Metabolism of Fatty Acid, Glycerol and a Monoglyceride Analogue by Rat Cardiac Myocytes and Perfused Hearts

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ABSTRACT

Studies have been conducted on the uptake and metabolism of unesterified fatty acid, free glycerol and 1-hexadecyl glyceryl ether by rat cardiac myocytes, and of fatty acid, intact triglyceride and the glyceryl ether by perfused rat hearts. Cardiac myocytes efficiently extracted, oxidized and esterified oleic acid, but demonstrated little ability to utilize free glycerol. Although the glyceryl ether was efficiently extracted by myocytes, it was neither hydrolyzed or esterified. The perfused heart also extracted and metabolized unesterified fatty acid, and the fatty acid released during lipolysis of circulating lipoprotein triglyceride. The glyceride glycerol, however, was largely recovered (90%) in the perfusate suggesting inefficient myocardial utilization of either free glycerol or partial glycerides. Myocardial extraction of glyceryl monoether was demonstrated, but the monoglyceride analogue was also unmetabolized by intact heart tissue. The results suggest that if monoglycerides are produced by the action of lipoprotein lipase on circulating triglycerides, reutilization of intact monoglycerides for higher glyceride synthesis is not a major fate of these products.
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INTRODUCTION

The intact heart can efficiently utilize the triglycerides of circulating chylomicrons and very low density lipoprotein (VLDL) (1-4). Available evidence suggests that these glycerides are partially or completely hydrolyzed at the capillary endothelial surface by the action of functional (membrane-supported) lipoprotein lipase (5,6). The released products are rapidly utilized by the tissue, since these do not appear to accumulate extracellularly during organ perfusion studies in vitro (6,7).

Free glycerol, resulting from the action of lipoprotein lipase, is not efficiently reutilized for glycerolipid resynthesis in heart muscle due to low levels of glycerokinase activity (8). Thus, synthesis of phospholipids and glycerides in heart muscle requires either the availability of α-glycerophosphate during glycolytic production of dihydroxyacetone phosphate, or reutilization of monoglyceride, as in the case of intestinal tissue. There are suggestive data (9) that, under certain conditions, the heart may extract intact glycerides. However, the fate of the extracted glyceride has not been determined. Thus, it has been difficult to assess whether monoglyceride formation and utilization in heart muscle may represent an important aspect of lipoprotein lipase activity in situ.

As an approach to assessing the possibility that intact glycerides may be utilized by heart tissue, the uptake and metabolism of 1-[1-^{14}C]hexadecyl glyceryl ether by the perfused rat heart and by rat cardiac myocytes have been compared to that of unesterified oleic acid, free glycerol and lipoprotein-associated triglyceride. These monoglyceride analogues have been effectively employed as model substrates for elucidating specific aspects of glycerolipid resynthesis in intestinal epithelial tissue. Since glyceryl monoethers are not hydrolyzed by lipases (10), their incorporation into di- and trialkoxy ether derivatives has been taken as a measure of monoglyceride metabolism in tissue (10, 11).

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MATERIALS AND METHODS

Materials

Trypsin (180 units/mg), collagenase (type 2, 136 units/mg) and lima bean trypsin inhibitor were obtained from Worthington Biochemicals (Freehold, NJ). Bovine serum albumin (fraction 5, fatty acid-poor) was purchased from Sigma Chemical Co., St. Louis, MO. Lipids (>99% purity) were from Supelco Corp., Bellefonte, PA, and labeled compounds were from Amersham-Searle Corp., Arlington Heights, IL. All other chemicals were of highest purity and purchased from Fisher Chemical Co., La Jolla, CA. Adult male Wistar rats were obtained from Charles River Laboratories (Wilmington, MA). The 1-[1-^{14}C]hexadecyl glyceryl monoether was kindly provided by Dr. R. Wykle,

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Lipoprotein Isolation and Labeling

Adult male rats (200-300 g) were allowed standard laboratory chow ad libitum, and were subjected to cannulation of the left thoracic lymphatic duct (12). Lymph was collected on ice for periods up to 24 hr during which animals were not provided food, but received a continuous intraduodenal infusion of 0.9% NaCl to maintain lymph flow. Large chylomicrons ($d<1.006$ g/ml) were removed by preparative ultracentrifugation of 3×10^6 g-avg min. The very low density lipoproteins (VLDL or small chylomicrons) were obtained at $d<1.006$ g/ml NaCl, dialyzed, and characterized by analysis of apolipoprotein composition using SDS-polyacrylamide disc electrophoresis (13) and analysis of lipid compositions (14).

The VLDL fraction was labeled with triglyceride in vitro by a modification of the method of Brenneman and Spector (15). This involved addition of glyceryl tri[1-^{14}C]oleate (30 μCi) and [2-^{3}H]glyceryl trioleate (150 μCi) to 30 g Celite and evaporation of solvents under nitrogen. Six ml of the VLDL preparation (0.5 mg protein/ml) were added to the Celite, the mixture was incubated for 2 hr at room temperature and the suspension was passed through 1.2-μm millipore filter. The filtered lipoprotein was washed with 1 mM albumin in saline to remove unesterified fatty acids resulting from the procedure and the resulting lipoprotein preparation contained 85-90% of the [^{14}C] and [^{3}H] labels in the core triglyceride fractions.

Heart Perfusions

Hearts from adult male rats were perfused by a modified Langandroff procedure in a closed recirculating apparatus, as described earlier (16). The perfusion media (20 ml) consisted of a modified Krebs bicarbonate buffer containing 0.5% albumin to which lipids were added as described for individual studies. The media was circulated in the apparatus for 5 min prior to insertion of heart. Aliquots of the perfusate were obtained at 0 and 45 min perfusion for analysis of $^{14}CO_2$ and lipids as described earlier (14,16,17). After perfusion, the coronaries were flushed with 5 ml of unlabeled media and hearts were blotted, weighed and homogenized in buffer for lipid extraction (18). For individual studies, perfusion media were prepared to contain: 400 μM unesterified fatty acid complexed to bovine serum albumin; freshly prepared lymph VLDL (400 μM fatty acid equivalent in triglycerides), containing [2-^{3}H]glyceryl tri[1-^{14}C]oleate; or 1-[1-^{14}C]hexadecyl glyceryl ether (60 μM) added in acetone to the albumin-containing media.

Preparation and Incubation of Heart Cells

The procedure for enzymatic dissociation of rat ventricular tissue was a modification of the described earlier (19). The buffered media for both tissue dissociation and resuspension of cells consisted of (g/l): NaCl, 6.8; KCL, 0.4; NaH_2PO, 0.21; Na_2HPO_4, 0.06; EDTA, 0.01; and glucose, 0.9. The pH was adjusted to 7.4 and osmolarity to 300 mOsm/l. Left ventricular tissue from adult rat hearts was minced and subjected to successive 20 min incubations in 2 ml 0.6% trypsin (2 x), 2 ml 0.3% trypsin inhibitor (1 x), and 2 ml 0.3% collagenase (4-5 x). Supernatants from each collagenase incubation were collected and maintained on ice; these were subsequently combined and filtered through 350-μm nylon mesh. The harvested cells were washed 2-3 times with buffer allowing cells to settle after each washing. These were resuspended in fresh buffer for cell counts and determination of viability (19).

For studies on lipid uptake and metabolism, the myocytes were diluted in 10 ml buffer containing 0.5% albumin to a final cell count 0.5×10^5 cells/ml in individual studies, incubation media were prepared to contain the following: 4 μmol [1-^{14}C]oleic acid (10 μCi) complexed to bovine serum albumin; 1.2 μmol [2-^{14}C]glycerol (50 μCi); 1.2 μmol 1-[1-^{14}C]-hexadecyl glyceryl ether (1 μCi) added in 5 μl acetone to the albumin-containing media;

Incubations were for 45 min at 37 C. Aliquots of the cell suspensions were obtained at 1 min and at 15-min intervals for determinations of $^{14}CO_2$ (20). Aliquots (1.5 ml) were also subjected to centrifugation for 4 min at 2 C to sediment cells, for direct isotopic analysis of cells and supernatant, or for lipid extraction and analysis of lipid fractions.

Analyses

$^{14}CO_2$ in the heart perfusion media and CO_2 trap (16), and in the myocyte incubation media, was assessed by the method of Cuppy and Crevasse (20). Lipids in heart perfusion and cell incubation media, and in the tissue preparations were extracted by the method of Folch et al. (18) using 20 vol of chloroform/methanol (2:1, v/v). After evaporation of the chloroform extract under N_2 and reextraction of lipids in hexane, aliquots of the hexane extract were subjected to thin layer silicic acid chromatography in either hexane/diethyl ether/acetic acid (80:16:2, v/v) or hexane/acetone/acetic

acid (89:11:3, v/v). The latter system has been effectively employed to separate alkoxy derivatives of glycerolipids (11). Lipid classes were identified by comigration with authentic standards. Individual silicic acid areas corresponding to cholesteryl esters, triglycerides (or alkoxy diglycerides), unesterified fatty acids, mono- and diglycerides (or alkoxy monoglycerides and unmetabolized glyceryl ether) and phospholipid were scraped into vials for subsequent isotope analysis by liquid scintillation spectrometry (11).

RESULTS

Heart Perfusion Studies

The comparative myocardial extraction and metabolism of free and esterified fatty acid, glyceride glycerol and the glyceryl monoether is summarized in Table 1. During the 45-min recirculating perfusion of 8 μmol (0.4 mM) albumin-bound oleic acid, 2.5 μmol, or 31.2 ± 4.0%, was extracted by the intact heart. Of this, 0.6 μmol, or 24.1 ± 2.8%, was completely oxidized to $^{14}CO_2$ and the remainder was accounted for as tissue lipid (33.1 ± 1.5%) and water-soluble metabolites (42.8 ± 4.0%). Ca. 91% of the lipid radioactivity from [1-^{14}C]-oleate was recovered as esterified lipids and primarily as triglyceride. These levels of tissue extraction, oxidation and distribution of unesterified fatty acids are comparable to those reported earlier (19,21) using an identical approach.

We have recently reported on studies using VLDL labeled in the core lipids with [2-^{3}H]-glyceryl tri[1-^{14}C]oleate (22). It was shown that the correlation coefficient of disappearance of triglyceride mass and label from VLDL during heart perfusion is 0.96 ± 0.04, demonstrating the utility of this in vitro labeled model for studies on triglyceride lipolysis.

In the present studies, perfusion of hearts with VLDL for 45 min resulted in lipolysis of 32.5 ± 1.6% of the available triglyceride fatty acid. Of this (2.6 μmol), 2.4 μmol, or 92.3%, was extracted by the heart and the remainder was recovered as unesterified fatty acid in the perfusing media. Ca. one-third (29.8%, 0.72 μmol) of the extracted oleic acid was oxidized to $^{14}CO_2$, and only 7.6% was recovered in tissue lipids (Table 1). The remainder was accounted for as water-soluble metabolites, presumably derived from oxidation of oleic acid. The distribution of the extracted oleate among tissue lipids was comparable to that observed during perfusion of unesterified oleic acid.

During lipolysis of VLDL triglycerides by

TABLE 1

Extraction and Metabolism of Oleic Acid, VLDL Triglyceride and Glyceryl Monoether by Perfused Rat Hearts

Substrate[a]	Lipolysis (μmol/g)	Tissue uptake (μmol/g)	Oxidation to CO_2 (μmol/g)	Distribution of tissue lipid radioactivity				
				UFA[b] (%)	TG (%)	MG-DG (%)	PL (%)	Other (%)
[1-^{14}C]Oleic acid (8 μmol)	–	2.500 ±0.32	0.60 ± 0.07	7 ±2	80 ±2	7 ±1	4 ±0.3	–
Glyceryl tri[1-^{14}C]oleate (8 μmol)	2.60 ± 0.13	2.420 ±0.13	0.72 ± 0.14	12 ±1	71 ±1	11 ±1	6 ±2	–
[2-^{3}H]Glyceryl trioleate (2.4 μmol)	0.89 ± 0.09	0.083 ±0.010	0	0 ±0	70 ±4	22 ±2	7 ±3	
1-[1-^{14}C]Hexadecyl glyceryl ether (1.2 μmol)	–	0.700 0.16	0	0 ±0	2 ± 0.5	4 ±0.1	0 ±0	94[c] ± 1

[a]Recirculating perfusion of intact rat heart was conducted for 45 min in a closed system (16), using the substrates indicated, in 20 ml Krebs buffer, pH 7.4, containing 0.5% bovine serum albumin. Preparation of the fatty acid and glyceryl monoether substrates are described under Methods. Labeled glyceryl trioleate was incorporated into VLDL core lipids as described under Methods. Data represent means from 4-6 studies ± SEM.

[b]Abbreviations: UFA, unesterified fatty acid; TG, triglyceride; MG-DG, mono- and diglycerides; PL, phospholipids.

[c]Recovered as authentic 1-hexadecyl glyceryl monoether.

the perfused heart, 0.89 μmol of [2-^{3}H] glycerol (37%) was hydrolyzed from [2-^{3}H] glyceryl trioleate. Of this, 90.3 ± 15.1% was recovered in the perfusate (Table 1) and only 5.3 ± 0.2% was accounted for in tissue lipids (Table 1). The remainder (4.0 ± 1.1%) was recovered as nonlipid glycerol radioactivity. Analysis of the distribution of lipid glycerol (Table 1) indicated that ca. 92% was as partial glycerides and triglyceride and the remainder as phospholipid glycerol. No radioactivity was recovered as unesterified fatty acid. Thus, overall, the ratio of VLDL triglyceride fatty acid release to glycerol release was 2.92, whereas the ratio of tissue extraction of fatty acid and glycerol was 28.9, or almost 10-fold. These data are also compatible with those reported earlier (21).

Recirculating perfusion of [1-^{14}C] hexadecylglyceryl-1-monoether for 45 min resulted in a extensive tissue extraction representing 58% of the available glyceride analogue. Based on analysis of the perfusates and tissues, there was no lipolysis of the ether (e.g., no unesterified hexadecyl alcohol or fatty acid) and there was no oxidation of the labeled hexadecyl moiety. Thus, the extracted label was completely recovered in tissue lipids, and of this, 94% was recovered as the unmetabolized ether. The remainder was distributed in chromatographic fractions corresponding to partial glycerides (4%) and triglycerides (2%).

Cell Studies

Studies with cardiac ventricular myocytes were conducted only for qualitative comparisons of tissue lipid extraction and metabolism. Since we have previously demonstrated that these cells are unable to metabolize VLDL triglycerides (21), comparative studies were conducted using unesterified oleic acid, glycerol and the glyceryl monoether. During 45-min incubations of cells (5 × 10^5 cells/ml) with [1-^{14}C] oleic acid, total cellular extraction of the fatty acid (Table 2) was 18.3 ± 2.2 nmol/mg protein (24.8 ± 3.1% of the available fatty acid). Of this uptake, 2.85 ± 0.40 nmol/mg protein, or ca. 16%, was oxidized to $^{14}CO_2$ by the end of incubation. As shown in Figure 1, the extracted fatty acid was rapidly esterified, primarily to triglycerides, during the course of incubation, and by 45 min, only 12% was recovered as unesterified fatty acid (Table 2).

When myocytes were incubated with [2-^{14}C] glycerol, only 0.4 ± 0.08% was extracted by the cells and none of this appeared as $^{14}CO_2$ (Table 2). Analysis of the cellular radioactivity after the 45-min incubation showed that 90% of the extracted glycerol was recovered as

TABLE 2

Uptake and Metabolism of Oleic Acid, Glycerol and Glyceryl Ether by Rat Cardiac Myocytes

Substrate[a]	Cellular uptake (nmol/mg protein)	Oxidation to Co_2 (nmol/mg protein)	Distribution of cellular lipid radioactivity				
			UFA[b] (%)	TG (%)	MG-DG (%)	PL (%)	Other (%)
[1-^{14}C] Oleic acid (4 μmol)	18.3 ±2.2	2.85 ± 0.40	12 ± 2	78 ±4	4 ± 0.3	8 ± 2	–
[2-^{14}C] Glycerol (1.2 μmol)	0.089 ±0.018	0	0 ± 0	42 ± 2	39 ± 2	19 ± 2	–
[1-^{14}C] Hexadecyl glyceryl ether (1.2 μmol)	3.45 ±0.02	0	1 ± 0.5	1 ± 0.2	0 ± 0	0 ± 0	98[c] ± 1

[a]Cardiac myocytes (5 × 10^5 cells; 53.9 mg protein; 10 ml) were incubated for 45 min at 37 C with 400 μM oleic acid albumin bound, 120 μM glycerol or 120 μM glyceryl monoether. Incubations were conducted in sealed vessels (95% O_2, 5% CO_2) containing hyamine hydroxide in center wells for analysis of $^{14}CO_2$ (20). Cells and media were individually analyzed for lipids or glycerol as described under Methods. Figures represent mean of 3-4 studies ± SEM.

[b]Abbreviations: UFA, unesterified fatty acid; TG, triglycerides; MG-DG, mono- and diglycerides; PL, phospholipids.

[c]Recovered as unmetabolized 1-hexadecyl glyceryl ether.

nonlipid glycerol. The 10% associated with tissue lipids (0.009 μmol/mg protein) was entirely as esterified lipids, of which ca. 80% was as partial glycerides and triglycerides (Table 2). The time course of esterification of these small amounts of glycerol into glycerides and phospholipid is shown in Figure 2.

Incubation of myocytes with the glyceryl monoether resulted in cellular extraction of 15.5 ± 0.1% (3.45 ± 0.02 nmol/mg protein) of the available glyceride analogue. Of this uptake, none was recovered as $^{14}CO_2$ during the 45-min incubation. As in the case of the perfused organ, the extracted radioactivity was completely recovered as tissue lipid, and of this, 98% was associated with the chromatographic area corresponding to the authentic glyceryl monoether.

DISCUSSION

Previous studies have demonstrated the low levels of heart glycerokinase, and the inefficient metabolism of free glycerol by myocardial tissue (8). This is also clearly demonstrated in the present studies using both the intact organ model and isolated cardiac myocytes. With cardiac myocytes, utilization of free glycerol was less than 0.5% of the available substrate (1.2 μmol). In contrast, incubations with unesterified oleic acid, at a level approximating a 3:1 ratio to glycerol, was efficiently extracted (24.8%) and rapidly incorporated into esterified lipids. Thus, the relative utilization of fatty acid to glycerol was 200:1, despite an initial concentration ratio of 3:1.

During the perfusion of hearts with VLDL containing [2-^{3}H]glyceryl trioleate, there was essentially no accumulation of [^{3}H] partial glycerides in the perfusion medium, and by 45 min, over 90% of the label appeared in the perfusate as water-soluble tritium. From these studies, it could not be determined whether total hydrolysis of triglyceride occurred by the action of membrane-associated LPL, or that partial glycerides were produced, extracted by the heart and subsequently hydrolyzed by tissue lipases. In either case, only 5.3% of the triglyceride glycerol released during perfusion was recovered in tissue lipids, and this was largely as partial glycerides and triglycerides.

In contrast to the inefficient utilization of glyceride glycerol by the intact heart, the released triglyceride fatty acid was efficiently extracted (92%) and utilized by perfused heart. Thus, at best, utilization of the triglyceride fatty acid was almost 29-fold that of the glycerol moiety. These data collectively suggest that either partial glycerides are not a major

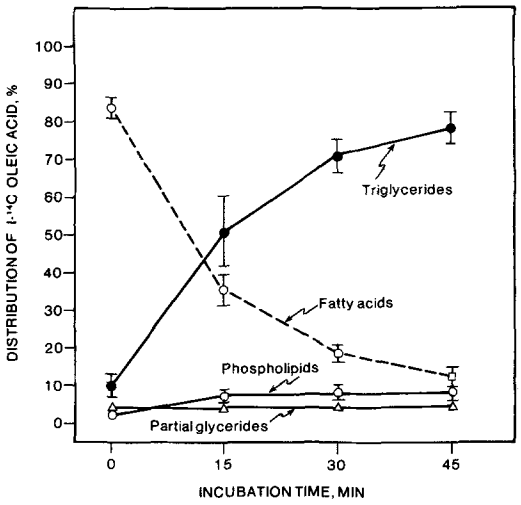

FIG. 1. Esterification and distribution of [1-^{14}C]-oleic acid during incubations with adult rat cardiac myocytes. Cells (5 X 10^5 cells/10 ml) were incubated with 4 μmol oleic acid complexed to bovine serum albumin for 45 min at 37 C in air. At the times indicated, aliquots were removed for reisolation of cells, lipid extraction and separation, and analysis of recovery and distribution of radioactivity.

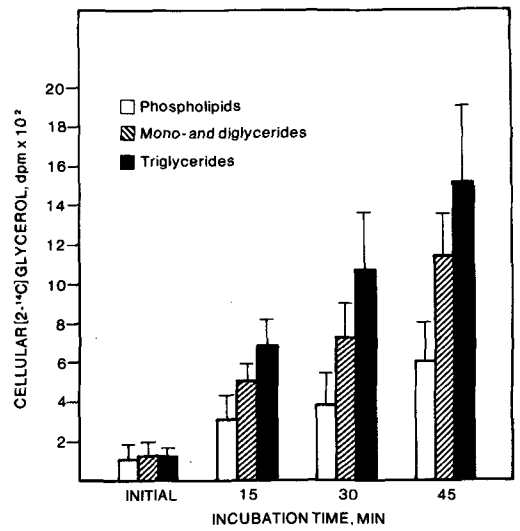

FIG. 2. Esterification and distribution of [2-^{14}C]-glycerol during incubations with rat cardiac myocytes. Cells (5 X 10^5 cells/10 ml) were incubated with 1.2 μmol glycerol for 45 min at 37 C in air. At the times indicated, aliquots were removed for reisolation of cells and lipid extraction, separation and distribution of radioactivity.

end product of LPL action in situ, or that, if they are produced and extracted by the heart, the glycerides are subsequently hydrolyzed rather than utilized by a monoacylglycerol

pathway.

Studies with the glyceryl monoether were predicated on their physicochemical similarity to monoglycerides and on their resistance to lipolysis in other model systems (10). Similar analogues have been effectively empolyed to elucidate specific aspects of the monoglyceride pathway in intestine (10,11). In the present studies, both the isolated cell model and the perfused heart were able to extract the glyceryl monoether from the respective media. With both tissue preparations, 94-98% of the tissue associated with radioactivity was recovered as unmetabolized ether, and at best, only 6% was incorporated into higher glyceride analogues. However, there is no direct evidence that the monoether was quantitatively internalized by cardiac cells or by the perfused heart.

Although the quantitative data from the tissue models empolyed in these studies should not be compared directly, the overall results suggest that cardiac tissue does not efficiently utilize either free glycerol or monoglycerides for subsequent formation of higher glycerides.

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