbit IgG) antiserum coupled to alkaline phosphatase (Sigma). After incubation for 2 h at 37 °C, the plates were washed five times with PBS/Tween and incubated with 100 μl/well of *p*-nitrophenyl phosphate (1 mg/ml) in 10% diethylamine buffer (pH 9.8). The developed colour was determined at 405 nm with a Dynatech micro-e.l.i.s.a. reader MR 600.

VLDL [^{3}H]triacylglycerol clearance

VLDL triacylglycerol clearance was evaluated in ether-anaesthetized rats injected through the jugular vein with about 0.3×10^6 d.p.m. of [^{3}H]triacylglycerol-labelled VLDL (0.1–0.5 mg of triacylglycerol in 0.3 ml of saline). Plasma VLDL clearance was monitored by sampling ~ 0.2 ml of tail blood at the indicated times. The blood samples were allowed to clot, and 75 μl of serum was counted for radioactivity in Lumax scintillation fluid. More than 96% of the plasma label could be accounted for as triacylglycerol, as verified by t.l.c. [in light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid, 65:35:1, by vol.] of the respective lipid extracts.

VLDL apoB clearance

VLDL apoB clearance was evaluated in ether-anaesthetized rats injected through the jugular vein with 60×10^6 c.p.m. of ^{125}I-VLDL (0.18 mg of protein, 0.9 mg of triacylglycerol) in 0.3 ml of saline, followed by sampling ~ 0.2 ml of tail blood at the indicated times. Dilution of the labelled VLDL by saline was made only before its injection into recipient rats. The blood samples were allowed to clot, and 20 μl serum samples were vortex-mixed with 60 μl of an aqueous solution containing 6.67% (w/v) SDS, 6.67% (v/v) mercaptoethanol and 10% (v/v) glycerol. The mixture was frozen at −20 °C for 1 h and then thawed at room temperature. After five cycles of freezing and thawing, a 30 μl portion was subjected to SDS/PAGE in 4%-acrylamide slab gels at a fixed current of 40 mA/gel. ApoB-100 and apoB-48 bands identified by autoradiography were sliced off the gel and counted in a γ-radiation counter. Whole plasma ^{125}I-apoB precipitation by propan-2-ol as previously described for rabbit VLDL [7] was found to result in appreciable amounts of non-B ^{125}I-apoproteins in the propan-2-ol precipitate of whole rat plasma.

Plasma apoC-III clearance

Plasma apoC-III clearance was evaluated in ether-anaesthetized rats injected through the jugular vein with 60×10^6 c.p.m. of ^{125}I-VLDL (0.18 mg of protein in 0.3 ml of saline). At 2 min after injection, 0.2 ml of tail blood was sampled and the animals were allowed to wake up. Additional tail blood samples were taken under light ether anaesthesia at 1.5 h, 3.5 h and 6 h after injection. The blood samples were allowed to clot, and 20 μl samples of serum were extracted with propan-2-ol, followed by isoelectric focusing as described under 'Plasma apoC'. The label in apoC-III isoforms was determined by counting the radioactivity of the respective bands. Repeated blood sampling as described here was found not to affect plasma apoC-III clearance, as verified by comparing the apoC-III label remaining in plasma in animals bled once at either 3.5 h or 6 h after injection of label.

Tissue uptake of VLDL [^{3}H]triacylglycerol

Unless otherwise stated, ether-anaesthetized rats were injected with [^{3}H]triacylglycerol-labelled VLDL (0.3×10^6 d.p.m., 0.1–0.5 mg of triacylglycerol) through the jugular vein, followed by excising about 1 g of the respective tissues at the indicated times. The tissue samples were washed in 300 ml of cold saline, wiped, weighed, extracted in 20 ml of chloroform/methanol (1:1, v/v) for at least 48 h, and the organic phase was counted for radioactivity in toluene-based scintillation fluid. The extraction yield was 92.5 ± 1.8% of the overall tissue counts.

Net hepatic uptake of [^{3}H]triacylglycerol-labelled VLDL was determined by perfusing the liver with 20 ml of cold saline before its sampling and subtracting the fraction of the tracer still remaining in the extracellular space by using [U-^{14}C]sucrose, co-injected with the labelled lipoprotein, as an extracellular marker. The amount of tracer remaining in the extracellular space for each time point of the uptake curve was calculated by multiplying the [U-^{14}C]sucrose counted in the aqueous phase of the liver extract by the ^{3}H/^{14}C ratio in the respective blood sample drawn just before perfusing the liver, or the ^{3}H/^{14}C ratio in the injected mixture (for the 15 s time period of the uptake curve).

Assays

Lipoprotein lipase was assayed essentially as previously described [8]. The reaction mixture (200 μl) consisted of 5 μl of post-heparin plasma as an enzyme source, and Intralipid (2 mg of triacylglycerol/assay) sonicated with glycerol tri[9,10-^{3}H]oleate (0.5×10^6 d.p.m./assay) as a substrate. Lipoprotein lipase activity was defined as the difference between the activity measured in the presence and absence of 0.75 M NaCl. Triacylglycerols were determined with a Boehringer Mannheim kit (cat no. 126039). Protein was determined as described by Bradford [9].

Materials

MEDICA 16 was synthesized as previously described [2]. [9,10-^{3}H]Palmitic acid, glycerol tri[9,10-^{3}H]oleate and [U-^{14}C]sucrose

were from ARC, St. Louis, MO, U.S.A. $Na^{125}I$ was from Nuclear Research Center, Negev, Israel.

RESULTS

The effect of MEDICA 16 on plasma VLDL catabolism was evaluated by monitoring the plasma clearance of triacylglycerol-labelled VLDL prepared in a non-treated non-fasting rat and injected into non-treated or MEDICA 16-treated non-fasting animals. As shown in Figure 1, plasma VLDL-triacylglycerol clearance was significantly enhanced in MEDICA 16-treated animals, being characterized by an initial fast clearance phase resulting in elimination of 68 % of the label within the first 2 min after its injection, as compared with 10 % for non-treated rats. Similar results have been observed in 14 similar experiments employing 3–8 rats in each treatment group. Thus, VLDL-triacylglycerol fractional clearance rate (FCR) values for

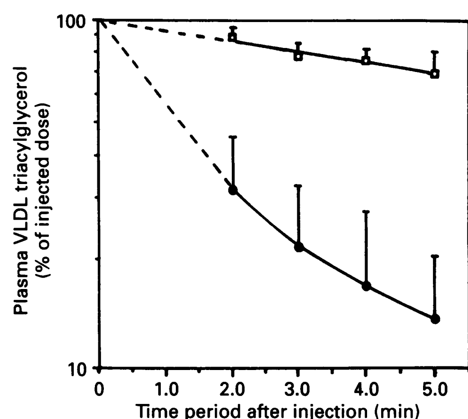

Figure 1 VLDL-triacylglycerol clearance in non-treated and MEDICA 16-treated rats

VLDL-triacylglycerol clearance was determined in non-treated (□) and MEDICA 16-treated (●) rats as described in the Methods section. The 100% value for the injected dose was derived by linear extrapolation of the semi-logarithmic plot for non-treated rats to zero time [23]. Results are means ± S.D. ($n = 5$).

MEDICA 16-treated and non-treated rats ranged from 0.43 to 0.69 min^{-1} (mean 0.58 min^{-1}) and from 0.050 to 0.14 min^{-1} (mean 0.069) respectively. An increased clearance rate of VLDL triacylglycerol was similarly observed in MEDICA 16-treated non-fasting rats injected with triacylglycerol-labelled VLDL derived from fasting animals. The FCR values for MEDICA 16-treated and non-treated rats under these conditions were 0.91 ± 0.29 min^{-1} ($n = 6$) and 0.10 ± 0.03 min^{-1} ($n = 6$) respectively. It is noteworthy that the initial enhanced clearance of plasma VLDL triacylglycerol in treated animals was too fast to be measured experimentally (Figure 1). Initial FCR values for treated animals were thus calculated by interpolating the first experimental point determined in treated animals to the 100 % intercept value derived from the linear regression line of plasma VLDL-triacylglycerol clearance in non-treated animals (Figure 1). Hence FCR values for MEDICA 16-treated animals should be considered as lower limits for clearance rates of plasma VLDL triacylglycerol.

Increase in plasma VLDL catabolism could also be verified by monitoring the clearance of plasma VLDL apoB in MEDICA 16-treated rats injected with ^{125}I-VLDL prepared in a non-treated animal. As shown in Figure 2, the initial clearance of VLDL apoB-100 and apoB-48 in non-treated rats followed first-order kinetics, having FCR values of 0.025 and 0.040 min^{-1} for the two apoB species respectively, as compared with 0.19 and 0.29 min^{-1} for MEDICA 16-treated rats. It is noteworthy, however, that the FCR values of both apoB species in non-treated as well as in MEDICA 16-treated rats were lower than the respective FCR values for plasma VLDL triacylglycerol. Thus, although the initial fast clearance phase of VLDL triacylglycerol observed in MEDICA 16-treated rats resulted in elimination of 61 % of the injected dose within the first 2 min, only 45 % and 31 % of the injected apoB-48 and apoB-100 respectively were cleared within the first 2 min (Figure 2).

VLDL-triacylglycerol uptake by various tissues in MEDICA 16-treated and non-treated rats is shown in Table 1. No significant differences in uptake between treated and nontreated rats could be observed in heart muscle or epididymal fat. Similarly, no significant differences in uptake were observed in skeletal muscle, bone marrow, spleen, intestine, adrenal, lung and kidney (results not shown). In contrast, the hepatic uptake of VLDL triacylglycerol was significantly enhanced in MEDICA 16-treated rats

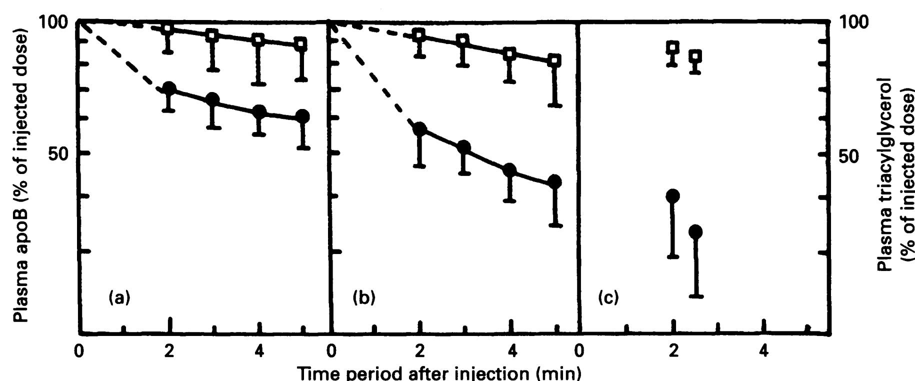

Figure 2 VLDL-apoB clearance in non-treated and MEDICA 16-treated rats

VLDL-apoB-48 (**a**), VLDL-apoB-100 (**b**) and VLDL-triacylglycerol (**c**) clearance was determined in non-treated (□) and MEDICA 16-treated (●) rats as described in the Methods section. The rats were first injected through the left jugular vein with [^3H]triacylglycerol-labelled VLDL, followed by sampling tail blood at 2 and 2.5 min. ^{125}I-VLDL was then injected through the right jugular vein, followed by sampling tail blood as indicated. The 100% values of the injected apoBs were derived by linear extrapolation of the semi-logarithmic plot for nontreated rats to zero time. Results are means ± S.D. ($n = 8$).

Table 1 Tissue uptake of VLDL triacylglycerol in MEDICA 16-treated rats

[^{3}H]Triacylglycerol-labelled VLDL was injected at zero time, and tissues were sampled at the indicated time periods and processed as described in the Methods section. Results are means ± S.D. for the number of animals in parentheses: *significantly different from the respective value for non-treated rats ($P < 0.05$).

Tissue	Uptake period (min)	Uptake (% of injected dose/1 g of tissue)	
		Non-treated	MEDICA 16-treated
Epididymal fat	3.0	0.323 ± 0.164 (4)	0.357 ± 0.070 (4)
Heart muscle	0.5	0.364 ± 0.055 (3)	0.294 ± 0.029 (3)
	3.5	0.409 ± 0.061 (4)	0.513 ± 0.137 (4)
Liver	3.7	0.996 ± 0.253 (4)	2.690 ± 0.637 (4)*

(Table 1). In light of the difference in liver weights between MEDICA 16-treated and non-treated rats (15.2 ± 2.0 g and 13.5 ± 1.7 g respectively), the calculated uptake values for the whole organ during the first 3.5 min uptake period amounted to 41.7% and 13.4% of the injected dose respectively. This 3-fold difference is a lower limit only, since uptake values in Table 1 include tracer counts present in the extracellular space, which may be expected to be higher in non-treated animals as a result of their lower plasma clearance rate (Figure 1).

The enhanced hepatic uptake of VLDL triacylglycerol in MEDICA 16-treated rats was further characterized by determining the net hepatic uptake as a function of time. As shown in Figure 3, net hepatic uptake of VLDL triacylglycerol in non-treated rats started only at about 4 min after injection of the label. In contrast, hepatic uptake of VLDL triacylglycerol in MEDICA 16-treated rats was already evident at the shortest sampling period employed, amounting to 17% of the injected dose at 15 s and being practically completed within the first 4 min after injection of the label. The overall hepatic extraction was about 50% of the injected dose in both treated and non-treated animals.

The role played by the liver in the enhanced clearance of VLDL triacylglycerol in MEDICA 16-treated rats was further pursued by evaluating the clearance rates in functionally hepatectomized rats. As shown in Table 2, functional hepatectomy did not affect VLDL-triacylglycerol catabolism in non-treated rats within a time period of 5 min after injection of the label. However, the FCR of VLDL triacylglycerol in functionally hepatectomized MEDICA 16-treated rats was 8.4-fold lower than in non-hepatectomized treated rats and approached the respective FCR values determined in non-treated animals. It is noteworthy that VLDL-triacylglycerol clearance in hepatectomized treated animals was still 1.8-fold faster than the respective clearance rate in non-treated rats (Table 2), thus indicating that extrahepatic factors could possibly contribute to increased plasma VLDL clearance by MEDICA 16. Indeed, lipolysis *in vitro* of Intralipid by post-heparin-plasma lipoprotein lipase was increased 1.7–1.9-fold in the presence of 30% or 80% (v/v) plasma derived from MEDICA 16-treated rats, compared with that of nontreated animals. This difference could not be accounted for by direct activation of lipoprotein lipase by MEDICA 16, as the drug was actually found to inhibit the lipase non-competitively, with $K_{i,\mathrm{intercept}}$ and $K_{i,\mathrm{slope}}$ values of 0.25 mM and 0.75 mM respectively.

Enhancement of plasma VLDL clearance and its hepatic uptake by MEDICA 16 could have been mediated by plasma

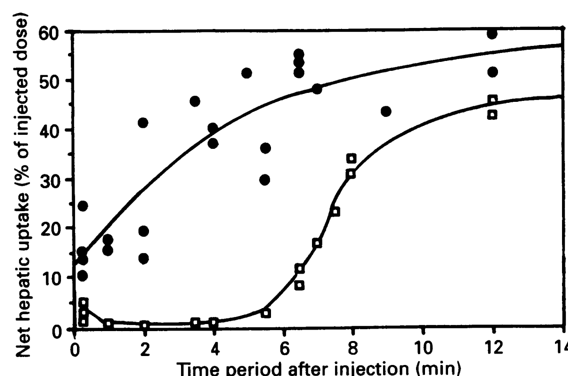

Figure 3 Net hepatic uptake of VLDL-triacylglycerol in non-treated and MEDICA 16-treated rats

Net hepatic uptake of VLDL-triacylglycerol in non-treated (□) and MEDICA 16-treated (●) rats was determined as described in Methods section. Net hepatic uptake during the first 15 s was determined by injecting the VLDL label through the portal vein. The portal vein was first cannulated, and 1.4 ml of blood was aspirated into a 2 ml syringe containing 0.3 μl of the VLDL label. The VLDL was allowed to equilibrate with the aspirated blood for 30 s, and was then injected within 5 s.

Table 2 Clearance of VLDL triacylglycerol in MEDICA 16-treated functionally hepatectomized rats

Functional hepatectomy was carried out by ligating the portal bundle as previously described [20]. Plasma VLDL-[^{3}H]triacylglycerol clearance in MEDICA 16-treated and nontreated rats was evaluated as in Figure 1. Results are means ± S.D. for the numbers of animals in parentheses: * significantly different from the respective value for non-hepatectomized rats ($P < 0.05$); † significantly different from the respective value for non-treated rats ($P < 0.05$).

Rats	FCR (min^{-1})	
	Non-treated	MEDICA 16-treated
Non-hepatectomized	0.050 ± 0.012 (3)	0.650 ± 0.250 (3)†
Hepatectomized	0.048 ± 0.018 (6)	0.086 ± 0.037 (6)*

Table 3 Plasma apoC in MEDICA 16-treated rats

The content of plasma apoC isoforms and apoC-III mass were determined as described in the Methods section. Results in (**a**) are presented in relative densitometric units, where the total apoC content in non-treated rats is taken as 100. Results are means ± S.D. ($n = 5$): * significantly different from the respective value for non-treated rats ($P < 0.05$).

	Plasma apoC	
	Non-treated	MEDICA 16-treated
(a) Plasma apoC isoforms (relative densitometric units)		
apoC-II	20.5 ± 7.5	9.3 ± 4.2
apoC-III$_0$	50.9 ± 9.1	6.0 ± 0.7*
apoC-III$_{1,2}$	10.0 ± 3.4	1.1 ± 0.7*
apoC-III$_3$	18.6 ± 2.6	15.3 ± 6.0
(b) Plasma apoC-III mass (mg %)	27.8 ± 8.0	9.5 ± 1.5*

apoC-III depletion [10–22] induced by MEDICA 16 treatment [23]. Indeed, as shown in Table 3, MEDICA 16 treatment resulted in a significant depletion of densitometrically determined

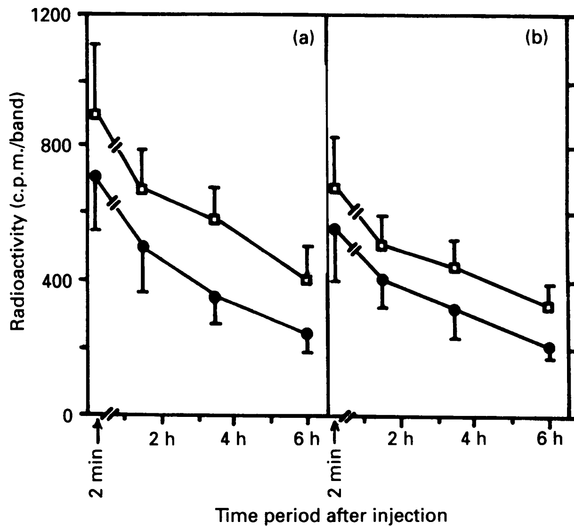

Figure 4 Plasma apoC-III clearance in non-treated and MEDICA 16-treated rats

Plasma apoC-III_0 (a) and apoC-III_{1-3} (b) clearance was determined in non-treated (□) and MEDICA 16-treated rats (●) as described in the Methods section. Results are means ± S.D.: $n = 9$ for 2 min and 6 h samples; $n = 3$ for 1.5 h samples; $n = 6$ for 3.5 h samples.

plasma apoC-III, with a concomitant 1.6-fold increase in the apoC-II/apoC-III ratio. The observed decrease in plasma apoC-III was further confirmed by evaluating plasma apoC-III mass in non-treated and treated animals by e.l.i.s.a (Table 3). It is noteworthy that, in light of the very low K_m of lipoprotein lipase for its apoC-II activator, the decreased levels of plasma apoC-II in MEDICA 16-treated rats could still leave the lipoprotein lipase saturated by plasma apoC-II while being deinhibited by the decreased levels of plasma apoC-III.

The decrease in plasma apoC-III could have resulted from enhancement of its clearance, inhibition of its liver production, or both. To evaluate plasma apoC-III clearance, ^{125}I-apoC-III-labelled VLDL was injected into MEDICA 16-treated and non-treated rats, and the radioactivity in plasma apoC-III_0 and apoC-$III_{1,3}$ was studied as a function of time. It is noteworthy that, in light of the rapid exchange of apoC-III between plasma lipoproteins, VLDL just served here as a vehicle for apoC-III delivery, and the clearance rates observed relate to plasma apoC-III rather than VLDL apoC-III. As shown in Figure 4, the steady-state clearance of plasma apoC-III isoforms was not affected by MEDICA 16 treatment. In light of the lower level of plasma apoC-III in treated rats, the sustained clearance rates imply a decrease in the production and turnover of plasma apoC-III induced by MEDICA 16.

DISCUSSION

Treatment with MEDICA 16 of rats fed *ad libitum* or meal-fed was previously reported to result in decreased plasma VLDL triacylglycerol and VLDL cholesterol, being ascribed to inhibition of liver lipogenesis and cholesterogenesis by MEDICA 16 and its CoA thioester [2–4]. Since the hypolipidaemic effect with respect to VLDL was sustained under conditions of starvation, where the production of VLDL was already suppressed and could not be further inhibited by MEDICA 16 treatment [3], the observed hypolipidaemic effect implied activation of VLDL catabolism by MEDICA 16, in addition to inhibition of its liver production. Activation of VLDL catabolism by MEDICA 16 could also be indirectly implied by the previously reported activation of catabolism of plasma chylomicrons by MEDICA 16 treatment [23]. Short-term treatment with MEDICA 16 was indeed found here to result in an 8-fold increase in the FCR of plasma VLDL triacylglycerol and VLDL apoB (Figures 1 and 2). Since no effect on chylomicron-remnants clearance has been previously observed in MEDICA 16-treated animals [23], the effect of the drug on the clearance rate of triacylglycerol-rich lipoproteins present in the VLDL fraction is concerned with VLDL proper. The roles played by activation of plasma VLDL catabolism or inhibition of liver VLDL production in the overall hypolipidaemic effect of MEDICA 16 are presumably dependent on the prevailing dietary, physiological and hormonal setting which determines the flux of liver lipogenesis and cholesterogenesis.

Enhancement of plasma VLDL clearance by MEDICA 16 treatment may essentially be accounted for by activation of its hepatic uptake. Indeed, functional hepatectomy was found here practically to eliminate the increase in plasma VLDL clearance induced by MEDICA 16 (Table 2). Moreover, MEDICA 16 treatment was found here to activate directly the hepatic uptake of plasma VLDL under conditions close to single-pass liver perfusion (Figure 3). Furthermore, enhancement of plasma VLDL apoB clearance may implicate MEDICA 16 in enhancing the clearance of the VLDL particle as such, rather than its individual constituents. These lines of evidence may thus indicate that, in contrast with VLDL catabolism in non-treated animals, that prevailing in MEDICA 16-treated animals may be ascribed to premature hepatic uptake of whole VLDL particles before their extrahepatic lipolysis to VLDL remnants.

However, some lines of evidence may indicate that, in addition to premature hepatic uptake of VLDL particles, an extraheptic site of action may be involved in enhancing plasma VLDL clearance by MEDICA 16. Thus the FCR of plasma VLDL apoB was always observed to be significantly lower than that of plasma VLDL-triacylglycerol. Although this difference in clearances could be expected in non-treated animals, in light of the consecutive nature of VLDL catabolism being initiated by its intravascular lipolysis before hepatic uptake of the resulting VLDL remnants, premature hepatic uptake of VLDL particles could be expected to result in similar clearance rates for the apoB and triacylglycerol constituents. Also, whereas 35–50% of the injected triacylglycerol was found to be cleared from plasma during the first 1 min after its injection into MEDICA 16-treated animals, only 17% of the injected dose could be found to accumulate in the liver during that period of time (Figure 3). Furthermore, the FCR of plasma VLDL triacylglycerol in hepatectomized treated animals was 7.5-fold lower as compared with treated non-hepatectomized rats, but still 1.8-fold higher than in non-treated animals (Table 2), thus pointing to an extrahepatic site for MEDICA 16 action related to the catabolic fate of the triacylglycerol constituent of VLDL. The 1.8-fold activation of post-heparin-plasma lipolytic activity induced by MEDICA 16 treatment may account for the extrahepatic site of MEDICA 16 action.

ApoC-III has been repeatedly reported to inhibit metabolism of plasma triacylglycerol-rich lipoprotein as a result of inhibition of both their liver uptake [10–15] mediated by the apoB,E, apoE or related receptors, as well as their intravascular lipolysis by lipoprotein lipase [16–19]. The 3-fold decrease in plasma apoC-III induced by MEDICA 16 treatment (Table 3), due to suppression of apoC-III production (Figure 4), may thus offer the primary cause for activation of catabolism of plasma VLDL as well as

chylomicrons [23] in MEDICA 16-treated rats. Furthermore, hypertriglyceridaemia with decreased VLDL FCR induced by the human apoC-III transgene in mice [21] has recently been reported to mainly reflect inhibition of tissue uptake of VLDL rather than inhibition of lipoprotein lipase activity [22]. Similarly, hypotriglyceridaemia with increased plasma VLDL FCR induced here by pharmacological decrease in plasma apoC-III seems to result mainly from activation of hepatic uptake of VLDL rather than of lipoprotein lipase activity. Premature hepatic uptake of plasma triacylglyerol-rich lipoproteins due to suppression of apoC-III production may thus serve as a potential target for hypolipidaemic drugs.

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