Pattern of response to feeding and fasting of heparin-releasable lipoprotein lipase in rat cardiomyocytes

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The regulatory events whereby the amount of secreted heart lipoprotein lipase decreases post-prandially and increases during fasting are unclear. We examined whether the nutritional state influenced the lipolytic activities that hydrolyze tri-, di-, and monoacylglycerol as membrane-associated enzyme in rat cardiomyocytes. Properties of triacylglycerol lipase are typical of lipoprotein lipase whereas diacylglycerol and monoacylglycerol lipase activities hydrolyze the products of lipoprotein lipase action. We observed that: (1) membrane-bound activity levels assayed at the cell boundary were high for MAGL and much lower for TAGL and DAGL, regardless of whether cells originated from fasted or fed rats; (2) the stimulatory effects of serum were likewise similar in the fasted and the fed states; (3) isolated cardiomyocytes exhibited no constitutive secretion of active enzyme; and (4) factors determining the variations in amounts of heparin-releasable enzyme in response to nutritional changes appeared to be related to the pre-existing high (in the fasted state) or low (in the fed state) intracellular content in enzymatic activities, supporting the proposal that the secretion of active lipoprotein lipase involves disruption of intracellular vesicles and exocytosis of the enzyme, without its accumulation in the plasma membrane. On a functional basis, the results emphasize the heterogenous nature of the LPL enzymatic complex.

Keywords: heart lipoprotein lipase; heparin; serum; nutritional state; rat

Introduction

In the heart, lipoprotein lipase (LPL) is important for supplying cells, mainly cardiomyocytes, with fatty acids that can support most of the energy requirement of the heart muscle.[1] LPL hydrolyzes triacylglycerol transported in the plasma by chylomicrons and by very low density lipoproteins[2]; it preferentially splits the ester bond at the position *sn*-1 of its substrate and is also active toward other fatty esters.[3] This relatively broad substrate specificity is consistent with the assay in post-heparin heart effluents of three lipolytic activities that are located at the same endothelial site, where they catalyze the sequential degradation of triacylglycerol.[4] Triacylglycerol lipase (TAGL) exhibits all of the highly regulated properties of LPL, whereas diacylglycerol (DAGL) and monoacylglycerol (MAGL) lipase activities exhibit a lesser functional dependence on nutritional stimuli.[5]

LPL is synthesized in particularly abundant quantities in parenchymal cells of adipose and muscular tissues. The enzyme appears to be localized in an inactive form in the endoplasmic reticulum and becomes activated via linkage of N-linked oligosaccharide chains in the Golgi apparatus.[6] LPL is regulated in a tissue-specific manner: post-prandially, LPL activity is elevated in adipose tissue compared with heart and muscle, whereas the reverse is true during fasting.[7] The mechanism of the physiological dependence of LPL on the feeding/fasting cycle is poorly understood. The functional fraction of the enzyme exerts its role at the luminal surface of the capillary endothelial cells, from which it can be released by heparin.[8] Whereas numerous recent studies have dealt with the properties of the enzyme either at its intracellular sites or in post-heparin effluents, little is known about the mechanism

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of the enzyme secretion. It has been postulated that the heparin-releasable functional enzyme originates from a plasma membrane pool in equilibrium with an intracellular pool of enzyme contained in transport vesicles.[9] In the present study, we investigated the enzyme fraction associated with the plasma membrane of cardiomyocytes isolated from the adult rat heart, and we evaluated the influence of the nutritional state on this enzyme pool.

Materials and methods

Isolation of cardiomyocytes

Female Sprague-Dawley rats (300 g) from IFFA-CREDO (69210 L'Arbresle, France) were used. They were housed at 22° C in a controlled light-dark environment. They had free access to water and a standard rat diet (UAR, 91000 Villemoisson sur Orge, France) containing, by wt, 5% lipid, 49.5% carbohydrate, and 23.5% protein. Animals were allowed to acclimate to their environmental conditions for at least 2 weeks before the experiments. They were killed either at the end of the dark period (fed animals) or after a 24-hr fast by cervical dislocation under light anesthesia (pentobarbital 25 mg/kg body wt, intraperitonally). Cardiomyocytes were immediately isolated according to published procedures.[9,10] Hearts were perfused via the aorta, without recycling, with Ca^{2+}-free Joklik-minimum essential medium (pH 7.4) supplemented with 1 mmol/L carnitine (Serva, Heidelberg, Germany) and 1.2 mmol/L $MgSO_4$ (buffer 1).[11] The perfusion medium, maintained at 37° C, was continuously oxygenated by a stream of O_2/CO_2 (95/5, vol/vol). The nonrecirculating perfusion was continued for 5 min under a hydrostatic pressure of 58 mm Hg. Then, buffer 1 was supplemented with defatted albumin (0.1%, wt/vol; Boehringer, Mannheim, Germany) and 0.5 mg/mL collagenase (Type I, 280 mU/mg, Sigma Chemical Co., St. Louis, MO, USA; buffer 2). Hearts were perfused for 15 min with recirculation of 25 mL of buffer 2, then minced and rinsed twice with buffer 1 supplemented with 1.5 mmol/L $CaCl_2$ and 1% (wt/vol) albumin (buffer 3).[12] Heart tissue residues were incubated for 10 min in buffer 2 (5 mL/g wet tissue) containing 1 mg/mL collagenase, and the dispersed cells were sieved through a nylon mesh (250 μm). The cell suspensions were pooled and centrifuged at 50*g* for 2 min. The pellets were resuspended in buffer 1. To remove cell debris and Ca^{2+}-intolerant cells, isolated cardiomyocytes were allowed to settle twice for 10 min in buffer 3 containing 4% (wt/vol) albumin.[13] The final cell pellet was resuspended in buffer 3 and the suspension was used as the enzyme source. Cardiomyocytes were counted in a hemocytometric chamber with a light microscope. Cells were considered to be viable if simultaneously they appeared with their typical rod shape and excluded trypan blue dye. During lipolytic assays, cellular integrity was checked by measuring the amount of lactate dehydrogenase (LDH) activity[14] released in the incubation medium relative to the total activity contained in the homogenized cells. The inactivating effect of collagenase (1 mg/mL) was tested for 10 min at 37° C on the heparin-released enzyme and was found to be <12% for TAGL and DAGL, and <7% for MAGL; it was <10% for all cell-bound activities.

Assay of lipolytic activities

All activities were assayed in duplicate for 15 min at 37° C in Fain's medium[15] containing 20 mmol/L Hepes (Sigma) instead of sodium phosphate, to which was added 1% (wt/vol) defatted albumin (B grade, Boehringer) at pH 8.0 (buffer A). For the assay of TAGL activity, emulsified tri-[^{3}H] oleoylglycerol (1 mmol/L) was used as the substrate and the reaction was started by adding 0.05 mL (~30 μg protein) of cardiomyocyte suspension (about 0.9 × 10^6 cells) or 0.05 mL of heart perfusate as the enzyme sources, in a final volume of 0.5 mL at 37° C. Unless otherwise stated, TAGL assays were performed in the presence (10% by vol.) of serum from fasted rats inactivated at 55° C for 20 min. After incubation, the amount of released [^{3}H] oleic acid was extracted and quantified using a liquid-liquid partition system as previously described.[10] The difference between the rates of release of fatty acids in serum-activated and in basal media expressed serum-dependent TAGL, typical of LPL activity. DAGL and MAGL activities were assayed in buffer A containing emulsified di-[^{3}H], or mono-[^{3}H] oleoylglycerol as the substrate at a final concentration of 1 mmol/L (pH 7.4); no serum was added as cofactor, unless otherwise stated. After incubation, the media were treated as for the TAGL assay. Cardiomyocytes in suspension hydrolyzed tri-, di-, and mono-[^{3}H] oleoylglycerol according to zero order kinetics for at least 15 min. One milliunit (mU) of lipase activity corresponds to the release of one nmol of acid per min. Enzymatic activities are expressed as means ± SEM and the significance of the differences between means was determined by Student's *t* test, with *P* values <0.05 considered as significant.

Materials

Tri-[9,10-^{3}H] oleoylglycerol (1.0 Ci/mmol) and [9,10-^{3}H] oleic acid (10 Ci/mmol) were purchased from Amersham (Buckinghamshire, U.K.). Di-[9,10-^{3}H] oleoylglycerol, and mono-[9,10-^{3}H] oleoylglycerol were synthetized in the laboratory by conventional methods and purified by a two-step procedure involving chromatographies on Florisil[16] and on thin-layer silicagel plates. The substrates were >98% radiochemically pure as established by scanning in an automatic thin-layer chromatography linear radioactivity analyzer (LS 283-2, Berthold, Wildbad, Germany). Dioleoylglycerol had a composition of about 40% 1,2- and 60% 1,3-diacyl-*sn*-glycerols. Monooleoylglycerol was >90% 1(3)-monoleoyl-*sn*-glycerol. The substrate emulsions containing ^{3}H-labeled acylglycerols were prepared by sonication at room temperature with a Branson sonicator (model B-12, Heat System-Ultrasonics, Plainview, N.Y. USA) with four bursts lasting 15 sec each, at setting 4 (approx. 30 W).

Results

The number of cardiomyocytes isolated from one rat heart amounted to an average of 10^7 cells (n = 15) equivalent to ~ 65 ± 11 mg (dry wt). On the basis of a wet wt:dry wt ratio of 4.35,[17] and a mean wet wt of 0.9 ± 0.2 g (n = 15) per heart, the yield in cardiomyocytes was 30%. Cell viability was 76 ± 8.4% (n = 18). Cells spontaneously beating at low frequency were <3%. During incubation, cardiomyocytes released LDH activity in amounts representing approximatively 0.5% per min of the total LDH cell content.

Cardiomyocytes in suspension displayed TAGL, DAGL, and MAGL activities that were essentially cell-bound, as inferred from the very low amounts of activities spontaneously released during incubation, and assayed in the cell-free medium; the latter amounted

Table 1 Cell-bound tri-, di-, and monoacylglycerol lipase activities in suspensions of cardiomyocytes isolated from rat in the fed or fasted states

Feeding state	Hydrolyzing activities (mU/10^6 cells) [a] toward		
	Triacylglycerol[b]	Dioleoylglycerol	Monooleoylglycerol
Fasted	3.1 ± 1.4 (10)	2.8 ± 0.3 (10)	80.1 ± 4.1 (10)
Fed	3.3 ± 0.7 (18)	2.6 ± 0.5 (14)	71.2 ± 15.1 (10)

[a]Results are means ± SEM for the numbers of experiments given in parentheses.
[b]TAGL activity was measured in the presence of inactivated rat serum (10% by vol.) as protein cofactor.

for TAGL, DAGL, and MAGL, respectively, to 1.0, 0.9, and 1.6% per min in the fasting state, and 0.6, 0.6, and 1% per min in the fed state, relative to the total amount of each activity assayed in the complete (cells + fluid) incubation mixture. These percentages are in the range of those expressing the LDH release (~ 0.5% per min) in the same samples, and therefore are likely to reflect cell damage during incubation rather than any secretory process.

As shown in *Table 1,* cell-bound activity levels were maximum for MAGL and much lower for TAGL and DAGL, without regard to whether cells originated from fasted or fed rats. Compared to basal (no serum) values, cell-bound TAGL activity was stimulated seven- to ten-fold by serum as compared to approximately four-fold for DAGL and two-fold for MAGL *(Figure 1);* again there was no significant difference in stimulation factor as a function of the nutritional state for any of the three activities. In no case were the stimulatory effects associated with the release of enzyme.

By contrast, addition of heparin to the cell suspension *(Figure 2)* caused the immediate release in the medium of amounts of activities that were significantly higher in the fasted than in the fed state for the three activities. Regardless of the nutritional state, MAGL activity was released to a much lesser extent than TAGL and DAGL.

Discussion

During short-term incubation, isolated cardiomyocytes hydrolyzed tri-, di-, and monoacylglycerol exposed to the cells in the emulsified state. In agreement with other studies using various heart tissue preparations as

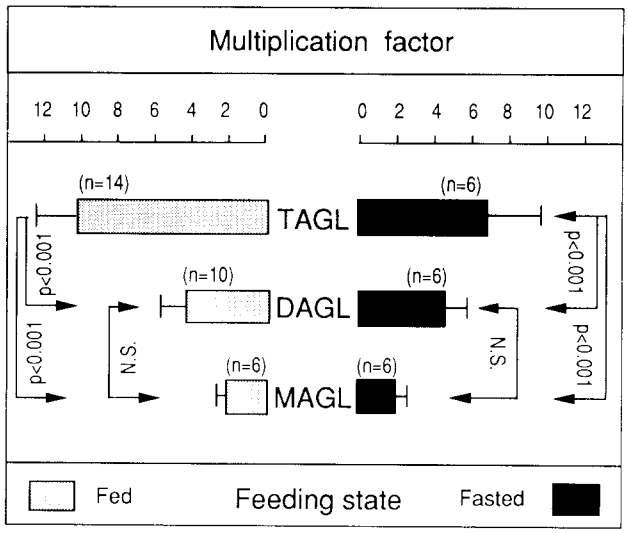

Figure 1 Effect of rat serum on trioleoylglycerol (TAGL), dioleoylglycerol (DAGL) and monooleoylglycerol (MAGL) lipase activities of isolated rat cardiomyocytes. Rat serum was a pool of serum of fasted rats. Cells were obtained from rats fasted for 24 h or from fed rats. The multiplication factor was defined as the ratio of enzyme activity measured with added serum (10 % by volume) to that without serum, taken as baseline (zero) value. Baseline values (mU/10^6 cells) in fed and fasted states were respectively: 2.4 ± 0.8 and 2.8 ± 0.9 for TAGL, 2.6 ± 0.4 and 2.1 ± 0.2 for DAGL and 60 ± 10 and 78 ± 41 for MAGL activities. Values are means ± SE from 6–14 determinations.

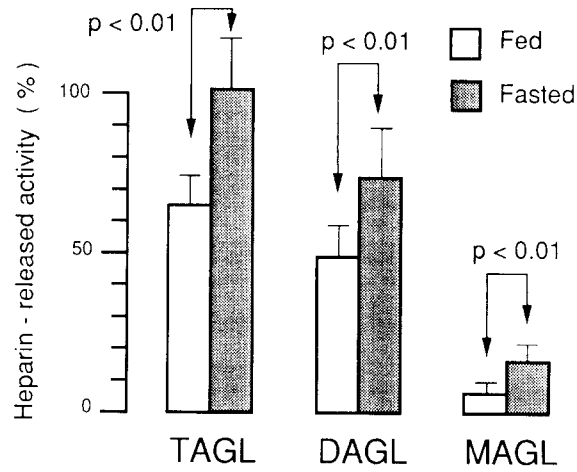

Figure 2 Effect of heparin on the release of tri-, di- and monoacylglycerol lipase activities from rat cardiomyocytes as a function of the nutritional state. Cardiomyocytes were isolated from hearts of fed (n = 5) and fasted (n = 5) rats. Each individual cell suspension was used for determinations in quadriplicate of cell-bound and heparin-released activities. Cells were incubated for 10 min at 37° C with or without heparin (16 U/ml); after centrifugation (90 g for 3 min), the supernatant was removed and used for assays of TAGL DAGL and MAGL activities. Each amount of heparin-released activity was compared to the corresponding amount of cell-bound activity, defined as the difference between total (cells + fluid) and spontaneously (no heparin)-released activity. Basal levels (mU/10^6 cells) of cell-bound activity, taken as 100 %, were respectively in the fed and fasted states: 2.5 ± 0.8 and 3.0 ± 0.4 for TAGL, 2.9 ± 0.8 and 4.1 ± 0.5 for DAGL, and 92 ± 7 and 93 ± 4 for MAGL. Differences between means were analyzed using the Student's t-test.

the enzyme sources, we found that TAGL assayed at the cell surface was stimulated several-fold by serum, a property typical of LPL; DAGL and MAGL were also stimulated, but to a lesser extent, as was the case in post-heparin effluents.[5] In contrast with results in isolated adipocytes,[10] the fed-to-fasted transition influenced neither the basal levels of membrane-bound TAGL, DAGL, and MAGL, nor the extent to which serum stimulated any of these activities. These observations suggest that: (1) activities assayed at a given time as membrane-bound enzyme in cardiomyocytes do not reflect the amounts of functional enzyme; the latter amounts, equated with the amounts of activities assayed in post-heparin heart effluents, were found to be 3.5-fold higher for TAGL and DAGL, and 1.9-fold higher for MAGL in fasted compared with fed rats[5]; (2) under the experimental conditions, isolated cardiomyocytes exhibit no constitutive secretion of active enzyme, in agreement with observations made with mesenchymal rat heart cells in culture[18]; (3) factors determining feeding state-dependent activity changes in post-heparin effluents appear to be related to the metabolic state of the cells, i.e., their pre-existing high (in the fasted state) or low (in the fed state) intracellular content in immediately heparin-releasable enzyme. This supports, for cardiomyocytes isolated from adult animals, the proposal essentially derived from studies with cultured adipose cells, that potentially active LPL is stored within intracellular vesicles that disrupt in response to heparin and supply the enzyme for secretion.[19,20]

Taken together, the results suggest that among the factors that control the secretion of functional LPL activity in rat heart, one type should modulate the amount of potentially active intravesicular enzyme so that it is high or low in the fasted or fed states, respectively; whether this control takes place pre-,[21] post-translationally,[19,20,22] or both is still uncertain. Another type of factor(s) appears to trigger the export of the stored activities according to the needs of the tissue; whether the latter stimulus is physiologically heparin (that does not cross plasma membranes) or some heparin-like component is unclear.

Confirming earlier results obtained with post-heparin heart effluents,[4,5] MAGL was released by heparin in much lower amounts than TAGL and DAGL, suggesting that the major part of MAGL activity assayed as membrane-bound enzyme *(Table 1)* might correspond to a distinct heart lipase hydrolyzing specifically monoacylglycerol.[23] On a functional basis, the results therefore emphasize the heterogenous nature of the LPL enzymatic complex.

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